



(51) International Patent Classification:

A61K 47/60 (2017.01) A61P 11/00 (2006.01)
A61P1/00 (2006.01)

(21) International Application Number:

PCT/US20 19/042823

(22) International Filing Date:

22 July 2019 (22.07.2019)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/702,273	23 July 2018	(23.07.2018)	U S
62/723,347	27 August 2018	(27.08.2018)	U S
62/732,538	17 September 2018	(17.09.2018)	U S
62/774,726	03 December 2018	(03. 12.2018)	U S
62/847,236	13 May 2019	(13.05.2019)	U S

(71) Applicant: SIENNA BIOPHARMACEUTICALS, INC.

[US/US]; 30699 Russell Ranch Road, Suite 140, Westlake
Village, California 91362 (US).

(72) Inventors: TRAVERSA, Silvio; c/o Sienna Biopharma-

ceuticals, Inc., 30699 Russell Ranch Road, Suite 140, West-
lake Village, California 91362 (US). MAINERO, Valenti-
na; c/o Sienna Biopharmaceuticals, Inc., 30699 Russell

Ranch Road, Suite 140, Westlake Village, California 91362
(US). BAGNOD, Raffaella; c/o Sienna Biopharmaceuti-
cals, Inc., 30699 Russell Ranch Road, Suite 140, Westlake
Village, California 91362 (US). HARRIS, Todd James;
c/o Sienna Biopharmaceuticals, Inc., 30699 Russell Ranch
Road, Suite 140, Westlake Village, California 91362 (US).
RAVA ROSSA, Luisa Bertarione; c/o Sienna Biopharma-
ceuticals, Inc., 30699 Russell Ranch Road, Suite 140, West-
lake Village, California 91362 (US).

(74) Agent: CULLMAN, Louis C. et al.; K&L Gates LLP, 1

Park Plaza, Twelfth Floor, Irvine, California 92614 (US).

(81) Designated States (unless otherwise indicated, for every
kind of national protection available):

AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available):

ARIPO (BW, GH,

(54) Title: USES OF POLYMER CONJUGATES OF INDOLOCARBAZOLE COMPOUNDS WITH REDUCED EXPOSURE

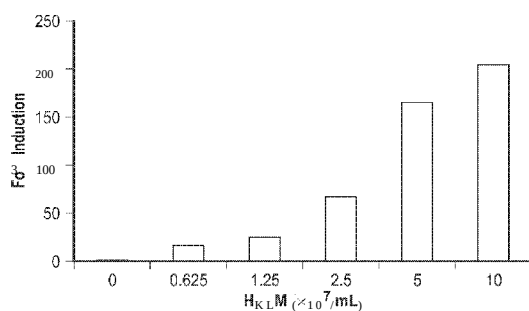
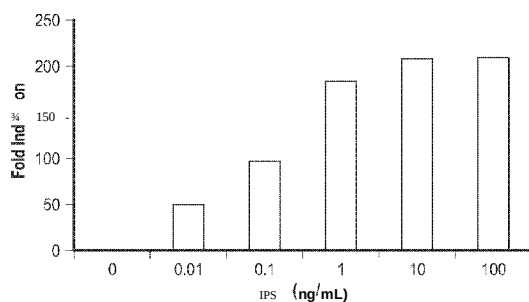


FIG. 1

(57) Abstract: Disclosed herein are therapeutic uses of polymer con-
jugates, including SNA-120 and SNA-125, comprising an active agent
linked to a polymer, wherein the active agent comprises an indolocar-
bazole compound or derivative thereof. The disclosed polymer conju-
gates reduce exposure of the active agent at non-target sites.

GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

**USES OF POLYMER CONJUGATES OF INDOLOCARBAZOLE COMPOUNDS WITH
REDUCED EXPOSURE
INCORPORATION BY REFERENCE**

[1] This application claims the priority of U.S. Provisional Patent Application No. 62/847,236, filed May 13, 2019, U.S. Provisional Patent Application No. 62/774,726, filed December 3, 2018, U.S. Provisional Patent Application No. 62/732,538, filed September 17, 2018, U.S. Provisional Patent Application No. 62/723,347, filed August 27, 2018, and U.S. Provisional Patent Application No. 62/702,273, filed July 23, 2018, the entire content of each of which is incorporated herein by reference.

FIELD

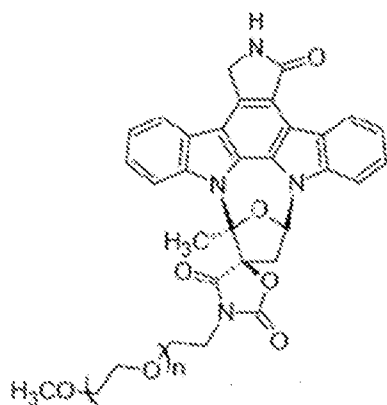
[2] Disclosed herein are therapeutic uses of polymer conjugates, comprising indolocarbazole active agents linked to polymers.

BACKGROUND

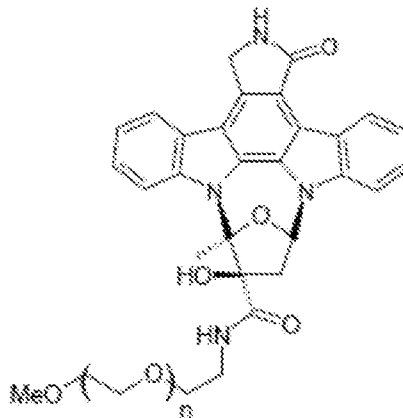
[3] Polymer conjugates of indolocarbazoles, including SNA-120 (also known as CT-327) and SNA-125 (also known as CT-340) have been described for possible therapeutic use in the prevention, alleviation and treatment of certain kinase-associated pathologies. Additional clinical indications may be found.

SUMMARY OF EMBODIMENTS

[4] In several embodiments, new uses for the polymer conjugates, SNA-120 and SNA-125, shown below are provided.



SNA-120



SNA-125

[5] Effective delivery of pharmacologically active agents may be hindered by unwanted exposure of those agents to non-desired locations (such as the systemic circulation and/or lymphatic system). For example, topical agents useful in treating various skin disorders may result in toxic side effects because of systemic exposure. One issue with delivering compositions comprising one or more active agents topically (or non-topically) is the concern that such agents need to be delivered in an amount and at a location sufficient to have a

therapeutic effect. At the same time however, exposure (e.g., absorption or longevity of the composition in the systemic circulation, lymphatic system, or other non-targeted sites) may not be desirable for multiple reasons, including, but not limited to, safety reasons. There remains an unmet need for compounds with reduced exposure at non-target sites that result in a clinically therapeutic effect.

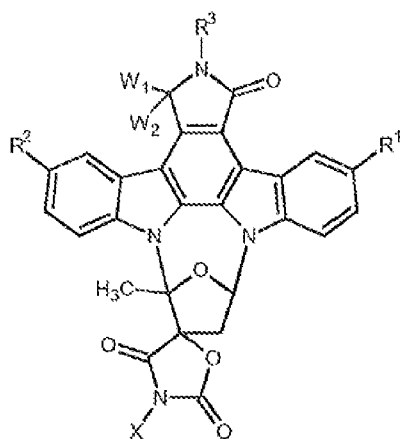
[6] In several embodiments of the invention, the compositions described herein are both therapeutically efficacious and minimize non-target (e.g., systemic or bloodstream) exposure. In some embodiments, the active agents are PEGylated or otherwise coupled to large molecules, and surprisingly, are effective in crossing biological membranes such that the active agents are effectively delivered to the target location. Although inflammatory skin conditions are disclosed in several embodiments, other embodiments are used to treat non-dermal inflammation, as well as other several conditions (e.g., those conditions that would benefit from treatment with reduced exposure at non-target sites). For example, in some embodiments, the compositions and technology described herein are used in the gastrointestinal and pulmonary systems. Ophthalmic treatments are provided in some embodiments. In yet other embodiments, compositions for treating joints are provided. Treatment of the nose and ear are provided in other embodiments. Inflammatory and non-inflammatory conditions are contemplated herein.

[7] Reduced exposure compounds and compositions are provided in several embodiments. "Reduced exposure" compounds are those compounds that, when delivered to a target location, are formulated to act at the target location with reduced exposure (e.g., entry and/or longevity) in non-target sites. Exposure is reduced as compared to active agents not formulated according to the embodiments described herein. As a non-limiting example, a PEGylated topical dermal active agent has reduced exposure to the bloodstream as compared to the active agent alone. Reduced exposure compounds include topical compounds that can be delivered to body surfaces and cavities such as the skin, eyes, ears, nose, mouth, vagina, rectum, etc., as well as oral (e.g., enteric coated) compounds for oral delivery that treat the gastrointestinal system (e.g., the GI lining), inhalants that treat the lungs, injections for joints, and other modes of delivery that target one location with the goal of reducing exposure to a non-desired site. Non-desired target sites include, for example, the systemic system, the lymphatic system, non-target tissue, etc. "Reduced exposure compositions" comprise or consist essentially of one or more "reduced exposure compounds."

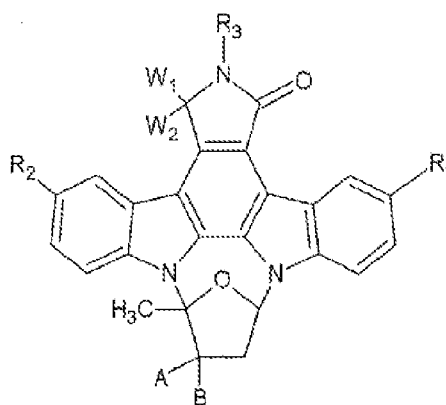
[8] Reduced exposure topical compositions are provided in many embodiments. In some embodiments, a reduced exposure composition is delivered orally, e.g., for treatment of the gastrointestinal system. The active agent remains in the lining of the gastrointestinal tract and is able to achieve pharmacological specificity. Because the active agent is conjugated

with PEG or another molecule as described herein, the active agent is absorbed more slowly into the non-target site (e.g., the systemic circulation and/or lymphatic system). In some cases, less or none of the active agent is absorbed into the non-target site (e.g., systemic circulation and/or lymphatic system). Further, once the composition enters the systemic circulation and/or lymphatic system, clearance (e.g., by the kidney) occurs at a much faster rate. One or more of the advantages of (i) *reduced* absorption into the non-target site (e.g., systemic circulation and/or lymphatic system), (ii) *slower* absorption into the non-target site (e.g., systemic circulation and/or lymphatic system), and (iii) *faster* clearance rates from the non-target site (e.g., systemic circulation and/or lymphatic system) are also achieved when using the compositions (formulated according to the methods described herein) for treating the eye (e.g., via eye drops), the lungs (e.g., via inhalants), the skin (e.g., via dermal topicals), joints (e.g., via injectables), nasal passageways, and the ear (such as the ear canal and other structures). Vaginal and rectal tissues are treated in some embodiments via, for example suppositories.

[9] In several embodiments, there is provided in a reduced exposure composition, a polymer conjugate comprising a warhead (e.g., at least one active agent) linked to a polymer, wherein the warhead comprises an indolocarbazole compound. In some embodiments, the polymer conjugate comprises an indolocarbazole compound of formula (I) or of formula (II):



formula (I)



formula (II)

wherein in formula (I) and (II)

R¹ and R² are the same or a different residue and are each independently selected from the group consisting of:

(a) hydrogen, halogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, hydroxy, lower alkoxy, carboxy, lower alcoxycarbonyl, acyl, nitro, carbamoyl, lower alkylaminocarbonyl, -

NR^5R^6 , wherein R^5 and R^6 are each independently selected from hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted aralkyl, substituted or unsubstituted lower alkylaminocarbonyl, substituted or unsubstituted lower arylaminocarbonyl, alkoxy carbonyl, carbamoyl, acyl or R^5 and R^6 are combined with a nitrogen atom to form a heterocyclic group,

(b) $-\text{CO}(\text{CH}_2)_j\text{R}^4$, wherein j is 1 to 6, and R^4 is selected from the group consisting of

(i) hydrogen, halogen, $-\text{N}_3$,

(ii) $-\text{NR}^5\text{R}^6$, wherein R^5 and R^6 are as defined above,

(iii) $-\text{SR}^7$, wherein R^7 is selected from the group consisting of hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted aralkyl, $-(\text{CH}_2)_a\text{CO}_2\text{R}^{10}$ (wherein a is 1 or 2, and wherein R^{10} is selected from the group consisting of hydrogen and substituted or unsubstituted lower alkyl) and $-(\text{CH}_2)_a\text{CO}_2\text{NR}^5\text{R}^6$,

(iv) $-\text{OR}^8$, $-\text{OCOR}^8$, wherein R^8 is selected from hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl

(c) $-\text{CH}(\text{OH})(\text{CH}_2)_j\text{R}^4$ wherein j and R^4 are as defined above;

(d) $-(\text{CH}_2)_d\text{CHR}^{11}\text{CO}_2\text{R}^{12}$ or $-(\text{CH}_2)_d\text{CHR}^{11}\text{CONR}^5\text{R}^6$, wherein d is 0 to 5, R^{11} is hydrogen, $-\text{CONR}^5\text{R}^6$, or $-\text{CO}_2\text{R}^{13}$, wherein R^{13} is hydrogen or a wherein substituted or unsubstituted lower alkyl, and R^{12} is hydrogen or a substituted or unsubstituted lower alkyl;

(e) $-(\text{CH}_2)_k\text{R}^{14}$ wherein k is 2 to 6 and R^{14} is halogen, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, $-\text{COOR}^{15}$, $-\text{OR}^{15}$, (wherein R^{15} is hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl or acyl), $-\text{SR}^7$ (wherein R^7 is as defined above), $-\text{CONR}^5\text{R}^6$, $-\text{NR}^5\text{R}^6$ (wherein R^5 and R^6 are as defined above) or $-\text{N}_3$;

(f) $-\text{CH}=\text{CH}(\text{CH}_2)_m\text{R}^{16}$, wherein m is 0 to 4, and R^{16} is hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, $-\text{COOR}^{15}$, $-\text{OR}^{15}$ (wherein R^{15} is as defined above) $-\text{CONR}^5\text{R}^6$ or $-\text{NR}^5\text{R}^6$ (wherein R^5 and R^6 are as defined above);

(g) $-\text{CH}=\text{C}(\text{CO}_2\text{R}^{12})_2$, wherein R^{12} is as defined above;

(h) $-\text{C}\equiv\text{C}(\text{CH}_2)_n\text{R}^{16}$, wherein n is 0 to 4 and R^{16} is as defined above;

(i) $-\text{CH}_2\text{OR}^{22}$, wherein R^{22} is tri-lower alkyl silyl in which the three lower alkyl groups are the same or different or wherein R^{22} has the same meaning as R^8

(j) $-\text{CH}(\text{SR}^{23})_2$ and $-\text{CH}_2-\text{SR}^7$ wherein R^{23} is lower alkyl, lower alkenyl or lower alkynyl and wherein R^7 is as defined above; and

R_3 is hydrogen, halogen, acyl, carbamoyl, substituted or unsubstituted lower alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted lower alkynyl or amino; and

W^1 and W^2 are independently hydrogen, hydroxy or W^1 and W^2 together represent oxygen;

and wherein in formula (I), X is a polymer moiety, either linear or branched,

and wherein in formula (II), A represents $-\text{U}-\text{X}'$ and B represents $-\text{L}^2-\text{Y}'$, wherein at least one of X' and Y' is a polymer moiety, either linear or branched, which is bound by L^1 and/or L^2 to the tetrahydrofuran ring of the compound of formula (II); L^1 and/or L^2 are a covalent chemical bond or a linker group;

when Y' is a polymer moiety, and X' is not a polymer, L^1 is a covalent chemical bond and X' is selected from the group consisting of

(a) hydrogen, lower hydroxyalkyl, acyl, carboxy, lower alkoxycarbonyl,

(b) $-\text{CONR}^{17a}\text{R}^{17b}$, wherein R^{17a} and R^{17b} are each independently selected from

(i) hydrogen, lower alkyl, lower alkenyl, lower alkynyl,

(ii) $-\text{CH}_2\text{R}^{18}$; wherein R^{18} is hydroxy,

or (iii) $-\text{NR}^{19}\text{R}^{20}$, wherein R^{19} or R^{20} are each independently selected from hydrogen, lower alkyl, lower alkenyl, lower alkynyl or R^{19} or R^{20} are independently the residue of an α -amino acid in which the hydroxy group of the carboxyl group is excluded, or R^{19} or R^{20} are combined with a nitrogen atom to form a heterocyclic group; and

(c) $-\text{CH}=\text{N}-\text{R}^{21}$, wherein R^{21} is hydroxy, lower alkoxy, amino, guanidino, or imidazolylamino;

when X' is a polymer moiety, and Y' is not a polymer, L^2 is a covalent chemical bond and Y' is selected from hydroxy, lower alkoxy, aralkyloxy, or acyloxy;

or a pharmaceutically acceptable salt of formula (I) and/or (II).

[10] The polymer moiety X , X' or/and Y' covalently attached to the indolocarbazole compound of formulae (I) and (II) has to be biocompatible, can be of natural or semi-synthetic or synthetic origin and can have a linear or branched structure. In some embodiments, the polymer moiety X , X' or/and Y' is selected from poly(alkylene oxides), in particular from (polyethylene) oxides. However, further exemplary polymers include without limitation polyacrylic acid, polyacrylates, polyacrylamide or N-alkyl derivatives thereof, polymethacrylic acid, polymethacrylates, polyethylacrylic acid, polyethylacrylates,

polyvinylpyrrolidone, poly(vinylalcohol), polyglycolic acid, polylactic acid, poly(lactic-co-glycolic) acid, dextran, chitosan, polyaminoacids, hydroxyethyl starch.

[11] In some embodiments, the polymer moiety X, X' or/and Y' is a polyethylene glycol (PEG) moiety, wherein the terminal OH group can optionally be modified e.g. with C₁-C₅ alkyl or C₁-C₅ acyl groups. In some embodiments, the terminal OH group is optionally modified with C₁-, C₂- or C₃-alkyl groups or C₁-, C₂- or C₃ groups. In some embodiments, the modified polyethylene glycol is a terminally alkoxy-substituted polyethylene glycol. In some embodiments, the polymer moiety is methoxy-polyethylene- glycol (mPEG).

[12] As used in this application, except as otherwise expressly provided herein, each of the following terms shall have the meaning set forth below.

[13] As used herein, the term “about” will be understood by one of ordinary skill in the art and will vary to some extent on the context in which it is used. As used herein when referring to a measurable value such as an amount, a temporal duration, and the like, the term “about” is meant to encompass variations of +20 % or +10 %, including ±5 %, ±1 %, and +0.1 % from the specified value, as such variations are appropriate to perform the disclosed methods.

[14] The term “body surface area” includes the face, scalp, intertriginous, and genital areas, exclusive of the mucosa, palms, and soles, of the subject.

[15] The term “lower alkyl”, when used alone or in combination with other groups, means a straight chained or branched lower alkyl group containing from 1-6 carbon atoms, preferably from 1-5, more preferably from 1-4 and especially preferably 1-3 or 1-2 carbon atoms. These groups include, in some embodiments, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, amyl, isoamyl, neopentyl, 1-ethylpropyl, hexyl, and the like. The lower alkyl moiety of the “lower alkoxy”, the “lower alkoxy carbonyl”, the “lower alkylaminocarbonyl”, “lower hydroxyalkyl” and of the “tri-lower alkylsilyl” groups has the same meaning as “lower alkyl” defined above.

[16] The “lower alkenyl” groups are defined as C₂-C₆ alkenyl groups which may be straight chained or branched and may be in the Z or E form. Such groups include vinyl, propenyl, 1-butenyl, isobutenyl, 2-butenyl, 1-pentenyl, (Z)-2-pentenyl, (E)-2-pentenyl, (Z)-4-methyl-2-pentenyl, (E)-4-methyl-2-pentenyl, pentadienyl, e.g., 1,3 or 2,4-pentadienyl, and the like. In some embodiments, the C₂-C₆- alkenyl groups are C₂-C₅-, C₂-C₄-alkenyl groups. In other embodiments, the C₂-C₆- alkenyl groups are C₂-C₃-alkenyl groups.

[17] The term “lower alkynyl” groups refers to C₂-C₆-alkynyl groups which may be straight chained or branched and include ethynyl, propynyl, 1-butyne, 2-butyne, 1-pentyne, 2-pentyne, 3-methyl-1-pentyne, 3-pentyne, 1-hexyne, 2-hexyne, 3-hexyne and the like. In

some embodiments, C₂-C₆-alkynyl groups are C₂-C₅-, C₂-C₄-alkynyl groups. In other embodiments, C₂-C₆-alkynyl groups are C₂-C₃-alkynyl groups.

[18] The term “aryl” group refers to C₆-C₁₄-aryl groups which contain from 6 up to 14 ring carbon atoms. These groups may be mono-, bi- or tricyclic and are fused rings. In some embodiments, the aryl groups include phenyl, biphenyl, naphthyl, anthracenyl, phenanthrenyl and the like. The aryl moiety of the “arylcarbonyl” and the “arylaminocarbonyl” groups has the same meaning as defined above.

[19] The term “heteroaryl” groups may contain 1 to 3 heteroatoms independently selected from nitrogen, sulfur or oxygen and refers C₃-C₁₃-heteroaryl groups. These groups may be mono-, bi- or tricyclic. In some embodiments, the C₃-C₁₃ heteroaryl groups include heteroaromatics and saturated and partially saturated heterocyclic groups. These heterocyclics may be monocyclic, bicyclic, or tricyclic. In some embodiments, the 5 or 6-membered heterocyclic groups are thienyl, furyl, pyrrolyl, pyridyl, pyranyl, morpholinyl, pyrazinyl, methyl pyrrolyl, and pyridazinyl. The C₃-C₁₃-heteroaryl may be a bicyclic heterocyclic group. In some embodiments, the bicyclic heterocyclic groups are benzofuryl, benzothienyl, indolyl, imidazolyl, and pyrimidinyl. In some embodiments, the C₃-C₁₃-heteroaryls are furyl and pyridyl.

[20] The term “lower alkoxy” includes alkoxy groups containing from 1 to 6 carbon atoms, in some embodiments from 1 to 5, in other embodiments from 1 to 4 and in yet other embodiments 1 to 3 or 1 to 2 carbon atoms and may be straight chained or branched. These groups include methoxy, ethoxy, propoxy, butoxy, isopropoxy, tert-butoxy, pentoxy, hexoxy and the like.

[21] The term “acyl” includes lower alkanoyl containing 1 to 6 carbon atoms, in some embodiments from 1 to 5, from 1 to 4, from 1 to 3 or from 1 to 2 carbon atoms and may be straight chained or branched. These groups include, in some embodiments, formyl, acetyl, propionyl, butyryl, isobutyryl, tertiary butyryl, pentanoyl and hexanoyl. The acyl moiety of the “acyloxy” group has the same meaning as defined above.

[22] The term “halogen” includes fluoro, chloro, bromo, iodo, and the like.

[23] The term “aralkyl” group refers C₇-C₁₅-aralkyl wherein the alkyl group is substituted by an aryl. The alkyl group and aryl may be selected from the C₁-C₆ alkyl groups and the C₆-C₁₄-aryl groups as defined above, wherein the total number of carbon atoms is between 7 and 15. In some embodiments the C₇-C₁₅-aralkyl groups are benzyl, phenylethyl, phenylpropyl, phenylisopropyl, phenylbutyl, diphenylmethyl, 1,1 -diphenylethyl, 1,2-diphenylethyl. The aralkyl moiety of the “aralkyloxy” groups has the same meaning as defined above.

[24] The substituted lower alkyl, alkenyl and alkynyl groups have 1 to 3 independently selected substituents, such as lower alkyl, hydroxy, lower alkoxy, carboxyl, lower

alkoxycarbonyl, nitro, halogen, amino, mono- or di- lower alkylamino, dioxolane, dioxane, dithiolane, and dithione. The lower alkyl substituent moiety of the substituted lower alkyl, alkenyl and alkynyl groups, and the lower alkyl moiety of the lower alkoxy, the lower alkoxycarbonyl, and the mono- or di-lower alkylamino substituents of the substituted lower alkyl, alkenyl and alkynyl groups have the same meaning as "lower alkyl" defined above.

[25] The substituted aryl, the substituted heteroaryl and the substituted aralkyl groups each has 1 to 3 independently selected substituents, such as lower alkyl, hydroxy, lower alkoxy, carboxy, lower alkoxycarbonyl, nitro, amino, mono- or di-lower alkylamino, and halogen. The lower alkyl moiety of the lower alkyl, the lower alkoxy, the lower alkoxycarbonyl, and the mono- or di- lower alkylamino groups among the substituents has the same meaning as 'lower alkyl' defined above.

[26] The heterocyclic group formed by R^5 and R^6 combined with a nitrogen atom includes pyrrolidinyl, piperidinyl, piperidino, morpholinyl, morpholino, thiomorpholino, N-methylpiperazinyl, indolyl, and isoindolyl.

[27] In some embodiments, R^1 and R^2 are independently selected from the group consisting of hydrogen, halogen, nitro, $-CH_2OH$, $-(CH_2)_kR^{14}$, $-CH=CH(CH_2)_mR^{16}$, $-C\equiv C(CH_2)_nR^{15}$, $-CO(CH_2)_jR^4$ wherein R^4 is $-SR^7$, CH_2O -(substituted or unsubstituted) lower alkyl (wherein the substituted lower alkyl is in some embodiments methoxymethyl, methoxyethyl or ethoxymethyl), $-NR^5R^6$. In some embodiments, each of R^1 and R^2 is hydrogen.

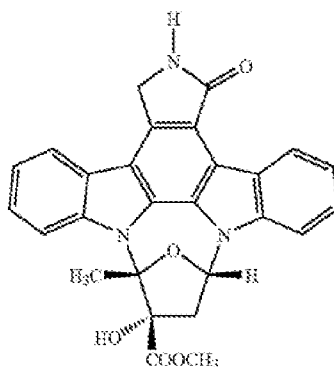
[28] In some embodiments of R^1 and R^2 , the residue R^{14} is selected from phenyl, pyridyl, imidazolyl, thiazolyl, tetrazolyl, $-COOR^{15}$, $-OR^{15}$ (wherein R^{15} is in some embodiments selected from hydrogen, methyl, ethyl, phenyl or acyl), $-SR^7$ (wherein R^7 is in some embodiments selected from substituted or unsubstituted lower alkyl, 2-thiazoline and pyridyl) and $-NR^5R^6$ (wherein R^5 and R^6 are in some embodiments selected from hydrogen, methyl, ethyl, phenyl, carbamoyl and lower alkylaminocarbonyl). Moreover, in some embodiments, the residue R^{16} is selected from hydrogen, methyl, ethyl, phenyl, imidazole, thiazole, tetrazole, $-COOR^{15}$, $-OR^{15}$ and $-NR^5R^6$ (wherein the residues R^{15} , R^5 and R^6 have the meanings as described above). In some embodiments of R^1 and R^2 , the residue R^7 is selected from the group consisting of substituted or unsubstituted lower alkyl, substituted or unsubstituted phenyl, pyridyl, pyrimidinyl, thiazole and tetrazole. Further, in some embodiments, k is 2, 3 or 4, j is 1 or 2 and m and n are independently 0 or 1.

[29] In some embodiments, R^3 is hydrogen or acetyl. Furthermore, in some embodiments, each W^1 and W^2 is hydrogen.

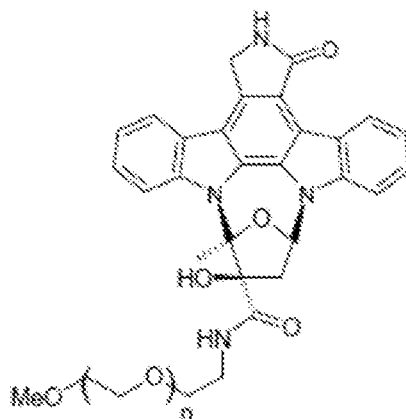
[30] In some embodiments, when Y' is a polymer moiety and X' is not a polymer moiety, X' is selected from carboxy, hydroxymethyl or a lower alkoxycarbonyl. In some embodiments X is selected from methoxycarbonyl.

[31] In some embodiments, when X' is a polymer moiety and Y' is not a polymer moiety, Y' is selected from hydroxy or acetyloxy.

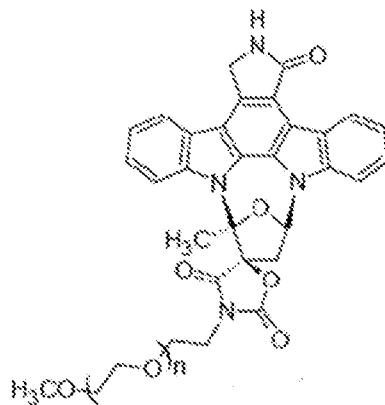
[32] In some embodiments, the warhead of the polymer conjugate is a derivative of K252a, which has the formula:



[33] In some embodiments, the polymer conjugate is SNA-125, wherein the composition has the formula:



[34] In some embodiments, the polymer conjugate is SNA-120, wherein the composition has the formula:



[35] The formulas depicted herein are not limited to any particular stereochemistry, and all stereoisomers and enantiomers thereof are included in this disclosure.

[36] Non-dermal (non-skin) inflammation or other conditions may also be treated in some embodiments with compositions comprising these compounds. Non-inflammatory conditions may also be treated with some embodiments.

[37] Local peripheral nerves in the skin play an important role in the pathogenesis of psoriasis, as the absence of neural input has been shown to lead to plaque clearance. Plaques in psoriasis patients with associated itch have elevated levels of NGF-immunoreactive keratinocytes and TrkA expression in innervating nerve fibers. The NGF/TrkA signaling pathway is important in neurogenic inflammation and keratinocyte hyperproliferation which contribute to psoriatic plaques, as well as itch. Without being bound by theory, SNA-120 is a topical tropomyosin receptor kinase A (TrkA) inhibitor that blocks nerve growth factor (NGF) signaling (i.e. SNA-120 selectively targets the NGF/TrkA signaling pathway), which plays an important role in the pathogenesis of psoriasis and itch.

[38] In some embodiments, the conjugates provided herein are useful in treating a dermal condition in a subject in need thereof. In some embodiments the dermal condition is pruritus, skin neoplasia, vascular tumor, inflammatory dermatosis, scarring, a bullous disease, UV irradiation therapy, alopecia areata, or vitiligo. In some embodiments, the pruritus is pruritus due to psoriasis, pruritus due to atopic dermatitis, or prurigo nodularis. In some embodiments, the skin neoplasia is squamous cell carcinoma, basal cell carcinoma, malignant melanoma, or malignant cutaneous lymphomas. In some embodiments, the vascular tumor is angiosarcoma, Kaposi's sarcoma, infantile hemangioma, or hemangioendothelioma. In some embodiments the inflammatory dermatosis is dermatitis, psoriasis, keloid, or rosacea. In some embodiments, the dermatitis is atopic dermatitis or irritant contact dermatitis. In some embodiments, the bullous disease is bullous pemphigoid or erythema multiforme.

[39] In some embodiments, the dermal condition is prurigo nodularis, postherpetic neuralgia, seborrheic dermatitis, lichen simplex chronicus, Grover's disease, neurodermatitis, bullous pemphigoid, or epidermolysis bullosa.

[40] In some embodiments, administration is once daily (e.g., once daily for 8 weeks or 12 weeks). In some embodiments, administration is twice daily (e.g., twice daily for 8 weeks or 12 weeks).

[41] In some embodiments, the subject has a weekly mean I-NRS score of at least 5 on the 11 point I-NRS scale.

[42] In some embodiments, the subject has a weekly mean I-NRS score of at least 5 on the 11 point I-NRS scale and the psoriasis is mild to moderate psoriasis (e.g., mild to moderate plaque psoriasis).

[43] In some embodiments, the subject has mild-to-moderate psoriasis with at least moderate itch.

[44] In some embodiments, the psoriasis is mild to moderate psoriasis.

[45] In some embodiments, the psoriasis is mild to moderate plaque psoriasis.

[46] In some embodiments, the conjugates provided herein are useful in treating a gastrointestinal condition in a subject in need thereof. In some embodiments, the gastrointestinal condition is ulcerative colitis, Crohn's disease, intestinal fibrosis, or colorectal cancer.

[47] In some embodiments, the conjugates provided herein are useful in treating an ocular condition in a subject in need thereof. In some embodiments, the ocular condition is are-related macular degeneration, diabetic retinopathy, edema, dry eye, uveitis, pterygia, conjunctivitis, graft versus host disease, glaucoma, or retinopathy of prematurity. In some embodiments, the age-related macular degeneration is wet age-related macular degeneration or dry age-related macular degeneration. In some embodiments, the edema is macular edema or corneal edema. In some embodiments, the macular edema is diabetic macular edema. In some embodiments, dry eye is spontaneous dry eye or aqueous deficiency dry eye. In some embodiments, the uveitis is juvenile idiopathic arthritis uveitis or posterior uveitis. In some embodiments, the conjunctivitis is vernal keratoconjunctivitis (allergic conjunctivitis). In some embodiments, the graft versus host disease is ophthalmic graft versus host disease. In some embodiments, the glaucoma is primary open angle glaucoma.

[48] In some embodiments, the conjugates provided herein are useful in treating a respiratory condition in a subject in need thereof. In some embodiments, the respiratory condition is asthma, chronic obstructive pulmonary disease, pulmonary fibrosis, or cough. In some embodiments the cough is chronic cough.

[49] In some embodiments, the conjugates provided herein are useful in treating rheumatoid arthritis, osteoarthritis, neuropathic pain, or neuropathic itch in a subject in need thereof.

[50] As described above, several embodiments disclosed herein provide reduced or minimized exposure (e.g., entry into and/or longevity in a non-target site such as the systemic circulation and/or lymphatic system). In some embodiments, exposure at a non-target site is less than 90%, 75%, 50%, 25%, 15%, 10%, 5% or 2% (or less) of the polymer conjugate as compared to a similar active entity that has not been produced according to the embodiments described herein. In some embodiments, desirable rate of clearance from the non-target site (e.g., systemic circulation and/or lymphatic system) for the compositions described herein is increased by at least 10%, 25%, 50%, or 75% or more as compared to non-conjugated controls. As an example, a PEGylated active agent described herein not

only penetrates the desired membranes to reach a desired target, but has reduced non-target exposure by at least 20-80% or more as compared to the non-PEGylated active agent. In some embodiments, blood concentrations measured post administration of the compositions described herein are less than about 0.1 ng/ml, less than 1 ng/ml, or less than 10 ng/ml after, e.g., 15 minutes, 30 minutes, 1 hour, 6 hours or 12 hours.

[51] In some embodiments, reduced exposure at non-target sites contributes to enhanced efficacy. Efficacy may be enhanced because lower concentrations/amounts/dosing schedules are required to achieve the same or similar therapeutic efficacy at the target site (because, for example, the active ingredient stays at the desired target site for a longer time). In one embodiment, concentrations/amounts/dosing schedules are reduced by 25%-75% or more.

[52] More rapid clearance rates of the active agent once in the non-target site(s) (such as systemic circulation and/or lymphatic system) are also beneficial because this may allow for a *higher* concentration or *more* doses to be delivered. This is especially beneficial for active agents in which a subject would benefit from a higher dose but cannot tolerate the higher dose due to toxicity at the non-target site (e.g., systemic toxicity). Faster clearance rates would permit the desired higher dose to be delivered according to the desired schedule. For example, a subject may be able to tolerate daily doses rather than weekly doses because of the reduced exposure.

[53] In some embodiments, the active agents of the compositions described herein (e.g., indolocarbazole compounds conjugated e.g., with PEG or other polymers) are measured in non-target sites (e.g., the systemic circulation and/or lymphatic system) at less than amounts found when the active agent is delivered without conjugation (e.g., less than 0.5%, 1% or 2% after 6 or 12 hours, as compared with 3-15% (e.g., 3-6%) when the active agent is delivered without conjugation). In some embodiments, the active agents of the compositions described herein (e.g., indolocarbazole compounds conjugated e.g., with PEG or other polymers) are measured in non-target sites (e.g., the systemic circulation and/or lymphatic system) at less than 0.5%, 1% or 2% after 3-24 hours, as compared to an amount 2-20 times greater when the active agent is delivered without conjugation.

[54] In some embodiments, clearance of the compositions (e.g., the conjugated polymer compounds) occurs within minutes of exposure to the non-target site (e.g., systemic circulation and/or lymphatic system), as opposed to hours. In other embodiments, 50% clearance of the conjugated polymer compounds occurs in less than 5 minutes, 15 minutes, 30 minutes, 1 hour, 6 hours, and 12 hours of exposure to the systemic circulation and/or lymphatic system. Clearance times of the conjugated polymer compounds are reduced by more than 25%, 50%, 75% and 90%, as compared to the non-conjugated active agents or

other formulations. These reduced clearance times are beneficial to reduce toxicity and undesired side effects.

[55] In some embodiments, an active agent may be increasingly toxic as it is metabolized in the non-target site (e.g., systemic circulation and/or lymphatic system) because the metabolites exhibit more toxicity than the original agent. Thus, faster clearance rates, in some cases even before the toxic metabolites are created, are especially beneficial.

[56] The term "active entity" as used herein should not be understood as limiting the participation of the polymer itself and/or the chemical linking moiety between the polymer and the warhead in defining the pharmacology of the polymer conjugate. In some embodiments, the polymer influences the selectivity and/or inhibitory activity of the polymer conjugate. In some embodiments, the chemical linking moiety between the polymer and warhead influences the selectivity and/or inhibitory activity of the polymer conjugate. In some embodiments, the polymer conjugates exhibit no change in selectivity or inhibitory activity against the therapeutic target in comparison with the unconjugated active agent. In some embodiments, the polymer conjugates exhibit a significant increase in selectivity against the therapeutic target in comparison with the unconjugated active agent. In some embodiments, the polymer conjugates exhibit a significant increase in inhibitory activity against the therapeutic target in comparison with the unconjugated active agent. In some embodiments, the polymer conjugates exhibit a significant increase in selectivity and inhibitory activity against the therapeutic target in comparison with the unconjugated active agent. In some embodiments, the increased selectivity and/or inhibitory activity of the polymer conjugate against the therapeutic target in comparison with the unconjugated active agent causes decrease in undesired biological effects. In some embodiments, the increased selectivity of the polymer conjugate is caused by an increase of the hydrodynamic volume resulting from the conjugated polymer chain. In some embodiments, the polymer chain creates a higher steric hindrance which allows discrimination among the diverse shapes and sizes of the binding sites of different proteins, thus improving selectivity with respect to the active agent alone.

[57] In several embodiments, various inflammatory skin diseases are treated. The inflammatory skin disease comprises, in some embodiments, psoriasis, psoriasis guttata, inverse psoriasis, pustular psoriasis, psoriatic erythroderma, acute febrile neutrophilic dermatosis, eczema, xerotic eczema, dyshidrotic eczema, vesicular palmar eczema, acne vulgaris, atopic dermatitis, contact dermatitis, allergic contact dermatitis, dermatomyositis, exfoliative dermatitis, hand eczema, pompholyx, keloids, rosacea, rosacea due to sarcoidosis, rosacea due to scleroderma, rosacea due to Sweet syndrome, rosacea due to systemic lupus erythematosus, rosacea due to urticaria, rosacea due to herpetic pain, Sweet's disease, neutrophilic hydradenitis, sterile pustule, drug rash, seborrheic dermatitis,

pityriasis rosea, Kikuchi's disease of the skin, pruritic urticarial papules and plaques of pregnancy, Stevens-Johnson syndrome and toxic epidermal necrolysis, tattoo reaction, Wells syndrome (eosinophilic cellulitis), reactive arthritis (Reiter syndrome), bowel-associated dermatosis-arthritis syndrome, rheumatoid neutrophilic dermatosis, neutrophilic eccrine hidradenitis, neutrophilic skin disease of dorsum of hand, balanitis circumscripta plasmacellularis, balanoposthitis, Behcet's disease, erythema annulare centrifugum, erythema dyschromicum perstans, erythema multiforme, granuloma annulare, dermatitis of hand, lichen nitidus, lichen planus, lichen sclerosus et atrophicus, lichen simplex chronicus, lichen spinulosus, nummular dermatitis, pyoderma gangrenosum, sarcoidosis, subcorneal pustular dermatosis, urticaria, and transient acantholytic dermatosis.

[58] In several embodiments, various skin neoplasias are treated. The skin neoplasia comprises, in some embodiments, squamous cell carcinoma, basal cell carcinoma, malignant melanoma, malignant cutaneous lymphoma, Kaposi's sarcoma, Merkel cell skin cancer, and non-melanoma skin cancer.

[59] In several embodiments, various vascular tumors are treated. The vascular tumor comprises, in some embodiments, hemangiomas, Kaposi's sarcoma, lymphangioma, glomangioma, angiosarcoma, hemangioendothelioma, and infantile hemangiomas.

[60] In several embodiments, various bullous diseases are treated. The bullous disease comprises, in some embodiments, bullous pemphigoid, erythema multiforme, dermatitis herpetiformis, epidermolysis bullosa acquisita, linear Immunoglobulin A disease, mucous membrane pemphigoid, pemphigoid gestationis, pemphigus foliaceus, and pemphigus vulgaris.

[61] In several embodiments, age-related macular degeneration is treated. In several embodiments, diabetic retinopathy is treated. In several embodiments, corneal edema is treated. In several embodiments, macular edema is treated. In several embodiments, dry eye is treated.

[62] In several embodiments, hair growth and cycling are modulated. In several embodiments, alopecia is treated.

[63] In several embodiments, the polymer conjugates are administered in combination with UV irradiation therapy.

[64] Also provided herein, in several embodiments, are polymer conjugates wherein the polymer is polyethylene glycol (PEG) or methoxy-polyethylene glycol (m-PEG). In several embodiments, there is provided a pharmaceutical composition comprising or consisting essentially of a polymer conjugate disclosed herein that is formulated for topical and non-topical administration. In several embodiments, methods of making and using the compositions described herein are provided.

[65] In several embodiments, the invention comprises a reduced exposure composition comprising at least one active entity linked to at least one polymer, wherein the composition has reduced exposure at a non-target site as compared to the active entity delivered without the polymer. The non-target site comprises the systemic system, the lymphatic system and/or another non-target tissue site in some embodiments.

[66] In some embodiments, the active entity comprises an indolocarbazole compound. In some embodiments, the active entity comprises a derivative of K252a. In some embodiments, the composition comprises SNA-125.

[67] The active entity binds to a tropomyosin-receptor-kinase A (TrkA) in some embodiments. The active entity binds to a Janus Kinase (JAK) family member in some embodiments. The active entity binds to one or more of Janus Kinase 1 (JAK1), Janus Kinase 2 (JAK2), Janus Kinase 3 (JAK3), and/or Tyrosine kinase 2 (TYK2) in some embodiments. The active entity binds to mitogen-activated protein kinase kinase (MAP2K) in some embodiments. The active entity binds to mitogen-activated protein kinase kinase 3 (MAP2K3) in some embodiments. The binding may be partially or fully inhibitory or not.

[68] In some embodiments, the conjugates provided herein (e.g., Formula (I), Formula (II), SNA-120 or SNA-125) selectively inhibit TrkA, the high-affinity receptor of NGF, that blocks nerve growth factor- (NGF) dependent over-activation of TRPV1 (transient receptor potential vanilloid 1), a key mediator of peripheral itch in psoriasis. Reducing the severity of itch is considered an important treatment goal for patients. Thus, in some embodiments, the conjugates provided herein (e.g., Formula (I), Formula (II), SNA-120 or SNA-125) are useful in treating itch (pruritus, e.g., moderate to severe pruritus) associated with psoriasis (e.g., moderate to severe psoriasis).

[69] In some embodiments, the polymer used in the reduced exposure compounds comprises polyethylene glycol (PEG) and/or methoxy-polyethylene glycol (m-PEG). In embodiments where the active entity has one or more carboxyl, hydroxyl, amino and/or sulfhydryl groups, the active entity is PEGylated (or conjugated/coupled to another polymer) at one or more of said carboxyl, hydroxyl, amino and/or sulfhydryl groups.

[70] The reduced exposure compositions described herein are formulated for topical administration in several embodiments. Inhalants, injectables, eye drops, nasal sprays, oral administration etc. are provided in some embodiments. In several embodiments, methods of treating one or more of the following are provided: non-dermal inflammation, inflammatory skin disease, vascular tumors, skin neoplasia, bullous diseases, age-related macular degeneration, diabetic retinopathy, corneal edema, macular edema, dry eye, alopecia, wounds, scars, autoimmune disorders, and cancerous or pre-cancerous lesions. Methods for modulating hair growth and cycling are provided in some embodiments. Kits comprising one

or more compounds and devices for administration (syringes, containers, inhalers, etc.), as well as instructions for use, are provided in certain embodiments.

[71] In some embodiments, the conjugates provided herein are provided as a topical nonsteroidal therapy (e.g., and ointment) to treat itch (pruritus, e.g., moderate to severe pruritus) associated with psoriasis (e.g., moderate to severe psoriasis) in a subject in need thereof.

[72] Compositions may be administered via at least two routes of administration, either simultaneously or sequentially according to some embodiments. In one embodiment, the composition is administered via a first (e.g. topical dermal) route to a subject, wherein the subject further receives an additional agent via a second (e.g., non-dermal) route to achieve synergetic effects.

[73] In several embodiments, the inventions comprises methods for reducing exposure of a composition at least one non-target site, wherein the method comprises applying a composition comprising at least one active entity linked to at least one polymer, wherein the combination of the active entity and polymer reduces exposure at the non-target site by more than 50% as compared to the active entity without the polymer. The composition may be applied topically, injected, inhaled, or administered orally. The non-target site includes non-target tissue at which pharmacological activity is not desired and/or not achieved. Non-target sites can include the bloodstream or systemic system. Non-target sites can also include the lymphatic system.

[74] In several embodiments, methods for treating one or more of an ophthalmic condition, a gastrointestinal condition, and a dermatological condition in a subject in need thereof are provided. In one embodiment, the composition comprises or consists essentially of a conjugate comprising or consists essentially of an active entity coupled (e.g., linked) to at least one polymer. Two, three or more active entities or two, three or more polymers may be used. The polymer can include, for example, polyethylene glycol (PEG) and/or methoxy-polyethylene glycol (m-PEG). There is provided, in several embodiments, any stereoisomer, enantiomer and/or salt of the conjugate. The active entity may be for example, an inhibitor, antagonist, or inverse agonist of a kinase that mediates the one or more ophthalmic, gastrointestinal, and dermatological conditions. In several embodiments, the active entity may be an indolocarbazole compound or a derivative thereof. In one embodiment, the active entity comprises SNA-120. In one embodiment, the active entity comprises SNA-125. In one embodiment, a pharmaceutically acceptable carrier formulated for delivering the conjugate to the target site is also provided. In several embodiments, the conjugate has reduced exposure at a non-target site as compared to the active entity delivered without the polymer. The non-target site includes for example the systemic system, the lymphatic system and/or other non-target tissue sites. In several embodiments, the non-target site comprises any site

at which pharmacological activity is not desired and/or not achieved. In one embodiment, the conjugate can advantageously traverse plasma membranes of cells at the target site, thereby promoting interactions between the active entity and the kinase. This traversal may include the crossing of cellular lipid bilayers to, e.g., distribute the active entity among both lipophilic and hydrophilic cellular compartments. Membranes include the lipid bilayer, plasma membrane and the nuclear membrane as examples. In several embodiments, the conjugate interacts with a kinase associated with the plasma membrane, cytoplasm and/or nucleus. The conjugate may exhibit a depot effect across cellular compartments, thereby reducing the dose of the active entity required to inhibit the kinase compared to the active entity without conjugation to the polymer.

[75] In several embodiments, the kinase may be one or more of tropomyosin receptor kinase A (TrkA), mitogen-activated protein kinase (MAPK), mitogen-activated protein kinase kinase (MAP2K), mitogen-activated protein kinase kinase 3 (MAP2K3), and a Janus Kinase (JAK) family member. In some embodiments, the JAK protein family includes JAK1, JAK2, JAK3, and Tyrosine kinase 2 (TYK2). In some embodiments, the composition prevents the activation of NF-Kappa B signaling. In some embodiments, the composition binds and/or inhibits the one or more kinases. In some embodiments, the active entity has one or more carboxyl, hydroxyl, amino and/or sulfhydryl groups. In one embodiment, at least one polymer is conjugated (e.g., PEGylated) to the active entity at the one or more carboxyl, hydroxyl, amino and/or sulfhydryl groups.

[76] In some embodiments, the reduced exposure composition may be formulated for topical, oral, local ocular (e.g., eye drop), inhalation, injection or suppository delivery. Topical, oral, injection, inhalation, local ocular, and suppository administration is provided in several embodiments. In several embodiments, the administration is daily. In the methods of treatment, effective amounts of the active entity are delivered to a subject (e.g., human or veterinary). In several embodiments, the composition may be administered via at least two routes of administration, either simultaneously or sequentially. In some embodiments, the composition is administered via a topical route to a subject, and the subject further receives an additional agent via a non-topical route. In some such embodiments, this co-administration achieves synergetic effects. The composition may further comprise one or more additional ingredients, such as, for example, a protective agent, an emollient, an astringent, a humectant, a sun screening agent, a sun tanning agent, a UV absorbing agent, an antibiotic agent, an anti-angiogenesis agent, a preventive or therapeutic agent for inflammatory bowel disease, a physiological cooling agent, an antifungal agent, an antiviral agent, an antiprotozoal agent, an anti-acne agent, an anesthetic agent, a steroidal anti-inflammatory agent, a non-steroidal anti-inflammatory agent, an antipruritic agent, an additional antioxidant agent, a chemotherapeutic agent, an anti-histamine agent, a vitamin or

vitamin complex, a hormone, an anti-dandruff agent, an anti-wrinkle agent, an anti-skin atrophy agent, a skin whitening agent, and/or a cleansing agent.

[77] In several embodiments, the active entity and/or conjugate may have a longer residence time within a cell or other tissue at the target site compared to the active entity without conjugation to the polymer. For example, the residence time of the active entity and/or conjugate within a cell or other tissue at the target site is, as compared to the active entity without conjugation to the polymer, (i) at least 25% (e.g., 25-50%, 50-75%, 75-100%, 100-150%, or higher and overlapping ranges therein) longer and/or (ii) at least 2-20 fold (e.g., 2-10 fold, 2-4 fold, 4-6 fold, 6-8 fold, 8-10 fold, 10-12 fold, 12-14 fold, 14-16 fold, 16-18 fold, 18-20 fold, 20-30 fold, 40-50 fold, 10-50 fold, 50-100 fold, and overlapping ranges therein) longer. In one embodiment, the residence time is over 100 fold longer.

[78] In some embodiments, a smaller dose of the conjugate may be needed to achieve a therapeutic effect comparable to the active entity without conjugation to the polymer. For example, in several embodiments, the dose of the conjugate needed to achieve a therapeutic effect comparable to the active entity without conjugation to the polymer is at least 10% (e.g., 10-15%, 15-20%, 20-25%, 25-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, 80-90%, 90-100%, 100%-125%, 125-150%, or higher and overlapping ranges therein) lower. In one embodiment, the dose is over 200% lower. In some embodiments, fewer doses and/or smaller doses of the conjugate are required as compared to the active entity delivered without the polymer.

[79] In several embodiments, the active entity and/or conjugate may have an increased concentration, activity and/or bioavailability within a cell or tissue at the target site compared to the active entity without conjugation to the polymer. In some such embodiments, the therapeutically effective amount of the active entity is at the target site. For example, the concentration, activity and/or bioavailability within a cell or other tissue at the target site is, as compared to the active entity without conjugation to the polymer, at least 2-20 fold (e.g., 2-4 fold, 4-6 fold, 6-8 fold, 8-10 fold, 10-12 fold, 14-16 fold, 18-20 fold, 20-30 fold, 30-40 fold, 40-50 fold, 50-100 fold, and overlapping ranges therein) greater than within a cell or tissue at a non-target site (e.g., the systemic system, the lymphatic system, the circulatory system, bone marrow). In one embodiment, the concentration, activity and/or bioavailability within a cell or tissue at the target site is over 100 fold greater.

[80] In several embodiments, the active entity and/or conjugate may have reduced concentration, activity and/or bioavailability within a cell or tissue at a non-target site compared to the active entity without conjugation to the polymer. In several embodiments, the active entity and/or conjugate is present at a biologically inactive concentration within a cell or tissue at a non-target site. In several embodiments, reduced concentration, activity and/or bioavailability within a cell or tissue at a non-target site (e.g., the systemic system, the

lymphatic system, bone marrow, the circulatory system) advantageously reduces toxicity and/or other side effects, such as, for example, immunosuppression. For example, in some embodiments, the active entity and/or conjugate has reduced systemic absorption and/or little or no systemic toxicity when the composition is formulated for oral delivery and is administered orally (e.g., a single administration, administration on a daily basis). In some embodiments, the composition displays minimal toxicity when administered topically (e.g., topical administration on a daily basis). In some embodiments, the reduced exposure composition reduces inflammation upon administration. In several embodiments, the active entity and/or conjugate inhibits the proliferation of keratinocytes.

[81] In several embodiments, the conjugate is amphiphilic and/or amphipathic. In some embodiments, the conjugate is more amphiphilic and/or amphipathic than the active entity without conjugation to the polymer. For example, in several embodiments, the conjugate, as compared to the active entity without conjugation to the polymer, is at least 25% (e.g., 20-25%, 25-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, 80-90%, 90-100%, 100%-125%, 125-150%, or higher and overlapping ranges therein) more amphiphilic. In one embodiment, the amphiphilicity is over 200% greater. Additionally, in some embodiments, the conjugate is more hydrophilic than the active entity without conjugation to the polymer. For example, in several embodiments, the conjugate, as compared to the active entity without conjugation to the polymer, is at least 25% (e.g., 20-25%, 25-30%, 30-40%, 40-50%, 50-60%, 60-70%, 70-80%, 80-90%, 90-100%, 100%-125%, 125-150%, or higher and overlapping ranges therein) more hydrophilic. In one embodiment, the hydrophilicity is over 200% greater. In some embodiments, the greater hydrophilicity of the conjugate advantageously facilitates one or more of: non-compartmentalization within a cell or tissue at the target site; access to and activity in both the lipid bilayer and the cytosol of the cell; access to and/or activity in both the lipid bilayer and the cytoplasm of the cell; and/or access to and/or activity across the lipid bilayer. In several embodiments, the conjugate exhibits greater access to the kinase (e.g., a JAK family protein and/or a STAT family protein) compared to the active entity without conjugation to the polymer.

[82] In several embodiments, the method of treatment and/or use of the compositions described herein are provided for modulating hair growth and cycling in a subject in need thereof.

[83] In several embodiments, the method of treatment and/or use of the compositions described herein are provided for the prophylaxis or treatment of one or more of the following in a subject in need thereof: a joint, an eye, alopecia, dry eye, corneal edema, macular edema, an autoimmune disorder, the gastrointestinal system, a lung, a vascular tumor, age-related macular degeneration, a cancerous or pre-cancerous lesion, a skin neoplasia, a bullous disease, a scar, a wound, diabetic retinopathy, non-dermal

inflammation, an inflammatory condition, an inflammatory skin condition, and/or an inflammatory skin disease.

[84] In several embodiments, the method of treatment and/or use of the compositions described herein are employed in combination with UV irradiation.

[85] In several embodiments, the method of treatment and/or use of the compositions described herein are provided for the prophylaxis or treatment of one or more of the following conditions: squamous cell carcinoma, basal cell carcinoma, malignant melanoma, malignant cutaneous lymphoma, Kaposi's sarcoma, Merkel cell skin cancer, non-melanoma skin cancer, bullous pemphigoid, erythema multiforme, dermatitis herpetiformis, epidermolysis bullosa acquisita, linear Immunoglobulin A disease, mucous membrane pemphigoid, pemphigoid gestationis, pemphigus foliaceus, pemphigus vulgaris, hemangiomas, Kaposi's sarcoma, lymphangioma, glomangioma, angiosarcoma, hemangioendothelioma, infantile hemangiomas, psoriasis, psoriasis guttata, inverse psoriasis, pustular psoriasis, psoriatic erythroderma, acute febrile neutrophilic dermatosis, eczema, xerotic eczema, dyshidrotic eczema, vesicular palmar eczema, acne vulgaris, atopic dermatitis, contact dermatitis, allergic contact dermatitis, dermatomyositis, exfoliative dermatitis, hand eczema, pompholyx, rosacea, rosacea due to sarcoidosis, rosacea due to scleroderma, rosacea due to Sweet syndrome, rosacea due to systemic lupus erythematosus, rosacea due to urticaria, rosacea due to herpetic pain, Sweet's disease, neutrophilic hydradenitis, sterile pustule, drug rash, seborrheic dermatitis, pityriasis rosea, Kikuchi's disease of the skin, pruritic urticarial papules and plaques of pregnancy, Stevens-Johnson syndrome and toxic epidermal necrolysis, tattoo reaction, Wells syndrome (eosinophilic cellulitis), reactive arthritis (Reiter syndrome), bowel-associated dermatosis-arthritis syndrome, rheumatoid neutrophilic dermatosis, neutrophilic eccrine hidradenitis, neutrophilic skin disease of dorsum of hand, balanitis circumscripta plasmacellularis, balanoposthitis, Behcet's disease, erythema annulare centrifugum, erythema dyschromicum perstans, erythema multiforme, granuloma annulare, dermatitis of hand, lichen nitidus, lichen planus, lichen sclerosus et atrophicus, lichen simplex chronicus, lichen spinulosus, nummular dermatitis, pyoderma gangrenosum, sarcoidosis, subcorneal pustular dermatosis, urticaria, skin fibrosis, and transient acantholytic dermatosis, alopecia, alopecia areata, androgenic alopecia, and/or dry eye.

[86] In several embodiments, a method for synthesizing SNA-125 is provided. In some embodiments, the method comprises the hydrolysis of K252a to K252b followed by the coupling of K252b to methoxy polyethylene glycol amine (mPEG amine). In several embodiments, the hydrolysis K252a to K252b may performed in the presence of at least one of lithium hydroxide and tetrahydrofuran. In some embodiments, the coupling of K252b to mPEG amine is performed in the presence of at least one of TBTU, 4-methylmorpholine, and

dichloromethane, thereby resulting in the formation of an amide bond between the K252b and the mPEG.

[87] In one aspect, provided herein are methods of treating a gastrointestinal system condition in a subject in need thereof, comprising administering to the subject a compound, or a pharmaceutically acceptable salt thereof, comprising an active entity linked to at least one polymer.

[88] In another aspect, provided herein are methods of treating multifocal inflammation caused by a gastrointestinal system condition in a subject in need thereof, comprising administering to the subject a compound, or a pharmaceutically acceptable salt thereof, comprising an active entity linked to at least one polymer.

[89] In another aspect, provided herein are methods of treating edema caused by a gastrointestinal system condition in a subject in need thereof, comprising administering to the subject a compound, or a pharmaceutically acceptable salt thereof, comprising an active entity linked to at least one polymer.

[90] In another aspect, provided herein are methods of treating mucosal necrosis caused by a gastrointestinal system condition in a subject in need thereof, comprising administering to the subject a compound, or a pharmaceutically acceptable salt thereof, comprising an active entity linked to at least one polymer.

[91] In another aspect, provided herein are methods of treating edema caused by a gastrointestinal system condition in a subject in need thereof, comprising administering to the subject a compound, or a pharmaceutically acceptable salt thereof, comprising an active entity linked to at least one polymer.

[92] In another aspect, provided herein are methods of treating blood in the stool caused by a gastrointestinal system condition in a subject in need thereof, comprising administering to the subject a compound, or a pharmaceutically acceptable salt thereof, comprising an active entity linked to at least one polymer.

[93] In another aspect, provided herein are methods of treating diarrhea caused by a gastrointestinal system condition in a subject in need thereof, comprising administering to the subject a compound, or a pharmaceutically acceptable salt thereof, comprising an active entity linked to at least one polymer.

[94] In another aspect, provided herein are methods of treating body weight loss caused by a gastrointestinal system condition in a subject in need thereof, comprising administering to the subject a compound, or a pharmaceutically acceptable salt thereof, comprising an active entity linked to at least one polymer.

[95] In another aspect, provided herein are methods of treating a dermal condition in a subject in need thereof, comprising administering to the subject a compound, or a

pharmaceutically acceptable salt thereof, comprising an active entity linked to at least one polymer.

[96] In another aspect, provided herein are methods of reducing the psoriasis area and severity index (PASI) score of a subject suffering from a dermal condition, comprising administering to the subject a compound, or a pharmaceutically acceptable salt thereof, comprising an active entity linked to at least one polymer.

[97] In another aspect, provided herein are methods of reducing the body surface area affected by a dermal condition in a subject affected thereby, comprising administering to the subject a compound, or a pharmaceutically acceptable salt thereof, comprising an active entity linked to at least one polymer.

[98] In another aspect, provided herein are methods of reducing the investigator global assessment (IGA) score of a subject suffering from a dermal condition, comprising administering to the subject a compound, or a pharmaceutically acceptable salt thereof, comprising an active entity linked to at least one polymer.

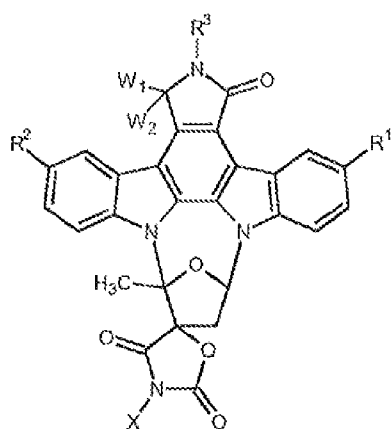
[99] In another aspect, provided herein are methods of maintaining a plasma concentration of a compound or a metabolite thereof at or below a threshold level in a subject suffering from a dermal condition, comprising administering to the subject the compound, or a pharmaceutically acceptable salt thereof, wherein:

the compound comprises an active entity linked to at least one polymer;

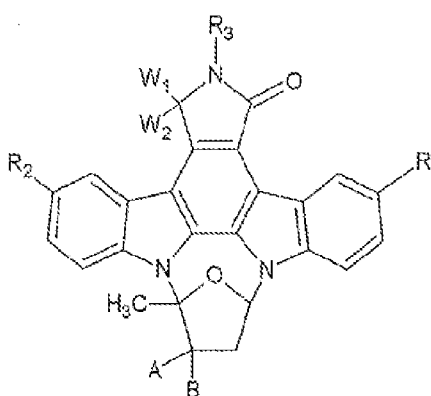
the compound, or a pharmaceutically acceptable salt thereof, treats the dermal condition; and

the threshold level is about 5.0 ng/ml.

[100] In some embodiments of these aspects, the compound is a compound of Formula (I) or of Formula (II):



formula (I)



formula (II)

or a pharmaceutically acceptable salt thereof,

wherein in formula (I) and (II)

R^1 and R^2 are the same or a different residue and are each independently selected from the group consisting of:

(a) hydrogen, halogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, hydroxy, lower alkoxy, carboxy, lower alkoxycarbonyl, acyl, nitro, carbamoyl, lower alkylaminocarbonyl, $-NR^5R^6$, wherein R^5 and R^6 are each independently selected from hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted aralkyl, substituted or unsubstituted lower alkylaminocarbonyl, substituted or unsubstituted lower arylaminocarbonyl, alkoxycarbonyl, carbamoyl, acyl or R^5 and R^6 are combined with a nitrogen atom to form a heterocyclic group,

(b) $-\text{CO}(\text{CH}_2)_j\text{R}^4$, wherein j is 1 to 6, and R^4 is selected from the group consisting of

(i) hydrogen, halogen, $-\text{N}_3$,

(ii) $-\text{NR}^5\text{R}^6$, wherein R^5 and R^6 are as defined above,

(iii) $-\text{SR}^7$, wherein R^7 is selected from the group consisting of hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted aralkyl, $-(\text{CH}_2)_a\text{CO}_2\text{R}^{10}$ (wherein a is 1 or 2, and wherein R^{10} is selected from the group consisting of hydrogen and substituted or unsubstituted lower alkyl) and $-(\text{CH}_2)_a\text{CO}_2\text{NR}^5\text{R}^6$,

(iv) $-\text{OR}^8$, $-\text{OCOR}^8$, wherein R^8 is selected from hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl

(c) $-\text{CH}(\text{OH})(\text{CH}_2)_j\text{R}^4$ wherein j and R^4 are as defined above;

(d) $-(\text{CH}_2)_d\text{CHR}^{11}\text{CO}_2\text{R}^{12}$ or $-(\text{CH}_2)_d\text{CHR}^{11}\text{CONR}^5\text{R}^6$, wherein d is 0 to 5, R^{11} is hydrogen, $-\text{CONR}^5\text{R}^6$, or $-\text{CO}_2\text{R}^{13}$, wherein R^{13} is hydrogen or a wherein substituted or unsubstituted lower alkyl, and R^{12} is hydrogen or a substituted or unsubstituted lower alkyl;

(e) $-(\text{CH}_2)_k\text{R}^{14}$ wherein k is 2 to 6 and R^{14} is halogen, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, $-\text{COOR}^{15}$, $-\text{OR}^{15}$, (wherein R^{15} is hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted

aryl, substituted or unsubstituted heteroaryl or acyl), $-\text{SR}^7$ (wherein R^7 is as defined above), $-\text{CONR}^5\text{R}^6$, $-\text{NR}^5\text{R}^6$ (wherein R^5 and R^6 are as defined above) or $-\text{N}_3$;

(f) $-\text{CH}=\text{CH}(\text{CH}_2)_m\text{R}^{16}$, wherein m is 0 to 4, and R^{16} is hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, $-\text{COOR}^{15}$, $-\text{OR}^{15}$ (wherein R^{15} is as defined above) - CONR^5R^6 or $-\text{NR}^5\text{R}^6$ (wherein R^5 and R^6 are as defined above);

(g) $-\text{CH}=\text{C}(\text{C}_2\text{R}^{12})_2$, wherein R^{12} is as defined above;

(h) $-\text{C}\equiv\text{C}(\text{CH}_2)_n\text{R}^{16}$, wherein n is 0 to 4 and R^{16} is as defined above;

(i) $-\text{CH}_2\text{OR}^{22}$, wherein R^{22} is tri-lower alkyl silyl in which the three lower alkyl groups are the same or different or wherein R^{22} has the same meaning as R^8

(j) $-\text{CH}(\text{SR}^{23})_2$ and $-\text{CH}_2-\text{SR}^7$ wherein R^{23} is lower alkyl, lower alkenyl or lower alkynyl and wherein R^7 is as defined above; and

R_3 is hydrogen, halogen, acyl, carbamoyl, substituted or unsubstituted lower alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted lower alkynyl or amino; and W^1 and W^2 are independently hydrogen, hydroxy or W^1 and W^2 together represent oxygen;

and wherein in formula (I), X is a polymer moiety, either linear or branched,

and wherein in formula (II), A represents $-\text{U}-\text{X}'$ and B represents $-\text{L}^2-\text{Y}'$, wherein at least one of X' and Y' is a polymer moiety, either linear or branched, which is bound by L^1 and/or L^2 to the tetrahydrofuran ring of the compound of formula (II); L^1 and/or L^2 are a covalent chemical bond or a linker group;

when Y' is a polymer moiety, and X' is not a polymer, L^1 is a covalent chemical bond and X' is selected from the group consisting of

(a) hydrogen, lower hydroxyalkyl, acyl, carboxy, lower alkoxycarbonyl,

(b) $-\text{CONR}^{17a}\text{R}^{17b}$, wherein R^{17a} and R^{17b} are each independently selected from

(i) hydrogen, lower alkyl, lower alkenyl, lower alkynyl,

(ii) $-\text{CH}_2\text{R}^{18}$; wherein R^{18} is hydroxy,

or (iii) $-\text{NR}^{19}\text{R}^{20}$, wherein R^{19} or R^{20} are each independently selected from hydrogen, lower alkyl, lower alkenyl, lower alkynyl or R^{19} or R^{20} are independently the residue of an α -amino acid in which the hydroxy group of the carboxyl group is excluded, or R^{19} or R^{20} are combined with a nitrogen atom to form a heterocyclic group; and

(c) $-\text{CH}=\text{N}-\text{R}^{21}$, wherein R^{21} is hydroxy, lower alkoxy, amino, guanidino, or imidazolylamino; and

when X' is a polymer moiety, and Y' is not a polymer, L² is a covalent chemical bond and Y' is selected from hydroxy, lower alkoxy, aralkyloxy, or acyloxy.

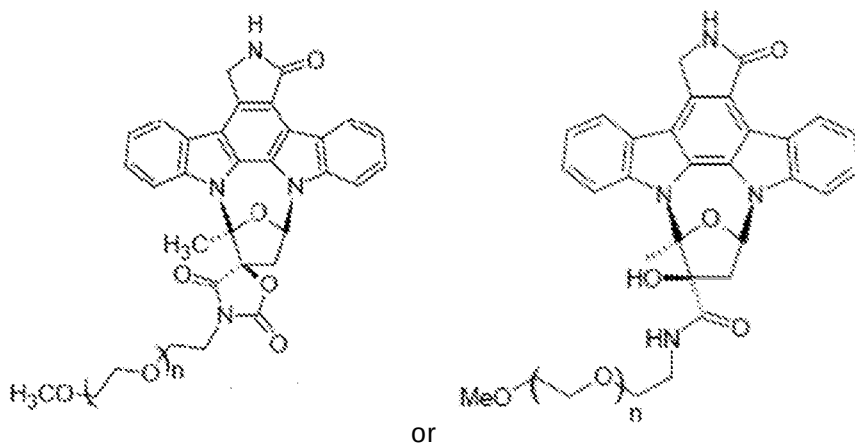
[101] In some embodiments, the compound is a compound of Formula (I).

[102] In some embodiments, the compound is a compound of Formula (II).

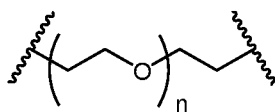
[103] In some embodiments: R¹ is hydrogen, halogen, or lower alkyl; R² is hydrogen, halogen, or lower alkyl; R₃ is hydrogen, halogen, or lower alkyl; W¹ is hydrogen or hydroxy; W² is hydrogen or hydroxy; or W¹ and W² together represent oxygen; X is a linear polymer moiety or a branched polymer moiety; A represents -L¹-X'; B represents -L²-Y'; L¹ is a covalent chemical bond or a linker group; X' is a polymer moiety; L² is a covalent chemical bond; and Y' is hydroxy, lower alkoxy, or aralkyloxy.

[104] In some embodiments, the polymer moiety has a molecular weight of about 500 to about 10,000 Da.

[105] In some embodiments, the compound is

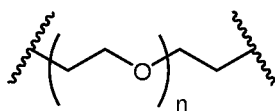


or a pharmaceutically acceptable salt thereof,
wherein



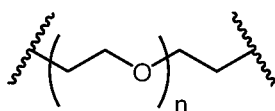
has a molecular weight of about 500 to about 10,000 Da.

[106] In some embodiments,



has a molecular weight of about 1000-3000 Da.

[107] In some embodiments,



has a molecular weight of about 2000 Da.

[108] In some embodiments, the compound is SNA-120, or a pharmaceutically acceptable salt thereof.

[109] In some embodiments, the compound is SNA-125, or a pharmaceutically acceptable salt thereof.

[110] In some embodiments, the gastrointestinal system condition is inflammatory bowel disease, irritable bowel syndrome, small intestinal bacterial overgrowth, Crohn's disease, colitis, intestinal fibrosis, or colorectal cancer.

[111] In some embodiments, the gastrointestinal system is colitis.

[112] In some embodiments, the colitis is ulcerative colitis, collagenous colitis, lymphocytic colitis, ischemic colitis, diversion colitis, or indeterminate colitis.

[113] In some embodiments, the gastrointestinal system condition is ulcerative colitis, Crohn's disease, intestinal fibrosis, or colorectal cancer.

[114] In some embodiments, the dermal condition is pruritus, skin neoplasia, vascular tumor, inflammatory dermatosis, scarring, a bullous disease, a dermal condition caused by ultra-violet irradiation therapy, alopecia areata, or vitiligo.

[115] In some embodiments: pruritus is pruritus due to psoriasis, pruritus due to atopic dermatitis, or pruritus due to prurigo nodularis; skin neoplasia is squamous cell carcinoma, basal cell carcinoma, malignant melanoma, or malignant cutaneous lymphoma; vascular tumor is angiosarcoma, kaposi's sarcoma, infantile hemangioma, or hemangioendothelioma; inflammatory dermatosis is dermatitis, psoriasis, keloid, or rosacea; and the bullous disease is bullous pemphigoid or erythema multiforme.

[116] In some embodiments, dermatitis is atopic dermatitis or irritant contact dermatitis.

[117] In some embodiments, the dermal condition comprises pruritic psoriatic plaques, pruritis associated with psoriasis vulgaris, psoriasis or dermatitis.

[118] In some embodiments, the administration comprises topical administration.

[119] In some embodiments: the subject is administered an ointment comprising about 0.5 % w/w of the compound, or a pharmaceutically acceptable salt thereof; and the ointment is administered twice a day.

[120] In some embodiments: the subject is administered an ointment comprising about 0.5 % w/w of the compound, or a pharmaceutically acceptable salt thereof; and the ointment is administered at a frequency of about every 12 hours.

[121] In some embodiments: the subject is administered an ointment comprising about 0.5 % w/w of the compound; the compound is SNA-120, or a pharmaceutically acceptable salt thereof; and the ointment is administered twice a day.

[122] In some embodiments: the subject is administered an ointment comprising about 0.5 % w/w of the compound; the compound is SNA-120, or a pharmaceutically acceptable salt thereof; and the ointment is administered at a frequency of about every 12 hours.

[123] In some embodiments: the subject is administered an ointment comprising about 0.5 % w/w of the compound; the compound is SNA-120, or a pharmaceutically acceptable salt thereof; a plasma concentration of the compound, or a metabolite thereof, is maintained at or below 5.0 ng/mL in the subject; and the ointment is administered twice a day.

[124] In some embodiments: the subject is administered an ointment comprising about 0.5 % w/w of the compound; the compound is SNA-120, or a pharmaceutically acceptable salt thereof; a plasma concentration of the compound, or a metabolite thereof, is maintained at or below 5.0 ng/mL in the subject; and the ointment is administered at a frequency of about every 12 hours.

[125] In some embodiments, the dermal condition affects at least about 20 % of the body surface area of the subject.

[126] In some embodiments, the ointment is administered to at least about 20 % of the body surface area of the subject affected by the dermal condition.

[127] In some embodiments, a dosage of the compound is topically administered to the subject twice in a day.

[128] In some embodiments, a dosage of the compound is topically administered to the subject at a frequency of about every 12 hours.

[129] In some embodiments, the daily dosage of the compound is up to about 100 mg.

[130] In some embodiments, the compound is formulated in an ointment.

[131] In some embodiments, the ointment comprises about 0.5 % w/w of the compound.

[132] In some embodiments, about 7 g to about 14 g of an ointment is administered daily, and the ointment comprises about 0.5 % w/w of the compound.

[133] In some embodiments, the compound is administered daily for about four weeks.

[134] In some embodiments, the compound is administered daily for at least four weeks.

[135] In some embodiments, the compound is administered once or twice daily for about four weeks, and the PASI score of the subject is reduced by about 50 % to about 75 % as compared to the PASI score of the subject without administration of the compound.

[136] In some embodiments, about 30 mg to about 100 mg of the compound is administered.

[137] In some embodiments, the threshold plasma concentration is about 2.5 ng/mL.

[138] In some embodiments, the threshold plasma concentration is about 0.5 ng/mL.

[139] In some embodiments, the compound is administered twice in a day.

[140] In some embodiments, the compound is administered once in a day.

[141] In another aspect, provided herein are methods of treating pruritus associated with psoriasis in a subject in need thereof, comprising topically administering twice daily to the subject an ointment comprising 0.5 % w/w of SNA-120.

[142] In some embodiments of these aspects, the ointment is administered at a frequency of about every 12 hours.

[143] In another aspect, provided herein are methods of improving the histological characteristics of lesional psoriasis skin in a subject in need thereof, comprising administering to the subject an effective amount of SNA-125.

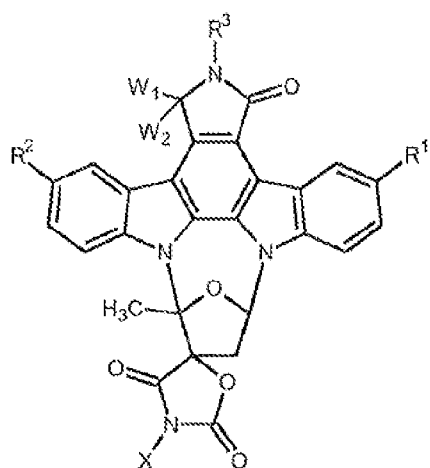
[144] In another aspect, provided herein are methods of improving the molecular signature of lesional psoriasis skin in a subject in need thereof, comprising administering to the subject an effective amount of SNA-125.

[145] In another aspect, provided herein are methods of treating psoriasis in a subject in need thereof, comprising administering to the subject an effective amount of SNA-125.

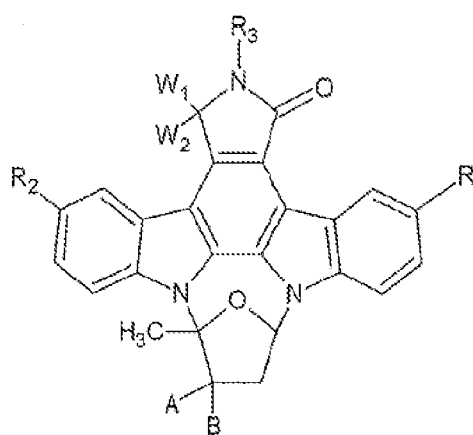
[146] In some embodiments of these methods, the SNA-125 is formulated in a composition.

[147] In some embodiments of these methods, the composition is administered topically and the composition is a pharmaceutical composition that is a cream, an ointment, a lotion, or a gel.

[148] In another aspect, provided herein are methods of treating a condition in a subject in need thereof, comprising administering to the subject an effective amount of a compound of Formula (I) or of Formula (II):



formula (I)



formula (II)

or a pharmaceutically acceptable salt thereof,
wherein in formula (I) and (II)

R^1 and R^2 are the same or a different residue and are each independently selected from the group consisting of:

(a) hydrogen, halogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, hydroxy, lower alkoxy, carboxy, lower alkoxycarbonyl, acyl, nitro, carbamoyl, lower alkylaminocarbonyl, $-NR^5R^6$, wherein R^5 and R^6 are each independently selected from hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted aralkyl, substituted or unsubstituted lower alkylaminocarbonyl, substituted or unsubstituted lower arylaminocarbonyl, alkoxycarbonyl, carbamoyl, acyl or R^5 and R^6 are combined with a nitrogen atom to form a heterocyclic group,

(b) $-\text{CO}(\text{CH}_2)_j\text{R}^4$, wherein j is 1 to 6, and R^4 is selected from the group consisting of

(i) hydrogen, halogen, $-\text{N}_3$,

(ii) $-\text{NR}^5\text{R}^6$, wherein R^5 and R^6 are as defined above,

(iii) $-\text{SR}^7$, wherein R^7 is selected from the group consisting of hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted aralkyl, $-(\text{CH}_2)_a\text{CO}_2\text{R}^{10}$ (wherein a is 1 or 2, and wherein R^{10} is selected from the group consisting of hydrogen and substituted or unsubstituted lower alkyl) and $-(\text{CH}_2)_a\text{CO}_2\text{NR}^5\text{R}^6$,

(iv) $-\text{OR}^8$, $-\text{OCOR}^8$, wherein R^8 is selected from hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl

(c) $-\text{CH}(\text{OH})(\text{CH}_2)_j\text{R}^4$ wherein j and R^4 are as defined above;

(d) $-(\text{CH}_2)_d\text{CHR}^{11}\text{CO}_2\text{R}^{12}$ or $-(\text{CH}_2)_d\text{CHR}^{11}\text{CONR}^5\text{R}^6$, wherein d is 0 to 5, R^{11} is hydrogen, $-\text{CONR}^5\text{R}^6$, or $-\text{CO}_2\text{R}^{13}$, wherein R^{13} is hydrogen or a wherein substituted or unsubstituted lower alkyl, and R^{12} is hydrogen or a substituted or unsubstituted lower alkyl;

(e) $-(\text{CH}_2)_k\text{R}^{14}$ wherein k is 2 to 6 and R^{14} is halogen, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, $-\text{COOR}^{15}$, $-\text{OR}^{15}$, (wherein R^{15} is hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl or acyl), $-\text{SR}^7$ (wherein R^7 is as defined above), $-\text{CONR}^5\text{R}^6$, $-\text{NR}^5\text{R}^6$ (wherein R^5 and R^6 are as defined above) or $-\text{N}_3$;

(f) $-\text{CH}=\text{CH}(\text{CH}_2)_m\text{R}^{16}$, wherein m is 0 to 4, and R^{16} is hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, $-\text{COOR}^{15}$, $-\text{OR}^{15}$, $-\text{SR}^7$, $-\text{CONR}^5\text{R}^6$, $-\text{NR}^5\text{R}^6$ (wherein R^5 and R^6 are as defined above) or $-\text{N}_3$;

OR¹⁵ (wherein R¹⁵ is as defined above) -CONR⁵R⁶ or -NR⁵R⁶ (wherein R⁵ and R⁶ are as defined above);

(g) -CH=C(CO₂R¹²)₂, wherein R¹² is as defined above;

(h) -C≡C(CH₂)_nR¹⁶, wherein n is 0 to 4 and R¹⁶ is as defined above;

(i) -CH₂OR²², wherein R²² is tri-lower alkyl silyl in which the three lower alkyl groups are the same or different or wherein R²² has the same meaning as R⁸

(j) -CH(SR²³)₂ and -CH₂-SR⁷ wherein R²³ is lower alkyl, lower alkenyl or lower alkynyl and wherein R⁷ is as defined above; and

R₃ is hydrogen, halogen, acyl, carbamoyl, substituted or unsubstituted lower alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted lower alkynyl or amino; and W¹ and W² are independently hydrogen, hydroxy or W¹ and W² together represent oxygen;

and wherein in formula (I), X is a polymer moiety, either linear or branched,

and wherein in formula (II), A represents -U-X' and B represents -L²-Y', wherein at least one of X and Y' is a polymer moiety, either linear or branched, which is bound by L¹ and/or L² to the tetrahydrofuran ring of the compound of formula (II); L¹ and/or L² are a covalent chemical bond or a linker group;

when Y' is a polymer moiety, and X' is not a polymer, L¹ is a covalent chemical bond and X is selected from the group consisting of

(a) hydrogen, lower hydroxyalkyl, acyl, carboxy, lower alkoxy carbonyl,

(b) -CONR^{17a}R^{17b}, wherein R^{17a} and R^{17b} are each independently selected from

(i) hydrogen, lower alkyl, lower alkenyl, lower alkynyl,

(ii) -CH₂R¹⁸; wherein R¹⁸ is hydroxy,

or (iii) -NR¹⁹R²⁰, wherein R¹⁹ or R²⁰ are each independently selected from hydrogen, lower alkyl, lower alkenyl, lower alkynyl or R¹⁹ or R²⁰ are independently the residue of an α-amino acid in which the hydroxy group of the carboxyl group is excluded, or R¹⁹ or R²⁰ are combined with a nitrogen atom to form a heterocyclic group; and

(c) -CH=N-R²¹, wherein R²¹ is hydroxy, lower alkoxy, amino, guanidino, or imidazolylamino; and

when X is a polymer moiety, and Y' is not a polymer, L² is a covalent chemical bond and Y' is selected from hydroxy, lower alkoxy, aralkyloxy, or acyloxy.

[149] In some embodiments of the aspects provided herein, the condition is a dermal condition, a gastrointestinal condition, an ophthalmological condition, a respiratory condition, or a combination thereof.

[150] In some embodiments of the aspects provided herein, the dermal condition is pruritus (e.g., pruritus due to psoriasis, pruritus due to atopic dermatitis, or prurigo nodularis), skin neoplasia (e.g., squamous cell carcinoma, basal cell carcinoma, malignant melanoma, or malignant cutaneous lymphoma), vascular tumor (e.g., angiosarcoma, Kaposi's sarcoma,

infantile hemangioma, or hemangioendothelioma), inflammatory dermatosis (e.g., dermatitis (e.g., atopic dermatitis or irritant contact dermatitis), psoriasis, keloid, or rosacea), scarring, a bullous disease (e.g., bullous pemphigoid or erythema multiforme), UV irradiation therapy, alopecia areata, vitiligo, dry skin pruritus in the elderly, warts, or a combination thereof.

[151] In some embodiments of the aspects provided herein, the dermal condition is angiosarcoma, Kaposi's sarcoma, infantile hemangioma, hemangioendothelioma, irritant contact dermatitis, rosacea, bullous pemphigoid, erythema multiforme, UV irradiation therapy, alopecia areata, vitiligo, or a combination thereof.

[152] In some embodiments of the aspects provided herein, the dermal condition is pruritus, prurigo nodularis, postherpetic neuralgia, seborrheic dermatitis, lichen simplex chronicus, Grover's disease, neurodermatitis, pemphigoid, bullous pemphigoid, epidermolysis bullosa, alopecia areata, atopic dermatitis, chronic actinic dermatitis, chronic mucocutaneous candidiasis, cutaneous t-cell lymphoma, dermatomyositis, erythema multiforme, graft-versus-host disease (cutaneous), hypereosinophilic syndrome, lupus erythematosus, mastocytosis and mast cell disease, STING vasculopathy, palmoplantar pustulosis, polyarteritis nodosa, psoriasis, vitiligo, dry skin pruritus in the elderly, warts, or a combination thereof.

[153] In some embodiments of the aspects provided herein, the dermal condition is pruritus due to intrahepatic cholestasis, posthepatic cholestasis, drug-induced cholestasis, chronic renal failure, dialysis treatment, diabetes mellitus, systemic parasitosis, Sjogren's syndrome, AIDS, drug eruption, pregnancy, a psychogenic condition, sclerosis multiplex, stroke, delusion of parasitosis, lymphoma, mastocytosis, polycythemia vera, iron-deficiency anemia, hyperthyroidism, hyperparathyroidism, brain tumor, myxoedema, acute eczema, chronic eczema, monetary eczema, sebaceous deficiency eczema, contact dermatitis, atopic dermatitis, seborrheic dermatitis, neurodermatitis, acute prurigo (strophus, urticaria, lichen urticatus), subacute prurigo, chronic prurigo (nodular prurigo), systemic skin pruritus, focal skin pruritus, or a combination thereof.

[154] In some embodiments of the aspects provided herein, the gastrointestinal condition is ulcerative colitis, Crohn's disease, intestinal fibrosis, colorectal cancer, irritable bowel syndrome, visceral pain, or a combination thereof.

[155] In some embodiments of the aspects provided herein, the ophthalmological condition is age-related macular degeneration (e.g., wet age-related macular degeneration or dry age-related macular degeneration), diabetic retinopathy, edema (e.g., macular edema (e.g., diabetic macular edema) or corneal edema), dry eye (e.g., spontaneous dry eye or aqueous deficiency dry eye), uveitis (e.g., juvenile idiopathic arthritis uveitis or posterior uveitis), pterygia, conjunctivitis (e.g., vernal keratoconjunctivitis (allergic conjunctivitis) or atopic keratoconjunctivitis), graft versus host disease (e.g., ophthalmic graft versus host disease),

glaucoma (e.g., primary open angle glaucoma), retinopathy of prematurity, or a combination thereof.

[156] In some embodiments of the aspects provided herein, the ophthalmological condition is age-related macular degeneration (e.g., wet age-related macular degeneration or dry age-related macular degeneration), edema (e.g., macular edema (e.g., diabetic macular edema) or corneal edema), dry eye (e.g., spontaneous dry eye or aqueous deficiency dry eye), juvenile idiopathic arthritis uveitis, posterior uveitis, pterygia, conjunctivitis (e.g., vernal keratoconjunctivitis (allergic conjunctivitis) or atopic keratoconjunctivitis), ophthalmic graft versus host disease, glaucoma (e.g., primary open angle glaucoma), retinopathy of prematurity, diabetic retinopathy, or a combination thereof.

[157] In some embodiments of the aspects provided herein, the respiratory condition is asthma (e.g., T_H2 asthma or non-T_H2 asthma), chronic obstructive pulmonary disease, pulmonary fibrosis (e.g., idiopathic pulmonary fibrosis), pulmonary hypertension, sinusitis with nasal polyposis, allergic rhinitis, cough (e.g., chronic cough), lymphangioleiomyomatosis, or a combination thereof.

[158] In some embodiments of the aspects provided herein, the condition is rheumatoid arthritis, osteoarthritis, neuropathic pain, neuropathic itch, or a combination thereof.

BRIEF DESCRIPTION OF THE FIGURES

[159] The Figures below are illustrative for some embodiments and should not be construed as overly limiting.

[160] Figure 1 depicts luciferase activity of THP1 cells stimulated with a concentration range of HKLM or LPS, calculated relative to unstimulated cells.

[161] Figure 2 depicts plasma levels after intravenous dosing.

[162] Figure 3 depicts body weight versus day of study - males.

[163] Figure 4 depicts body weight versus day of study - males.

[164] Figure 5 depicts plasma levels after intravenous dosing - Day 1.

[165] Figure 6 depicts plasma levels after intravenous dosing - Week 2.

[166] Figure 7 depicts body weight versus day of study - Males.

[167] Figure 8 depicts body weight versus day of study - Males.

[168] Figure 9 depicts body weight versus day of study (Main phase) - Males.

[169] Figure 10 depicts body weight versus day of study (Main phase) - Males.

[170] Figure 11 depicts normalized body weight trends.

[171] Figure 12 depicts scar formation; SEI of saline-injected scars (Group 1, Left Ears). Values are expressed as mean $\pm$ StdDev.

[172] Figure 13 depicts scar formation; SEI of vehicle-treated scars (Group 1, Right Ears). Values are expressed as mean $\pm$ StdDev.

[173] Figure 14 depicts scar formation; SEI of CT340-injected scars (Group 2). Values are expressed as mean $\pm$ StdDev.

[174] Figure 15 depicts Scar Formation; SEI of CT340 topical-dosed scars (Group 3). Values are expressed as mean $\pm$ StdDev.

[175] Figure 16 depicts Scar Formation; SEI of TACA-treated scars (Group 4). Values are expressed as mean $\pm$ StdDev.

[176] Figure 17 depicts scar formation; summary of SEI. Values are expressed as mean $\pm$ StdDev.

[177] Figure 18 depicts scar formation following intra-lesion injections with CT340 or TACA.

[178] Figure 19 depicts scar formation following topical dosing with CT340

[179] Figure 20 depicts scar formation SEI of CT340-treated scars, Values are expressed as mean $\pm$ StdDev.

[180] Figure 21 depicts scar inflammation scores following intra-lesion injections with CT340 or TACA. Values are expressed as mean $\pm$ StdDev.

[181] Figure 22 depicts scar inflammation scores following topical dosing with CT340, Values are expressed as mean $\pm$ StdDev.

[182] Figure 23 depicts the inhibition of proliferation by K252a, CT327 and CT340. Column bar graphs of proliferation assay results expressed as Absorbance at 570 nm with reference at 650 nm.

[183] Figure 24 depicts the inhibition of proliferation by K252a, CT327 and CT340. Column bar graphs of proliferation assay results expressed as Absorbance at 570 nm with reference at 650 nm.

[184] Figure 25 depicts the inhibition of proliferation by K252a, CT327 and CT340. Column bar graphs of proliferation assay results expressed as Absorbance at 570 nm with reference at 650 nm.

[185] Figure 26 depicts a JAK2 vs Staurosporine concentration-%inhibition curve used to derive the slope (1.853), R^2 (1.00), and IC_{50} (4.30E-10).

[186] Figure 27 depicts a JAK2 vs CT340 concentration-%inhibition curve used to derive the slope (1.147), R^2 (1.00), and IC_{50} (1.35E-07).

[187] Figure 28 depicts a JAK3 vs Staurosporine concentration-%inhibition curve used to derive the slope (1.597), R^2 (1.00), and IC_{50} (2.78E-10).

[188] Figure 29 depicts a JAK3 vs CT340 concentration-%inhibition curve used to derive the slope (1.164), R^2 (1.00), and IC_{50} (3.87E-08).

[189] Figure 30 depicts a PDGFRb vs Staurosporine concentration-%inhibition curve used to derive the slope (2.900), R^2 (1.00), and IC_{50} (3.87E-10).

[190] Figure 31 depicts a PDGFRb vs CT340 concentration-%inhibition curve used to derive the slope (1.165), R^2 (1.00), and IC_{50} (1.12E-07).

[191] Figure 32 depicts a TRKA vs Staurosporine concentration-%inhibition curve used to derive the slope (2.106), R^2 (1.00), and IC_{50} (5.02E-10).

[192] Figure 33 depicts a TRKA vs CT340 concentration-%inhibition curve used to derive the slope (1.159), R^2 (1.00), and IC_{50} (2.55E-08).

[193] Figure 34 depicts a MAP2K1 vs Staurosporine concentration-%inhibition curve used to derive the slope (1.287), R^2 (1.00), and IC_{50} (1.39E-09).

[194] Figure 35 depicts a MAP2K1 vs CT340 concentration-%inhibition curve used to derive the slope (1.434), R^2 (1.00), and IC_{50} (1.96E-08).

[195] Figure 36 depicts a MAP2K3 vs Staurosporine concentration-%inhibition curve used to derive the slope (1.402), R^2 (1.00), and IC_{50} (1.13E-09).

[196] Figure 37 depicts a MAP2K3 vs CT340 concentration-%inhibition curve used to derive the slope (1.411), R^2 (1.00), and IC_{50} (1.26E-08).

[197] Figure 38 depicts a TAK1-TAB1 vs Staurosporine concentration-%inhibition curve used to derive the slope (1.369), R^2 (.98), and IC_{50} (4.14E-08).

[198] Figure 39 depicts a TAK1-TAB1 vs CT340 concentration-%inhibition curve used to derive the slope (1.480), R^2 (.98), and IC_{50} (2.19E-07).

[199] Figure 40 depicts CT327 effect on Capsaicin responses. Inhibition of capsaicin responses in DRG neurons by CT327 was between $35.6 \pm 3.0\%$ (1 nM CT327), and $57 \pm 5.3\%$ (10 μ M CT327). Results are given as mean (percent inhibition) $\pm$ s.e.m. P values were obtained from comparison between percent inhibition of each dose with control. Further, for 10 pM vs 10 nM, $P=0.008$, 10 pM vs 1 nM $P=0.001$, and 1 pM vs 1 nM $P=0.007$.

[200] Figure 41 depicts CT340 effect on Capsaicin responses. Inhibition of capsaicin responses in DRG neurons by CT340 was between $35.6 \pm 3.7\%$ (1 nM CT340), and $57.4 \pm 4.3\%$ (10 pM CT340). Results are given as mean (percent inhibition) $\pm$ s.e.m. 10 pM vs 10 nM, $P=0.01$, 10 pM vs 1 nM, $P=0.0009$; 1 pM vs 1 nM, $P=0.04$.

[201] Figure 42 depicts GW441756 effect on Capsaicin responses. Inhibition of capsaicin responses in DRG neurons by GW441756 was between $39.8 \pm 4.1\%$ (1 nM GW441756), and $54.4 \pm 7.4\%$ (1.5 pM GW441756). $IC_{50}=15$ pM. Results are given as mean (percent inhibition) $\pm$ s.e.m.

[202] Figure 43 depicts anti-NGF antibody effect on Capsaicin responses. Inhibition of capsaicin responses in DRG neurons by anti-NGF was between $37.4 \pm 7.7\%$ (100 ng/ml), and $63.3 \pm 9.7\%$ (10 pg/ml). Results are given as mean (percent inhibition) $\pm$ s.e.m.

[203] Figure 44 depicts the effect of compound incubation on neurite length. Treatment with CT327 and CT340 at 1 nM, 10 nM, 100 nM, 1 pM and 10 pM concentrations did not affect neurite length of neurons compared with control. Treatment with GW441756 resulted

in vesiculation and reduced neurite length at the higher concentrations of 1 μ M and 10 μ M. Anti-NGF antibody treatment at 1 and 10 μ g/ml concentrations also did not affect neurite length. Neurite lengths were normalized to controls and are given as mean percent of control $\pm$ s.e.m.

[204] Figure 45 depicts the effect of CT327 incubation on neurite length. 24 hour Incubation with CT327 did not have any effect on neurite length. Neurite lengths are expressed as mean percent of control $\pm$ s.e.m.

[205] Figure 46 depicts the effect of CT340 incubation on neurite length. 24 hour Incubation with CT340 did not significantly affect neurite length compared to control. Neurite lengths are expressed as mean percent of control $\pm$ s.e.m.

[206] Figure 47 depicts the effect of GW441756 incubation on neurite length. Commercial TrkA inhibitor GW441756 had no effect on neurite length at concentrations up to 100 nM concentration, but appeared to diminish neurite length at 1 μ M ($P=0.09$ n.s.) and 10 μ M ($*P=0.03$) concentrations, with vesiculation of neurites (see Figure 44). Neurons treated with 0.33% ethanol (solvent for GW441756) had similar neurite length compared with NGF-treated controls. Neurite lengths are expressed as mean percent of control $\pm$ s.e.m.

[207] Figure 48 depicts the effect of anti-NGF antibody incubation on neurite length. Neurons treated with anti-NGF at 1 or 10 μ g/ml did not show a significant change in neurite length compared to control. Neurite lengths are expressed as mean percent of control $\pm$ s.e.m.

[208] Figure 49 depicts TrkA/Gap43 immunostaining in DRG neurons: A) Merged image showing co-localization of TrkA and Gap43 immunostaining in DRG neuron; B) Gap43 was strongly localized in cell bodies and neurites; C) TrkA immunostaining was observed to be densely localized in the cell bodies, while neurites were very faint.

[209] Figure 50 depicts representative PGP9.5-immunoreactive intra-epithelial nerve fibres (IEFN) (arrowed) in untreated control skin (top panel) and treated skin region (bottom panel) mini-pig skin using an antibody dilution of 1:40,000, magnification x40.

[210] Figure 51 depicts a scatter plot showing the PGP9.5 intra epithelial fibre counts in untreated and treated mini-pig skin from the various groups. The median value is indicated. C, control; L, low dose; M, medium dose; H high dose; R, recovery; ut untreated area; t treated area.

[211] Figure 52 depicts the BioMAP profile of SNA-120 in the Diversity PLUS Panel. The X-axis lists the quantitative protein-based biomarker readouts measured in each system. The Y-axis represents a log-transformed ratio of the biomarker readouts for the drug-treated sample ($n = 1$) over vehicle controls ($n \geq 6$). The grey region around the Y-axis represents the 95% significance envelope generated from historical vehicle controls. Biomarker activities are annotated when 2 or more consecutive concentrations change in the same

direction relative to vehicle controls, are outside of the significance envelope, and have at least one concentration with an effect size $> 20\%$ ($|\log_{10} \text{ratio}| > 0.1$). Biomarker key activities are described as modulated if these activities increase in some systems, but decrease in others. Cytotoxicity is indicated on the profile plot by a thin black arrow above the X-axis, and antiproliferative effects are indicated by a thick grey arrow. Cytotoxicity and antiproliferative arrows only require one concentration to meet the indicated threshold for profile annotation. Other BioMAP profiles disclosed herein are also depicted in a similar manner.

[212] Figure 53 depicts a Reference Benchmark Overlay of SNA-120 and Benchmark SR221 1. Common biomarker readouts are annotated when the readout for both profiles is outside of the significance envelope with an effect size $> 20\%$ ($|\log_{10} \text{ratio}| > 0.1$) in the same direction.

[213] Figure 54 depicts the changes in secretion of (a) IL-17F (b) IgG, (c) IL-17A, and (d) TNFa in the BioMAP BT system mediated by SNA-120 (3.1 μM), SNA-125 (3.9 μM), Tofacitinib (3.3 pM), Apremilast (3.3 pM), SR221 1 (3.3 pM), and Cyclosporin A (3.3 pM).

[214] Figure 55 depicts an overlay of SNA-120 (28 pM) and GSK690693 (10 pM), which was the top similarity match from a search of the BioMAP Reference Database of $> 4,000$ agents for SNA-120 (28 pM). Common biomarker readouts are annotated when the readout for both profiles is outside of the significance envelope with an effect size $> 20\%$ ($|\log_{10} \text{ratio}| > 0.1$) in the same direction. Similarity search results are filtered and ranked. Profiles are identified as having mechanistically relevant similarity if the Pearson's correlation coefficient is ≥ 0.7 .

[215] Figure 56 depicts Mechanism HeatMAP Analysis for SNA-120. HeatMAP analysis of the 148 biomarker readouts (rows) within the Diversity PLUS panel by SNA-120 in comparison to 19 consensus mechanism class profiles (columns). Horizontal grey lines separate the 12 Diversity PLUS systems, while the vertical grey line separates SNA-120 from the 19 consensus mechanism profiles. Biomarker activities outside of the significance envelope are red if protein levels are increased, blue if protein levels are decreased and white if levels are within the envelope or unchanged. Darker shades of color represent greater change in biomarker activity relative to vehicle control.

[216] Figure 57 depicts clustering of test agent profiles following pairwise correlation analysis and clustering of the most similar profiles. Each colored circle represents the BioMAP profile of a compound at a specific concentration, with larger circles representing higher concentrations.

[217] Figure 58 depicts the BioMAP profile of SNA-125 in the Diversity PLUS Panel. The X-axis lists the quantitative protein-based biomarker readouts measured in each system. The Y-axis represents a log-transformed ratio of the biomarker readouts for the drug-treated

sample ($n = 1$) over vehicle controls ($n \geq 6$). The grey region around the Y-axis represents the 95% significance envelope generated from historical vehicle controls. Biomarker activities are annotated when 2 or more consecutive concentrations change in the same direction relative to vehicle controls, are outside of the significance envelope, and have at least one concentration with an effect size $> 20\%$ ($|\log_{10} \text{ratio}| > 0.1$). Biomarker key activities are described as modulated if these activities increase in some systems, but decrease in others. Cytotoxicity is indicated on the profile plot by a thin black arrow above the X-axis, and antiproliferative effects are indicated by a thick grey arrow. Cytotoxicity and antiproliferative arrows only require one concentration to meet the indicated threshold for profile annotation. Other BioMAP profiles disclosed herein are also depicted in a similar manner.

[218] Figure 59 depicts a Reference Benchmark Overlay of SNA-125 and Benchmark Tofacitinib. Common biomarker readouts are annotated when the readout for both profiles is outside of the significance envelope with an effect size $> 20\%$ ($|\log_{10} \text{ratio}| > 0.1$) in the same direction.

[219] Figure 60 depicts an overlay of SNA-125 ($3.9 \mu\text{M}$) and SB203580 ($10 \mu\text{M}$), which was the top similarity match from a search of the BioMAP Reference Database of $> 4,000$ agents for SNA-125 ($3.9 \mu\text{M}$). Common biomarker readouts are annotated when the readout for both profiles is outside of the significance envelope with an effect size $> 20\%$ ($|\log_{10} \text{ratio}| > 0.1$) in the same direction. Similarity search results are filtered and ranked. Profiles are identified as having mechanistically relevant similarity if the Pearson's correlation coefficient is ≥ 0.7 .

[220] Figure 61 depicts Mechanism HeatMAP Analysis for SNA-125. HeatMAP analysis of the 148 biomarker readouts (rows) within the Diversity PLUS panel by SNA-125 in comparison to 19 consensus mechanism class profiles (columns). Horizontal grey lines separate the 12 Diversity PLUS systems, while the vertical grey line separates SNA-125 from the 19 consensus mechanism profiles. Biomarker activities outside of the significance envelope are red if protein levels are increased, blue if protein levels are decreased and white if levels are within the envelope or unchanged. Darker shades of color represent greater change in biomarker activity relative to vehicle control.

[221] Figure 62 depicts the BioMAP profile of SNA-125 in the Diversity PLUS Panel. The X-axis lists the quantitative protein-based biomarker readouts measured in each system. The Y-axis represents a log-transformed ratio of the biomarker readouts for the drug-treated sample ($n = 1$) over vehicle controls ($n \geq 6$). The grey region around the Y-axis represents the 95% significance envelope generated from historical vehicle controls. Biomarker activities are annotated when 2 or more consecutive concentrations change in the same direction relative to vehicle controls, are outside of the significance envelope, and have at

least one concentration with an effect size $> 20\%$ ($|\log_{10} \text{ratio}| > 0.1$). Biomarker key activities are described as modulated if these activities increase in some systems, but decrease in others. Cytotoxicity is indicated on the profile plot by a thin black arrow above the X-axis, and antiproliferative effects are indicated by a thick grey arrow. Cytotoxicity and antiproliferative arrows only require one concentration to meet the indicated threshold for profile annotation. Other BioMAP profiles disclosed herein are also depicted in a similar manner.

[222] Figure 63 depicts a Reference Benchmark Overlay of SNA-125 and Benchmark K252a. Common biomarker readouts are annotated when the readout for both profiles is outside of the significance envelope with an effect size $> 20\%$ ($|\log_{10} \text{ratio}| > 0.1$) in the same direction.

[223] Figure 64 depicts an overlay of SNA-125 (30 μM) and IKK 16 (370 nM), which was the top similarity match from a search of the BioMAP Reference Database of $> 4,000$ agents for SNA-125 (30 μM). Common biomarker readouts are annotated when the readout for both profiles is outside of the significance envelope with an effect size $> 20\%$ ($|\log_{10} \text{ratio}| > 0.1$) in the same direction. Similarity search results are filtered and ranked. Profiles are identified as having mechanistically relevant similarity if the Pearson's correlation coefficient is ≥ 0.7 .

[224] Figure 65 depicts Mechanism HeatMAP Analysis for SNA-125. HeatMAP analysis of the 148 biomarker readouts (rows) within the Diversity PLUS panel by SNA-125 in comparison to 19 consensus mechanism class profiles (columns). Horizontal grey lines separate the 12 Diversity PLUS systems, while the vertical grey line separates SNA-125 from the 19 consensus mechanism profiles. Biomarker activities outside of the significance envelope are red if protein levels are increased, blue if protein levels are decreased and white if levels are within the envelope or unchanged. Darker shades of color represent greater change in biomarker activity relative to vehicle control.

[225] Figure 66 depicts a BioMAP profile overlay of SNA-125 (10 μM), Methotrexate 10 μM , and Tofacitinib (10 μM).

[226] Figure 67 depicts the design of the IMQ-induced psoriasis mouse study. Animal shaving, IMQ cream application, treatment, left ear biopsy punch, body weight measurements, ear thickness measurements, psoriasis clinical scoring, and termination were performed at the indicated time points.

[227] Figure 68 depicts the change in animal body weight throughout the IMQ-induced psoriasis mouse study.

[228] Figure 69 depicts the changes in the total psoriasis score throughout the IMQ-induced psoriasis mouse study. The difference between SNA-125 at 5% and the vehicle is statistically significant from day 7. The differences between SNA-125 at 0.5% and 1% and the vehicle are statistically significant on day 10.

[229] Figure 70 depicts the changes in the Erythema score throughout the IMQ-induced psoriasis mouse study. SNA-125 at 5% is statistically significant from the vehicle from day 7. SNA-125 at 0.5% and 1% are statistically significant on day 10 only.

[230] Figure 71 depicts the changes in the plaque score throughout the IMQ-induced psoriasis mouse study. SNA-125 at 5% is statistically significant from the vehicle on day 10.

[231] Figure 72 depicts the changes in the punctate redness/scabbing score throughout the IMQ-induced psoriasis mouse study.

[232] Figure 73 depicts the changes in spleen thickness throughout the IMQ-induced psoriasis mouse study

[233] Figure 74 depicts the changes in ear thickness throughout the IMQ-induced psoriasis mouse study.

[234] Figure 75 depicts the levels of cytokines (a) IL-22, (b) IL-17A, (c) IL17F, and (d) TNFa, in ear samples at Day 4.

[235] Figure 76 depicts CT327 calibration curves in rat plasma used to determine pharmacokinetic plasma levels (dotted lines represent upper and lower confidence limits).

[236] Figure 77 depicts mean plasma concentration-time after dose profile of CT327 (SNA-120) after a single intravenous administration at 18 mg/kg. Open symbols represent the average measured values ($\pm 95\%$ CI, vertical bar), while filled-in symbols represent interpolated values.

[237] Figure 78 depicts the (A) colon dissection diagram and (B) fields and scoring order employed in the oxazolone-induced colitis mouse study.

[238] Figure 79 depicts the effect of SNA-125, SNA-352, tofacitinib, and prednisolone on the body weight of animals challenged with oxazolone. Compounds or vehicle controls were dosed BID as indicated intracecally (IC) or orally (PO). Figure 79A depicts percent body weight change from Day -1 to Day 4 of the study. Figure 79B depicts the area under the curve (AUC) of the percent weight change depicted in Figure 79A.

[239] Figure 80 depicts the effect of SNA-125, SNA-352, tofacitinib, and prednisolone on the body weight of animals challenged with oxazolone according to last observation carried forward analysis. Compounds or vehicle controls were dosed BID as indicated intracecally (IC) or orally (PO). Figure 80A depicts percent body weight change from Day -1 to Day 4 of the study. Figure 80B depicts the area under the curve (AUC) of the percent weight change depicted in Figure 80A.

[240] Figure 81 depicts the effect of SNA-125, SNA-352, tofacitinib, and prednisolone on the Day 2 endoscopy score of animals challenged with oxazolone by (A) bar chart and (B) dot plot. Compounds or vehicle controls were dosed BID as indicated intracecally (IC) or orally (PO).

[241] Figure 82 depicts the effect of SNA-125, SNA-352, tofacitinib, and prednisolone on the Day 2 stool consistency score of animals challenged with oxazolone by (A) bar chart and (B) dot plot. Compounds or vehicle controls were dosed BID as indicated intracecally (IC) or orally (PO).

[242] Figure 83 depicts the effect of SNA-125, SNA-352, tofacitinib, and prednisolone on the Day 4 endoscopy score of animals challenged with oxazolone by (A) bar chart and (B) dot plot. Compounds or vehicle controls were dosed BID as indicated intracecally (IC) or orally (PO).

[243] Figure 84 depicts the effect of SNA-125, SNA-352, tofacitinib, and prednisolone on the Day 4 stool consistency score of animals challenged with oxazolone by (A) bar chart and (B) dot plot. Compounds or vehicle controls were dosed BID as indicated intracecally (IC) or orally (PO).

[244] Figure 85 depicts the effect of SNA-125, SNA-352, tofacitinib, and prednisolone on the disease activity index (DAI) score of animals at (A) Day 2 and (B) Day 4 following challenge with oxazolone. Compounds or vehicle controls were dosed BID as indicated intracecally (IC) or orally (PO).

[245] Figure 86 depicts the effect of SNA-125, SNA-352, tofacitinib, and prednisolone on the colon weight/length ratio of animals challenged with oxazolone. Compounds or vehicle controls were dosed BID as indicated intracecally (IC) or orally (PO).

[246] Figure 87 depicts the effect of SNA-125, SNA-352, tofacitinib, and prednisolone on the colon inflammation histopathology scores of animals challenged with oxazolone. Compounds or vehicle controls were dosed BID as indicated intracecally (IC) or orally (PO). Group means with standard error of the mean (SEM) bars are depicted.

[247] Figure 88 depicts the effect of SNA-125, SNA-352, tofacitinib, and prednisolone on the colon edema histopathology scores of animals challenged with oxazolone. Compounds or vehicle controls were dosed BID as indicated intracecally (IC) or orally (PO). Group means with standard error of the mean (SEM) bars are depicted.

[248] Figure 89 depicts the effect of SNA-125, SNA-352, tofacitinib, and prednisolone on the colon mucosal necrosis/loss histopathology scores of animals challenged with oxazolone. Compounds or vehicle controls were dosed BID as indicated intracecally (IC) or orally (PO). Group means with standard error of the mean (SEM) bars are depicted.

[249] Figure 90 depicts the effect of SNA-125, SNA-352, tofacitinib, and prednisolone on the summed colon histopathology scores of animals challenged with oxazolone. Compounds or vehicle controls were dosed BID as indicated intracecally (IC) or orally (PO). Group means with standard error of the mean (SEM) bars are depicted.

[250] Figure 91 depicts representative control animal H&E-stained colon histopathology micrographs at 40x and 100x magnifications.

[251] Figure 92 depicts representative H&E-stained colon histopathology micrographs at 40x and 100x magnifications for animals administered BID (A) Vehicle PO, (B) 15 mg/kg Tofacitinib PO, (C) 1 mg/kg Prednisolone PO, (D) 400 mg/kg SNA-125 PO, and (E) 400 mg/kg SNA-352 PO. Moderate inflammation (unfilled black arrows), edema (filled red arrows) and multifocal ulceration (brackets) are indicated.

[252] Figure 93 depicts representative H&E-stained colon histopathology micrographs at 40x and 100x magnifications for animals administered BID (A) Vehicle IC, (B) 1 mg/kg Tofacitinib IC, (C) 400 mg/kg SNA-125 IC, and (D) 400 mg/kg SNA-352 IC.

[253] Figure 94 depicts the effect of SNA-125, SNA-352, tofacitinib, and prednisolone on the proin levels of IFN γ in colon tissue homogenate supernatants of animals following challenge with oxazolone. Compounds or vehicle controls were dosed BID as indicated intracecally (IC) or orally (PO). Group means with standard error of the mean (SEM) bars are depicted.

[254] Figure 95 depicts the effect of SNA-125, SNA-352, tofacitinib, and prednisolone on the protein levels of TNF α in colon tissue homogenate supernatants of animals following challenge with oxazolone. Compounds or vehicle controls were dosed BID as indicated intracecally (IC) or orally (PO). Group means with standard error of the mean (SEM) bars are depicted, with outliers removed (A), or present (B).

[255] Figure 96 depicts the effect of SNA-125, SNA-352, tofacitinib, and prednisolone on the protein levels of IL-6 in colon tissue homogenate supernatants of animals following challenge with oxazolone. Compounds or vehicle controls were dosed BID as indicated intracecally (IC) or orally (PO). Group means with standard error of the mean (SEM) bars are depicted, with outliers removed (A), or present (B).

[256] Figure 97 depicts the effect of SNA-125, SNA-352, tofacitinib, and prednisolone on the protein levels of IL-10 in colon tissue homogenate supernatants of animals following challenge with oxazolone. Compounds or vehicle controls were dosed BID as indicated intracecally (IC) or orally (PO). Group means with standard error of the mean (SEM) bars are depicted, with outliers removed (A), or present (B).

[257] Figure 98 depicts a schematic showing how the IMQ-induced psoriasis study was performed.

[258] Figure 99 depicts the total psoriasis clinical scores over time for all groups (A), the SNA-101 group (B), the SNA-125 group (C), and the SNA-352 group (D). The mean score for each group is displayed for each day $\pm$ SEM.

[259] Figure 100 depicts the erythema scores over time for all groups (A), the SNA-101 group (B), the SNA-125 group (C), and the SNA-352 group (D). The mean score for each group is displayed for each day $\pm$ SEM.

[260] Figure 101 depicts the plaque scores over time for all groups (A), the SNA-101 group (B), the SNA-125 group (C), and the SNA-352 group (D). The mean score for each group is displayed for each day $\pm$ SEM.

[261] Figure 102 depicts the punctate redness/scabbing scores over time for all groups (A), the SNA-101 group (B), the SNA-125 group (C), and the SNA-352 group (D). The mean score for each group is displayed for each day $\pm$ SEM.

[262] Figure 103A depicts the weight of spleens upon experimental termination on day 10. Mean spleen weight for each group is displayed $\pm$ SEM. Figure 103B depicts left ear thickness as measured with a caliper on days 0, 4, 6, 8, and 10. Mean thickness for each group is displayed for each day $\pm$ SEM. Figure 103C depicts the daily weight of mice. Body weight changes are displayed for each day as a percent of their weight measured on day 0. Mean values for each group are displayed $\pm$ SEM.

[263] Figure 104 depicts the levels of IL-17F (A), TNF- α (B), IL-22 (C), and IL-17A (D) as measured in left ears biopunched on day 4. After tissue homogenization, the cytokine levels in tissue lysates were measured via multiplex and then normalized with total protein amounts. Mean values for each group are displayed $\pm$ SEM.

[264] Figure 105 depicts a schematic of the IL-23-induced psoriasis mouse model study.

[265] Figure 106 depicts the effect SNA-120 and SNA-352 in an IL-23-induced psoriasis mouse model. Figure 106A depicts the total psoriasis clinical scores for each group over time. The mean score for each group is displayed for each day $\pm$ SEM. Figure 106B depicts the right ear thickness of each group at the indicated time points. Mean thickness for each group is displayed for each day $\pm$ SEM. Figure 106C depicts body weight of each group over the course of the study. Body weight changes are displayed for each day as a percent of their weight measured on day 0. Mean values for each group are displayed $\pm$ SEM.

[266] Figure 107 depicts the inhibition of VEGF-induced proliferation following treatment with SNA-125 (A), SNA-352 (B), SNA-103 (C), and motesanib diphosphate (D). Data are presented as mean corrected counts per minute (CCPM) $\pm$ SEM, with $n=6$ for (A)-(C) and with $n=4$ for (D).

[267] Figure 108 depicts representative Day 2 endoscopy images of naive control, vehicle control (PO), vehicle control (IC), and tofacitinib (15 mg/kg PO) animals. Animals underwent video endoscopy on Day 2 and colitis severity was scored on a scale of 0-4. Images were captured from each animal during the procedure and representative images from each treatment group are presented.

[268] Figure 109 depicts representative Day 2 endoscopy images of tofacitinib (15 mg/kg IC), prednisolone (1mg/kg PO), SNA-125 (400 mg/kg PO), and SNA-352 (400mg/kg PO) animals. Animals underwent video endoscopy on Day 2 and colitis severity was scored on a

scale of 0-4. Images were captured from each animal during the procedure and representative images from each treatment group are presented.

[269] Figure 110 depicts representative Day 2 endoscopy images of SNA-125 (400 mg/kg IC) and SNA352 (400 mg/kg IC) animals. Animals underwent video endoscopy on Day 2 and colitis severity was scored on a scale of 0-4. n=8-15 per group. Images were captured from each animal during the procedure and representative images from each treatment group are presented.

[270] Figure 111 depicts representative Day 4 endoscopy images of naive control, vehicle control (PO), vehicle control (IC), and tofacitinib (15 mg/kg PO) animals. Animals underwent video endoscopy on Day 4 and colitis severity was scored on a scale of 0-4. n=8-15 per group. Images were captured from each animal during the procedure and representative images from each treatment group are presented.

[271] Figure 112 depicts representative Day 4 endoscopy images of tofacitinib (15 mg/kg IC), prednisolone (1mg/kg PO), SNA-125 (400 mg/kg PO), and SNA-352 (400mg/kg PO) animals. Animals underwent video endoscopy on Day 4 and colitis severity was scored on a scale of 0-4. n=8-15 per group. Images were captured from each animal during the procedure and representative images from each treatment group are presented.

[272] Figure 113 depicts representative Day 4 endoscopy images of SNA-125 (400 mg/kg IC) and SNA352 (400 mg/kg IC) animals. Animals underwent video endoscopy on Day 4 and colitis severity was scored on a scale of 0-4. n=8-15 per group. Images were captured from each animal during the procedure and representative images from each treatment group are presented.

[273] Figure 114 depicts Percent Body Weight Change Relative to Day 0 (All Groups). Animals were weighed daily and their percent body weight relative to Day 0 was calculated. The AUC was calculated using the trapezoidal rule transformation. Statistically significant differences were determined by one-way ANOVA with Tukey's multiple comparisons test used to compare all groups to one another. Only significant differences between naive and vehicle controls are shown. ***p<0.005; ****p<0.001. Data is presented as mean $\pm$ SEM. n=8-15 per group.

[274] Figure 115 depicts Percent Body Weight Change Relative to Naive Animals (All Groups). Animals were weighed daily and their percent body weight relative to naive animals was calculated. The AUC was calculated using the trapezoidal rule transformation. Statistically significant differences were determined by one-way ANOVA with Tukey's multiple comparisons test used to compare all groups to one another. Only significant differences between naive and vehicle controls are shown. **p<0.01; ***p<0.005. Data is presented as mean $\pm$ SEM. n=8-15 per group.

[275] Figure 116 depicts Composite DAI Score (Day 0 Body Weight Change; All Groups). Animals were assigned a composite DAI score based on body weight change relative to Day 0, and presence/severity of diarrhea and/or blood in stool on a daily basis. The AUC was calculated using the trapezoidal rule transformation. Statistically significant differences were determined by one-way ANOVA with Tukey's multiple comparisons test used to compare all groups to one another. Only significant differences between naive and vehicle controls are shown. * $p < 0.05$; ** $p < 0.01$; Data is presented as mean $\pm$ SEM. $n = 8-15$ per group.

[276] Figure 117 depicts Composite DAI Score (Naive Animal Body Weight Change; All Groups). Animals were assigned a composite DAI score based on body weight change relative to naive animals, and presence/severity of diarrhea and/or blood in stool on a daily basis. The AUC was calculated using the trapezoidal rule transformation. Statistically significant differences were determined by one-way ANOVA with Tukey's multiple comparisons test used to compare all groups to one another. Only significant differences between naive and vehicle controls are shown. *** $p < 0.005$; **** $p < 0.001$. Data is presented as mean $\pm$ SEM. $n = 8-15$ per group.

[277] Figure 118 depicts Body Weight Change Relative to Day 0 DAI Score (All Groups). Animals were assigned a score based on body weight change relative to Day 0 on a daily basis. The AUC was calculated using the trapezoidal rule transformation. Statistically significant differences were determined by one-way ANOVA with Tukey's multiple comparisons test used to compare all groups to one another. Data is presented as mean $\pm$ SEM. $n = 8-15$ per group.

[278] Figure 119 depicts Body Weight Change Relative to Naive Animals DAI Score (All Groups). Animals were assigned a score based on body weight change relative to naive animals on a daily basis. The AUC was calculated using the trapezoidal rule transformation. Statistically significant differences were determined by one-way ANOVA with Tukey's multiple comparisons test used to compare all groups to one another. Only significant differences between naive and vehicle controls are shown. ** $p < 0.01$; *** $p < 0.005$. Data is presented as mean $\pm$ SEM. $n = 8-15$ per group.

[279] Figure 120 depicts Diarrhea DAI Score (All Groups). Animals were assigned a score based on the presence and severity of diarrhea on a daily basis. The AUC was calculated using the trapezoidal rule transformation. Statistically significant differences were determined by one-way ANOVA with Tukey's multiple comparisons test used to compare all groups to one another. Only significant differences between naive and vehicle controls are shown. * $p < 0.05$; ** $p < 0.01$. Data is presented as mean $\pm$ SEM. $n = 8-15$ per group.

[280] Figure 121 depicts Blood in Stool DAI Score (All Groups). Animals were assigned a score based on the presence and severity of bloody stool on a daily basis. The AUC was calculated using the trapezoidal rule transformation. Statistically significant differences were

determined by one-way ANOVA with Tukey's multiple comparisons test used to compare all groups to one another. Only significant differences between either naive and vehicle controls or between vehicle controls and test-treatment groups are shown. # is as compared to vehicle controls. # $p < 0.05$. Data is presented as mean $\pm$ SEM. $n = 8-15$ per group.

[281] Figure 122 depicts Colitis Severity Score. Animals underwent video endoscopy on Days 10, 21, and 34 to assess colon inflammation and were assigned a colitis severity score. Statistically significant differences were assessed by Kruskal-Wallis test with Dunn's multiple comparisons test used to compare all groups to one another; significance between naive and vehicle control groups only are noted on the figure. *** $p < 0.005$; **** $p < 0.001$. Data is presented as mean $\pm$ SEM. $n = 8-15$ per group.

[282] Figure 123 depicts Stool Consistency Score. Animals underwent video endoscopy on Days 10, 21, and 34 to assess colon inflammation and were assigned a stool consistency score. Animals with no stool present at endoscopy were not assigned a score. Statistically significant differences were assessed by Kruskal-Wallis test with Dunn's multiple comparisons test used to compare all groups to one another; significance between naive and vehicle control groups only are noted on the figure. * $p < 0.05$; ** $p < 0.01$. Data is presented as mean $\pm$ SEM. $n = 8-15$ per group.

[283] Figure 124 depicts Composite Endoscopy DAI Score. Animals underwent video endoscopy on Days 10, 21, and 34 to assess colon inflammation and were assigned colitis severity and stool consistency scores; these scores were combined with the score assigned to each animal based on body weight loss in order to determine the endoscopy DAI score. Animals with no stool present at endoscopy were not assigned a score for this parameter. Statistically significant differences were assessed by Kruskal-Wallis test with Dunn's multiple comparisons test used to compare all groups to one another; significance between naive and vehicle control groups only are noted on the figure. * $p < 0.05$; ** $p < 0.01$; **** $p < 0.001$. Data is presented as mean $\pm$ SEM. $n = 8-15$ per group.

[284] Figure 125 depicts Colon Weight:Length Ratio at Euthanasia. Animals were euthanized on Day 34 and the colon was weighed and measured; the colon weight:length ratio was calculated. Significant differences between groups were determined by one-way ANOVA with Tukey's multiple comparisons test used to compare all groups to one another. Only significant differences between naive and vehicle controls are shown. **** $p < 0.001$. Data is presented as mean $\pm$ SEM. $n = 8-15$ per group.

[285] Figure 126 depicts Colon Cytokine IFN γ at Euthanasia. Animals were euthanized on Day 34 and the most proximal 1.0 cm of colon was snap-frozen for downstream analysis by multiplex. Significant differences between groups were determined by one-way ANOVA with Holm-Sidak's multiple comparisons test used to compare all groups to one another. Only significant differences between naive and vehicle controls are shown. Sample values that

were out-of-range on the low end of the curve were entered as 50% of the lowest standard; if on the high end of the curve, they were entered as the highest standard value. Data is presented as mean $\pm$ SEM. n=8-15 per group.

[286] Figure 127 depicts Colon Cytokine IL-6 at Euthanasia. Animals were euthanized on Day 34 and the most proximal 1.0 cm of colon was snap-frozen for downstream analysis by multiplex. Significant differences between groups were determined by one-way ANOVA with Holm-Sidak's multiple comparisons test used to compare all groups to one another. Only significant differences between naive and vehicle controls are shown. Sample values that were out-of-range on the low end of the curve were entered as 50% of the lowest standard; if on the high end of the curve, they were entered as the highest standard value. Data is presented as mean $\pm$ SEM. n=8-15 per group.

[287] Figure 128 depicts Colon Cytokine IL-10 at Euthanasia. Animals were euthanized on Day 34 and the most proximal 1.0 cm of colon was snap-frozen for downstream analysis by multiplex. Significant differences between groups were determined by one-way ANOVA with Holm-Sidak's multiple comparisons test used to compare all groups to one another. Only significant differences between naive and vehicle controls are shown. Sample values that were out-of-range on the low end of the curve were entered as 50% of the lowest standard; if on the high end of the curve, they were entered as the highest standard value. Data is presented as mean $\pm$ SEM. n=8-15 per group.

[288] Figure 129 depicts Colon Cytokine TNFa at Euthanasia. Animals were euthanized on Day 34 and the most proximal 1.0 cm of colon was snap-frozen for downstream analysis by multiplex. Significant differences between groups were determined by one-way ANOVA with Holm-Sidak's multiple comparisons test used to compare all groups to one another. Only significant differences between naive and vehicle controls are shown. Sample values that were out-of-range on the low end of the curve were entered as 50% of the lowest standard; if on the high end of the curve, they were entered as the highest standard value. Data is presented as mean $\pm$ SEM. n=8-15 per group.

[289] Figure 130 depicts Plasma CRP at Euthanasia. Animals were euthanized on Day 34. Blood was collected and was processed by centrifugation for plasma; an aliquot of plasma was analyzed for CRP. Significant differences between groups were determined by one-way ANOVA with Holm-Sidak's multiple comparisons test used to compare all groups to one another. Only significant differences between naive and vehicle controls are shown. Sample values that were out-of-range on the low end of the curve were entered as 50% of the lowest standard; if on the high end of the curve, they were entered as the highest standard value. Data is presented as mean $\pm$ SEM. n=8-15 per group.

[290] Figure 131 depicts Inflammation Scores for colon sections. Untreated control animals that did not receive DSS had minimal scattered background inflammation. All other animals

were given DSS and had varying degrees of inflammation, with some regions more severely affected than others. When comparing animals treated from day 0, treatment significantly affected inflammation (one-way ANOVA, $p < 0.0001$). Similarly, treatment starting at day 10 also significantly affected inflammation (one-way ANOVA, $p = 0.0152$). Individual groups were compared in both analyses using a Tukey's multiple comparisons test. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$.

[291] Figure 132 depicts Edema Scores for colon sections. Untreated control animals that did not receive DSS had minimal scattered background edema. All other animals were given DSS and had varying degrees of edema, with some regions more severely affected than others. When comparing animals treated from day 0, treatment significantly affected edema (one-way ANOVA, $p < 0.0001$). Treatment starting at day 10 approached significance (one-way ANOVA, $p = 0.0697$). Individual groups were compared in both analyses using a Tukey's multiple comparisons test. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$.

[292] Figure 133 depicts Mucosal Necrosis/Loss Scores for colon sections. Untreated control animals that did not receive DSS had no necrosis. All other animals were given DSS and had varying degrees of necrosis, with some regions more severely affected than others. When comparing animals treated from day 0, treatment significantly affected necrosis (one-way ANOVA, $p < 0.0001$). Treatment starting at day 10 approached significance (one-way ANOVA, $p = 0.0543$). Individual groups were compared in both analyses using a Tukey's multiple comparisons test. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$.

[293] Figure 134 depicts Sum Scores for colon sections. Sum scores (Inflammation + Edema + Mucosal Necrosis) varied in all animals administered DSS. When comparing animals treated from day 0, treatment significantly affected the sum score (one-way ANOVA, $p < 0.0001$). Similarly, treatment starting at day 10 significantly affected the sum score (one-way ANOVA, $p = 0.0161$). Individual groups were compared in both analyses using a Tukey's multiple comparisons test. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$.

[294] Figure 135 shows representatives of two possible scenarios for the assignment of test fields as referred to in Example 28.

[295] Figure 136 shows H&E and immunohistochemistry (IHC) results of Example 28 from a single subject in panel display; D1 NLS = day 1 non-lesional skin, D1 LS = day 1 lesional skin.

[296] Figure 137 shows the aggregate epidermal thickness (mm) results for treated lesional skin at Day 12 in Example 28; D1 NL = day 1 non-lesional skin, D1 LS = day 1 lesional skin.

[297] Figure 138 shows post-treatment lesional skin compared to baseline lesional skin of psoriasis transcriptome as referred to in Example 28 (left = down-regulated, right = up-regulated).

[298] Figure 139 shows the the relative percent improvement in disease transcriptome of day 12 lesional tissue versus day 1 lesional tissue as referred to in Example 28. Histogram groupings are, left to right, IMP1 (2 % SNA-125), IMP2 (0.2 % SNA-125), IMP3 (0.5 % SNA-120), and IMP4 (vehicle).

[299] Figure 140 shows post-treatment lesional skin compared to baseline lesional skin of psoriasis transcriptome in responders as referred to in Example 28 (left = down-regulated, right = up-regulated).

[300] Figure 141 shows in life assessment stool consistency scores for day 2 of Example 29.

[301] Figure 142 shows in life assessment stool consistency scores for day 4 of Example 29.

[302] Figure 143 shows in life assessment colitis severity by endoscopy scores for day 2 of Example 29.

[303] Figure 144 shows in life assessment colitis severity by endoscopy scores for day 4 of Example 29.

[304] Figure 145 shows in life assessment composite endoscopy DAI scores for day 2 of Example 29.

[305] Figure 146 shows in life assessment composite endoscopy DAI scores for day 4 of Example 29.

[306] Figure 147 shows terminal assessment body weight change results of Example 29.

[307] Figure 148 shows terminal assessment colon weight to length ratio results of Example 29.

[308] Figure 149 shows terminal assessment composite DAI scores of Example 29.

DETAILED DESCRIPTION

Platform Technology

[309] Several embodiments relate to the use of agents that were developed using Applicant's proprietary Low Systemic Exposure™ ("LSE™") platform technology to generate LSE molecules (also generally referred to herein as polymer conjugates or compositions). In several embodiments, the LSE platform creates polymer conjugates optimized for topical applications. In several embodiments, the polymer conjugates developed by LSE or more generally the reduced exposure technology exhibit enhanced penetration. In still further embodiments, the enhanced penetration leads to delivery of a high local concentration of the drug. In further embodiments, the polymer conjugates show a limited non-target absorption upon topical administration due to their increased molecular size and amphiphilicity and/or

amphipathicity. In still further embodiments, side-effects are minimized by limiting or eliminating non-target (e.g., systemic) absorption.

[310] In several embodiments of the reduced exposure compositions/compounds, the polymer conjugate comprises a "warhead" linked to a polymer. In some embodiments, the warhead is a pharmacologically active entity selected according to the particular target or pathway of interest. As discussed herein, there are also provided, in several embodiments, polymer conjugates for use in the treatment of conditions (including but not limited to inflammatory skin diseases). In several embodiments, the polymer is directly coupled to the warhead without a separate chemical linking moiety between the polymer and the warhead; such direct coupling may involve without limitation ester, ether, acetal, ketal, vinyl ether, carbamate, urea, amine, amide, enamine, imine, oxime, amidine, iminoester, carbonate, orthoester, phosphonate, phosphinate, sulfonate, sulfinat, sulfide, sulfate, disulfide, sulfonamide, sulfonamide, thioester, aryl, silane, siloxane, heterocycles, thiocarbonate, thiocarbamate, and phosphonamide bonds. In several embodiments, the linker is a separate chemical linking moiety between the polymer and the warhead. In several embodiments, the polymer is polyethylene glycol (PEG), wherein the terminal OH group can optionally be modified e.g. with C1-C5 alkyl or C1-C5 acyl groups, e.g., with C1-, C2- or C3-alkyl groups or C1-, C2- or C3 groups. In several embodiments, the modified PEG is a terminally alkoxy-substituted PEG. In several embodiments, the modified PEG is a methoxy-PEG (mPEG). In some embodiments, the polymer has a molecular weight ranging from about 100 to about 100,000 Da. In some embodiments, the polymer is polydisperse with respect to molecular weight (e.g., has a distribution of molecular weights) and the indicated molecular weight of the polymer represents an average molecular weight. In other embodiments, the polymer has a molecular weight ranging from about 200 to about 50,000 Da. In several embodiments, the polymer has a molecular weight ranging from about 500 to about 10,000 Da (e.g., 500-1000, 1000-2000, 2000-3000, 3000-5000, 5000-7000, 7000-10,000 Da, and overlapping ranges therein).

[311] In several embodiments, the polymer is a short-chain PEG, and in some embodiments a terminally alkoxy-substituted PEG, such as a mPEG with a molecular weight ranging from about 200 to about 4,000 Da, from about 400 to about 3,000 Da, from about 500 to about 2,000 Da, from about 700 to about 3,000 Da, from about 900 to about 4,000 Da, or from about 1,000 to about 5,000 Da. In several embodiments, the short-chain PEG or mPEG has an average molecular weight of about 1,000-3,000 Da. (e.g., 2,000 Da).

[312] In some embodiments, the polymer is a long-chain PEG. The long-chain PEG may be a terminally alkoxy-substituted PEG, such as methoxy-substituted PEG, with a molecular weight ranging greater than about 4,000 Da. In several embodiments, the molecular weight ranges from about 4,500-10,000 Da (e.g., 4,500 to about 5,500 Da). In several embodiments,

the long-chain PEG or mPEG has an average molecular weight of about 2,000 Da or of about 5,000 Da. In several embodiments, the polymer is of natural or semi-synthetic or synthetic origin. In several embodiments, the polymer has a linear or branched structure. In several embodiments, the polymer is selected from poly(alkylene oxides) or from (polyethylene) oxides. In several embodiments, the polymer selected may include, without limitation, one or more of the following: polyacrylic acid, polyacrylates, polyacrylamide or N-alkyl derivatives thereof, polymethacrylic acid, polymethacrylates, polyethylacrylic acid, polyethylacrylates, polyvinylpyrrolidone, poly(vinylalcohol), polyglycolic acid, polylactic acid, poly(lactic-co-glycolic) acid, dextran, chitosan, and hydroxyethyl starch.

[313] In an embodiment, the polymer conjugates provided herein are administered to the skin by topical application. In one embodiment, the polymer conjugates provided herein treat inflammatory skin diseases. In one embodiment, the polymer conjugates provided herein treat skin neoplasias. In one embodiment, the polymer conjugates provided herein treat bullous diseases.

[314] In one embodiment, active agents useful for stimulating hair follicles (for hair growth) are provided as oral applications or topical applications for the scalp. Hair removal agents and anti-acne agents are provided in other embodiments. Hair growth, hair removal and anti-acne therapies can all involve active agents that, if exposed to the non-target site (e.g., systemic circulation and/or lymphatic system) for long periods, result in toxicity or undesired side effects. Thus, the reduced exposure compositions described herein provides benefits for these applications as well. In one embodiment, the polymer conjugates provided herein modulate hair growth and cycling. In one embodiment, the polymer conjugates provided herein treat alopecia.

[315] In alternative embodiments, the polymer conjugates configured for reduced exposure are administered to other areas of the body besides the skin. For example, in one embodiment, administration comprises treatment of the lung and respiratory conditions via inhalation of the polymer conjugates. Eye drops are provided in some embodiments to treat eye inflammation or ophthalmic disorders and diseases. Treatment to the joints to treat inflammation or other joint conditions is also provided. In yet another embodiment, administration comprises treatment of the gastro-intestinal tract via, for example, an enteric coated capsule comprising the polymer conjugates taken orally. Reduced exposure provides benefits in these applications. Applications for the nose and ear, such as inhalants, ointments and drops are provided in several embodiments. Treatment to the nasal passage to treat allergies or allergic rhinitis is also provided. Vaginal and rectal compounds are provided in some embodiments, including as suppositories, creams, ointments, etc. In one embodiment, the polymer conjugates provided herein treat vascular tumors. In one embodiment, the polymer conjugates provided herein treat diabetic retinopathy. In one

embodiment, the polymer conjugates provided herein treat macular edema. In one embodiment, the polymer conjugates provided herein treat corneal edema. In one embodiment, the polymer conjugates provided herein treat age-related macular degeneration.

[316] In some embodiments, conjugating the warhead to a polymer (e.g., PEG) in the disclosed molecular weight ranges may slow diffusion of the molecule in the tissue, thereby potentially increasing residence time of the molecule in the target tissue, e.g. epidermis and dermis for skin, associated epithelial and sub-epithelial layers in other topical surfaces like gut, eye, mucosa, lungs etc. This “depot” effect may also lead to lower concentrations needing to be applied or for products to be applied with lower frequency, or both.

[317] In other embodiments, conjugating the warhead to a polymer (e.g., PEG) in the disclosed molecular weight ranges may be useful in reducing the diffusion or extravasation of the molecule out of the circulatory system after it enters it via injection and or diffusion from the target tissue. Indeed, changes in the tissue distribution of polymer conjugates compared to unconjugated drug have been observed in IV injection studies. In general, the unconjugated drug tends to have a long half-life and a volume of distribution within tissues AND blood, suggesting that the unconjugated drug extravasates out of the blood vessels into the tissue prior to being cleared. Whereas, in some instances, the PEGylated drug has a volume of distribution that is largely restricted to the blood, indicating that very little extravasation occurs with the polymer conjugates prior to being renally cleared. This reduced extravasation may explain at least in part the observed shorter half-life for the polymer conjugates.

[318] The compositions described herein may be combined with other modalities to achieve synergic effects. These other modalities include, but are not limited to, energy delivery (such as laser, radiofrequency, ultrasound, microwave, etc.), thermal therapy, light therapy, radiation, intravenous chemotherapy, and others. In some embodiments, the compositions are applied with pressure, heat, massage etc. to facilitate localization to the desired target site. In some embodiments, the compositions are administered in combination with one or more additional therapeutics that may not be reduced exposure compounds. In some embodiments, the compositions are administered in combination with UV irradiation therapy.

[319] Santi et al. state that “permanent PEGylation is generally not applicable to small-molecule drugs because the bulky carrier usually prevents their binding to targets and cell penetration.” (Proceedings of the National Academy of Sciences 109.16 (2012): 6211-6216). Further, Nakagami et al. state that “hydrophilic polymers on the surface of particles... prevents the close interactions between particles and target cell membranes, inhibiting the cellular uptake and, subsequently, preventing endosomal escape. All of these

factors combine to decrease the biological efficacy of PEGylated particles" (Gene therapy (2013): 2-4). In several embodiments, the polymer conjugate exhibits unexpected permeability across the plasma membrane. In several embodiments, the polymer conjugate exhibits unexpected permeability across the nuclear membrane. In several embodiments, the polymer conjugate exhibits unexpected permeability across both the nuclear and plasma membranes. Accordingly, in Example 9, two polymer conjugates, SNA-125 and SNA-120, were surprisingly shown to penetrate the keratinocyte cellular membrane and interact with the target kinases intracellularly within the cytoplasm, thereby leading to inhibition of proliferation of keratinocytes in a non-toxic manner.

[320] The reduced exposure compounds, comprising a hydrophobic drug conjugated to a short chain PEG, exhibit surprising accessibility across cellular compartments, compared to the unconjugated drug. This accessibility is thought to result for the amphipathic nature of the conjugate, allowing it to traverse and distribute evenly among both lipophilic and hydrophilic cellular compartments. Accordingly, the conjugate can cross and reside within the lipid bilayer of the cell membrane, accumulate within the cytosol, and even traverse the nuclear envelope - thereby providing access both membrane, cytosolic and nuclear molecular targets. This property of the reduced exposure compounds result in excellent depo'ing, longer residence times within target cells, and relative non-compartmentalization. Consequently, these compounds are biologically active at lower concentrations and require less frequent dosing - thereby reducing potential drug toxicity.

Polymer Conjugates of Indolocarbazole Compounds

[321] In several embodiments, the warhead employed in the LSE polymer conjugate is an indolocarbazole compound.

[322] Angiogenesis, the process of sprouting new blood vessels from existing vasculature, and arteriogenesis, the remodeling of small vessels into larger conduit vessels, are both physiologically important aspects of vascular growth in adult tissues. These processes of vascular growth are required for beneficial processes such as tissue repair, wound healing, recovery from tissue ischemia and menstrual cycling. They are also required for the development of pathological conditions such as the growth of neoplasias, diabetic retinopathy, rheumatoid arthritis, psoriasis, certain forms of macular degeneration, and certain inflammatory pathologies. The inhibition of vascular growth in these contexts has also shown beneficial effects in preclinical animal models. For example, inhibition of angiogenesis by blocking vascular endothelial growth factor or its receptor has resulted in inhibition of tumor growth and in retinopathy. Also, the development of pathological pannus tissue in rheumatoid arthritis involves angiogenesis and might be blocked by inhibitors of angiogenesis. Certain diseases are known to be associated with deregulated angiogenesis, for example ocular neovascularization, such as retinopathies (including diabetic retinopathy),

age-related macular degeneration, psoriasis, hemangioblastoma, hemangioma, arteriosclerosis, inflammatory disease, such as a rheumatoid or rheumatic inflammatory disease, especially arthritis (including rheumatoid arthritis), or other chronic inflammatory disorders, such as chronic asthma, arterial or post- transplantational atherosclerosis, endometriosis, and neoplastic diseases, for example so-called solid tumors and liquid tumors (such as leukemias).

[323] There is provided, in several embodiments, methods of treating a vascular tumor in a subject, the method comprising administering to the subject an effective amount of a polymer conjugate, wherein the warhead is an indolocarbazole compound. Non-limiting examples of vascular tumors include hemangiomas, Kaposi's sarcoma, lymphangioma, glomangioma, angiosarcoma, hemangioendothelioma, and infantile hemangiomas.

[324] While blood vessels in healthy adults are largely quiescent, adult skin retains the capacity for rapid initiation of angiogenesis during tissue repair and in numerous diseases including inflammatory skin diseases such as psoriasis, many types of dermatitis, blistering diseases, cutaneous neoplasias including squamous cell carcinomas, malignant melanomas, and Kaposi's sarcomas, and proliferative hemangiomas of childhood. Angiogenesis in the skin is also implicated in a number of other diseases that are characterized by macroscopically visible, prominent blood vessels, including rosacea and basal cell carcinoma.

[325] There is provided, in several embodiments, methods of treating a skin neoplasia in a subject, the method comprising administering to the subject an effective amount of a polymer conjugate, wherein the warhead is an indolocarbazole compound. Non-limiting examples of skin neoplasias include squamous cell carcinoma, basal cell carcinoma, malignant melanoma, malignant cutaneous lymphoma, Kaposi's sarcoma, Merkel cell skin cancer, and non-melanoma skin cancer.

[326] Increased vascular permeability is one of the earliest manifestations of inflammation, resulting in extravasation of protein-rich plasma into the effected tissue. Acute vascular permeability allows the deposition of circulating plasma matrix proteins including fibrin and fibronectin (FN) which facilitate cell migration in the inflamed area. This process also provides an access point for immune cells and immunoglobulins to enter the tissue and fight foreign antigens (Nagy et al, Cold Spring Harb. Perspect. Med. 2:a006544, 2012). Conversely, chronic vascular hyperpermeability is suggested to sustain the inflammatory response and retard resolution, further promoting the development of chronic inflammation (Nagy et al, Cold Spring Harb. Perspect. Med. 2:a006544, 2012; Costa et al, Angiogenesis 10:149-166, 2007). This type of vascular hyperpermeability underlies the pathogenesis of a large number of chronic disorders including rheumatoid arthritis (RA), psoriasis, ocular

disease, cancer and chronic wounds (Nagy et al, Cold Spring Harb. Perspect. Med. 2:a006544, 2012; Costa et al, Angiogenesis 10:149-166, 2007).

[327] There is provided, in several embodiments, methods of treating an inflammatory skin disease in a subject, the method comprising administering to the subject an effective amount of a polymer conjugate, wherein the warhead is an indolocarbazole compound. Non-limiting examples of inflammatory skin diseases include psoriasis, psoriasis guttata, inverse psoriasis, pustular psoriasis, psoriatic erythroderma, acute febrile neutrophilic dermatosis, eczema, xerotic eczema, dyshidrotic eczema, vesicular palmar eczema, acne vulgaris, atopic dermatitis, contact dermatitis, allergic contact dermatitis, dermatomyositis, exfoliative dermatitis, hand eczema, pompholyx, keloids, rosacea, rosacea due to sarcoidosis, rosacea due to scleroderma, rosacea due to Sweet syndrome, rosacea due to systemic lupus erythematosus, rosacea due to urticaria, rosacea due to herpetic pain, Sweet's disease, neutrophilic hydradenitis, sterile pustule, drug rash, seborrheic dermatitis, pityriasis rosea, Kikuchi's disease of the skin, pruritic urticarial papules and plaques of pregnancy, Stevens-Johnson syndrome and toxic epidermal necrolysis, tattoo reaction, Wells syndrome (eosinophilic cellulitis), reactive arthritis (Reiter syndrome), bowel-associated dermatosis-arthritis syndrome, rheumatoid neutrophilic dermatosis, neutrophilic eccrine hidradenitis, neutrophilic skin disease of dorsum of hand, balanitis circumscripta plasmacellularis, balanoposthitis, Behcet's disease, erythema annulare centrifugum, erythema dyschromicum perstans, erythema multiforme, granuloma annulare, dermatitis of hand, lichen nitidus, lichen planus, lichen sclerosus et atrophicus, lichen simplex chronicus, lichen spinulosus, nummular dermatitis, pyoderma gangrenosum, sarcoidosis, subcorneal pustular dermatosis, urticaria, and transient acantholytic dermatosis.

[328] Bullous diseases are skin disorders characterized by blistering that often have an autoimmune etiology. Two bullous diseases are bullous pemphigoid and erythema multiforme. Bullous pemphigoid is a subepidermal disorder which manifests as subepidermal blisters with a dermal infiltrate of neutrophils and eosinophils. Erythema multiforme is an inflammatory eruption characterized by symmetric erythematous, edematous, or bullous lesions of the skin or mucous membranes. There is provided, in several embodiments, methods of treating a bullous disease in a subject, the method comprising administering to the subject an effective amount of a polymer conjugate, wherein the warhead is an indolocarbazole compound. Non-limiting examples of bullous diseases include bullous pemphigoid, erythema multiforme, dermatitis herpetiformis, epidermolysis bullosa acquisita, linear Immunoglobulin A disease, mucous membrane pemphigoid, pemphigoid gestationis, pemphigus foliaceus, and pemphigus vulgaris.

[329] There is evidence to suggest that increased expression of angiogenic factors is a central cause of proliferative diabetic retinopathy (PDR). In this condition, and others such as

retinopathy of prematurity, sickle cell retinopathy, age-related macular degeneration, retina vein occlusion and Eales disease, preretinal vascularisation is a major cause of blindness. New blood vessels grow from the inner retinal vasculature into the vitreous humour. This can cause visual loss by vitreous haemorrhage and/or tractional retinal detachment due to contraction of the fibrous tissue associated with the new blood vessels.

[330] There is provided, in several embodiments, methods of treating age-related macular degeneration in a subject, the method comprising administering to the subject an effective amount of a polymer conjugate, wherein the warhead is an indolocarbazole compound. There is provided, in several embodiments, methods of treating diabetic retinopathy in a subject, the method comprising administering to the subject an effective amount of a polymer conjugate, wherein the warhead is an indolocarbazole compound. There is provided, in several embodiments, methods of treating corneal edema in a subject, the method comprising administering to the subject an effective amount of a polymer conjugate, wherein the warhead is an indolocarbazole compound. There is provided, in several embodiments, methods of treating macular edema in a subject, the method comprising administering to the subject an effective amount of a polymer conjugate, wherein the warhead is an indolocarbazole compound. There is provided, in several embodiments, methods of treating dry eye in a subject, the method comprising administering to the subject an effective amount of a polymer conjugate, wherein the warhead is an indolocarbazole compound.

[331] The process of new hair growth, whether as part of the natural hair cycle or as a result of a treatment to encourage hair growth, relies on numerous cross-talking signal pathways to bring about the processes necessary for hair growth. These principal processes are: cell proliferation of the dermal papilla, cell migration to form the appropriate structures, and angiogenesis to form blood supply routes to the new hair follicle. There is provided, in several embodiments, methods of modulating hair growth and cycling in a subject, the method comprising administering to the subject an effective amount of a polymer conjugate, wherein the warhead is an indolocarbazole compound. There is provided, in several embodiments, methods of treating alopecia in a subject, the method comprising administering to the subject an effective amount of a polymer conjugate, wherein the warhead is an indolocarbazole compound.

[332] There is provided, in several embodiments, a combination therapy, the combination therapy comprising administering to the subject an effective amount of a polymer conjugate in conjunction with UV irradiation therapy, wherein the warhead is an indolocarbazole compound.

[333] A growing body of research suggests that dry eye is the result of an underlying cytokine and receptor-mediated inflammatory process. There is provided, in several

embodiments, methods of treating dry eye in a subject, the method comprising administering to the subject an effective amount of a polymer conjugate, wherein the warhead is an indolocarbazole compound. In some embodiments the composition is formulated as an eye drop. In some embodiments, one or two drops of the composition are used per application. In other embodiments, three or four drops of the composition are used per application. In additional embodiments, six drops of the composition are used per application. In some embodiments, the composition is applied for a period of 60 seconds before flushing. In other embodiments, the composition is applied for a period of 120 seconds before flushing. In additional embodiments, the composition is applied for a period of 360 seconds before flushing. In some embodiments, the composition may be administered one or more times a day. In some embodiments, the composition is administered daily. In some embodiments, the composition may be administered once a week.

[334] In some embodiments, alopecia is treated. Non-limiting examples include androgenic alopecia and alopecia areata. Androgenic alopecia (also known as hereditary baldness, male pattern baldness, and seborrheic alopecia) is a non-scarring hair loss of telogen hairs caused by an excessive androgen effect in genetically susceptible men and women. Alopecia areata is known to be associated with autoimmune activities; hence, topically administered immunomodulatory compounds demonstrate efficacy for treating that type of hair loss.

[335] There is provided, in several embodiments, methods of treating an alopecia in a subject, the method comprising administering to the subject an effective amount of a polymer conjugate, wherein the warhead is an indolocarbazole compound. In some embodiments, hair regeneration compositions are in the form of a liquid. In other embodiments, hair regeneration compositions are in the form of a lotion. In additional embodiments, hair regeneration compositions are in the form of a cream. In some embodiments, hair regeneration compositions are in the form of a gel. In other embodiments, the hair regeneration composition is administered twice daily. In other embodiments, the hair regeneration composition is administered one daily. In additional embodiments, the hair regeneration composition is administered once weekly. In some embodiments, the hair regeneration composition is administered directly to the scalp. In some embodiments, the hair regeneration composition is administered directly non-scalp areas.

[336] Allergic inflammatory diseases are characterized by an immune response against a sensitizing agent, such as an allergen, resulting in the release of inflammatory mediators that recruit cells involved in inflammation in a subject, potentially leading to tissue damage and sometimes death. Allergic inflammatory diseases of the eye, skin, upper and lower airways, and gastrointestinal tract, lung, including, but not limited to, atopic dermatitis, atopic keratoconjunctivitis, allergic conjunctivitis, asthma, and allergic rhinitis. There is provided, in

several embodiments, methods of treating an allergic inflammatory disease in a subject, the method comprising administering to the subject an effective amount of a polymer conjugate, wherein the warhead is an indolocarbazole compound.

[337] There is also provided, in several embodiments, methods of treating the following conditions in a subject, the method comprising administering to the subject an effective amount of a polymer conjugate, wherein the warhead is an indolocarbazole compound: nail dystrophy; seborrheic keratosis; androgenic alopecia; contact dermatitis; actinic keratosis; acne; asthma; eczema (atopic dermatitis); onychomycosis; sinusitis; allergic rhinitis; rosacea; COPD; pruritus; early AMD; urticaria; diabetic retinopathy; psoriasis; alopecia areata; dry eye; vitiligo; glaucoma; late AMD; ulcerative colitis; Crohn's disease; ocular rosacea; hair growth and cycling; skin neoplasias; squamous cell carcinoma; basal cell carcinoma; malignant melanoma; malignant cutaneous lymphomas; vascular tumors; angiosarcoma; kaposi's sarcoma; infantile hemangiomas; hemangioendothelioma; inflammatory dermatoses; dermatitis (atopic, contact); psoriasis; keloids; rosacea; bullous diseases; bullous pemphigoid; erythema multiforme; UV irradiation therapy; age-related macular degeneration; diabetic retinopathy; macular and corneal edema.

[338] There is also provided, in several embodiments, methods of treating a respiratory disease in a subject via delivery of the polymer conjugates (wherein the warhead is an indolocarbazole compound) to the lungs and/or airways. Delivery routes may include for example intratracheal instillation or inhalation. The formulation may include liquids, nebulized or aerosolized liquids or suspensions, dry powder, nanocomposites, nanoparticles or microparticles, etc. Respiratory disorders, include treatable obstructive, restrictive or inflammatory airways diseases of whatever type, etiology, or pathogenesis. Non-limiting examples of respiratory conditions include: acute bronchitis; acute laryngotracheal bronchitis; arachidic bronchitis; catarrhal bronchitis; croupus bronchitis; dry bronchitis; infectious asthmatic bronchitis; productive bronchitis; staphylococcus or streptococcal bronchitis; vesicular bronchitis; cylindric bronchiectasis; sacculated bronchiectasis; fusiform bronchiectasis; capillary bronchiectasis; cystic bronchiectasis; dry bronchiectasis; follicular bronchiectasis; chronic obstructive pulmonary disease (COPD), chronic obstructive lung disease (COLD), chronic obstructive airways disease (COAD) or small airways obstruction of whatever type, etiology, or pathogenesis, in particular chronic bronchitis, pulmonary emphysema, bronchiectasis, cystic fibrosis, bronchiolitis obliterans, organizing pneumonia (BOOP), chronic organizing pneumonia (COP), bronchiolitis fibrosa obliterans, follicular bronchiolitis or dyspnea associated therewith; cough of whatever type, etiology, or pathogenesis in particular idiopathic cough or cough associated with gastro-esophageal reflux disease (GERD), drugs, bronchial hyper-responsivity, asthma, COPD, COLD, COAD, bronchitis, bronchiectasis, pulmonary eosinophilic syndromes, pneumoconiosis, interstitial

lung disease, pulmonary fibrosis, aspiration disorders, rhinitis, laryngitis or pharyngitis; pulmonary eosinophilic syndromes of whatever type, etiology, or pathogenesis, in particular acute eosinophilic pneumonia (idiopathic or due to drugs or parasites), simple pulmonary eosinophilia, Loeffler's syndrome, tropical pulmonary eosinophilia, chronic eosinophilic pneumonia, allergic bronchopulmonary mycosis, allergic bronchopulmonary aspergillosis (ABPA), Churg-Strauss syndrome or idiopathic hypereosinophilic syndrome; asthma of whatever type, etiology, or pathogenesis, in particular asthma that is a member selected from the group consisting of atopic asthma, non-atopic asthma, allergic asthma, atopic bronchial IgE-mediated asthma, bronchial asthma, essential asthma, true asthma, intrinsic asthma caused by pathophysiologic disturbances, extrinsic asthma caused by environmental factors, essential asthma of unknown or inapparent cause, non-atopic asthma, bronchitic asthma, emphysematous asthma, exercise-induced asthma, allergen induced asthma, cold air induced asthma, occupational asthma, infective asthma caused by bacterial, fungal, protozoal, or viral infection, non-allergic asthma, incipient asthma and wheezy infant syndrome; alveolar hemorrhage of whatever type, etiology, or pathogenesis, in particular a member of the group consisting of idiopathic pulmonary hemosiderosis, alveolar hemorrhage due to drugs or other exogenous agents, alveolar hemorrhage associated with HIV or bone marrow transplant or autoimmune alveolar hemorrhage (e.g. associated with systemic lupus erythematosus, Goodpasture's syndrome, Wegener's granulomatosis, microscopic polyangiitis, Churg-Strauss syndrome, pauci-immune glomerulonephritis); pneumoconiosis of whatever type, etiology, or pathogenesis, in particular pneumoconiosis that is a member selected from the group consisting of aluminosis or bauxite workers' disease, anthracosis or miners' asthma, asbestosis or steam-fitters' asthma, chalicosis or flint disease, ptilosis caused by inhaling the dust from ostrich feathers, siderosis caused by the inhalation of iron particles, silicosis or grinders' disease, byssinosis or cotton-dust asthma and talc pneumoconiosis; interstitial lung diseases (ILD) or pulmonary fibrosis of whatever type, etiology, or pathogenesis, in particular idiopathic pulmonary fibrosis, cryptogenic fibrosing alveolitis, fibrosing alveolitis, ILD or pulmonary fibrosis associated with connective tissue disease (systemic lupus erythematosus, mixed connective tissue disease, polymyositis, dermatomyositis, Sjorgen's syndrome, systemic sclerosis, scleroderma, rheumatoid arthritis), usual interstitial pneumonia (UIP), desquamative interstitial pneumonia (DIP), granulomatous lung disease, sarcoidosis, Wegener's granulomatosis, histiocytosis X, Langerhan's cell granulomatosis, hypersensitivity pneumonitis, extrinsic allergic alveolitis, silicosis, chronic eosinophilic pneumonia, lymphangiomyomatosis, drug-induced ILD or pulmonary fibrosis, radiation-induced ILD or pulmonary fibrosis, alveolar proteinosis, graft-versus-host-disease (GVHD), lung transplant rejection, ILD or pulmonary fibrosis due to environmental/occupational exposure, BOOP, COP, bronchiolitis fibrosa obliterans, follicular

bronchiolitis, idiopathic acute interstitial pneumonitis (Hamman Rich syndrome) or alveolar hemorrhage syndromes; seasonal allergic rhinitis or perennial allergic rhinitis or sinusitis of whatever type, etiology, or pathogenesis, in particular sinusitis that is a member selected from the group consisting of purulent or nonpurulent sinusitis, acute or chronic sinusitis and ethmoid, frontal, maxillary, or sphenoid sinusitis; Acute Respiratory Distress Syndrome (ARDS), adult respiratory distress syndrome or acute lung injury of whatever type, etiology, or pathogenesis; progressive massive fibrosis (PMF); pulmonary hypertension of whatever type, etiology or pathogenesis including primary pulmonary hypertension, essential hypertension, pulmonary hypertension secondary to congestive heart failure, pulmonary hypertension secondary to COPD, pulmonary venous hypertension, pulmonary arterial hypertension and hypoxia-induced pulmonary hypertension. Respiratory disorders also include, in some embodiments, malignancies and tumors of the respiratory system, non-limiting examples of which include lung adenocarcinoma, squamous cell carcinoma, large cell carcinoma, bronchioloalveolar carcinoma (BAC), pulmonary adenocarcinoma (AIS), non-small-cell carcinoma, small cell carcinoma, and mesothelioma.

[339] Compositions comprising an indolocarbazole compound are used, in several embodiments, as inhibitors, antagonists, and inverse agonists of TkrA, Jak3, and/or MAP2K3. Several embodiments relate to polymer conjugates of an indolocarbazole compound, optimized for topical applications while also minimizing side-effects caused by exposure at non-target sites (e.g., systemic absorption). Non-topical applications are provided in other embodiments.

[340] In several embodiments, the warhead of the polymer conjugate is an indolocarbazole compound or derivative thereof. There is also provided, in several embodiments, methods of treating an inflammatory skin disease in a subject, the method comprising administering to the subject an effective amount of an LSE polymer conjugate wherein the warhead is an indolocarbazole compound. In some embodiments, the warhead of the LSE polymer conjugate is a derivative of K252a. In some embodiments, the LSE polymer conjugate is SNA-125.

[341] There is also provided, in several embodiments, methods of treating a vascular tumor in a subject, the method comprising administering to the subject an effective amount of an LSE polymer conjugate wherein the warhead is an indolocarbazole compound. In some embodiments, the warhead of the LSE polymer conjugate is a derivative of K252a. In some embodiments, the LSE polymer conjugate is SNA-125.

[342] There is also provided, in several embodiments, methods of treating a skin neoplasia in a subject, the method comprising administering to the subject an effective amount of an LSE polymer conjugate wherein the warhead is an indolocarbazole compound. In some

embodiments, the warhead of the LSE polymer conjugate is a derivative of K252a. In some embodiments, the LSE polymer conjugate is SNA-125.

[343] There is also provided, in several embodiments, methods of modulating hair and growth cycling in a subject, the method comprising administering to the subject an effective amount of an LSE polymer conjugate wherein the warhead is an indolocarbazole compound. In some embodiments, the warhead of the LSE polymer conjugate is a derivative of K252a. In some embodiments, the LSE polymer conjugate is SNA-125.

[344] There is also provided, in several embodiments, methods of treating alopecia in a subject, the method comprising administering to the subject an effective amount of an LSE polymer conjugate wherein the warhead is an indolocarbazole compound. In some embodiments, the warhead of the LSE polymer conjugate is a derivative of K252a. In some embodiments, the LSE polymer conjugate is SNA-125.

[345] There is also provided, in several embodiments, methods of treating a bullous disease in a subject, the method comprising administering to the subject an effective amount of an LSE polymer conjugate wherein the warhead is an indolocarbazole compound. In some embodiments, the warhead of the LSE polymer conjugate is a derivative of K252a. In some embodiments, the LSE polymer conjugate is SNA-125.

[346] There is also provided, in several embodiments, methods of treating dry eye, diabetic retinopathy, macular edema, corneal edema, and/or age-related macular degeneration in a subject, the method comprising administering to the subject an effective amount of an LSE polymer conjugate wherein the warhead is an indolocarbazole compound. In some embodiments, the warhead of the LSE polymer conjugate is a derivative of K252a. In some embodiments, the LSE polymer conjugate is SNA-125.

[347] There is also provided, in several embodiments, a combination therapy, the combination therapy comprising administering to the subject an effective amount of an LSE polymer conjugate in conjunction with UV irradiation therapy and wherein the warhead is an indolocarbazole compound. In some embodiments, the warhead of the LSE polymer conjugate is a derivative of K252a. In some embodiments, the LSE polymer conjugate is SNA-125.

[348] In some embodiments, for indolocarbazole compounds having one amino group, the compound is modified (e.g., PEGylated) at that location (e.g., a PEG or modified PEG is linked to the compound by reaction with the amino group). If two or more amino groups are present, either location is PEGylated in some embodiments. In other embodiments, the amino group located the furthest away from the moieties interacting with the target is used. In some embodiments, the amino group that causes the least hindrance on activity is used (whether or not it is located the furthest away from the moieties interacting with the target). The effect of conjugation on the activity of the compound can be determined based on

various methods, such as bioassays, mass spectroscopy, surface plasmon resonance, in vivo assays, clinical assays, and predictive in silico modeling programs.

[349] In some embodiments, for indolocarbazole compounds having one sulfhydryl group, the compound is modified (e.g., PEGylated) at that location. If two or more sulfhydryl groups are present, either location is PEGylated in some embodiments. In other embodiments, the sulfhydryl group located the furthest away from the moieties interacting with the target is used. In some embodiments, the sulfhydryl group that causes the least hindrance on activity is used (whether or not it is located the furthest way from the moieties interacting with the target).

[350] In some embodiments, for indolocarbazole compounds having one hydroxyl group, the compound is modified (e.g., PEGylated) at that location. If two or more hydroxyl groups are present, either location is PEGylated in some embodiments. In other embodiments, the hydroxyl group located the furthest away from the moieties interacting with the target is used. In some embodiments, the hydroxyl group that causes the least hindrance on activity is used (whether or not it is located the furthest way from the moieties interacting with the target).

[351] In some embodiments, for indolocarbazole compounds having one carboxyl group, the compound is modified (e.g., PEGylated) at that location. If two or more carboxyl groups are present, either location is PEGylated in some embodiments. In other embodiments, the carboxyl group located the furthest away from the moieties interacting with the target is used. In some embodiments, the carboxyl group that causes the least hindrance on activity is used (whether or not it is located the furthest way from the moieties interacting with the target).

[352] In some embodiments, for indolocarbazole compounds having two or more carboxyl, hydroxyl, amino and/or sulfhydryl groups, the compound is modified (e.g., PEGylated) at the site furthest away from the active site. In some embodiments, the site that causes the least hindrance on activity is used (whether or not it is located the furthest way from the moieties interacting with the target).

[353] Methods for conjugating the PEG or modified PEG to an indolocarbazole compound, through reaction between functional groups (or functionalized groups), including reaction with the above-mentioned functional groups (amino, sulfhydryl, hydroxyl, carboxyl) are used in several embodiments. Methods of conjugation can be found for example in "Bioconjugate Techniques" (3rd Edition) 2013 by Greg T. Hermanson (<http://www.sciencedirect.com/science/book/9780123822390>); incorporated herein in its entirety by reference. Although PEGylation is used as an example, other polymers are used in some embodiments.

[354] An existing carboxylic moiety (-COOH) can be conjugated to PEG-amine through formation of an amide bond using any one of several possible coupling agents (including, e.g., TBTU, HBTU, HOBt, DCC, and N-hydroxysuccinimide). An existing amino group (-NH₂) can be conjugated to PEG-COOH through formation of an amide bond using any one of several possible coupling agents (including, e.g., TBTU, HBTU, HOBt, DCC, and N-hydroxysuccinimide). An existing hydroxyl moiety (-OH) can be conjugated to PEG-halide through formation of an ether bond in presence of a strong base (including, e.g. NaH, KH, and n-BuLi). These conjugation sites and chemistries are neither exhaustive nor limiting, and are included herein as examples only, and not intended to limit the scope of the embodiments described herein.

[355] Identifying a conjugation site and developing a conjugation strategy and/or chemistry does not require that all the atoms and the structures of the starting compound are maintained. Once the active part of the compound has been identified or hypothesized, some atoms, groups and structures of the compound can be removed or modified while maintaining sufficient or similar target site binding and activity in several embodiments.

[356] Suitable protecting groups, in some embodiments, are for protecting functional groups during the conjugation of warhead and polymer. Various protecting groups as well as suitable means and conditions for protecting and deprotecting the substituents are used in several embodiments. The means and conditions of protecting and deprotecting employed depend on the nature of the involved functional groups. Protecting groups for hydroxy-, amino-, and/or carboxy residues are selected in several embodiments from acetonide, ethylidene methoxymethyl, 2-methoxyethoxymethyl, benzyloxymethyl, tetrahydropyranyl, methyl, ethyl, isopropyl, t-butyl, benzyl, triphenylmethyl, t-butyl dimethylsilyl, triphenylsilyl, methoxycarbonyl, t-butyloxycarbonyl, benzyloxycarbonyl, fluorenylmethoxycarbonyl, acetyl, benzoyl, toluenesulfonyl, dimethoxybenzyl, nitrophenyloxycarbonyl, nitrobenzyloxycarbonyl, allyl, fluorenylmethyl, tetrahydrofuranyl, phenacyl, acetol, phenyl, trimethylsilyl, pyrrolidyl, indolyl, hydrazino and other protecting groups such as those that can be found in Greene T. W., et al., *Protective Groups in Organic Synthesis*, 4th ed., John Wiley and Son, New York, N.Y. (2007); incorporated herein in its entirety by reference. The reagents and conditions of protecting and deprotecting reactions are in particular selected for their suitability at selectively attaching and removing the protecting group without adversely affecting the rest of the compound.

[357] The polymer conjugates disclosed herein may also be prepared as pharmaceutically acceptable salts including salts of inorganic acids such as hydrochloric, hydroiodic, hydrobromic, phosphoric, metaphosphoric, nitric acid and sulfuric acids as well as salts of organic acids, such as tartaric, acetic, citric, malic, benzoic, glycolic, gluconic, succinic, aryl sulfonic, (e.g., p-toluene sulfonic acids, benzenesulfonic), phosphoric, malonic, and the like.

Suitable acids for formation of pharmaceutically acceptable salts are used in some embodiments. Further, pharmaceutically acceptable salts of compounds may be formed with a pharmaceutically acceptable cation. Pharmaceutically acceptable cations include, but are not limited to, alkali cations (Li^+ , Na^+ , K^+), earth alkali cations (Mg^{2+} , Ca^{2+} , Ba^{2+}), ammonium and organic cations, such as quaternary ammonium cations.

Synthesis of Polymer Conjugates

[358] The description above is not intended to be limiting and should be viewed as an example to guide the manufacture of the other compounds identified herein. The polymer conjugates may also be made as described in US Patent Nos. 8,673,347 and 8,926,955, both herein incorporated by reference. Several embodiments provide a method for the production of polymer conjugates of the active agents that result in a highly pure reaction product, obtained in high and consistent yields.

[359] In one embodiment, the conjugation reaction of the process to synthesize a conjugate polymer compound is catalysed by a base in an organic solvent. The base may be a strong base. In one embodiment, the base is selected from the group of alkali metal hydrides, tertiary amines and/or alkoxide. In another embodiment, the base catalysing the polymer conjugation reaction is sodium hydride. Other bases, such as sodium methoxide, or triethylamine can also be used. In several embodiments, the molar ratio of the base catalyst to the compound is between about 1:1 and about 4:1, about 1:1 to about 1.5:1 and about 1:1. The reaction may be carried out in an organic solvent, such as in anhydrous conditions (e.g., in a dry organic solvent). The water content in the solution mixture of the conjugation process may be equal or less than 200 ppm. The organic solvent may be selected from the group of dichloromethane, chloroform, N,N-dimethylformamide. In certain embodiments, the organic solvent is dichloromethane or anhydrous dichloromethane.

[360] The conjugation reaction may be carried out under inert gas atmosphere, such as nitrogen or argon atmosphere. The reaction of the process may be carried out at a temperature of about -10° to about 60° C, about 0° to about 25° C or at room temperature after an initial step at 0° C.

[361] Following the production of the target compound, the polymer conjugate may then be separated and purified from the reaction mixture. In one embodiment, the compound is obtained by purification of the crude mixture by flash chromatography. An automated gradient flash purification system may be used and may be equipped with a suitable column and solvent. The purification method may be selected from reverse phase and direct phase columns and the conditioning/elution solvent may be selected from dichloromethane, water, methanol, acetonitrile, ammonium formate buffer solution at different mixture ratios. In one embodiment, the compound is purified by a reverse phase flash chromatography equipped with a C18 cartridge and the purification is carried out by gradient elution with

acetonitrile/water. In one embodiment, the compound is purified by a normal phase flash chromatography.

[362] The product may then be dried e.g. over sodium sulphate and filtered off and the solvent is removed by evaporation under reduced pressure at 25° C. Purification of the target product is carried out in several embodiments. After the purification step the resultant polymer compound has a purity of at least about 95%, about 96%, about 97%, about 98%, about 98.5%, about 99% or about 99.5%. The disclosed process results in an overall mass yield of the compound from about 40% to about 98% by weight, or from about 50% to about 95% by weight based on the weight of a reactant compound.

[363] In several embodiments, the polymer moiety which is covalently attached to the active entity is biocompatible, can be of natural or semi-synthetic or synthetic origin and can have a linear or branched structure. The polymer may be selected from poly(alkylene oxides), or from (polyethylene) oxides. However, other polymers include without limitation polyacrylic acid, polyacrylates, polyacrylamide or N-alkyl derivatives thereof, polymethacrylic acid, polymethacrylates, polyethylacrylic acid, polyethylacrylates, polyvinylpyrrolidone, poly(vinylalcohol), polyglycolic acid, polylactic acid, poly(lactic-co-glycolic) acid, dextran, chitosan, hydroxyethyl starch.

[364] In some embodiments, the above-mentioned polymer moiety can carry an amino functional end-group or can be functionalized to carry an amino functional end-group. Hence, the polymer moiety can be an amino-activated polymer of general formula X—NH₂.

[365] The reaction of formation of the compositions identified herein may be carried out at a temperature of about 10° to about 60° C., about 15° to about 25° C. or at room temperature. The polymer moiety X may be a polyethylene glycol (PEG) moiety, wherein the terminal OH group can optionally be modified e.g. with C1-C5 alkyl or C1-C5 acyl groups, such as with C1-, C2- or C3-alkyl groups or C1-, C2- or C3 groups. The modified polyethylene glycol may be a terminally alkoxy-substituted polyethylene glycol, including a methoxy-polyethylene-glycol (mPEG).

[366] In other aspects, the conjugated polymer compounds may be used as active agents in a topical medicament useful for the prevention, alleviation and/or treatment of dermal pathologies. It has been shown that the conjugated polymer compounds described herein are very advantageously used as topical medicament since they do not show adverse or toxic effects (e.g. irritation) when dermally administered or any phototoxic effect (e.g. photomutagenicity, phototoxicity or photosensitisation) (as shown in the studies described in the following examples).

[367] The dermal pathologies for such treatment may be pathologies characterized by hyperproliferation of the keratinocytes, such as psoriasis, atopic dermatitis, chronic eczema,

acne, pityriasis rubra pilaris, keloids, hypertrophic scars and skin tumors, such as keratoacanthoma, squamous cell carcinoma, basal cell carcinoma.

[368] The compounds disclosed herein or pharmaceutically acceptable salts thereof can be administered as they are, or in the form of various pharmaceutical compositions according to the pharmacological activity and the purpose of administration. Yet another aspect is a pharmaceutical composition comprising an effective amount of at least one polymer conjugate optionally together with pharmaceutically acceptable carriers, adjuvants, diluents or/and additives. Pharmaceutical carriers, adjuvants, diluents or/and additives are applied in the formulation of the pharmaceutical composition comprising a compound of embodiments identified herein.

[369] The disclosed compounds can be employed as the sole active agent in a pharmaceutical composition. Alternatively, the polymer conjugates may be used in combination with one or several further active agents, e.g. other active pharmaceutical agents in the treatment of the conditions described herein.

[370] In particular, the polymer conjugate compounds may be used in combination with at least one endogenous angiogenesis inhibitor, for example and not restricted to, angioarrestin, angiostatin (plasminogen fragment), antiangiogenic antithrombin III, cartilage-derived inhibitor (CDI), CD59 complement fragment, endostatin (collagen XVIII fragment), fibronectin fragment, Gro-beta, heparinases, heparin hexasaccharide fragment, human chorionic gonadotropin (hCG), interferon alpha/beta/gamma, interferon inducible protein (IP-10), Interleukin-12, kringle 5 (plasminogen fragment), metalloproteinase inhibitors (TIMPs), 2-methoxyestradiol, placental ribonuclease inhibitor, plasminogen activator inhibitor, platelet factor-4 (PF4), prolactin 16 kD fragment, proliferin-related protein (PRP), retinoids, tetrahydrocortisol-S, thrombospondin-1 (TSP-1), transforming growth factor-beta or (TGF-b), vasculostatin, and vasostatin (calreticulin fragment).

[371] In particular, the polymer conjugate compounds may be used in combination with at least one steroidal anti-inflammatory drug and/or one further agent capable of inhibiting an early mediator of the inflammatory cytokine cascade, e.g. an antagonist or inhibitor of a cytokine selected from the group consisting of TNF, IL-1 α , IL-1 β , IL-1 γ , IL-8, MIP-1 α , MIP-1 β , MIP-2, MIP-3, MIP-4, MIP-5, MIP-6, MIP-7, MIP-8, MIP-9, MIP-10, MIP-11, MIP-12, MIP-13, MIP-14, MIP-15, MIP-16, MIP-17, MIP-18, MIP-19, MIP-20, MIP-21, MIP-22, MIP-23, MIP-24, MIP-25, MIP-26, MIP-27, MIP-28, MIP-29, MIP-30, MIP-31, MIP-32, MIP-33, MIP-34, MIP-35, MIP-36, MIP-37, MIP-38, MIP-39, MIP-40, MIP-41, MIP-42, MIP-43, MIP-44, MIP-45, MIP-46, MIP-47, MIP-48, MIP-49, MIP-50, MIP-51, MIP-52, MIP-53, MIP-54, MIP-55, MIP-56, MIP-57, MIP-58, MIP-59, MIP-60, MIP-61, MIP-62, MIP-63, MIP-64, MIP-65, MIP-66, MIP-67, MIP-68, MIP-69, MIP-70, MIP-71, MIP-72, MIP-73, MIP-74, MIP-75, MIP-76, MIP-77, MIP-78, MIP-79, MIP-80, MIP-81, MIP-82, MIP-83, MIP-84, MIP-85, MIP-86, MIP-87, MIP-88, MIP-89, MIP-90, MIP-91, MIP-92, MIP-93, MIP-94, MIP-95, MIP-96, MIP-97, MIP-98, MIP-99, MIP-100, MIP-101, MIP-102, MIP-103, MIP-104, MIP-105, MIP-106, MIP-107, MIP-108, MIP-109, MIP-110, MIP-111, MIP-112, MIP-113, MIP-114, MIP-115, MIP-116, MIP-117, MIP-118, MIP-119, MIP-120, MIP-121, MIP-122, MIP-123, MIP-124, MIP-125, MIP-126, MIP-127, MIP-128, MIP-129, MIP-130, MIP-131, MIP-132, MIP-133, MIP-134, MIP-135, MIP-136, MIP-137, MIP-138, MIP-139, MIP-140, MIP-141, MIP-142, MIP-143, MIP-144, MIP-145, MIP-146, MIP-147, MIP-148, MIP-149, MIP-150, MIP-151, MIP-152, MIP-153, MIP-154, MIP-155, MIP-156, MIP-157, MIP-158, MIP-159, MIP-160, MIP-161, MIP-162, MIP-163, MIP-164, MIP-165, MIP-166, MIP-167, MIP-168, MIP-169, MIP-170, MIP-171, MIP-172, MIP-173, MIP-174, MIP-175, MIP-176, MIP-177, MIP-178, MIP-179, MIP-180, MIP-181, MIP-182, MIP-183, MIP-184, MIP-185, MIP-186, MIP-187, MIP-188, MIP-189, MIP-190, MIP-191, MIP-192, MIP-193, MIP-194, MIP-195, MIP-196, MIP-197, MIP-198, MIP-199, MIP-200, MIP-201, MIP-202, MIP-203, MIP-204, MIP-205, MIP-206, MIP-207, MIP-208, MIP-209, MIP-210, MIP-211, MIP-212, MIP-213, MIP-214, MIP-215, MIP-216, MIP-217, MIP-218, MIP-219, MIP-220, MIP-221, MIP-222, MIP-223, MIP-224, MIP-225, MIP-226, MIP-227, MIP-228, MIP-229, MIP-230, MIP-231, MIP-232, MIP-233, MIP-234, MIP-235, MIP-236, MIP-237, MIP-238, MIP-239, MIP-240, MIP-241, MIP-242, MIP-243, MIP-244, MIP-245, MIP-246, MIP-247, MIP-248, MIP-249, MIP-250, MIP-251, MIP-252, MIP-253, MIP-254, MIP-255, MIP-256, MIP-257, MIP-258, MIP-259, MIP-260, MIP-261, MIP-262, MIP-263, MIP-264, MIP-265, MIP-266, MIP-267, MIP-268, MIP-269, MIP-270, MIP-271, MIP-272, MIP-273, MIP-274, MIP-275, MIP-276, MIP-277, MIP-278, MIP-279, MIP-280, MIP-281, MIP-282, MIP-283, MIP-284, MIP-285, MIP-286, MIP-287, MIP-288, MIP-289, MIP-290, MIP-291, MIP-292, MIP-293, MIP-294, MIP-295, MIP-296, MIP-297, MIP-298, MIP-299, MIP-300, MIP-301, MIP-302, MIP-303, MIP-304, MIP-305, MIP-306, MIP-307, MIP-308, MIP-309, MIP-310, MIP-311, MIP-312, MIP-313, MIP-314, MIP-315, MIP-316, MIP-317, MIP-318, MIP-319, MIP-320, MIP-321, MIP-322, MIP-323, MIP-324, MIP-325, MIP-326, MIP-327, MIP-328, MIP-329, MIP-330, MIP-331, MIP-332, MIP-333, MIP-334, MIP-335, MIP-336, MIP-337, MIP-338, MIP-339, MIP-340, MIP-341, MIP-342, MIP-343, MIP-344, MIP-345, MIP-346, MIP-347, MIP-348, MIP-349, MIP-350, MIP-351, MIP-352, MIP-353, MIP-354, MIP-355, MIP-356, MIP-357, MIP-358, MIP-359, MIP-360, MIP-361, MIP-362, MIP-363, MIP-364, MIP-365, MIP-366, MIP-367, MIP-368, MIP-369, MIP-370, MIP-371, MIP-372, MIP-373, MIP-374, MIP-375, MIP-376, MIP-377, MIP-378, MIP-379, MIP-380, MIP-381, MIP-382, MIP-383, MIP-384, MIP-385, MIP-386, MIP-387, MIP-388, MIP-389, MIP-390, MIP-391, MIP-392, MIP-393, MIP-394, MIP-395, MIP-396, MIP-397, MIP-398, MIP-399, MIP-400, MIP-401, MIP-402, MIP-403, MIP-404, MIP-405, MIP-406, MIP-407, MIP-408, MIP-409, MIP-410, MIP-411, MIP-412, MIP-413, MIP-414, MIP-415, MIP-416, MIP-417, MIP-418, MIP-419, MIP-420, MIP-421, MIP-422, MIP-423, MIP-424, MIP-425, MIP-426, MIP-427, MIP-428, MIP-429, MIP-430, MIP-431, MIP-432, MIP-433, MIP-434, MIP-435, MIP-436, MIP-437, MIP-438, MIP-439, MIP-440, MIP-441, MIP-442, MIP-443, MIP-444, MIP-445, MIP-446, MIP-447, MIP-448, MIP-449, MIP-450, MIP-451, MIP-452, MIP-453, MIP-454, MIP-455, MIP-456, MIP-457, MIP-458, MIP-459, MIP-460, MIP-461, MIP-462, MIP-463, MIP-464, MIP-465, MIP-466, MIP-467, MIP-468, MIP-469, MIP-470, MIP-471, MIP-472, MIP-473, MIP-474, MIP-475, MIP-476, MIP-477, MIP-478, MIP-479, MIP-480, MIP-481, MIP-482, MIP-483, MIP-484, MIP-485, MIP-486, MIP-487, MIP-488, MIP-489, MIP-490, MIP-491, MIP-492, MIP-493, MIP-494, MIP-495, MIP-496, MIP-497, MIP-498, MIP-499, MIP-500, MIP-501, MIP-502, MIP-503, MIP-504, MIP-505, MIP-506, MIP-507, MIP-508, MIP-509, MIP-510, MIP-511, MIP-512, MIP-513, MIP-514, MIP-515, MIP-516, MIP-517, MIP-518, MIP-519, MIP-520, MIP-521, MIP-522, MIP-523, MIP-524, MIP-525, MIP-526, MIP-527, MIP-528, MIP-529, MIP-530, MIP-531, MIP-532, MIP-533, MIP-534, MIP-535, MIP-536, MIP-537, MIP-538, MIP-539, MIP-540, MIP-541, MIP-542, MIP-543, MIP-544, MIP-545, MIP-546, MIP-547, MIP-548, MIP-549, MIP-550, MIP-551, MIP-552, MIP-553, MIP-554, MIP-555, MIP-556, MIP-557, MIP-558, MIP-559, MIP-560, MIP-561, MIP-562, MIP-563, MIP-564, MIP-565, MIP-566, MIP-567, MIP-568, MIP-569, MIP-570, MIP-571, MIP-572, MIP-573, MIP-574, MIP-575, MIP-576, MIP-577, MIP-578, MIP-579, MIP-580, MIP-581, MIP-582, MIP-583, MIP-584, MIP-585, MIP-586, MIP-587, MIP-588, MIP-589, MIP-590, MIP-591, MIP-592, MIP-593, MIP-594, MIP-595, MIP-596, MIP-597, MIP-598, MIP-599, MIP-600, MIP-601, MIP-602, MIP-603, MIP-604, MIP-605, MIP-606, MIP-607, MIP-608, MIP-609, MIP-610, MIP-611, MIP-612, MIP-613, MIP-614, MIP-615, MIP-616, MIP-617, MIP-618, MIP-619, MIP-620, MIP-621, MIP-622, MIP-623, MIP-624, MIP-625, MIP-626, MIP-627, MIP-628, MIP-629, MIP-630, MIP-631, MIP-632, MIP-633, MIP-634, MIP-635, MIP-636, MIP-637, MIP-638, MIP-639, MIP-640, MIP-641, MIP-642, MIP-643, MIP-644, MIP-645, MIP-646, MIP-647, MIP-648, MIP-649, MIP-650, MIP-651, MIP-652, MIP-653, MIP-654, MIP-655, MIP-656, MIP-657, MIP-658, MIP-659, MIP-660, MIP-661, MIP-662, MIP-663, MIP-664, MIP-665, MIP-666, MIP-667, MIP-668, MIP-669, MIP-670, MIP-671, MIP-672, MIP-673, MIP-674, MIP-675, MIP-676, MIP-677, MIP-678, MIP-679, MIP-680, MIP-681, MIP-682, MIP-683, MIP-684, MIP-685, MIP-686, MIP-687, MIP-688, MIP-689, MIP-690, MIP-691, MIP-692, MIP-693, MIP-694, MIP-695, MIP-696, MIP-697, MIP-698, MIP-699, MIP-700, MIP-701, MIP-702, MIP-703, MIP-704, MIP-705, MIP-706, MIP-707, MIP-708, MIP-709, MIP-710, MIP-711, MIP-712, MIP-713, MIP-714, MIP-715, MIP-716, MIP-717, MIP-718, MIP-719, MIP-720, MIP-721, MIP-722, MIP-723, MIP-724, MIP-725, MIP-726, MIP-727, MIP-728, MIP-729, MIP-730, MIP-731, MIP-732, MIP-733, MIP-734, MIP-735, MIP-736, MIP-737, MIP-738, MIP-739, MIP-740, MIP-741, MIP-742, MIP-743, MIP-744, MIP-745, MIP-746, MIP-747, MIP-748, MIP-749, MIP-750, MIP-751, MIP-752, MIP-753, MIP-754, MIP-755, MIP-756, MIP-757, MIP-758, MIP-759, MIP-760, MIP-761, MIP-762, MIP-763, MIP-764, MIP-765, MIP-766, MIP-767, MIP-768, MIP-769, MIP-770, MIP-771, MIP-772, MIP-773, MIP-774, MIP-775, MIP-776, MIP-777, MIP-778, MIP-779, MIP-780, MIP-781, MIP-782, MIP-783, MIP-784, MIP-785, MIP-786, MIP-787, MIP-788, MIP-789, MIP-790, MIP-791, MIP-792, MIP-793, MIP-794, MIP-795, MIP-796, MIP-797, MIP-798, MIP-799, MIP-800, MIP-801, MIP-802, MIP-803, MIP-804, MIP-805, MIP-806, MIP-807, MIP-808, MIP-809, MIP-810, MIP-811, MIP-812, MIP-813, MIP-814, MIP-815, MIP-816, MIP-817, MIP-818, MIP-819, MIP-820, MIP-821, MIP-822, MIP-823, MIP-824, MIP-825, MIP-826, MIP-827, MIP-828, MIP-829, MIP-830, MIP-831, MIP-832, MIP-833, MIP-834, MIP-835, MIP-836, MIP-837, MIP-838, MIP-839, MIP-840, MIP-841, MIP-842, MIP-843, MIP-844, MIP-845, MIP-846, MIP-847, MIP-848, MIP-849, MIP-850, MIP-851, MIP-852, MIP-853, MIP-854, MIP-855, MIP-856, MIP-857, MIP-858, MIP-859, MIP-860, MIP-861, MIP-862, MIP-863, MIP-864, MIP-865, MIP-866, MIP-867, MIP-868, MIP-869, MIP-870, MIP-871, MIP-872, MIP-873, MIP-874, MIP-875, MIP-876, MIP-877, MIP-878, MIP-879, MIP-880, MIP-881, MIP-882, MIP-883, MIP-884, MIP-885, MIP-886, MIP-887, MIP-888, MIP-889, MIP-890, MIP-891, MIP-892, MIP-893, MIP-894, MIP-895, MIP-896, MIP-897, MIP-898, MIP-899, MIP-900, MIP-901, MIP-902, MIP-903, MIP-904, MIP-905, MIP-906, MIP-907, MIP-908, MIP-909, MIP-910, MIP-911, MIP-912, MIP-913, MIP-914, MIP-915, MIP-916, MIP-917, MIP-918, MIP-919, MIP-920, MIP-921, MIP-922, MIP-923, MIP-924, MIP-925, MIP-926, MIP-927, MIP-928, MIP-929, MIP-930, MIP-931, MIP-932, MIP-933, MIP-934, MIP-935, MIP-936, MIP-937, MIP-938, MIP-939, MIP-940, MIP-941, MIP-942, MIP-943, MIP-944, MIP-945, MIP-946, MIP-947, MIP-948, MIP-949, MIP-950, MIP-951, MIP-952, MIP-953, MIP-954, MIP-955, MIP-956, MIP-957, MIP-958, MIP-959, MIP-960, MIP-961, MIP-962, MIP-963, MIP-964, MIP-965, MIP-966, MIP-967, MIP-968, MIP-969, MIP-970, MIP-971, MIP-972, MIP-973, MIP-974, MIP-975, MIP-976, MIP-977, MIP-978, MIP-979, MIP-980, MIP-981, MIP-982, MIP-983, MIP-984, MIP-985, MIP-986, MIP-987, MIP-988, MIP-989, MIP-990, MIP-991, MIP-992, MIP-993, MIP-994, MIP-995, MIP-996, MIP-997, MIP-998, MIP-999, MIP-1000, MIP-1001, MIP-1002, MIP-1003, MIP-1004, MIP-1005, MIP-1006, MIP-1007, MIP-1008, MIP-1009, MIP-1010, MIP-1011, MIP-1012, MIP-1013, MIP-1014, MIP-1015, MIP-1016, MIP-1017, MIP-1018, MIP-1019, MIP-1020, MIP-1021, MIP-1022, MIP-1023, MIP-1024, MIP-1025, MIP-1026, MIP-1027, MIP-1028, MIP-1029, MIP-1030, MIP-1031, MIP-1032, MIP-1033, MIP-1034, MIP-1035, MIP-1036, MIP-1037, MIP-1038, MIP-1039, MIP-1040, MIP-1041, MIP-1042, MIP-1043, MIP-1044, MIP-1045, MIP-1046, MIP-1047, MIP-1048, MIP-1049, MIP-1050, MIP-1051, MIP-1052, MIP-1053, MIP-1054, MIP-1055, MIP-1056, MIP-1057, MIP-1058, MIP-1059, MIP-1060, MIP-1061, MIP-1062, MIP-1063, MIP-1064, MIP-1065, MIP-1066, MIP-1067, MIP-1068, MIP-1069, MIP-1070, MIP-1071, MIP-1072, MIP-1073, MIP-1074, MIP-1075, MIP-1076, MIP-1077, MIP-1078, MIP-1079, MIP-1080, MIP-1081, MIP-1082, MIP-1083, MIP-1084, MIP-1085, MIP-1086, MIP-1087, MIP-1088, MIP-1089, MIP-1090, MIP-1091, MIP-1092, MIP-1093, MIP-1094, MIP-1095, MIP-1096, MIP-1097, MIP-1098, MIP-1099, MIP-1100, MIP-1101, MIP-1102, MIP-1103, MIP-1104, MIP-1105, MIP-1106, MIP-1107, MIP-1108, MIP-1109, MIP-1110, MIP-1111, MIP-1112, MIP-1113, MIP-1114, MIP-1115, MIP-1116, MIP-1117, MIP-1118, MIP-1119, MIP-1120, MIP-1121, MIP-1122, MIP-1123, MIP-1124, MIP-1125, MIP-1126, MIP-1127, MIP-1128, MIP-1129, MIP-1130, MIP-1131, MIP-1132, MIP-1133, MIP-1134, MIP-1135, MIP-1136, MIP-1137, MIP-1138, MIP-1139, MIP-1140, MIP-1141, MIP-1142, MIP-1143, MIP-1144, MIP-1145, MIP-1146, MIP-1147, MIP-1148, MIP-1149, MIP-1150, MIP-1151, MIP-1152, MIP-1153, MIP-1154, MIP-1155, MIP-1156, MIP-1157, MIP-1158, MIP-1159, MIP-1160, MIP-1161, MIP-1162, MIP-1163, MIP-1164, MIP-1165, MIP-1166, MIP-1167, MIP-1168, MIP-1169, MIP-1170, MIP-1171, MIP-1172, MIP-1173, MIP-1174, MIP-1175, MIP-1176, MIP-1177, MIP-1178, MIP-1179, MIP-1180, MIP-1181, MIP-1182, MIP-1183, MIP-1184, MIP-1185, MIP-1186, MIP-1187, MIP-1188, MIP-1189, MIP-1190, MIP-1191, MIP-1192, MIP-1193, MIP-1194, MIP-1195, MIP-1196, MIP-1197, MIP-1198, MIP-1199, MIP-1200, MIP-1201, MIP-1202, MIP-1203, MIP-1204, MIP-1205, MIP-1206, MIP-1207, MIP-1208, MIP-1209, MIP-1210, MIP-1211, MIP-1212, MIP-1213, MIP-1214, MIP-1215, MIP-1216, MIP-1217, MIP-1218, MIP-1219, MIP-1220, MIP-1221, MIP-1222, MIP-1223, MIP-1224, MIP-1225, MIP-1226, MIP-1227, MIP-1228, MIP-1229, MIP-1230, MIP-1231, MIP-1232, MIP-1233, MIP-1234, MIP-1235, MIP-1236, MIP-1237, MIP-1238, MIP-1239, MIP-1240, MIP-1241, MIP-1242, MIP-1243, MIP-1244, MIP-1245, MIP-1246, MIP-1247, MIP-1248, MIP-1249, MIP-1250, MIP-1251, MIP-1252, MIP-1253, MIP-1254, MIP-1255, MIP-1256, MIP-1257, MIP-1258, MIP-1259, MIP-1260, MIP-1261, MIP-1262, MIP-1263, MIP-1264, MIP-1265, MIP-1266, MIP-1267, MIP-1268, MIP-1269, MIP-1270, MIP-1271, MIP-1272, MIP-1273, MIP-1274, MIP-1275, MIP-1276, MIP-1277, MIP-1278, MIP-1279, MIP-1280, MIP-1281, MIP-1282, MIP-1283, MIP-1284, MIP-1285, MIP-1286, MIP-1287, MIP-1288, MIP-1289, MIP-1290, MIP-1291, MIP-1292, MIP-1293, MIP-1294, MIP-1295, MIP-1296, MIP-1297, MIP-1298, MIP-1299, MIP-1300, MIP-1301, MIP-1302, MIP-1303, MIP-1304, MIP-1305, MIP-1306, MIP-1307, MIP-1308, MIP-1309, MIP-1310, MIP-1311, MIP-1312, MIP-1313, MIP-1314, MIP-1315, MIP-1316, MIP-1317, MIP-1318, MIP-1319, MIP-1320, MIP-1321, MIP-1322, MIP-1323, MIP-1324, MIP-1325, MIP-1326, MIP-1327, MIP-1328, MIP-1329, MIP-1330, MIP-1331, MIP-1332, MIP-1333, MIP-1334, MIP-1335, MIP-1336, MIP-1337, MIP-1338, MIP-1339, MIP-1340, MIP-1341, MIP-1342, MIP-1343, MIP-1344, MIP-1345, MIP-1346, MIP-1347, MIP-1348, MIP-1349, MIP-1350, MIP-1351, MIP-1352, MIP-1353, MIP-1354, MIP-1355, MIP-1356, MIP-1357, MIP-1358, MIP-1359, MIP-1360, MIP-1361, MIP-1362, MIP-1363, MIP-1364, MIP-1365, MIP-1366, MIP-1367, MIP-1368, MIP-1369, MIP-1370, MIP-1371, MIP-1372, MIP-1373, MIP-1374, MIP-1375, MIP-1376, MIP-1377, MIP-1378, MIP-1379, MIP-1380, MIP-1381, MIP-1382, MIP-1383, MIP-1384, MIP-1385, MIP-1386, MIP-1387, MIP-1388, MIP-1389, MIP-1390, MIP-1391, MIP-1392, MIP-1393, MIP-1394, MIP-1395, MIP-1396, MIP-1397, MIP-1398, MIP-1399, MIP-1400, MIP-1401, MIP-1402, MIP-1403, MIP-1404, MIP-1405, MIP-1406, MIP-1407, MIP-1408, MIP-1409, MIP-1410, MIP-1411, MIP-1412, MIP-1413, MIP-1414, MIP-1415, MIP-1416, MIP-1417, MIP-1418, MIP-1419, MIP-1420, MIP-1421, MIP-1422, MIP-1423, MIP-1424, MIP-1425, MIP-1426, MIP-1427, MIP-1428, MIP-1429, MIP-1430, MIP-1431, MIP-1432, MIP-1433, MIP-1434, MIP-1435, MIP-1436, MIP-1437, MIP-1438, MIP-1439, MIP-1440, MIP-1441, MIP-1442, MIP-1443, MIP-1444, MIP-1445, MIP-1446, MIP-1447, MIP-1448, MIP-1449, MIP-1450, MIP-1451, MIP-1452, MIP-1453, MIP-1454, MIP-1455, MIP-1456, MIP-1457, MIP-1458, MIP-1459, MIP-1460, MIP-1461, MIP-1462, MIP-1463, MIP-1464, MIP-1465, MIP-1466, MIP-1467, MIP-1468, MIP-1469, MIP-1470, MIP-1471, MIP-1472, MIP-1473, MIP-1474, MIP-1475, MIP-1476, MIP-1477, MIP-1478, MIP-1479, MIP-1480, MIP-1481, MIP-1482, MIP-1483, MIP-1484, MIP-1485, MIP-1486, MIP-1487, MIP-1488, MIP-1489, MIP-1490, MIP-1491, MIP-1492, MIP-1493, MIP-1494, MIP-1495, MIP-1496, MIP-1497, MIP-1498, MIP-1499, MIP-1500, MIP-1501, MIP-1502, MIP-1503, MIP-1504, MIP-1505, MIP-1506, MIP-1507, MIP-1508, MIP-1509, MIP-1510, MIP-1511, MIP-1512, MIP-1513, MIP-1514, MIP-1515, MIP-1516, MIP-1517, MIP-1518, MIP-1519, MIP-1520, MIP-1521, MIP-1522, MIP-1523, MIP-1524, MIP-1525, MIP-1526, MIP-1527, MIP-1528, MIP-1529, MIP-1530, MIP-1531, MIP-1532, MIP-1533, MIP-1534, MIP-1535, MIP-1536, MIP-1537, MIP-1538, MIP-1539, MIP-1540, MIP-1541, MIP-1542, MIP-1543, MIP-1544, MIP-1545, MIP-1546, MIP-1547, MIP-1548, MIP-1549, MIP-1550, MIP-1551, MIP-1552, MIP-1553, MIP-1554, MIP-1555, MIP-1556, MIP-1557, MIP-1558, MIP-1559, MIP-1560, MIP-1561, MIP-1562, MIP-1563, MIP-1564, MIP-1565, MIP-1566, MIP-1567, MIP-1568, MIP-1569, MIP-1570, MIP-1571, MIP-1572, MIP-1573, MIP-1574, MIP-1575, MIP-1576, MIP-1577, MIP-1578, MIP-1579, MIP-1580, MIP-158

dexamethasone, dexamethasone acetate, dexamethasone sodium phosphate, diflorasone diacetate, difluocortolone valerate, fludrocortisone acetate, fludroxycortide, flumetasone pivalate, flunisolide, fluocinolone acetonide, fluocinonide, fluocortolone, fluorometholone, flurandrenolide, fluticasone propionate, halcinonide, halobetasol propionate, medrysone, methylprednisolone, methylprednisolone acetate, methylprednisolone sodium succinate, mometasone furoate, paramethasone acetate, prednisolone, prednisolone acetate, prednisolone sodium phosphate, prednisolone tebutate, prednisone, triamcinolone, triamcinolone acetate, triamcinolone acetonide, triamcinolone diacetate, triamcinolone hexacetonide. Useful antagonists or inhibitors of a cytokine are selected from infliximab, etanercept or adalimumab.

[372] The polymer conjugate compounds may be used in combination with at least one natural extract or essential oil which is anti-itching agent, for example and not restricted to, extracts of *Abelmoschus esculentus*, *Actaea alba*, *Aglaia odorata*, *Alkanna tinctoria*, *Althaea officinalis*, *Altingia excelsa*, *Andropogon virginicus*, *Aralia nudicaulis*, *Aralia racemosa*, *Argemone mexicana*, *Barleria prionitis*, *Camelia sinensis*, *Caesalpinia digyna*, *Campsis grandiflora*, *Carissa congesta*, *Carthamus oxyacantha*, *Cassia tora*, *Chrysanthemum indicum*, *Cimicifuga racemosa*, *Cinnamomum camphora*, *Clematis vitalba*, *Cuscuta reflexa*, *Diospyros peregrina*, *Enicostema axillare*, *Hammamelis virginiana*, *Jatropha multifida*, *Lavandula officinalis*, *Lavandula latifolia*, *Liquidambar orientalis*, *Lithospermum officinale*, *Madhuca longifolia*, *Martynia annua*, *Medicago sativa*, *Michelia champaca*, *Mikania glomerata*, *Mimosa pudica*, *Oryza sativa*, *Phaseolus vulgaris*, *Phyllanthus urinaria*, *Phyllanthus virgatus*, *Pistacia vera*, *Polygonum hydropiper*, *Quercus ilex*, *Rauvolfia caffra*, *Ricinus communis*, *Rubus idaeus*, *Sagittaria sagittifolia*, *Sandoricum koetjape*, *Sapindus mukorossi*, *Schleichera oleosa*, *Sesbania grandiflora*, *Spondias dulcis*, *Tilia* sp., *Toona ciliata*, *Tragia involucrata*, *Trichosanthes quinqueangulata*, *Vaccaria pyramidata*, *Ventilago madraspatana*, *Veratrum album* or *Xanthium strumarium* among others

[373] The polymer conjugate compounds may be used in combination with at least one synthetic compound or product of biotechnological origin which is an anti-itching agent, for example and not restricted to mepyramine (pyrilamine), antazoline, diphenhydramine, carbinoxamine, doxylamine, clemastine, dimenhydrinate, pheniramine, chlorphenamine (chlorpheniramine), dexchlorpheniramine, brompheniramine, triprolidine, cyclizine, chlorcyclizine, hydroxyzine, meclizine, cetirizine, levocetirizine, promethazine, thenaldine, alimemazine (trimeprazine), cyproheptadine, azatidine, ketotifen, acrivastine, astemizole, cetirizine, loratadine, desloratadine, mizolastine, terfenadine, fexofenadine, fexofenadine, azelastine, levocabastine, olopatadine, corticosteroids such as cortisone, hydrocortisone dexamethasone, prednisone; Neutragen™ [INCI: Water, Butylene Glycol, Dextran, Palmitoyl Tripeptide-8] marketed by Atrium Innovations/Unipex Group, Meliprene [INCI: Dextran,

Acetyl Heptapeptide-1] marketed by Institut Europeen de Biologie Cellulaire/Unipex Group, Skinasensyl™ [INCI: Acetyl Tetrapeptide-15] marketed by Laboratoires Serobiologiques/Cognis, SymSitive® 1609 [INCI: 4-t-Butylcyclohexanol] marketed by Symrise, Symbiocell™ [INCI: Extract from Cestrum Latifolium] marketed by BASF, Gatuline®/Derma-Sensitive [INCI: Octyldodecyl Myristate, Capparis Spinosa Fruit Extract] marketed by Gattefosse or MAXnolia [INCI: Magnolia Officinalis Bark Extract, Vitis Vinifera/Vitis Vinifera (Grape) Seed Extract, Tocopherol] marketed by Mibelle among others.

[374] The polymer conjugate compounds may be used in combination with at least one physiological cooling agent, for example and not restricted to menthone glycerol acetal, menthyl lactate, menthyl ethyl oxamate, substituted menthyl-3-carboxylic acid amides (e.g. menthyl-3-carboxylic acid N-ethylamide, Na-(L- menthanecarbonyl)glycine ethyl ester, 2-isopropyl-N-2,3-trimethylbutanamide, substituted cyclohexanecarboxylic acid amides, 3-menthoxypropane-1,2-diol, 2-hydroxyethyl menthyl carbonate, 2-hydroxypropyl menthyl carbonate, N- acetyl glycine menthyl ester, isopulegol, menthyl hydroxycarboxylic acid esters (e.g. menthyl 3-hydroxybutyrate), monomenthyl succinate, monomenthyl glutarate, 2-mercaptocyclodecanone, menthyl 2-pyrrolidin-5-onecarboxylate, 2,3-dihydroxy-p-menthane, 3,3,5-trimethylcyclohexanone glycerol ketal, 3-menthyl 3,6-di- and -trioxaalkanoates, 3-menthyl methoxyacetate and icilin.

[375] Further agents which can be used in combination with the polymer compounds are e.g. antagonists and/or inhibitors of RAGE, antagonists and/or inhibitors of HMGB1, antagonists and/or inhibitors of the interaction of a Toll-like receptor (TLR) with HMGB1, the functional N-terminal lectin-like domain (D1) of thrombomodulin and/or a synthetic double-stranded nucleic acid or nucleic acid analogue molecule with a bent shape structure as described in the international patent application WO 2006/002971 which is herein incorporated by reference.

[376] The compositions described herein may be administered by a physician or other professional. Patients may also be able to self-administer. In several embodiments, administration of the composition may be performed dermally, via, for example, ointments, creams, oils, liposomes or trans-dermal patches, or wherein the polymer conjugates are incorporated into liposomes.

[377] In some embodiments, at least one excipient is provided. Excipients can include a nonaqueous or aqueous carrier, and one or more agents selected from moisturizing agents, pH adjusting agents, strontium ions (Sr²⁺), deodorants, fragrances, chelating agents, preservatives, emulsifiers, thickeners, solubilizing agents, penetration enhancers, anti-irritants, colorants, surfactants, beneficial agents, pharmaceutical agents, and other components for use in connection with the compositions described herein (such as topical compositions for treatment of the skin). In several embodiments, the composition is an

anhydrous formulation to prevent skin irritation such as water-based irritant contact dermatitis or stinging sensation upon application to damaged skin. In another embodiment, the composition is formulated such that preservatives need not be employed (e.g., a preservative-free formulation) so as to avoid skin irritation associated with certain preservatives.

[378] To facilitate application, the composition may be provided as an ointment, an oil, a lotion, a paste, a powder, a gel, a foam, or a cream. The composition may also include additional ingredients such as a protective agent, an emollient, an astringent, a humectant, a sun screening agent, a sun tanning agent, a UV absorbing agent, an antibiotic agent, an antifungal agent, an antiviral agent, an antiprotozoal agent, an anti-acne agent, an anesthetic agent, a steroidal anti-inflammatory agent, a non-steroidal anti-inflammatory agent, an antipruritic agent, an additional antioxidant agent, a chemotherapeutic agent, an anti-histamine agent, a vitamin or vitamin complex, a hormone, an anti-dandruff agent, an anti-wrinkle agent, an anti-skin atrophy agent, a skin whitening agent, a cleansing agent, additional peptides, additional modified peptides, and combinations thereof. In a further embodiment, the composition may avoid irritants (such as animal or cellular-based materials) to avoid skin irritation.

[379] In some embodiments, the compositions may be administered by injection or infusion, in particular by intravenous, intramuscular, transmucosal, subcutaneous or intraperitoneal injection or infusion and/or by oral, topical, dermal, nasal, inhalation, aerosol and/or rectal application, etc.

[380] In a further embodiment, the compositions are administered reversibly immobilized on the surface of a medical device, in particular by binding, coating and/or embedding the compositions on a medical device, such as but not limited to, stents, catheters, surgical instruments, cannulae, cardiac valves, or vascular prostheses. After contacting the medical device with body fluid or body tissue, the reversibly immobilized compounds are liberated. Consequently, the coated medical devices act as drug delivery devices eluting the medicament, whereby the drug delivery kinetics can be controlled, providing an immediate release or a controlled, delayed or sustained drug delivery, for example.

[381] In some embodiments, the composition further comprises an enteric coating that resists degradation under the prevailing pH of the stomach and permits delivery to specific regions of the gastrointestinal tract.

[382] The pharmaceutical compositions may also be used for diagnostic or for therapeutic applications. For diagnostic applications, the compound may be present in a labelled form, e.g. in a form containing an isotope, e.g. a radioactive isotope or an isotope which may be detected by nuclear magnetic resonance. In some embodiments, a therapeutic application

is, in the case of a topical application, the prevention, alleviation and treatment of psoriasis and dermatitis.

[383] The concentrations of the compounds in the pharmaceutical composition can vary. The concentration will depend upon factors such as the total dosage of the drug to be administered, the chemical characteristics (e.g., hydrophobicity) of the compounds employed, the route of administration, the age, body weight and symptoms of a patient. The compounds typically are provided in an aqueous physiological buffer solution containing about 0.1 to 10% w/v compound for topical administration. Typical dose ranges are from about 1 µg to about 1 g/kg of body weight per day; a dose range may be from about 0.01 mg/kg to 100 mg/kg of body weight per day, or about 0.1 to 20 mg/kg once to four times per day. In some embodiments, the dosage of the drug to be administered is likely to depend on variables such as the type and extent of the progression of the disease or disorder, the overall health status of the particular patient, the relative biological efficacy of the selected compound and the formulation of the compound excipient, and its route of administration.

[384] Although the foregoing has been described in some detail by way of illustrations and examples for purposes of clarity and understanding, it will be understood by those of skill in the art that modifications can be made without departing from the spirit of the present disclosure. Therefore, it should be clearly understood that the forms disclosed herein are illustrative only and are not intended to limit the scope of the present disclosure, but rather to also cover all modification and alternatives coming within the true scope and spirit of the embodiments of the invention(s).

[385] Terms and phrases used in this application, and variations thereof, especially in the appended claims, unless otherwise expressly stated, should be construed as open ended as opposed to limiting. As examples of the foregoing, the term 'including' should be read to mean 'including, without limitation,' 'including but not limited to,' or the like.

[386] The indefinite article "a" or "an" does not exclude a plurality. The term "about" as used herein to, for example, define the values and ranges of molecular weights means that the indicated values and/or range limits can vary within $\pm 20\%$, e.g., within $\pm 10\%$. The use of "about" before a number includes the number itself. For example, "about 5" provides express support for "5".

[387] The phrases "active agent" and "active entity" are synonyms and can be used interchangeably.

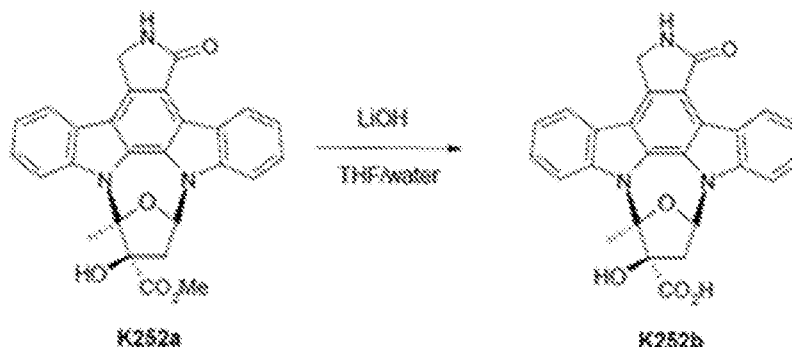
[388] The terms "CT340" and "SNA-125" are synonyms and can be used interchangeably. The terms "CT327" and "SNA-120" are synonyms and can be used interchangeably.

EXAMPLES

[389] Non-limiting examples are provided below.

Example 1: Synthesis of CT340

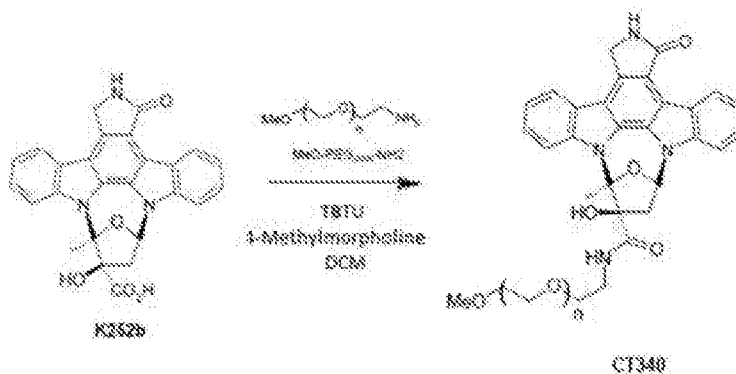
[390] Step 1: Hydrolysis to K252b



[391] 1 molar equivalent of K252a was dissolved in 7.1 vol of THF and the mixture was stirred for at least 30 minutes at ambient temperature ($\sim 20^{\circ}\text{C}$). A solution of 3 eq. $\text{LiOH}\cdot\text{H}_2\text{O}$ in 4 vol of highly purified water (with respect to K252a) was added to the pale yellow solution over ~ 4 minutes to give a biphasic system which was stirred for ~ 21 h at ambient temperature, after which an IPC test by HPLC indicated $<2\%$ a/a K252a.

[392] The majority of the solvent was distilled off under reduced pressure at $\sim 30^{\circ}\text{C}$. 3 vol of highly purified water were added to the residue and evaporation under reduced pressure at $\sim 30^{\circ}\text{C}$ was continued to remove the remainder of the solvent, resulting in a thick aqueous mixture. The mixture was cooled to $\sim 20^{\circ}\text{C}$ and 2N HCl-solution (approximately 3-3.5 vol) was added to adjust the pH to 2-3, affording a white suspension. The suspension was stirred for ~ 40 minutes at ambient temperature and the solid collected by suction filtration, washing twice with highly purified water. The solid was then tumble-dried on the rotary evaporator at $\sim 25^{\circ}\text{C}$ under reduced pressure. It was then slurried in 14.2 vol of ethyl acetate for ~ 1 h. The solid was collected by suction filtration, washing twice with heptane. The solid was then tumble-dried on the rotary evaporator at $\sim 25^{\circ}\text{C}$ under reduced pressure, affording K252b in essentially quantitative (uncorrected) yield.

[393] Step 2: Coupling



[394] 1 molar equivalent of K252b was pre-dried by suspending in 10 vol of dichloromethane and concentrating to dryness on the rotary evaporator at $\sim 25^{\circ}\text{C}$ then

holding under reduced pressure to afford a yellowish solid. The dried K252b was re-suspended in 160 vol of dichloromethane at ambient temperature (~20°C) and 2 molar equivalents of 4-methylmorpholine were added. The resulting suspension was stirred for ~35-45 minutes.

[395] 1 molar equivalent of PEG-amine was pre-dried by dissolving in dichloromethane (10 vol with respect to K252b) and concentrating to dryness on the rotary evaporator at ~25°C to give a white solid. The dried PEG-amine was dissolved in 50 vol of dichloromethane and this solution was added over ~20 minutes to the K252b/4-methylmorpholine suspension. The reaction mixture was stirred for ~20 minutes at ambient temperature and 1.7 eq. TBTU was then added in one portion. The reaction mixture was stirred for ~18-22h at ambient temperature, after which an IPC test by HPLC indicated <2% K252b.

[396] Methanol (0.01 vol) was added to quench the reaction and the mixture was stirred for ~1-1.5 hours at ambient temperature. 30 vol of saturated sodium bicarbonate solution were added and the biphasic mixture was then stirred for ~20 minutes. The phases were separated and the lower (organic) phase was washed with 15 vol of saturated sodium bicarbonate solution. The organic phase was concentrated to dryness under reduced pressure at ~25°C, protected from light.

[397] Step 3: Purification and Isolation

[398] The product from Step 2 is purified by preparative column chromatography using silica gel which has been pretreated with ethyl acetate containing 5% triethylamine. The Step 2 product was dissolved in dichloromethane/ethyl acetate for application to the column. Elution with ethyl acetate enabled residual K252a and UV-inactive species to be eluted (as monitored by TLC). The column was then conditioned with dichloromethane and eluted with dichloromethane/MeOH (98.5:1.5 → 95.5:4.5). Fractions were collected and analyzed by HPLC and TLC, and pooled according to their purity. Evaporation of the combined desired product fractions led to CT340 of purity >95% by HPLC area.

[399] Although the resulting material is of high chemical purity, a precipitation step is performed to transform it from a semisolid concentrate into a readily handled free-flowing solid.

[400] The concentrate was therefore dissolved in 4 vol of MeOH and this solution was added to chilled (~0°C) diethyl ether (20 vol) over 40-70 minutes to afford a white suspension that was stirred for further 1-2 hours at ~0°C. The solid product was filtered off, washing with diethyl ether (2 x 2 vol), to give CT340 Drug Substance in a typical yield of ~90%.

Example 2: CT340 Kinase Inhibition Panel

[401] K-252a is a potent inhibitor of multiple kinases. In the present study, the inhibitory activity of K-252a against selected common tyrosine kinases and serine/threonine kinases

was evaluated. A similar experiment has been conducted for evaluating the inhibitory activity of CT340.

[402] K-252a (Acros lot A020265401) was dissolved in DMSO to make a 1 mM stock solution which was then diluted with DMSO to obtain a 20 μ M solution, further diluted with the assay buffer to achieve a concentration of 0.8 μ M. K-252a was tested at a concentration of 200 nM. The preparation of CT340 and the reference compounds was conducted following a similar procedure. CT340 also was tested at a concentration of 200 nM. Staurosporine, 5-iodotubericidin, NK inhibitor II and SB202190 have been used as reference compounds.

[403] The kinase inhibition studies for K-252a and CT340 have been performed using standard assays for the respective kinase.

[404] The inhibitory activities of CT340 (200nM) against 89 tyrosine kinases are shown in Table 1 below. The inhibitory activities of CT340 (200nM) against 187 serine/threonine kinases are shown in Table 2. The readout value of reaction control (with ATP) was set as a 0% inhibition and the readout value of background (without ATP) was set as a 100% inhibition. The results clearly show a dramatic improvement in the selectivity of kinase inhibition of CT340 versus K-252a. The less other kinases are inhibited, the less toxic the molecule is likely to be.

TABLE 1 – Inhibitory activities of CT340 against 89 tyrosine kinases

Kinase	% Inhibition		Kinase	% Inhibition	
	CT340	K262a		CT340	K262a
	200 nM	200 nM		200 nM	200 nM
ABL	2,1	16,9	JAK1	17,2	102,7
ABL(T315I)	0,6	46,4	JAK2	80,1	102,0
ABL(E255K)	1,6	8,2	JAK3	90,4	101,0
ARG	1,3	11,2	TYK2	36,1	99,0
ACK	28,3	99,1	MET	-0,3	78,9
TNK1	14,0	99,7	MET(Y1236D)	1,1	94,9
ALK	2,8	93,8	RON	-0,3	64,3
LTK	6,1	98,2	MUSK	2,6	80,5
AXL	21,8	99,1	FLT3	84,8	100,6
MER	26,8	99,2	FMS	2,2	99,8
TYRO3	6,4	96,6	KIT	4,4	93,4
CSK	-0,8	43,8	KIT(T870D)	-0,4	91,1
CTK	-6,8	-6,0	KIT(V680G)	2,0	94,6
DDR2	29,8	99,7	PDGFRa	40,6	100,4
EGFR	1,9	49,8	PDGFRa(T874I)	76,2	99,0
EGFR(T790M)	-1,3	89,4	PDGFRa(V681D)	82,6	101,3
EGFR(L858R)	3,6	78,0	PDGFRb	88,0	100,3
HER2	-1,1	30,3	RET	21,9	99,6
HER4	-4,8	70,7	ROS	78,8	101,6
EphA1	0,0	82,8	BLK	8,6	93,9
EphA2	2,0	44,7	BRK	-3,4	72,8
EphA3	-8,4	60,1	FOR	6,8	98,1
EphA4	-7,7	36,3	FRK	2,0	82,8
EphA5	-3,1	61,1	FYN	4,6	76,1
EphA6	-11,6	24,3	HCK	16,8	91,6
EphA7	-2,6	68,6	LCK	7,8	89,8
EphA8	-6,2	16,4	LYNa	10,3	84,6
EphB1	-2,8	63,9	LYNb	7,9	78,6
EphB2	-3,2	10,4	SRC	0,0	80,0
EphB3	-4,3	0,6	SRM	-4,4	40,6
EphB4	-7,4	22,7	YES	4,1	86,3
FAK	-6,6	41,1	SYK	67,6	100,7
PYK2	4,3	92,0	ZAP70	30,8	98,7
FER	63,3	100,6	BMX	11,6	96,9
FES	3,1	80,9	BTX	-2,6	91,6
FGFR1	2,7	83,2	ITK	11,2	96,4
FGFR2	-4,9	89,0	TEC	10,6	96,1
FGFR3	2,3	84,4	TXK	6,3	69,3
FGFR3(K650E)	4,8	89,6	TIE2	-12,4	63,9
FGFR3(K650M)	8,1	84,3	TRKA	92,3	101,6
FGFR4	-6,0	22,0	TRKB	76,7	101,0
IGF1R	1,8	67,9	TRKC	91,1	99,0
INSR	3,6	86,8	FLT1	8,3	97,2
IRR	3,7	80,3	FLT4	63,4	99,6
			KDR	12,3	96,9

TABLE 2 – Inhibitory activities of CT340 against 187 serine/threonine kinases

Kinase	% Inhibition		Kinase	% Inhibition	
	CT340	K262a		CT340	K262a
	200 nM	200 nM		200 nM	200 nM
AKT1	-4,5	48,1	DAPK1	-0,6	93,0
AKT2	-3,0	23,3	DCAMKL2	-4,4	7,8
AKT3	-4,0	63,0	MAPKAPK2	-1,2	92,8
CRIK	6,3	92,5	MAPKAPK3	-7,5	32,8
ROCK1	-5,2	83,7	MAPKAPK5	-1,5	52,4
ROCK2	-2,8	86,8	skMLCK	7,3	89,1
MROCKa	-1,0	68,5	MNK1	15,8	97,4
PKAca	1,3	92,1	MNK2	67,1	101,7
PRKX	2,3	94,3	PIM1	1,5	89,9
PGK	12,5	89,2	PIM2	0,1	80,7
CGK2	86,8	101,3	PHKG1	93,0	100,7
POK1	41,2	99,9	PHKG2	13,6	101,8
PKCa	12,3	92,7	PKD1	65,1	103,1
PKCbi	4,0	82,2	PKD2	68,9	103,2
PKCg	7,3	84,9	PKD3	54,4	101,9
PKCd	7,9	88,4	CHK2	8,8	97,2
PKCa	10,9	77,5	TSSK1	25,6	101,9
PKCq	5,1	87,0	TSSK2	1,2	86,5
PKCz	-4,0	42,1	CDC2	4,3	87,9
PKCh	11,5	81,8	CDK2	26,4	97,9
PKCI	3,5	39,0	CDK3	2,3	82,6
PKN1	27,5	95,5	CDK4	4,2	99,5
MSK1	-0,5	91,5	CDC7	4,0	82,1
MSK2	-4,8	86,6	CDK6	28,3	93,3
p70S6K	9,3	91,7	DYRK2	2,1	97,0
RSK1	34,3	98,8	CLK1	53,0	100,7
RSK2	36,5	96,9	HIPK2	-4,1	87,9
RSK3	52,6	100,4	OSK3a	11,8	81,2
SGK	6,4	99,5	OSK3b	4,5	70,9
SGK2	-1,3	90,9	SRPK1	-2,4	-12,1
SGK3	-0,8	56,0	SRPK2	-5,5	-0,8
CaMK4	-4,0	6,7	AurA	85,2	100,8
CaMK1a	-3,3	60,3	AurB	12,1	96,6
CaMK2a	5,3	101,2	AurC	43,2	89,0
AMPKa1/b1/g1	13,0	97,6	IKKa	-3,9	20,0
BRSK1	26,2	98,6	IKKb	16,6	81,1
BRSK2	18,7	88,3	IKKa	4,0	96,4
CHK1	67,3	102,2	TBK1	37,8	100,5
MARK1	40,9	99,8	NEK1	-2,1	19,1
MARK2	40,7	100,7	NEK2	-2,2	6,5
MARK3	50,9	99,4	NEK6	0,2	-2,3
MELK	43,2	102,0	NEK7	-2,3	-1,8
MGC42105	1,0	20,3	PBK	62,3	98,7
Nuak1	78,7	100,8	PLK1	-2,0	58,0
PASK	-0,4	76,3	PLK2	1,8	-7,7

Kinase	% Inhibition		Kinase	% Inhibition	
	CT340	K252a		CT340	K252a
	200 nM	200 nM		200 nM	200 nM
PLK3	-10,5	-0,9	BRAF	-38,9	-55,6
TTK	12,6	93,5	BRAF(V600E)	10,7	-7,0
WNK1	7,1	-1,1	MAP4K2	38,4	98,0
IRAK1	5,7	96,9	HGK	97,9	100,4
IRAK4	9,5	98,0	PKCb2	9,4	91,1
BMPRI1A	1,8	42,1	WEE1	1,4	37,7
CK1a	-1,9	0,8	LIMK1	92,0	96,0
CK1d	0,7	7,1	LKB1	-7,0	21,4
CK1e	-2,6	1,0	PLK4	51,5	100,3
PAK2	-2,2	78,4	CK2a1	-8,3	-16,3
PAK6	5,7	94,8	PEK	5,4	12,0
MINK	94,0	101,8	PKR	5,7	28,4
MST4	-8,1	27,2	AMPKa2/b1/g1	19,3	99,5
Erk1	27,8	89,0	CaMK1d	-1,0	86,8
Erk2	40,8	94,0	CaMK2g	2,1	101,5
Erk5	6,7	67,6	CDK6	-1,2	94,0
JNK1	48,5	97,1	CDK7	-7,3	44,6
JNK2	11,7	91,9	CDK9	0,8	75,8
JNK3	18,5	94,3	CLK2	-8,5	85,8
p38a	-2,9	0,3	DDR1	41,4	97,3
p38b	-0,9	8,0	DYRK1A	0,6	96,4
p38g	13,6	65,8	DYRK1B	10,3	98,4
p38d	-0,8	23,1	DYRK3	9,7	100,5
MAP2K1	96,0	99,8	EEF2K	-5,6	-3,6
MAP2K2	93,3	99,7	HIPK1	-6,4	45,2
MAP2K3	99,4	100,1	HIPK3	0,9	60,0
MAP2K4	53,0	91,2	LOK	36,3	99,6
MAP2K5	88,5	89,2	MARK4	52,1	100,4
MAP2K6	100,0	100,0	MST1	75,3	100,2
MAP2K7	59,5	94,8	MST2	44,1	98,5
MAP3K1	43,0	1,2	MST3	-1,5	53,7
MAP3K2	51,7	97,9	NDR1	-2,4	82,0
MAP3K3	58,5	98,0	NEK9	-1,0	75,0
MAP3K4	57,5	52,2	p70S6Kb	2,2	73,9
MAP3K5	58,9	98,8	PAK1	5,7	85,3
TAK1	90,5	99,0	RSK4	50,4	99,8
COT	-3,5	10,0	SLK	18,3	97,8
MLK1	89,0	100,2	CaMK2b	-1,8	86,3
MLK2	33,6	95,8	CaMK2d	30,2	101,6
MLK3	87,7	99,0	CK1g1	-5,3	-2,5
DLK	-13,7	36,3	CK1g2	0,2	4,3
RAF1	44,4	36,9	PAK3	10,1	98,8

Kinase	% Inhibition	
	CT340	K262a
	200 nM	200 nM
PAK5	13,6	94,7
PDHK4	1,0	2,1
PDHK2	-9,3	-3,1
MRCKb	-5,8	79,2
CLK3	-8,1	-2,4
MSSK1	-2,9	54,9
CK1g3	0,7	2,2
PIK3CA/PIK3R1	11,0	44,6
TAOK2	-8,5	28,1
PIM3	32,0	96,4
Hespln	8,5	88,5
PAK4	9,9	92,8
MOS	10,4	41,4

Example 3: Efficacy Analysis of CT340 Using In Vitro Nf- κ B -Luciferase Reporter Gene Assays

[405] CT340 was tested in an in vitro cell-based assay to monitor the anti-inflammatory activity in a complex biological system. Inhibition of NF- κ B, a key regulator of the inflammatory response, was assayed using human monocytes (THP1) carrying a luciferase reporter gene driven by a tandem repeat of the NF- κ B consensus transcriptional response element. In addition, staurosporine, a broad spectrum kinase inhibitor, was used as comparator.

[406] For the setup of the assays, cell lines were stimulated for 6 or 24 h with a concentration range of 2 different stimuli: LPS and heat-killed *Listeria monocytogenes* (HKLM). Luciferase activity was measured as described below. Based on these data, for each stimulus, a concentration was chosen that resulted in a sub-optimal induction of luciferase activity.

[407] For assaying the effect of the inhibitors, cell lines were pre-cultured with the inhibitors, i.e. CT340 or staurosporine, or with a volume equivalent of DMSO. After 2 h, the pre-determined sub-optimal dose of stimuli were added, and incubation was continued for another 6 h. Luciferase activity was determined as described in the data sheet of the cell lines.

[408] To assay a potential toxic effect of the inhibitors and/or stimuli, the numbers of dead and viable cells were determined after the incubation period, by careful resuspending the cells, mixing them with trypan blue, and counting the number of blue and white cells in microscope counting chambers.

[409] THP1 cells were grown and stimulated for 24 h, as described on the data sheet of the cell line. Cells were stimulated with HKLM (0.625×10^7 cells/mL - 10×10^7 cells/mL) or LPS (0.01 - 100 ng/mL). Fold induction relative to unstimulated cells is depicted in Figure 1. Luciferase activity is dose dependently increased with both stimuli, with a maximum fold increase of 200-fold for both stimuli.

[410] A suboptimal concentration of HKLM and LPS was chosen for further experiments, being 2.5×10^7 cells/mL, and 1 ng/mL, respectively. Stimulation was furthermore confirmed when cells were stimulated for 6 h instead of 24 h.

Results of Pilot Experiment

[411] For this pilot experiment, 2 concentrations of inhibitors were tested that cover a broad range, being 100 μ M and 100 nM. As controls, cells were treated with 1% DMSO, a volume equivalent of DMSO for the cells treated with 100 pM inhibitor, or with medium only, as a control for cells treated with 100 nM inhibitor. Cells were pre-treated with inhibitor for 2 h, and subsequently stimulated with LPS or HKLM. Six or 22 h after addition of the stimuli, a culture supernatant sample was assayed for luciferase activity.

[412] Visual inspection of the cells after 8 h incubation revealed that cells treated with 100 pM staurosporine looked poor, whereas no irregularities were observed for the other conditions. After 24 h, the number of viable cells was counted. A significant increase in the percentage of dead cells was observed after addition of 100 pM staurosporine (data not shown), accompanied by an enormous decrease in the concentration of viable cells (Table 3). No significant numbers of dead cells were observed in the other conditions tested.

TABLE 3 - The relative concentration of viable THP1 cells cultured for 24 h with the inhibitors indicated

Inhibitors	Relative number of viable cells
control	100
DMSO 1%	92
100 μ M CT340	49
100 nM CT340	43
100 μ M Staurosporin	4
100 nM Staurosporin	81

[413] Luciferase activity was efficiently induced in cells stimulated for 8 or 24 h with HKLM or LPS (Table 4). Relative luciferase units are depicted in Table 5.

[414] Results obtained after 8 or 24 h are highly similar. Staurosporine results in substantial reduction of luciferase activity, in both concentrations used. In addition, pre-

incubation of cells with 100 μ M CT340 inhibits the luciferase activity, both in unstimulated, as well as in LPS or HKLM-stimulated cells.

TABLE 4 - Relative luciferase units of THP1 cells stimulated with HKLM or LPS for 8 or 24 h, relative to unstimulated cells.

Fold induction		
Stimuli	8 h	24 h
medium	1.0	1.0
HKLM	17.6	56.4
LPS	38.6	65.4

TABLE 5 - Relative luciferase units of THP1 cells treated with the inhibitors and stimuli indicated in the table

Relative luciferase units						
Inhibitors	8 h			24 h		
	Stimuli			Stimuli		
	Medium	HKLM	LPS	Medium	HKLM	LPS
control	880	15480	33990	3570	201500	233600
DMSO 1%	710	19190	25080	5090	1404330	1217830
100 μ M CT340	440	410	1070	660	16800	12860
100 nM CT340		11800	34860	3370	173860	235440
100 μ M Staurosporin	140	140	190	210	190	270
100 nM Staurosporin	820	2750	3580	1940	35720	29500

Results of Main Experiment

[415] Inhibitors were tested at 3 concentrations, being 100 μ M, 1 μ M and 10 nM. As controls, cells were treated with 1% DMSO (volume equivalent to 100 μ M inhibitor), 0.01% DMSO (volume equivalent to 1 μ M inhibitor), or with medium alone (control for cells treated with 10 nM inhibitor). Cells were pre- treated with inhibitor for 2 h, and subsequently stimulated with LPS or HKLM for 6 h.

[416] To assay potential toxic effect of the inhibitors and/or stimuli, the number of viable cells were determined after the incubation period. Addition of staurosporine (100 μ M and 1 μ M) had a consistent negative effect on the number of viable cells, whereas no consistent effects were observed for the other conditions tested (Table 6).

[417] Luciferase activity was effectively induced by both stimuli used, with an increase of 6.5- and 3.4-fold in LPS and HKLM stimulated cells, respectively. Relative luciferase units are depicted in Table 7.

TABLE 6 - Relative number of viable THP1 cells cultured for 8 h with the inhibitors and/or stimuli indicated.

Inhibitor	Relative number of viable cells		
	Medium	LPS	HKLM
medium	100	51	86
DMSO 1%	69	46	89
DMSO 0.01%	80	46	120
100 μ M CT340	40	97	123
1 μ M CT340	23	94	149
0.01 μ M CT340	71	109	120
100 μ M staurosporin	0	23	3
1 μ M staurosporin	57	20	11
0.01 μ M staurosporin	57	31	91

[418] Pre-treatment of cells with 100 μ M or 1 μ M staurosporine almost completely blocked the luciferase activity, both in non-stimulated cells, as well as in LPS or HKLM stimulated cells. Also 100 μ M CT340 efficiently reduced the luciferase activity, whereas lower concentrations (1 μ M or 0.01 μ M) did not have an effect.

TABLE 7 - Relative luciferase units of THP1 cells treated with the inhibitors and stimuli indicated in the table

Inhibitors	Relative luciferase units		
	Stimuli		
	Medium	LPS	HKLM
medium	590	3810	2030
DMSO 1%	340	1370	2210
DMSO 0.01%	370	2800	1790
100 μ M CT340	90	230	200
1 μ M CT340	320	5270	1870
0.01 μ M CT340	460	3110	1930
100 μ M staurosporin	60	100	110
1 μ M staurosporin	110	160	150
0.01 μ M staurosporin	480	6040	2140

Conclusions

[419] NF- κ B can efficiently and dose dependently be activated by LPS and heat killed *Listeria monocytogenes* (HKLM).

[420] 100 μ M and 1 μ M staurosporine appear toxic for THP1 cells, as the number of viable cells after culturing with this inhibitor is strongly and consistently reduced.

[421] No toxic effects were observed with 0.01 μ M staurosporine. This concentration however had no effect on NF- κ B activation.

[422] 100 μ M CT340 strongly and consistently blocks the activation of NF- κ B in both separate experiments performed, without consistently affecting cell viability. Therefore a specific anti-inflammatory effect of the compound in THP1 monocytes is demonstrated. This effect was not observed with 100-fold lower concentrations of the compound.

Example 4: Single Dose Toxicity and Pharmacokinetic Study in Rats
by Intravenous and Dermal Routes

[423] The toxicity and pharmacokinetic of CT340 were investigated when given by single intravenous and dermal administration to the Sprague Dawley rat.

[424] The toxicity features were investigated in 8 main groups (Groups 1-4 for intravenous dosing and Groups 5-8 for dermal dosing), each group comprising 2 male and 2 female rats. Animals were dosed at 10, 30 and 100 mg/kg for both administration routes (a dose volume of 5 ml/kg in physiological saline, for intravenous administration and Propylene glycol/DMSO/Water 10/40/50 for dermal administration). Animals of control groups (Groups 1 and 5) received the vehicle alone.

[425] *Intravenous dosing:* The test item was administered by intravenous bolus injection into the tail vein with an approximate speed of 3 ml/minute. The dose was administered to each animal at a dose volume of 5 ml/kg. Control animals received the vehicle alone at the same dose volume.

[426] *Dermal dosing:* The day before scheduled dosing the fur was removed from the dorsal surfaces of the trunk over an estimated area of at least 10% of the total body surface. Care was taken to avoid any damage or abrasion to the skin. The clipping procedure was repeated as necessary during the course of the study.

[427] The test item was applied uniformly over an area of approximately 10% of the total body surface area (approximately 5x7 cm). The dose was administered to each animal at a dose volume of 5 ml/kg. A patch of surgical gauze covered by a strip of synthetic film was placed over the treated site and the whole assembly held in place by encircling the trunk of the animal with a length of adhesive bandage, this forming a semi-occlusive barrier. The amount administered to individual animals was determined by their body weight, measured prior to dosing.

[428] After a period of 6 hours, the tape dressing was removed. The treated skin site was then gently washed free of any remaining test item using warm water.

[429] *Duration of treatment:* All animals were dosed once only. Main group animals were sacrificed on Day 8, after an observation period of 7 days. Satellite group animals were killed after the completion of bleeding procedures.

[430] *Pharmacokinetic results:* After single intravenous administration, animals were largely exposed to the drug. The AUCs [AUC_(0-t_{last})] were calculated as follows: -16715.4, 46918.2 and 149764 ng*h/ml in males for 10, 30 and 100 mg/kg respectively; -13027.7, 38084.9 and 142028 ng*h/ml in females for 10, 30 and 100 mg/kg respectively. AUCs increased proportionally with the dose and no major difference was observed between sexes.

[431] After single dermal administration, the amount of absorption was limited. The small amount found may be due to the low extent of absorption or high rate of elimination.

[432] Based on the above reported results, the maximum tolerated dose is then considered to be greater than 100 mg/kg for both dermal and intravenous administrations.

Results

[433] No clinical signs were observed after dosing and during the following observation period.

[434] No relevant body weight changes were noted during the study. At the end of the observation period, the body weight was within the ranges expected for this strain and age.

[435] Food consumption was unaffected by treatment.

[436] Terminal body weight of treated animals was unaffected by treatment. No toxicologically relevant variations of organ weights were recorded.

Toxicokinetics

[437] *Intravenous dosing:* The drug concentrations in plasma following single intravenous administration were determined up to 2 hours after dosing. Most of the values were obtained after dilution of plasma samples.

[438] The kinetic parameter values for the test item obtained after single intravenous administration (using mean plasma concentrations) are shown in Table 8, while graphs are presented in Figure 2.

TABLE 8 - Day 1 - Kinetic parameter values for CT340 (calculated on the mean; n=3)

Dose mg/kg	Gender & Group	AUC _(0-t_{last}) ng*h/ml	AUC _(0-t_{last}) /dose	t _{1/2} (h)
10	9M	16715.4	1672	0.36
30	10M	46918.2	1564	0.43
100	11M	149764	1498	0.39
10	9F	13027.7	1303	0.34
30	10F	38084.9	1269	0.34
100	11F	142028	1420	0.33

[439] The drug concentration-time profiles following single intravenous administration declined probably with a biphasic kinetic. Moreover, at the last time point observed, the

plasma mean concentrations were higher than the lower limit of quantification, at all dose levels:

- 10 mg/kg: approximately 138 and 88 fold in males and females respectively
- 30 mg/kg: approximately 434 and 282 fold in males and females respectively
- 100 mg/kg: approximately 1354 and 842 fold in males and females respectively

[440] With reference to the observed AUC [AUC_(0-tlast)], they increased proportionally with the dose and no major difference was observed between sexes.

[441] *Dermal dosing:* CT340 plasma levels were investigated up to 24 hours after dosing. Concentrations were over the limit of quantification in the following samples:

- 10 mg/kg: 4/21 samples in males and 10/21 samples in females
- 30 mg/kg: 8/21 samples in males and 11/21 samples in females
- 100 mg/kg: 19/21 samples in males and 16/21 samples in females

[442] The erratic absorption, as evidenced from the high variability, is likely to be due to the administration route.

[443] The amount of drug absorbed was limited. In fact, values observed were near the lower limit of quantification, as follows:

- 10 mg/kg: approximately 2 to 4 fold in males and 1 to 91 fold in females
- 30 mg/kg: approximately 1 to 11 fold in males and 1 to 48 fold in females
- 100 mg/kg: approximately 1 to 114 fold in males and 1 to 62 fold in females

[444] Due to the low amount of drug present in plasma, the kinetic data of the drug was not evaluated for the dermal route of administration.

Conclusions

[445] The toxicity of CT340 when given by single intravenous or dermal administration to rats at the dose levels of 10, 30 and 100 mg/kg, was investigated over a period of 7 days after dosing.

[446] No toxicologically relevant changes were observed during the in life phase of the study in the treatment groups when compared to the respective control groups. This applied both to intravenous and dermal routes of administration.

[447] Animals were largely exposed to the drug after intravenous dosing. The AUC_(0-tlast) increased proportionally with the dose and no major difference was observed between sexes.

[448] After single dermal administration, the amount of drug absorption was limited.

[449] The maximum tolerated dose is then considered to be greater than 100 mg/kg for both dermal and intravenous administrations.

Example 5: CT340 2 WEEK INTRA VENOUS TOXICITY STUDY IN RATS FOLLOWED BY A 2 WEEK RECOVERY PERIOD

Summary

[450] The toxicity of CT340 was investigated, when given by daily intravenous administration to the Sprague Dawley rat for a 2-week period followed by a 2-week recovery period.

[451] The toxicity was investigated in 4 groups (Groups 1-4), each group comprising 10 male and 10 female rats. Five additional animals/sex were included in control and high dose groups, for evaluation of recovery.

[452] The test item was dissolved in physiological saline (NaCl 0.9%) and treatment groups were dosed at 10, 30 and 100 mg/kg/day at the dose volume of 5 ml/kg and a speed of 3 ml/minute. The control group received the vehicle alone.

[453] Four satellite groups were additionally dosed at the same dose levels, for evaluation of toxicokinetics.

[454] The test item was administered by intravenous bolus injection into the tail vein with an approximate speed of 3 ml/minute. The dose was administered to each animal at a dose volume of 5 ml/kg. Control animals received the vehicle alone at the same dose volume. The dose was administered to each animal on the basis of the most recently recorded body weight and the volume administered was recorded for each animal.

[455] All animals were dosed once a day, 7 days a week, for a minimum of 2 consecutive weeks. Animals were dosed up until the day before necropsy, except for recovery animals which were not given any treatment during the recovery period.

[456] On Day 1 and in Week 2 of the study, blood samples were withdrawn from three animals/time point at the following time points: pre-dose, 5, 15, 45, 90 minutes and 24 hours after dosing.

[457] At the dose level of 10 mg/kg/day the severity of the changes observed was not sufficient to represent an adverse effect.

[458] Complete recovery occurred in animal treated at 100 mg/kg/day, after a 14-day recovery period.

[459] Based on the above results, it can be concluded that the No Observed Adverse Effect Level (NOAEL) can be defined to be 10 mg/kg body weight/day for both sexes.

[460] Finally, no cytogenetic effects were noted in the animals of the high group when compared to controls.

Results

[461] No mortality occurred during the study in the main groups of the study. Two death events in the satellite groups occurred after bleeding procedure on Day 2 (24 h) and were not dose-related. Since no mortality occurred in the main groups and clinical signs were limited during the whole treatment period, the deaths may be correlated to the bleeding procedure. Thus, the observed mortality was considered incidental.

[462] No statistically significant reductions of group mean body weight were recorded in the animals of treatment groups when compared to controls (Figures 3 and 4).

[463] Before the start of treatment, animals showing no ocular abnormality at the ophthalmoscopy were selected for the study. Both eyes of all live animals from the main groups were re-examined during the last week of the study and no significant findings were detected.

[464] For the dosing phase animals, no differences were recorded in terminal body weight of animals recorded at the end of treatment with the test item when compared to controls. For the recovery phase animals, no differences were recorded in terminal body weight of animals treated with the test item when compared to controls.

Kinetic behavior of CT340

[465] *Kinetic parameter calculation - Day 1:* The kinetic parameter values for the test item obtained after single intravenous administration (using mean plasma concentrations) are shown in Table 9, while graphs are presented in Figure 5.

TABLE 9 - Kinetic parameter values for CT340 (calculated on the mean; n=3)

Dose	Gender & Group	AUC (0-24h)	AUC (0-24h)	AUC (0-24h)/dose	$t_{1/2}$	MRT	λ_z	CL	V_D
mg/kg		ng*h/ml	ng*h/ml		(h)	(h)	(1/h)	ml/h/kg	ml/kg
10	6M	26039.7	26143.4	2604	3.54	1.08	0.195872	382.51	1953
30	7M	71548.6	71756	2385	3.24	0.97	0.207626	418.08	2014
100	8M	187060	187650	1877	3.24	1.04	0.213442	532.91	2497
10	6F	18365.5	18569.3	1837	4.14	1.53	0.167368	538.52	3218
30	7F	48273.5	48738.3	1609	4.13	1.39	0.167694	615.53	3706
100	8F	158048	158614	1580	3.41	1.01	0.20322	630.46	3102

[466] The drug concentration-time profiles following single intravenous administration appeared to decline with a biphasic kinetic (Figure 5).

[467] At the last time point observed, the plasma mean concentrations were slightly higher than the lower limit of quantification at all dose levels (approximately 5 to 28 fold in males and 8 to 26 fold in females). Moreover, the extrapolation was automatically fitted from 1.5 h (which means only with two points). For these reasons, the half-lives, slope (λ_z) and volume of distribution (V_D) reported in Table 9 could be considered only as an estimation and MRT (mean residence time) is reported as estimation of effective permanence in the body.

[468] The values of clearance, half-life and volume of distribution suggest that the drug is widely distributed and promptly eliminated from the body. The $AUC_{(0-\infty)}$, slightly decreased with dose and the clearance (CL) slightly increased with dose indicating that in the range of the doses studied the kinetic of the drug may be not linear. No major difference was observed between sexes.

[469] *Kinetic parameter calculation - Week 2:* The kinetic parameter values for the test item obtained after repeated intravenous administration (using mean plasma concentrations) are shown in Table 10, while graphs are presented in Figure 6.

TABLE 10-Week 2-Kinetic parameter values for CT340 (calculated on the mean; n=3)

Dose mg/kg	Gender & Group	AUC _(0-last) ng*h/ml	AUC _{(0-inf) Day1/ AUC_{(0-last) Week 2}}
10	6M	25259.8	1.03
30	7M	52637.8	1.36
100	8M	275350	0.68
10	6F	19931.6	0.93
30	7F	53305.2	0.91
100	8F	217466	0.73

[470] AUC calculation with trapezoidal rule was considered to be a good estimation.

[471] The ratio between the AUC_(0-inf) of Day 1 and AUC_(0-last) of Week 2 was calculated as the 24 hour ($t_{1/2}$) the interval (τ) between administrations. The ratio gave a qualitative indication that drug accumulation might occur at the high dose in both sexes. Being the AUCs of each dose/sex calculated with a single mean value for each bleeding time, statistical tests were not applicable to confirm this issue.

Discussion and Conclusions

[472] The toxicity of CT340 was investigated, when given at the dose levels of 10, 30 and 100 mg/kg/day by daily intravenous administration to the Sprague Dawley rat for a 2-week period followed by a 2-week recovery period.

GENERAL TOXICITY

[473] No statistically significant reductions of group mean body weight were recorded in the animals of treatment groups of the dosing phase when compared to controls.

[474] During the recovery period, no mortality or toxicologically relevant clinical signs were recorded and the body weight was within the expected range for this strain and age of animals. Variations observed in the food consumption during recovery period were not considered toxicologically relevant.

[475] Any changes observed in haematology, clinical chemistry and urinalysis parameter at the end of treatment were no longer observed at the end of recovery period.

[476] No treatment-related changes were noted at *post mortem* macroscopic and microscopic examination.

[477] The above finding suggested a complete recovery of the treatment-related changes seen at the end of the main phase of the study.

GENETIC TOXICITY

[478] Following treatment with the test item, no increase in the incidence of micronucleated PCEs over the vehicle control group was observed in CT340 treated group.

[479] Analysis of the ratio of mature to immature erythrocytes and the proportion of immature erythrocytes among total erythrocytes showed that the test item did not induce inhibitory effects on erythropoietic cell division.

[480] No statistically significant heterogeneity in response in any group. No statistically significant increase in the incidence of micronucleated PCEs was observed over the vehicle control group.

KINETIC BEHAVIOR

[481] The values of clearance, half-life and volume of distribution suggest that the drug is widely distributed and promptly eliminated from the body. The AUC (C_{0-in_0}), slightly decreased with dose and the Clearance slightly increased with dose, indicating that in the range of the doses studied, the kinetics of the drug may be not linear. No major difference was observed between sexes.

CONCLUSIONS

[482] At the dose level of 10 mg/kg/day, treatment-related and dose-related changes were not sufficient to represent an adverse effect.

[483] Complete recovery occurred in animal treated at 100 mg/kg/day, after a 14-day recovery period.

[484] Based on the above results, it can be concluded that the No Observed Adverse Effect Level (NOAEL) can be defined to be 10 mg/kg body weight/day for both sexes.

[485] Finally, no cytogenetic effects were noted in the animals of the high group when compared to controls.

Example 6: CT340 Single Dose Dermal Tolerance/Toxicity and Pharmacokinetic Study in Minipigs

Summary

[486] The pharmacokinetics, local dermal tolerance and toxicity of CT340 after a single dermal administration (6 hour exposure period) were investigated in the minipig during an observation period of 7 days in order to select dose levels for subsequent studies.

[487] Three groups, each of 2 male and 2 female minipigs (Groups 2, 3 and 4) were treated by dermal application of 0.5 mL/kg of the test item at the dose levels of 10, 30 and 100 mg/kg over an area of the dorsal skin of approximately 25 x 20 cm, previously clipped.

[488] The test item was applied evenly over the prepared skin of animals of the relevant group (Groups 2, 3 and 4) at a dose volume of 0.5 mL/kg. The required amount of formulation was spread evenly over the skin of the prepared site by gentle massage over an area of approximately 25x20 cm. Control animals (Group 1) received the vehicle alone at the

same dose volume, in the same manner. The treated skin site was covered using a patch of surgical gauze and the whole assembly held in position by encircling the trunk of the animal with a cotton jacket. This semi-occlusive dressing was removed approximately 6 hours later and the treated site was cleaned by washing with a piece of surgical gauze soaked with warm water, removing any residual test item.

[489] All animals were treated once only, followed by an observation period of 7 days.

[490] Plasma levels of CT340 were slightly >LLOQ (lower limit of quantitation = 4.5 ng/mL) only in 1 male and 1 female receiving 30 mg/kg and in 2 males receiving 100 mg/kg. These values were reported between 1 and 24 hour post-dose. No measurable plasma levels were found in male animals dosed at 10 mg/kg and in female animals dosed at 10 and 100 mg/kg. Due to the minimal absorption observed, in the majority of cases the calculation of the exposure (AUC) and of other kinetic parameters was not possible.

[491] A $C_{\max}$ of 8.74 ng/mL was measured in the single male of Group 3, while a $C_{\max}$ of 9.85 ng/mL was measured in the single female of the same group with a $T_{\max}$ of 2 and 1 hour in the male and female animals, respectively.

[492] Mean $C_{\max}$ was 7.735 ng/mL in the males of Group 4, with a $T_{\max}$ of 2 hours. $AUC_{0-\infty}$, calculable for 1 male dosed at 100 mg/kg, was 538.918 ng-h/mL.

[493] The results indicate that the test item CT340 is locally well tolerated after a dermal application at concentrations of 20, 60 and 200 mg/mL over the intact skin of the minipig.

[494] No signs of potential treatment-related adverse effects of the test item were observed at any of the dose levels investigated (10, 30 and 100 mg/kg/day).

[495] Absorption through the dermal route was minimal only at the mid and high dose levels (30 and 100 mg/kg/day). It appeared to be slightly higher in the males.

[496] On the basis of these results, the maximum tolerated dose in this study may be considered to be greater than 100 mg/kg for a single dermal administration in minipigs.

[497] The high dose level of 100 mg/kg at a concentration of 200 mg/mL may be considered as the high dose level to be selected for a subsequent repeated dose study.

Results

[498] No clinical signs were observed during the study.

[499] No signs of irritation were observed at treated sites in any animal during the 7 day observation period.

[500] Body weights were within the expected range for this strain and age of animals (Figures 7 and 8).

[501] Food consumption was not affected by treatment.

[502] No treatment-related signs were observed at clinical examination performed at the end of treatment.

[503] There were no treatment-related changes observed in the weight of the organs.

[504] No changes were observed in treated and untreated skin sites of control and treated animals during macroscopic observations. Animals killed at termination did not show macroscopic findings related to the administration of the test item.

Toxicokinetic analysis

[505] Plasma samples were obtained from male and female minipigs before dosing (pre-dose) and 0.5, 1, 2, 4, 6, 8 and 24 hours after the start of dosing on Day 1 of the study, following a single dermal administration of CT340 at the dose levels of 10, 30 and 100 mg/kg/day.

[506] Plasma levels of CT340 were slightly >LLOQ (lower limit of quantitation = 4.5 ng/mL) only in 1 male and 1 female receiving 30 mg/kg and in 2 males receiving 100 mg/kg/day. These values were reported between 1 and 24 hour post-dose. No measurable plasma levels were found in male animals dosed at 10 mg/kg and in female animals dosed at 10 and 100 mg/kg.

[507] Due to the minimal absorption observed, in the majority of cases the calculation of the exposure (AUC) and of other kinetic parameters was not possible. Only when more than 2 consecutive time points values were available some parameters were calculable.

[508] A C_{max} of 8.74 ng/mL was measured in the single male of Group 3, while a C_{max} of 9.85 ng/mL was measured in the single female of the same group. T_{max} were of 2 and 1 hour in the male and female animals, respectively.

[509] Mean C_{max} was 7.735 ng/mL in the males of Group 4, with a T_{max} of 2 hours. $AUC_{0-\infty}$, calculable for 1 male dosed at 100 mg/kg, was 538.918 ng-h/mL.

[510] No detectable levels were measured for animals treated with the vehicle alone. Absorption appeared to be slightly higher in males.

Conclusions

[511] The pharmacokinetics, local dermal tolerance and toxicity of CT340 after a single dermal administration (6 hour exposure period) at the dosages of 10, 30 and 100 mg (concentrations: 20, 60 and 200 mg/mL) were investigated in the minipig during an observation period of 7 days in order to select dose levels for subsequent studies.

[512] There were no clinical signs or other findings of toxicological importance noted in any animals during the study. In addition, no irritation of the treated skin site was observed during the study.

[513] Minimal absorption through the dermal route was detected at the mid and high dose levels (30 and 100 mg/kg). Absorption appeared to be slightly higher in the males.

[514] The above results indicate that the test item CT340, was locally and systemically well tolerated following a single dermal application to the minipig at all the dosages tested.

[515] Therefore, the maximum tolerated dose in this study may be considered to be greater than 100 mg/kg for a single dermal administration in minipigs. The high dose level of 100

mg/kg at a concentration of 200 mg/mL may be considered as the high dose level to be selected for a subsequent repeated dose study.

Example 7: CT340 2 Week Dermal Tolerance/Toxicity Study in Minipigs Followed By A 2 Week Recovery Period

[516] The local tolerance, potential systemic effect and the toxicokinetic profile of the test item, CT340, after repeated daily dermal administration were investigated in the minipig during a period of at least 14 consecutive days and recovery from any treatment-related effects during a recovery period of 2 weeks.

[517] Three groups each of 3 male and 3 female minipigs (Groups 2, 3 and 4) were treated by dermal application of 5.5 g of the test item formulations at concentrations of 20, 60 and 200 mg/mL (to achieve dose levels of 110, 330 and 1100 mg of active ingredient/animal/day, corresponding to 0.22, 0.66 and 2.2 mg/cm²/day and to approximately 7, 21 and 69 mg of active ingredient/kg/day) over an area of the dorsal skin of approximately 25 x 20 cm, previously clipped. A fourth similarly constituted group (Group 1) received the vehicle alone (Propylene glycol 20%, DMSO 20%, Benzyl alcohol 1%, Purified water) and acted as a control. One additional animal per sex was allocated to Groups 1 and 4 for 2 weeks of recovery.

[518] The test item formulations were applied evenly over the prepared skin of animals of the relevant group. A 5.5 g aliquot of each test item formulation was administered and spread evenly over the skin of the prepared site by gentle massage, over an area of approximately 20 x 25 cm in animals of the relevant group. Control animals (Group 1) received the vehicle alone (control item) at the same dose volume, in the same manner.

[519] The treated skin site was covered using a patch of surgical gauze and the whole assembly held in position by encircling the trunk of the animal with a cotton jacket. This semi-occlusive dressing was removed approximately 6 hours later and the treated site was cleaned by washing with a piece surgical gauze soaked with warm water, removing any residual substance.

[520] All animals of the main phase were dosed once a day, for a minimum of 14 consecutive days, up until the day before necropsy. No treatment was given to recovery animals during the recovery period.

[521] No plasma levels of CT340 greater than the LLOQ (lower limit of quantitation = 4.5 ng/mL) were detected for animals treated with the test item at concentrations of 20, 60 and 200 mg/mL on Day 1 or Day 14.

[522] No signs of potential treatment-related effects of the test item were observed at any of the dose levels investigated (approximately 110, 330 and 1100 mg/animal/day).

[523] On the basis of the above results, CT340 was considered reasonably tolerated at the application site and not systemically toxic in the minipig following a repeated dermal

(epicutaneous) administration over a period of at least 14 consecutive days, when administered at concentrations of 20, 60 and 200 mg/ml_ (corresponding to dose levels of approximately 110, 330 and 1100 mg/animal/day).

[524] No absorption through the dermal (epicutaneous) route was observed.

Results

[525] No treatment-related clinical signs were observed during the study.

[526] There was no effect of treatment on body weight. Body weights recorded during the treatment and recovery periods and terminal body weight were within the expected range for this strain and age of animals. No differences were observed among the groups (Figures 9 and 10).

[527] Food consumption was not affected by treatment.

[528] Before the start of treatment, animals showing no ocular abnormality at the ophthalmoscopy were selected for the study. Both eyes of all animals from each group and sex were re-examined during Week 2 of treatment. No findings were detected during the ophthalmoscopic examinations performed during the study.

[529] With regards to hematology analyses, no changes of toxicological significance were observed. No changes were recorded for coagulation parameters

[530] With regards to clinical chemistry analyses, no changes of toxicological relevance were observed.

[531] No changes of toxicological relevance were observed with regards to urine and feces analyses.

[532] No plasma levels of CT340 greater than the LLOQ (lower limit of quantitation = 4.5 ng/mL) were detected following treatment with the test item at concentrations of 20, 60 and 200 mg/ml_ on Day 1 or Day 14.

[533] There were no changes in organ weights which were considered to be treatment-related.

[534] No treatment-related macroscopic changes were noted in final sacrifice animals.

[535] All observed changes are suggested to be incidental, having a comparable incidence in control and treated groups, and/or characteristically seen in untreated Gottingen minipigs of the same age in our laboratory.

[536] No treatment-related macroscopic changes were noted in recovery sacrifice animals.

[537] No treatment-related changes were noted during microscopic observations.

[538] All observed changes are suggested to be incidental, having a comparable incidence in control and treated groups, and/or characteristically seen in untreated Gottingen minipigs of the same age in our laboratory.

Conclusions

[539] The local tolerance, potential systemic effect and the toxicokinetic profile of the test item, CT340, after repeated daily dermal administration were investigated in the minipig during a period of at least 14 consecutive days and recovery from any treatment-related effects during a recovery period of 2 weeks.

[540] No treatment-related clinical signs or other changes indicating a systemic effect of the test item were observed in any animal during the treatment or recovery periods.

[541] Microscopic examination carried out at the end of treatment did not show any treatment-related findings.

[542] No absorption through the dermal route was observed.

[543] On the basis of the above results, CT340 was considered reasonably tolerated at the application site and not systemically toxic in the minipig following a repeated dermal (epicutaneous) administration over a period of at least 14 consecutive days, when administered at concentrations of 20, 60 and 200 mg/ml (corresponding to dose levels of 110, 330 and 1100 mg of active ingredient/animal/day, i.e. 0.22, 0.66 and 2.2 mg/cm²/day and approximately 7, 21 and 69 mg of active ingredient/kg/day).

Example 8: Effects of CT340 in a Model of Hypertrophic Scar Formation in NZW

Rabbits

[544] The objective of this study was to assess the effect of CT340 on hypertrophic scar formation in NZW rabbits. Four wounds were surgically created in each ear, scars were allowed to develop, and the effects of test article on hypertrophic scars were determined by photomicroscopy and histopathology.

[545] Surgical wounds were induced bilaterally as four circular lesions per ear down to the bare cartilage on the ventral side of each ear. Treatment started on Day 26, after confirming the complete formation of hypertrophic scars. Animals were dosed via intra-lesional injections into each scar or by topical application with the test article, CT340. Dosing was repeated once weekly for a total of three doses. Test article efficacy in scar reduction was assessed using the Scar Elevation Index (SEI) and by histopathology.

[546] The SEI of scars dosed with intra-lesional injection or by topical application of CT340 did not differ significantly from scars dosed with vehicle. Scars dosed with the positive control article (TACA) showed a significant decrease in SEI when compared to scars injected with CT340 or with saline ($P < 0.005$).

[547] Scars injected with vehicle or CT340 exhibited moderate inflammation. Dosing scars with intra-lesion injection of TACA significantly decreased inflammation.

[548] Daily topical dosing of scars with CT340 significantly decreased the level of scar inflammation. This decrease was significantly lower when compared to TACA-injected scars.

[549] In summary, while there was no difference between vehicle and CT340 in the reduction of SEI in this study, daily topical dosing of scars with CT340 reduced the level of inflammation.

Study Design

[550] The study consisted of four groups of six female NZW rabbits each as shown in Table 11 below.

TABLE 11 - Summary of Study Design

Group	Animal No. (female)	Day 0	Days 0 - 26	Days 26 – 43		Other Procedures		
		Wound Induction	Procedure	Treatment			Route of Dosing	
				Left	Right			
1	151-156	Surgical wound induction (4/ear, bilateral, 7 mm in diameter, removal of perichondrium)	Dressing application and change (1x/week)	Saline	Vehicle 2	Left ear: Intra-lesional, q1w Right ear: Topical, QD	Days 0 – 43 <ul style="list-style-type: none">• Body weight: q1w• Photography: q1w• Clinical observations: QD Day 43 (terminal) <ul style="list-style-type: none">• Sacrifice• Photograph, collect, and fix ears Histology <ul style="list-style-type: none">• Section, stain with H&E, trichrome• Measure lesions (SEI)	
2	251-256			Injectable formulation of CT340(200mg/mL)		Intra-lesional injection (10 µL/scar, on Days 26, 33, and 40)		
3	351-356			Topical formulation of CT340 (5% semi-solid)		Topical: QD (10µL/scar) from Day 26 to Day42		
4	451-456			Triamcinolone 40 mg/mL (TACA)		Intra-lesional injection (10 µL/scar, on Days 26, 33, and 40)		

[551] On Day 0, surgical wounds were induced bilaterally (both ears) as four circular lesions per ear (i.e., 8/animal), down to the bare cartilage on the ventral side of each ear, using a 7-mm dermal biopsy punch, with removal of perichondrium. The wounds were dressed using polyurethane dressing (e.g., Tegaderm); dressing was changed once weekly for twenty-six (26) days.

[552] On Day 26, animals were assigned to study groups of six rabbits each. Animals of Groups 1 (right ear only) and 3 were dosed via topical application. Animals of Groups 1 (left ear only), 2, and 4 were dosed via intra-lesional injection into each scar (10 µL/scar). Animals of Groups 1 - 4 (respectively) were dosed with test and control article into both ears with vehicle; CT340 at 200 mg/mL; CT340 at 5%; or TACA at 40 mg/mL. For Groups 1 (left ear), 2, and 4, dosing was repeated once weekly for a total of 3 doses; Groups 1 (right ear) and 3 were dosed daily for 17 days. Throughout the in-life, the wounds were photographed once weekly; clinical observations were recorded at least once daily; and body weights were recorded once weekly.

[553] On Day 43 animals were sacrificed and weighed. Both ears of each animal were collected, photographed, and fixed in 10% neutral buffered formalin (NBF). Fixed lesions were processed for histopathology, including staining with hematoxylin and eosin (H&E) and

Masson's trichrome. Slides were evaluated by a board-certified veterinary pathologist, including lesion measurement using a scar elevation index (SEI).

Materials and Methods

[554] The test article (CT340) was provided to the laboratory in a powder form and stored frozen at -80 °C pending use. The first control article was the vehicle used to formulate the topical formulation of CT340. The control article consisted of 25% Transcutol (diethylene glycol monoethyl ether) and 75% 1,2-propanediol. The vehicle was formulated from components Transcutol P (Gattefosse Catalog 3260JV1) and 1,2-propanediol (Fluka Catalog 82281). The second control article was triamcinolone acetonide (TACA). This control article is a synthetic glucocorticoid corticosteroid with anti-inflammatory action. The third control article was sterile saline, intended to be identical to the vehicle used to formulate the injectable formulation of CT340.

Hypertrophic Scar Formation Model

[555] The model used in this study was based on that of Morris et al. (1997; *Plast. Reconstr. Surg.* 100: 674-81) and Kloeters et al. (2007; *Wound Rep. Reg.* 15: S40 - S45). Wounds were induced bilaterally (on both ears) on the respective Day 0 (2 or 3 February 2011) as follows.

[556] Prior to surgery, animals were anesthetized isoflurane inhalation per Testing Facility SOP.

[557] Four wounds were created down to the bare cartilage on the ventral side of each ear using a 7-mm dermal biopsy punch. The depth of wound was a factor; shallower lesions do not afford the proper degree of scarring.

[558] The cartilage was meticulously nicked without full dissection, as the latter results in noninterpretable histology.

[559] Epidermis, dermis, and perichondrium were thoroughly removed using a dissecting or surgical microscope. Complete removal of the perichondrial layer was mandatory.

[560] Any bleeding was treated by manual compression; electro-cauterization was not needed.

[561] At the end of surgery, a liquid adhesive (e.g., Mastisol; Ferndale Laboratories, Inc., Ferndale, MI) was applied to the surrounding skin followed by wound coverage with a polyurethane dressing (Tegaderm; 3M Health Care, St. Paul, MN). The polyurethane dressing remained attached at all times to ensure a moist wound environment.

[562] The dressing was changed (at least) once weekly through Day 26. Wounds were examined every day for signs of infection as well as for epithelialization progress on gross examination. By the time treatment was initiated (Day 26), scar formation was prominent.

[563] Following group assignment, animals of Group 1 served as negative controls and were dosed with vehicle as follows: the left ears were dosed with Control Article 3 (saline)

via intra-lesional injection, and the right ears were dosed topically with Control Article 1 (25% Transcutol:75% 1,2-propanediol). Dosing of the left ears was repeated once weekly for a total of 3 doses; dosing of the right ears was repeated once daily for a total of 17 doses. Animals of Groups 2 and 4 were dosed via intra-lesional injection of each scar (10 pL/scar); dosing was repeated once weekly for a total of 3 doses. Animals of Group 3 were dosed topically; dosing was repeated once daily for a total of 17 doses. Injections were administered as 10 pL/wound of test or control article using a Hamilton syringe with 27-gauge needles; topical application was performed by applying 10 pL using a micropipette.

Histopathology

Scar Formation

[564] The NBF-fixed tissues were processed for histopathologic evaluation. Tissues were dehydrated, embedded in paraffin, sectioned at 3- to 5-µm thicknesses, and stained with H&E and with Masson's trichrome. Slides were evaluated via light microscopy by a board-certified veterinary pathologist. Ear lesions (8/animal) were assessed using the scar elevation index (SEI) described by Morris et al. (1997). In brief, the SEI measures the ratio of total scar connective tissue area to the area of underlying dermis.

[565] The scar area was measured in 1 or 2 sections per scar. Measurements were made digitally. A computerized visual imaging system was used to photograph the areas of interest using an Olympus (Tokyo, Japan) camera, with the image digitized by using MicroSuite Basic Edition Software (Tokyo, Japan). For a cross-section of each scar, measurements of the Maximum Scar Elevation (MSE), the external perimeter of the newly formed hypertrophied dermis (P-h), and the perimeter of the underlying dermis (P-d) were collected. The software automatically calculates the area bounded by each perimeter; A-h, and A-d, respectively. The SEI was calculated according to the following formula:

$$\text{SEI} = \text{A-h} + \text{A-d} / \text{A-d}$$

[566] When two sections were analyzed, the SEI was calculated from the section with the highest MSE.

Scar Inflammation

[567] Approximately 2 levels from each ear wound were examined histopathologically for each animal. The incidence and severity of scar inflammation were scored using the accepted industry 5-point scoring system, as shown in Table 11a.

TABLE 11a

Score	Class	Description
0	Normal	No inflammation.
1	Trace/minimal	Barely detectable to a low number of scattered neutrophils.
2	Mild	Low numbers of scattered neutrophils present in the tissue.
3	Moderate	Increased numbers of cells, often forming distinct foci or accumulations of neutrophils.
4	Severe	Extensive areas densely infiltrated with large numbers of neutrophils.

Results

Acclimation

[568] There were no clinical signs of abnormality during the acclimation period. All animals were released for use in the study at the end of the acclimation period.

Mortality

[569] There was one unexpected death: Rabbit 56 died 11 minutes after the surgery.

Clinical Observations

[570] Clinical observations were recorded daily. During the in-life period, none of the wounds showed any sign of infection. Scars were fully formed by Day 26, when dosing was initiated.

Body Weight

[571] Group mean normalized (to pre-dose) body weight values are plotted in Figure 11. All rabbits gained weight during the course of the study; adverse effects were not apparent following the surgery or treatments.

[572] Starting on Day 33, and until the end of the in-life period, animals dosed with TACA gained significantly lower ($P < 0.01$) body weight when compared to the vehicle or test article treated animals. The lower rate of increase in body weight is a known side effect of TACA in rodents and rabbits.

Histopathology

Scar Formation

[573] Scar formation results are summarized in Table 12 and in Figures 12 through 20. The perimeters of 188 scars were measured, and the SEI of each scar was calculated. Quantitative analysis of the scar area demonstrated decreases in SEI in TACA-treated scars (Group 4) when compared to the vehicle- and CT340-treated scars (1.41 ± 0.11 vs. 1.82 ± 0.25 and 1.73 ± 0.16 for the left ears of Group 1 and Group 2, respectively). Treating scars with topical application of CT340 had no effect on the scar area when compared to the vehicle-treated scars (1.62 ± 0.18 vs. 1.69 ± 0.18 for Group 3 vs. the right ears of Group 1, respectively). Treating scars by topical application or intra-lesional injection of CT340 had no effect on the scar area (1.73 ± 0.16 vs. 1.62 ± 0.18 for Groups 2 and 3, respectively).

[574] Statistical analysis using the Student's t-test was performed to compare the TACA-injected scars to CT340- and saline-injected scars. The differences in the SEI values were significant ($P < 0.005$); no significant differences were found when comparing the SEI of CT340-injected scars to that of saline-injected scars.

TABLE 12 - Summary of Scar Elevation Index (SEI), Groups 1 - 4

Group No.	Left Ear Scars	
	Mean	StdDev.
1-Left Ears	1.82	0.25
1-Right Ears	1.69	0.18
2	1.73	0.16
3	1.62	0.16
4	1.41	0.11

Histopathology

[575] Inflammation was composed of a variable inflammatory response consisting of an admixture of mononuclear cells, lymphoid cells, macrophages, and neutrophils in the wound site. Fibrosis and granulation tissues also were present. All wounds were morphologically similar, but somewhat differentiated based upon severity of the lesion, size of the scar, and presence of inflammation. There were clear differences in the severity of the inflammation between groups. Group 1 left (saline) and right (vehicle 2) ears did not differ with regard to inflammation, and there was marked inflammation at the lesion sites in both groups. In Group 4 (treated with TACA), there was very little inflammation, consistent with the expected activity of TACA in this model. While intra-lesion injection of CT340 had little effect on inflammation, there was marked reduction in inflammation in scars treated daily by topical application of CT340, similar to that seen with TACA. This observation suggests that topical application of 5% CT340 has marked anti-inflammatory effects under the conditions of this study.

Scar Inflammation

[576] Scar inflammation scoring is presented in Table 13 and plotted in Figures 21 and 22. Vehicle-treated scars (topical application or intra-lesion injection) exhibited moderate inflammation, with mean scores of 2.94 and 3.36 (respectively). Intra-lesion injection of scars with TACA, the positive control article, reduced the level of scar inflammation. Scars treated with TACA displayed significant ($P < 0.003$) decreases in inflammation scores when compared to saline- and CT340-injected scars (scores of 1.46 ± 0.22 , 2.94 ± 0.73 and 3.02 ± 0.34 , respectively).

[577] Daily topical dosing of scars with CT340 reduced the level of scar inflammation. Scars treated with topical CT340 displayed significant ($P < 0.001$) decreases in inflammation scores when compared to topical application of vehicle (right ears of Group 1) (scores of 2.03 ± 0.49 and 3.35 ± 1.44 , respectively). The inflammation scores of scars topically treated with CT340 were compared to intra-lesion injection of scars with TACA. Scars treated with TACA displayed significant ($P < 0.05$) decreases in inflammation scores when compared to scars treated by daily topical application of CT340 (scores of 1.46 ± 0.22 and 2.03 ± 0.49 , respectively).

TABLE 13 - Summary of Scar Inflammation Scores, Groups 1 - 7

Group No.	Left Ear Scars	
	Mean	StdDev.
1-Left	3.94	0.73
1-Right	3.35	1.44
2	3.02	0.34
3	2.03	0.49
4	1.46	0.22

Discussion and Conclusions

[578] The SEI of scars dosed with intra-lesional injection or by topical application of CT340 did not differ significantly from that of scars dosed with vehicle. Scars dosed with the positive control article (TACA) showed significant decreases in SEI when compared to scars injected with CT340 or with saline ($P < 0.005$).

[579] Scars injected with vehicle or CT340 exhibited moderate inflammation. Dosing scars with intra-lesion injection of TACA significantly decreased inflammation.

[580] Daily topical dosing of scars with CT340 significantly decreased the level of scar inflammation. This decrease was significantly lower when compared to TACA-injected scars.

[581] In summary, while there was no difference between vehicle and CT340 in the reduction of SEI in this study, daily topical dosing of scars with CT340 reduced the level of inflammation. As seen in scars treated with TACA, the reduction of inflammation plays a role in the reduction of SEI.

Example 9: CT340 *in vitro* activity testing in comparison to K252a and CT327:**Anti-proliferative activity on neonatal Human Epidermal Keratinocytes**

[582] The purpose of this study was to evaluate the *in vitro* activity of CT340 in comparison with K252a and CT327. The *in vitro* activity was evaluated as ability to inhibit proliferation on neonatal Human Epidermal Keratinocytes.

Materials and methods***Cell Preparation***

[583] One or more 70-80% confluent T-75 culture flasks (according to the number of cells needed to perform the experiment) are rinsed one time with 9 ml of Trypsin/EDTA solution according to the manufacturer instructions. 3 ml of Trypsin/EDTA is then added for the second time to detach cells. After approximately 10 minutes at 37°C, cell detachment is complete and Trypsin is blocked with 9 ml of Trypsin Neutralizer solution. Cells are transferred to a sterile 15 ml conical tube, filled with other 9 ml of Trypsin neutralizer and centrifuged at 180 x g for 7 minutes. The supernatant is removed and the cell pellet is resuspended in 12 ml of supplemented medium. Vital cell count with Trypan Blue is performed and cells are diluted to seed one or more 96-well plates with 1.5×10^3 cells/well in 100 μ L. Cells are incubated for 18h in a 37°C, 5% CO₂, humidified cell culture incubator.

The day after, 10 μ L of test item solution at the appropriate concentration are added to each well (10 μ L/well of Complete Growth medium are added to control wells).

[584] The plate(s) are then incubated for 24h, 48h, 72h and 96h in a humidified incubator at 37°C with 5% CO₂ and analyzed with MTT at each time-point (Time 0 is analyzed just after cell treatment with test item solution).

Solution for cell treatment preparation

[585] A K252a 10 mM stock solution is prepared in DMSO and stored at -20°C. Solutions for cell treatment are freshly prepared just before use by serial dilution of stock solution in complete growth medium.

[586] CT340 and CT327 10 mM stock solutions are prepared in DMSO and stored at -20°C. Solutions for cell treatment are freshly prepared just before use by serial dilution of stock solution in complete growth medium. Only for the third experiment a 10 mM starting solution is prepared in complete growth medium just before cell treatment. This solution is then further diluted in the same medium to obtain the desired concentrations to be tested. Complete growth medium is used as positive control.

MTT viability assay

[587] At designed time-points after addition of the test item (K252a, CT327, CT340) 15 μ L of Dye Solution is added to each well. The plate(s) are then incubated at 37°C, 5% CO₂ for up to 4 hours. After incubation, 100 μ L of stop solution is added to each well and plate(s) are left at 37°C for 1 hour. The content of the wells is mixed to get a uniformly colored solution with a multichannel pipette and plate is read using a 96- well plate reader by setting the wavelength at 570 nm (reference 650 nm). Results are expressed as optical density units (OD).

Data representation and statistical analysis

[588] Data are represented in bar columns as mean OD₅₇₀₋₆₅₀ (Optical Density) $\pm$ SEM (Standard Error of the Mean) of the results obtained from six wells.

[589] In order to compare selected pairs of data sets, One-way ANOVA followed by either Dunnett's post test (control column data: negative control - Complete Growth Medium) or Bonferroni's post test among pairs of selected data sets is the statistical analysis performed.

[590] K252a, CT327 or CT340 concentration data having a post test p value < 0.05 if compared to control wells data are considered as significantly inhibiting the proliferation of human epidermal keratinocytes.

[591] The IC₅₀ value was calculated fitting data on a sigmoidal dose-response curve with variable slope.

[592] GraphPad Prism Software was used for data representation, statistical analysis and curve fitting.

Results

[593] Three experiments were been performed to evaluate the effect of different concentrations of the test items at different time-points.

Proliferation Assay Setup with K252a, CT340 and CT327 - 1st Experiment

[594] In a first experiment K252a, CT340 and CT327 were tested at the following concentrations: 25 nM, 50 nM, 100 nM, 200 nM and 500 nM. MTT is performed at 24 h, 48 h, 72 h and 96 h. The results of the experiment performed on neonatal HEK cell line to evaluate the ability of the compounds to inhibit proliferation are reported in Figure 23.

[595] K252a shows a good concentration-response curve after contact time 48 h, 72 h and 96 h and is only partially effective after contact time 24h. CT327 and CT340 partially inhibit proliferation at time 24 h and 96 h but without a concentration-dependent response. The IC_{50} values for K252a at time-points 48 h, 72 h and 96 h, respectively, are 150.8 nM ($R^2=0.9326$), 101.4 nM ($R^2= 0.9532$) and 84.36 nM ($R^2= 0.9626$).

Proliferation Assay Setup with K252a, CT340 and CT327 -2nd Experiment

[596] In the second experiment CT327 and CT340 were tested at different concentrations than K252a in order to evaluate if higher concentrations of the molecules are able to inhibit the proliferation of HEK cell line. K252a was tested at the same concentrations of the previous experiment while CT327 and CT340 were tested at the following concentrations: 100 μ M, 50 μ M, 25 μ M, 5 μ M, 1 μ M, 500 nM, 200 nM, 100 nM, 50 nM and 25 nM.

[597] MTT assays were performed only at time 72 h. The results of the experiment performed on neonatal HEK cell line to evaluate the ability of the compounds to inhibit proliferation are reported in Figure 24.

[598] As in the 1st Experiment, K252a shows a good concentration-response curve after a contact time of 72 h, with an IC_{50} value of 171 nM ($r^2= 0.9823$). Furthermore, increased concentrations of CT327 and CT340 cause inhibited proliferation at time 72 h with a partial concentration-dependent response. Unfortunately, the 1% DMSO concentration present in the wells with the higher concentrations of CT327 and CT340 did not allow for statistically significant relevance and the IC_{50} value could not be calculated.

Proliferation Assay Final Experiment with K252a, CT340 and CT327

[599] A third experiment was performed in order to identify an IC_{50} value for CT340 and CT327 in the absence of DMSO in the final wells. For this reason, 10 mM starting solutions of CT340 and CT327 were prepared by dissolving them directly in complete growth medium just before cell treatment. These solutions were then further diluted in the same medium to obtain the desired concentrations to be tested.

[600] K252a was tested at the same concentrations of the previous experiments while CT327 was tested at the following concentrations: 500 μ M, 100 μ M, 50 μ M, 25 μ M, 5 μ M, 1

μM , 500 nM, and 50 nM. CT340 was tested at the following concentrations: 500 μM , 100 μM , 50 μM , 25 μM , 5 μM , 1 μM , 500 nM, 200 nM and 100 nM.

[601] MTT assays were performed at time 72 h, 96 h and 120 h. The results of experiments performed in neonatal HEK cells to evaluate the ability of the compounds to inhibit proliferation are reported in Figure 25.

[602] K252a showed a good concentration-dependent response for all three time points analyzed. Furthermore, increased concentrations of CT327 and CT340 inhibit proliferation at time points 72 h, 96 h and 120 h with a concentration-dependent response.

[603] IC_{50} for K252a for the time points 72 h, 96 h and 120 h are 98.31 nM ($R^2=0.9590$), 40.04 nM ($R^2=0.9630$) and 32.88 nM ($R^2=0.9899$), respectively.

[604] The IC_{50} values for CT327 and CT340 are the following:

CT327

T72h $\text{IC}_{50}= 21.93 \mu\text{M}$ ($R^2=0.9179$)

T96h $\text{IC}_{50}= 18.57 \mu\text{M}$ ($R^2=0.9281$)

T120h $\text{IC}_{50}= 17.51 \mu\text{M}$ ($R^2=0.9876$)

CT340

T72h $\text{IC}_{50}= 15.57 \mu\text{M}$ ($R^2=0.9263$)

T96h $\text{IC}_{50}= 14.55 \mu\text{M}$ ($R^2=0.9528$)

T120h $\text{IC}_{50}= 11.47 \mu\text{M}$ ($R^2=0.9915$)

[605] The IC_{50} values obtained show that CT327 and CT340 maintain the ability to inhibit proliferation of HEK cells, even though employed at higher concentrations if compared to the unconjugated molecule. Furthermore, CT340 appears to be slightly more active than CT327.

Conclusions

[606] The reported experiments confirm that both CT327 and CT340 maintain the K252a ability to inhibit the proliferation of HEK cells. This implies that they are able to pass the cell membrane and to interact with target kinases to elicit their effect.

[607] Comparing the IC_{50} values calculated in these experiments, it can be observed that the PEG moiety partially modifies the interaction with the pharmacological targets. Moreover, PEGylated molecules need longer contact time to show a noticeable effect. Indeed, when the contact time is 24 and 48 hours, K252a is already active while CT340 and CT327 are not. However, the loss of activity due to PEGylation was expected and is acceptable if suitably counterbalanced by benefits in terms of a reduced systemic toxicity and a dramatically improved selectivity in terms of kinase inhibition profile (as described in other examples herein).

[608] The final experiment provided a comparison the IC₅₀ values of CT340 and CT327, with the results suggesting that the presence of the amide linkage in CT340 results in a slightly more active molecule with lower IC₅₀ values than CT327 at all the tested time points.

Example 10: IC₅₀ Determination study of CT340 against 7 kinases

[609] The purpose of this study was to evaluate CT340 inhibition of JAK2, JAK3, PDGFR β , TRKA, MAP2K1, MAP2K3, TAK1-TAB1. Staurosporine was evaluated as well as a control. The CT340 test concentrations used were the following: 10, 3, 1, 0.3, 0.1, 0.03, 0.01, 0.003, 0.001, and 0.0003 μ M.

Materials and Methods

[610] The test compound was dissolved in and diluted with dimethylsulfoxide (DMSO) to achieve 100-fold higher concentration. Then the solution was further 25-fold diluted with assay buffer to make the final test compound solution. Reference compounds for assay control were prepared similarly.

[611] STK-ELISA procedure:

1) The 10 μ L of x4 compound solution, 10 μ L of x4 Substrate/ATP/Metal solution, and 20 μ L of x2 kinase solution were prepared with assay buffer (15 mM Tris-HCl, 0.01% Tween-20, 2 mM DTT, pH7.5) and mixed and incubated in a well of polypropylene 96 well microplate for 1 hour at room temperature.

2) 120 μ L of 40 mM EDTA solution (pH 7.5) was added to the well, and then 120 μ L of the mixture was transferred to the well of ELISA plate (as shown in Table 13a).

3) After 30 minutes incubation, the well was washed 4 times, and blocked with blocking buffer containing 0.1% BSA.

4) 100 pL of the first antibody (as shown below) solution was added to the well and incubated for 30 minutes.

5) After 4 times washing of the well, 100 pL of the second antibody (as shown in Table 13a) solution was added to the well, and incubated for 30 minutes.

6) After washing the well, 100 pL of TMB solution was added and incubated for 5 minutes. To stop the HRP reaction, 100 pL of 0.1 M sulfuric acid was added.

7) The kinase reaction was evaluated by the absorbance at 450 nm of the well.

TABLE 13a

Kinase	ELISA plate and Antibody
MAP2K1	ELISA plate: Glutathione coated 96 well plate (NUNC) 1st Ab: Anti-phospho p44/42 MAPK (Thr202/Tyr204) (E10) monoclonal antibody (Cell Signaling Technology) 2nd Ab: HRP labeled goat anti-mouse IgG Ab (Zymed)
MAP2K3	ELISA plate: Glutathione coated 96 well plate (NUNC) 1st Ab: Anti-phospho p38 MAPK (Thr180/Tyr182) (28B10) monoclonal antibody (Cell Signaling Technology) 2nd Ab: HRP labeled goat anti-mouse IgG Ab (Zymed)
TAK1-TAB1	ELISA plate: Glutathione coated 96 well plate (NUNC) 1st Ab: Anti-phospho MKN7(Ser271/Thr275) antibody (Cell Signaling Technology) 2nd Ab: HRP labeled goat anti-rabbit IgG Ab (Zymed)

[612] Off-chip Mobility Shift Assay (MSA):

1) The 5 μ L of x4 compound solution, 5 μ L of x4 Substrate/ATP/Metal solution, and 10 μ L of x2 kinase solution were prepared with assay buffer (20 mM HEPES, 0.01% Triton X- 100, 2 mM DTT, pH7.5) and mixed and incubated in a well of polypropylene 384 well microplate for 1 hour at room temperature.

2) 60 μ L of Termination Buffer (QuickScout Screening Assist MSA; Carna Biosciences) was added to the well.

3) The reaction mixture was applied to LabChip3000 system (Caliper Life Science), and the product and substrate peptide peaks were separated and quantitated.

4) The kinase reaction was evaluated by the product ratio calculated from peak heights of product(P) and substrate(S) peptides ($P/(P+S)$).

[613] The reaction conditions employed for the seven kinase assays are depicted in Table 13b.

TABLE 13b

Kinase	Platform	Substrate		ATP (μ M)		Metal		Positive control
		Name	μ M	K _m	Assay	Name	μ M	
JAK2	MSA	Srctide	1000	13	10	Mg	5	Staurosporine
JAK3	MSA	Srctide	1000	3.5	5	Mg	5	Staurosporine
PDGFR β	MSA	CSKtide	1000	23	25	Mg	5	Staurosporine
TRKA	MSA	CSKtide	1000	65	75	Mg	5	Staurosporine
MAP2K1	STK-ELISA	Erk2	100	11	10	Mg	5	Staurosporine
MAP2K3	STK-ELISA	p38 α (8-352)	100	0.36	0.5	Mg	10	Staurosporine
TAK1-TAB1	STK-ELISA	MAP2K7	180	10	10	Mg	10	Staurosporine

[614] The readout value of reaction control (complete reaction mixture) was set as a 0% inhibition, and the readout value of background (Enzyme(-)) was set as a 100% inhibition, then the percent inhibition of each test solution was calculated.

[615] IC₅₀ value was calculated from concentration vs. %Inhibition curves by fitting to a four parameter logistic curve.

Results

[616] The CT340 and staurosporine concentration vs. %Inhibition curves are depicted in Figures 26 to 39, and the IC₅₀ determinations for both compounds are shown in Table 14 below.

TABLE 14 - IC₅₀ Determination

Kinase	IC ₅₀	
	CT340	Staurosporine
JAK2	1.35E-07	4.30E-10
JAK3	3.87E-08	2.78E-10
PDGFR β	1.12E-07	3.87E-10
TRKA	2.55E-08	5.02E-10
MAP2K1	1.96E-08	1.39E-09
MAP2K3	1.26E-08	1.13E-09
TAK1-TAB1	2.19E-07	4.14E-08

Example 11: Examination of CT327 and CT340 in pain and nerve regeneration

[617] This study evaluated the effect on nociception and neurite outgrowth of the highly selective TrkA inhibitors CT327 and CT340, using: 1) a functional calcium imaging assay to measure inhibition of capsaicin responses; and 2) neurite outgrowth assay in cultured sensory (DRG) neurons. The effects of CT327 and CT340 were compared with a commercially available TrkA inhibitor (GW441756) and an anti-NGF antibody

Background

[618] Nerve Growth Factor (NGF) is a homodimer, which following binding results in dimerization and autophosphorylation of its high affinity receptor TrkA. Phosphorylation of TrkA increases the catalytic activity of the kinase domain and creates binding sites for SH2 domain containing cytoplasmic proteins. These proteins initiate the activation of several signal transduction pathways such as PLC γ , ras, PB kinase/AKT, and Raf/MEK/ERK.

[619] Increased NGF released from inflammatory cells and tissues during inflammation and injury results in hyperalgesia that can last from several hours to days via TrkA activation on sensory nerve terminals. This activates a multitude of downstream signalling pathways involving MAP kinases (ERK), PBK, and PLC, which are involved in pathological conditions. NGF binding to TrkA also potentiates the heat and capsaicin receptor TRPV1 through phosphorylation of TRPV1 channels by serine/threonine kinases, PKC, PKA, and calcium/calmodulin-dependent kinase II and tyrosine kinase c-Src.

[620] TRPV1 is a plasma membrane bound ion channel expressed by nociceptors involved in thermosensation, which is activated by noxious heat (>43°C), capsaicin, low pH, the inflammatory mediators arachidonic acid and bradykinin leading to the perception of pain and thermal hypersensitivity. The sensitivity of the capsaicin receptor (TRPV1) is modulated by NGF in rodent and human DRG neurons. TRPV1 expression and function has been found to be up-regulated by NGF in vitro and in clinical conditions of hypersensitivity. Responses of cultured DRG neurons to capsaicin thus provide an in vitro model for NGF-TrkA pathway signalling, as used in this study.

[621] While NGF influences the survival of developing primary sensory neurons mainly through its high affinity receptor TrkA, its role in mature DRG neurons is restricted to regulating their sensitivity and neuropeptide expression, but not survival, or neurite length. However, systemic blockade of NGF in clinical conditions has been associated with positive sensory symptoms and accelerated arthritis, and generalized loss of protective sensation remains a concern with long-term treatment; these may be avoided by regional topical delivery for cutaneous hypersensitivity disorders. Here we describe the effectiveness of the compounds CT327 and CT340 designed to block NGF-TrkA signalling in an in vitro model of NGF induced hypersensitivity in adult rat DRG neurons, and demonstrate the inhibition of capsaicin responses without affecting neurite length. Treatment with the TrkA inhibitors

CT327 and CT340 resulted in dose-related inhibition of capsaicin responses with IC_{50} = 10 nM, but no effect on neurite length in cultured DRG neurons. These results suggest that CT327 and CT340 could produce pain relief without affecting the integrity or regeneration of nociceptor fibers.

Materials and Methods

Neuron cultures

[622] Bilateral DRG from 11 adult rats were microdissected from cervical, thoracic, lumbar and sacral levels and enzyme digested in 0.2% collagenase/0.5% dispase in Ham's F12 medium for 3 hours (previously described in Anand et al, 2010). Enzyme digested tissue was mechanically dissociated in BSF2 medium (Ham's F12 containing 2% heat inactivated fetal calf serum, 60 ng/ml progesterone, 0.16 µg/ml sodium selenite, 0.1 mg/ml transferrin, 16 µg/ml putrescine, 10 pg/ml insulin, 3 mg/ml BSA, 100 pg/ml each penicillin/streptomycin), containing 1 mg/ml soy-bean trypsin inhibitor. The resulting neuronal suspension was plated on collagen (from rat tail, 50 pg/ml), and laminin (20 pg/ml) coated glass bottomed petri dishes (MatTek, USA) at 1000 cells per dish in 200 µl BSF2 medium; after 30 minutes 2 ml warm BSF2 medium containing human NGF (Sigma U.K. 100 ng/ml) was added to the cultures, which were incubated at 37 °C in a humid environment. Creablis TrkA inhibitors CT327, CT340 and commercially available TrkA inhibitor GW441756 (BML-1364, Enzo Life Sciences, Switzerland,), were dissolved in DMSO to make stock solutions of 4.5 mM (CT327), and 4.7 mM (CT 340), 30 mM (GW441756) aliquoted and stored at -20 °C, until use. Intermediate dilutions of CT327 and CT340 were prepared in sterile distilled water, and of GW441756 in ethanol, at 500x final concentration. Anti-NGF antibody (1 mg/ml, anti human/mouse), was obtained commercially (L148M, Exalpha Biologicals), aliquoted and stored at -20 °C until use.

Functional assay - Calcium Imaging

[623] 48 hours after plating, neurons were incubated with 2 µM Fura2 AM (1 hour, 37 °C), in phenol red free HEPES buffered HBSS (Hanks balanced salt solution) containing 0.1 % BSA, pH 7.3, followed by HBSS wash and de-esterification for 20 minutes. Phase bright, growing neurons were identified and alternately stimulated with u.v. excitation of 340 and 380 nm wavelength and transmitted light every 2 seconds (100 msec duration), and images were acquired with a Hamamatsu Orea ER FW cooled CCD camera using Kinetic Imaging software. Neurons with a stable baseline ratio of 340/380 (bound/unbound calcium) were stimulated with 200 nM capsaicin (20-30 seconds to identify capsaicin sensitive neurons which indicated calcium influx by increased 340/380 ratio; this was followed by washout and a rest period of 30 minutes. Test compounds were applied from stock solutions (500x final concentration- 4 µl/2ml), followed 10 minutes later by 1 µM capsaicin, and responses were measured as the difference from baseline to peak ratio, and expressed as a percentage

response (ratio of second (1 μ M capsaicin)/first (200 nM capsaicin) response), and compared with NGF treated controls (no test compound applied between capsaicin stimuli). Percent inhibition was calculated for each group and compared with controls. Student's t test was used to compare between groups; $P < 0.05$ was considered statistically significant.

Morphological assay - Neurite length measurement

[624] 24 hours after plating, neurons were treated with CT327, CT340, GW441756 or anti-NGF (L148M, Exalpha), for 24 hours and fixed with 4% PFA for 15 minutes, permeabilized with methanol (-20 °C, 3 minutes), prior to immunostaining with mouse monoclonal antibody to Gap43 (1 :200, Sigma, UK) for 45 minutes followed by secondary antibody Alexa 488 (1:200, Molecular Probes); the glass bottom coverslips were detached and mounted on glass slides, and TIFF images were acquired with a Zeiss inverted microscope equipped with standard FITC optics, for image analysis and neurite measurement using Metamorph software. Neurons with neurites greater than twice the cell body diameter were identified, and only those neurons which were clearly identifiable were used for analysis. The longest neurite length was measured for each neuron and the average length calculated for each group using Excel software. 30- 50 neurons were analyzed for each concentration in each of 3 experiments.

Results

Effect on capsaicin responses

[625] The effect the test and control compounds on capsaicin responses is depicted in Figures 40 to 43. Neurons acutely treated with CT327 or CT340 demonstrated dose-related inhibition of capsaicin responses with IC_{50} value of 10 nM for both compounds, normalised to controls. The TrkA inhibitor GW441756 (BMLE1364) had an IC_{50} value of 15 nM, while the anti-NGF antibody had an IC_{50} value of 1 pg/ml. Note that 23.8 ± 5.3 % reduction ($n=8$ neurons), was observed due to desensitization, as reported previously (see Anand et al, 2010); further inhibition in the presence of CT327 and CT340 was normalized to controls. For CT327, the goodness of fit, $R^2=0.78$, and for CT340, $R^2=0.68$ (using Graph Pad Prism 5.0 software.). The IC_{50} for morphine and Gabapentin in this bioassay were 1 μ M and 100 nM, respectively.

Effect on Neurite length

[626] Test compounds were added 24 hours after plating. 24 hour treatment with CT327 and CT340 did not show any difference in neurite length compared to control (100 ng/ml NGF) (Figures 44 to 46). As depicted in Figure 47, treatment with commercial TrkA inhibitor GW441756 showed some vesiculation at 1 μ M concentration and reduction of neurite length (n.s.), which were both increased at the higher concentration of 10 μ M ($p=0.03$). As depicted in Figure 48, treatment with the anti-NGF antibody at 1 and 10 pg/ml concentrations did not significantly alter neurite length compared to NGF treated controls.

[627] Separate neuronal cultures that had been treated with test compounds and fixed as for neurite length analysis were incubated with polyclonal TrkA antibody combined with mouse anti-Gap43 to confirm that TrkA positive neurons were not eliminated following treatment with CT327 and CT340. TrkA/Gap43 immunofluorescence confirmed that TrkA positive neurons were present in the treated groups (Figure 49).

Conclusions

[628] This study used an *in vitro* model of NGF-induced neuronal hypersensitivity to assess the effects of the TrkA inhibitors on neuronal responses to capsaicin, and on neurite length. Acute treatment with both CT327 and CT340 resulted in functional inhibition of capsaicin responses with IC_{50} = 10 nM, indicating that these small molecule TrkA inhibitors are promising candidates for targeting neuronal hypersensitivity. The commercial TrkA inhibitor GW441756 and the NGF antibody were used for comparison, and acute treatment with both agents showed inhibition of capsaicin responses.

[629] The morphological findings of this study indicate that CT327 and CT340, at the concentrations used here for functional effects, did not affect neurite length, indicating lack of a toxic effect. The commercial TrkA inhibitor GW441756, used for comparison, also did not have any effect on neurite length at 1, 10 and 100 nM concentrations, but reduced neurite length at the higher concentrations of 1 and 10 μ M with vesiculation, indicating possible structural effects at these concentrations. Anti-NGF antibody treatment at 1 and 10 μ g/ml concentrations did not significantly affect neurite length.

[630] In conclusion, CT327 and CT340 are potent inhibitors of NGF/TrkA-dependent capsaicin responses in cultured sensory neurons. Topical treatment with TrkA inhibitors may provide pain relief with the efficacy but without the safety liability of systemic NGF blockade.

Example 12: Investigation of intra-epidermal nerve fibers following epicutaneous 2-week repeated treatment of mini-pig skin with CT340

[631] Mini-pig skin tissues from treated and untreated areas of control (vehicle) and active animal groups of the study described in Example 7 were pre-fixed in benzoquinone (BQ) and frozen embedded were used in this study (see Table 15).

TABLE 15 - Tissues studied

Group Number	Treatment (mg API/animal/day)	Level
1	0	Control
2	110	Low
3	330	Medium
4	1100	High
Control (recovery group)	0	Control (recovery group)
High dose (recovery group)	1100	High (recovery group)

[632] Tissue sections (15µm thick) were collected onto poly-L-lysine (Sigma, Poole, UK) coated glass slides. Endogenous peroxidase was blocked by incubation in industrial methylated spirits (IMS) containing 0.3% w/v hydrogen peroxide for thirty minutes. After rehydration with PBS buffer, sections were incubated overnight with primary antibody at final dilutions listed above. Sites of primary antibody attachment were revealed using nickel-enhanced, avidin-biotin peroxidase (ABC - Vector Laboratories, Peterborough, UK) as described. Sections were counter-stained for nuclei in 0.1% w/v aqueous neutral red, dehydrated and mounted in xylene-based mountant (DPX; BDH/Merck, Poole, UK), prior to photomicrography.

[633] PGP9.5 intra-epidermal fibers were counted at optimal titre of 1:40,000 and results expressed as fibers/mm. The nerve fibers present in first 2 mm of each section fibers were counted.

[634] Optimal dilution for PGP9.5 staining in the pig tissue was 1:40,000. Intra-epidermal nerve fibers were seen, and immunoreactive fibers were also present in arrector pili and deeper dermal nerve fascicles (Figure 50).

[635] There was a tendency for fewer IENF in treated skin (Figure 51) but no statistically significant decrease. There was no difference in the PGP9.5 fiber counts of the recovery animals that received vehicle or high dose for 2 consecutive weeks and studied 2 weeks after last administration.

[636] Epicutaneous 2-week repeated treatment of mini-pig skin with CT340 at doses of 2%, 6% and 20% w/v (corresponding to 0.1, 0.33 and 1.1 g of CT340/animal/day) did not have a significant effect on the numbers of intra-epidermal nerve fibers immunostained with the gold-standard pan-neuronal marker PGP9.5.

Example 13: BioMAP Platform Analysis of SNA-120

Aim of Study

[637] The goal of this study was to characterize SNA-120 in the BioMAP Diversity PLUS panel of 12 human primary cell-based systems. These systems are designed to model complex human tissue and disease biology of the vasculature, skin, lung and inflammatory tissues. Quantitative measurements of biomarker activities across this broad panel, along with comparative analysis of the biological activities of known bioactive agents in the BioMAP reference database are used to predict the safety, efficacy and function of these test agents.

Overview of BioMAP Technology Platform

[638] BioMAP panels consist of human primary cell-based systems designed to model different aspects of the human body in an in vitro format. The 12 systems in the Diversity PLUS panel allow test agent characterization in an unbiased way across a broad set of systems modeling various human disease states. BioMAP systems are constructed with one

or more primary cell types from healthy human donors, with stimuli (such as cytokines or growth factors) added to capture relevant signaling networks that naturally occur in human tissue or pathological conditions. Vascular biology is modeled in both a Th1 (3C system) and a Th2 (4H system) inflammatory environment, as well as in a Th1 inflammatory state specific to arterial smooth muscle cells (CASM3C system). Additional systems recapitulate aspects of the systemic immune response including monocyte-driven Th1 inflammation (LPS system) or T cell stimulation (SAg system), chronic Th1 inflammation driven by macrophage activation (/Mphg system) and the T cell-dependent activation of B cells that occurs in germinal centers (BT system). The BE3C system (Th1) and the BF4T system (Th2) represent airway inflammation of the lung, while the MyoF system models myofibroblast-lung tissue remodeling. Lastly, skin biology is addressed in the KF3CT system modeling Th1 cutaneous inflammation and the HDF3CGF system modeling wound healing.

[639] Each test agent generates a signature BioMAP profile that is created from the changes in protein biomarker readouts within individual system environments. Biomarker readouts (7 - 17 per system) are selected for therapeutic and biological relevance, are predictive for disease outcomes or specific drug effects and are validated using agents with known mechanism of action (MoA). Each readout is measured quantitatively by immune-based methods that detect protein (e.g., ELISA) or functional assays that measure proliferation and viability. BioMAP readouts are diverse and include cell surface receptors, cytokines, chemokines, matrix molecules and enzymes. In total, the Diversity PLUS panel contains 148 biomarker readouts that capture biological changes that occur within the physiological context of the particular BioMAP system.

[640] Using custom-designed software containing data mining tools, a BioMAP profile can be compared against a proprietary reference database of > 4,000 BioMAP profiles of bioactive agents (biologies, approved drugs, chemicals and experimental agents) to classify and identify the most similar profiles. This robust data platform allows rapid evaluation and interpretation of BioMAP profiles by performing the unbiased mathematical identification of similar activities. Specific BioMAP activities have been correlated to in vivo biology, and multiparameter BioMAP profiles have been used to distinguish compounds based on MoA and target selectivity and can provide a predictive signature for in vivo toxicological outcomes (e.g., vascular toxicity, developmental toxicity, etc.) across diverse physiological systems.

Materials and Methods

Test Agent

[641] SNA-120 was profiled in the BioMAP Diversity PLUS panel at concentrations of 28000 nM, 9200 nM, 3100 nM, and 1000 nM. SR221 1 was employed as the benchmark compound.

Methods for Diversity PLUS

[642] Human primary cells in BioMAP systems are used at early passage (passage 4 or earlier) to minimize adaptation to cell culture conditions and preserve physiological signaling responses. All cells are from a pool of multiple donors ($n = 2$ to 6), commercially purchased and handled according to the recommendations of the manufacturers. Human blood derived CD14+ monocytes are differentiated into macrophages *in vitro* before being added to the /Mphg system. Abbreviations are used as follows: Human umbilical vein endothelial cells (HUVEC), Peripheral blood mononuclear cells (PBMC), Human neonatal dermal fibroblasts (HDFn), B cell receptor (BCR), T cell receptor (TCR) and Toll-like receptor (TLR).

[643] Cell types and stimuli used in each system are as follows: 3C system [HUVEC + (IL-1 β , TNFa and IFNy)], 4H system [HUVEC + (IL-4 and histamine)], LPS system [PBMC and HUVEC + LPS (TLR4 ligand)], SAg system [PBMC and HUVEC + TCR ligands], BT system [CD19+ B cells and PBMC + (α -IgM and TCR ligands)], BF4T system [bronchial epithelial cells and HDFn + (TNFa and IL-4)], BE3C system [bronchial epithelial cells + (IL-1 β , TNFa and IFNy)], CASM3C system [coronary artery smooth muscle cells + (IL-1 β , TNFa and IFNy)], HDF3CGF system [HDFn + (IL-1 β , TNFa, IFNy, EGF, bFGF and PDGF-BB)], KF3CT system [keratinocytes and HDFn + (IL-1 β , TNFa and IFNy)], MyoF system [differentiated lung myofibroblasts + (TNFa and TGF β 3)] and /Mphg system [HUVEC and M1 macrophages + Zymosan (TLR2 ligand)].

[644] Systems are derived from either single cell types or co-culture systems. Adherent cell types are cultured in 96 or 384-well plates until confluence, followed by the addition of PBMC (SAg and LPS systems). The BT system consists of CD19+ B cells co-cultured with PBMC and stimulated with a BCR activator and low levels of TCR stimulation. Test agents prepared in either DMSO (small molecules; final concentration $\leq 0.1\%$) or PBS (biologies) are added at the indicated concentrations 1-hr before stimulation, and remain in culture for 24-hrs or as otherwise indicated (48-hrs, MyoF system; 72-hrs, BT system (soluble readouts); 168-hrs, BT system (secreted IgG)). Each plate contains drug controls (e.g., legacy control test agent colchicine at 1.1 μ M), negative controls (e.g., non-stimulated conditions) and vehicle controls (e.g., 0.1% DMSO) appropriate for each system. Direct ELISA is used to measure biomarker levels of cell-associated and cell membrane targets. Soluble factors from supernatants are quantified using either HTRF® detection, bead-based multiplex immunoassay or capture ELISA. Overt adverse effects of test agents on cell proliferation and viability (cytotoxicity) are detected by sulforhodamine B (SRB) staining, for adherent cells, and alamarBlue® reduction for cells in suspension. For proliferation assays, individual cell types are cultured at subconfluence and measured at time points optimized for each system (48-hrs: 3C and CASM3C systems; 72-hrs: BT and HDF3CGF systems; 96-hrs: SAg system). Cytotoxicity for adherent cells is measured by SRB (24-hrs: 3C, 4H, LPS, SAg, BF4T, BE3C, CASM3C,

HDF3CGF, KF3CT, and IMphg systems; 48-hrs: MyoF system), and by alamarBlue staining for cells in suspension (24-hrs: SAg system; 42-hrs: BT system) at the time points indicated. Additional information can be found in previous descriptions.

Data Analysis

[645] Biomarker measurements in a test agent-treated sample are divided by the average of control samples (at least 6 vehicle controls from the same plate) to generate a ratio that is then log₁₀ transformed. Significance prediction envelopes are calculated using historical vehicle control data at a 95% confidence interval.

Profile Analysis

[646] Biomarker activities are annotated when 2 or more consecutive concentrations change in the same direction relative to vehicle controls, are outside of the significance envelope and have at least one concentration with an effect size > 20% ($|\log_{10} \text{ratio}| > 0.1$). Biomarker key activities are described as modulated if these activities increase in some systems, but decrease in others. Cytotoxic conditions are noted when total protein levels decrease by more than 50% ($\log_{10} \text{ratio}$ of SRB or alamarBlue levels < -0.3) and are indicated by a thin black arrow above the X-axis. A compound is considered to have broad cytotoxicity when cytotoxicity is detected in 3 or more systems. Concentrations of test agents with detectable broad cytotoxicity are excluded from biomarker activity annotation and downstream benchmarking, similarity search and cluster analysis. Antiproliferative effects are defined by an SRB or alamarBlue log₁₀ ratio value < -0.1 from cells plated at a lower density and are indicated by grey arrows above the X-axis. Cytotoxicity and antiproliferative arrows only require one concentration to meet the indicated threshold for profile annotation.

Benchmark Analysis

[647] Common biomarker readouts are annotated when the readout for both profiles is outside of the significance envelope with an effect size > 20% in the same direction. Differentiating biomarkers are annotated when one profile has a readout outside of the significance envelope with an effect size > 20%, and the readout for the other profile is either inside the envelope or in the opposite direction. Unless specified, the top non-cytotoxic concentration of both the test agent and benchmark agent are included in the benchmark overlay analysis.

Similarity Analysis

[648] Common biomarker readouts are annotated when the readout for both profiles is outside of the significance envelope with an effect size > 20% in the same direction. Concentrations of test agents that have 3 or more detectable systems with cytotoxicity are excluded from similarity analysis. Concentrations of test agents that have 1 - 2 systems with detectable cytotoxicity will be included in the similarity search analysis, along with an overlay of the database match with the top concentration of the test agent. This will be followed by

an additional overlay of the next highest concentration of the test agent containing no systems with detectable cytotoxicity and the respective database match. To determine the extent of similarity between BioMAP profiles of compounds run in the Diversity PLUS panel, we have developed a custom similarity metric (BioMAP Z-Standard) that is a combinatorial approach that has improved performance in mechanism classification of reference agents compared to other measures tested (including Pearson's and Spearman's correlation coefficients). This approach more effectively accounts for variations in the number of data points, systems, active biomarker readouts and the amplitude of biomarker readout changes that are characteristic features of BioMAP profiles. A Pearson's correlation coefficient (r) is first generated to measure the linear association between two profiles that is based on the similarity in the direction and magnitude of the relationship. Since the Pearson's correlation can be influenced by the magnitude of any biomarker activity, a per-system weighted average Tanimoto metric is used as a filter to account for underrepresentation of less robust systems. The Tanimoto metric does not consider the amplitude of biomarker activity, but addresses whether the identity and number of readouts are in common on a weighted, per system basis. A real-value Tanimoto metric is calculated first by normalizing each profile to

the unit vector (e.g., $A = \frac{A}{\|A\|}$) and then applying the following formula: $\frac{A \cdot B}{\|A\| + \|B\| - A \cdot B}$, where A and B are the 2 profile vectors. Then, it is incorporated into a system weighted-

averaged real-value Tanimoto metric in this calculation: $\frac{\sum W_i \cdot T_i}{\sum W_i}$. The calculation uses the real-value Tanimoto score for each i th system (T_i) and the weight of each i th system (W_i). W_i

is calculated for each system in the following formula: $\frac{1}{1 + e^{-100 * (lr - 0.09)}}$, where lr is the largest absolute value of the ratios from the 2 profiles being compared. Based on the optimal performance of reference compounds, profiles are identified as having mechanistically relevant similarity if the Pearson's correlation coefficient (r) ≥ 0.7 . Finally, a Fisher r -to- z -transformation is used to calculate a z -score to convert a short tail distribution into a normal

distribution as follows: $z = 0.5 \log^{\text{TM}} \frac{1+r}{1-r}$. Then the BioMAP Z-Standard, which adjusts for the number of common readouts (CR), is generated according to the following formula: $\text{Z-Standard} = z \sqrt{CR-3}$. A $\log_e$ BioMAP Z-Standard value corresponds to a higher confidence level, and this is the metric used to rank similarity results.

Cluster Analysis

[649] Cluster analysis (function similarity map) uses the results of pairwise correlation analysis to project the “proximity” of agent profiles from multi-dimensional space into two dimensions. Functional clustering of the agent profiles generated during this analysis uses Pearson correlation values for pairwise comparisons of the profiles for each agent at each concentration, and then subjects the pairwise correlation data to multidimensional scaling. Profiles that are similar with a Pearson’s correlation coefficient ($r \geq 0.7$) are connected by lines. Agents that do not cluster with one another are interpreted as mechanistically distinct. This analysis is performed for projects with 3 or more agents tested. Cytotoxic concentrations are excluded from cluster analysis.

Mechanism HeatMAP Analysis

[650] Mechanism HeatMAP analysis provides a visualization of the test compound and 19 consensus mechanisms allowing comparison of biomarker activities across all compound concentrations and consensus mechanisms. The synthetic consensus profiles used in the Mechanism HeatMAP analysis are representative BioMAP profiles of the average of multiple compounds from structurally distinct chemical classes. Profiles were calculated by averaging the values for each biomarker endpoint for all profiles selected (multiple agents at different concentrations) to build the consensus mechanism profile.[8] Biomarker activities are colored in the heatmap for consensus mechanisms and compounds when they have expression relative to vehicle controls outside of the significance envelope. Red represents increased protein expression, blue represents decreased expression and white indicates levels that were unchanged or within filtering conditions. Darker shades of color represent greater change in biomarker activity relative to vehicle control. The Mechanism HeatMAP was prepared using R and the gplots package for R.

Assay Acceptance Criteria

[651] A BioMAP assay includes the multi-parameter data sets generated by the BioMAP platform for agents tested in the systems that make up the Diversity PLUS panel. Assays contain drug controls (e.g., legacy control test agent colchicine), negative controls (e.g., non-stimulated conditions), and vehicle controls (e.g., DMSO) appropriate for each system. BioMAP assays are plate-based, and data acceptance criteria depend on both plate performance (% CV of vehicle control wells) and system performance across historical controls for that system. The QA/QC Pearson Test is performed by first establishing the 1% false negative Pearson cutoff from the reference dataset of historical positive controls. The process iterates through every profile of system biomarker readouts in the positive control reference dataset, calculating Pearson values between each profile and the mean of the remaining profiles in the dataset. The overall number of Pearson values used to determine the 1% false negative cutoff is the total number of profiles present in the reference dataset. The Pearson value at the one percentile of all values calculated is the 1% false negative

Pearson cutoff. A system will pass if the Pearson value between the experimental plate's negative control or drug control profile and the mean of the historical control profiles in the reference dataset exceeds this 1% false negative Pearson cutoff. Overall assays are accepted when each individual system passes the Pearson test and 95% of all project plates have % CV <20%.

Results

BioMAP Profile

[652] Figure 52 depicts the BioMAP profile of SNA-120 in the Diversity PLUS Panel. SNA-120 was found to be active with 21 annotated readouts, mediating changes in key biomarker activities listed by biological and disease classifications in Table 16 below. SNA-120 impacted inflammation-related activities (decreased E-selectin, sTNFa, MIP-1a, IL-8, IL-1a), immunomodulatory activities (decreased sIgG, sIL-10; increased CD69), tissue remodeling activities (decreased TIMP-2, tPA, MMP-1, PAI-1, Collagen III, uPAR, MMP-9), and hemostasis-related activities (decreased TF). SNA-120 is antiproliferative to endothelial cells, fibroblasts, and T cells (as indicated by grey arrows in Figure 52). There are no cytotoxic impacts detected at the concentration range tested.

TABLE 16 - Key Biomarker Activities Impacted by SNA-120

Biological and Disease Relevance Category	Decreased Activity	Increased Activity
Inflammation-related activities	MIP-1, Esel, IL8, IL1a, sTNFa	
Immunomodulatory activities	sIgG, sIL-10	CD69
Tissue remodeling activities	uPAR, Col-III, MMP1, MMP9, PAI-I, TIMP2, tPA	
Hemostasis-related activities	TF	

Reference Benchmark Overlay

[653] Figure 53 depicts an overlay of SNA-120 at 28 μ M and the selected reference benchmark SR221.1 at 10 pM. SR221.1 is an antagonist of retinoic acid receptor related nuclear receptor gamma. There are 12 common activities that are annotated within the following systems: 3C (Prolif), LPS (CD40), SAg (Prolif), BT (SIL-17A, SIL-17F, sIL-2, sIL-6, sIgG, sTNFa), CASM3C (TF), HDF3CGF (Prolif 72), and IMphg (sIL-10).

[654] Differentiating biomarkers (not shown) are defined when one profile has a readout outside of the significance envelope with an effect size > 20% ($|\log_{10} \text{ratio}| > 0.1$), and the readout for the other profile is either inside the envelope or in the opposite direction. There are 33 differentiating activities between the two compounds: 3C (HLA-DR, TF, uPAR), 4H (MCP-1, uPAR), LPS (CD69, sPGE2, sTNFa), SAg (CD69), BF4T (Eotaxin 3), BE3C (MMP-

1, MMP-9, PAI-1, tPA), CASM3C (HLA-DR, IL-6, LDLR, TM, uPAR), HDF3CGF (Collagen I, Collagen III, EGFR, M-CSF, MIG, PAI-1, VCAM-1), MyoF (IL-8, MMP-1, VCAM-1), and IMphg (CD69, MCP-1, MIP-1a, VCAM-1).

BT System Secretion Profiles

[655] Figure 54 depicts changes in the secretion of IL-17F, IgG, IL-17A, and TNF α in the BioMAP BT system mediated by SNA-120 (3.1 μ M), Tofacitinib (3.3 pM), Apremilast (3.3 pM), SR221 1 (3.3 pM), and Cyclosporin A (3.3 pM). Tofacitinib was found to be more active than SNA-120 in decreasing IL-17F secretion, displaying an activity similar to SR221 1 (Figure 54A). Surprisingly, SNA-120 was found to be as active as tofacitinib in decreasing IL-17A secretion (Figure 54C). SR221 1 decreased IL-17A secretion as expected, while Apremilast increased IL-17A secretion.

Top Database Search Result for SNA-120

[656] In a search for mathematically similar compound profiles from the BioMAP reference database, SNA-120 (28 pM) was most similar to GSK690693 (10 pM) (Pearson's correlation, $r = 0.733$). The Pearson's correlation coefficient between these two profiles is above our determined threshold ($r \geq 0.7$) indicating these compounds share mechanistically relevant similarity. GSK690693 is an ATP-competitive pan-Akt kinase inhibitor that also exhibits some inhibition for AMPK, PKA and PAK and PKC isoforms. Figure 55 depicts an overlay of SNA-120 (28 pM) and GSK690693 (10 pM).

[657] There are 15 common activities that are annotated within the following systems: LPS (SPGE2, sTNF α), SAg (Prolif), BT (SIL-17A, SIL-17F, sIL-2, sIL-6, sIgG, sTNF α), BE3C (MMP-1, MMP-9, PAI-1), HDF3CGF (PAI-1), and IMphg (CD69, sIL-10).

Top BioSeek Reference Database Matches for SNA-120

[658] Table 17 depicts the top 3 similarity matches from a search of the BioMAP Reference Database of > 4,000 agents for each concentration of SNA-120. The similarity between agents is determined using a combinatorial approach that accounts for the characteristics of BioMAP profiles by filtering (Tanimoto metric) and ranking (BioMAP Z-Standard) the Pearson's correlation coefficient between two profiles. Profiles are identified as having mechanistically relevant similarity if the Pearson's correlation coefficient is ≥ 0.7 .

TABLE 17 - Top BioMAP Reference Database Matches for SNA-120

Ref. ID	Compound Name	BioMAP z-Score	Pearson's Corr.	# of Common Readouts	Reference Match (Mechanism Class)
25 μ M	GS-4860582, 10 μ M	11.268	0.728	148	Abl Inhibitor
	87K_4805, 370 nM	11.038	0.724	148	Lck Inhibitor
	Filgrastim, 10 μ M	10.973	0.722	148	JAK1 Inhibitor
5 μ M	Berberine, 19 μ M	7.713	0.595	148	PI3K Inhibitor
	NO2A-118, 3.8 μ M	7.043	0.530	148	MEK1/2 Inhibitor
	EGFR/ERK2/MEK1/4 Inhibitor, 1.1 μ M	7.032	0.528	148	EGFR Inhibitor
0.1 μ M	Doxercicofenol, 3.1 μ M	6.381	0.508	132	Vitamin D2 Analog
	Doxercicofenol, 8.0 μ M	6.057	0.488	132	Vitamin D2 Analog
	Tusamycin, 14 nM	5.601	0.454	148	Antisense Agent
1 μ M	Sonastatin, 1.1 μ M	5.094	0.396	148	Src Family Inhibitor
	Selenic, 4.3 nM	4.882	0.296	148	Vitamin C Recovator Agent
	FNu 2a, 1 μ M	3.443	0.358	148	Cytokine

[659] The Pearson's correlation coefficient between profiles that is above our determined threshold ($r \geq 0.7$) indicates these compounds share mechanistically relevant similarity. For profiles with a Pearson's correlation coefficient below our determined threshold ($r < 0.7$), the relevance of the similarity is unknown.

Mechanism HeatMAP Analysis of SNA-120

[660] Figure 56 depicts Mechanism HeatMAP Analysis of SNA-120, with the 148 biomarker readouts within the Diversity PLUS panel compared to 19 consensus mechanism class profiles. This analysis informs on the regulatory mechanisms controlling increases or decreases in each of the biomarker readouts.

Clustering of Project Profiles

[661] Figure 57 depicts a clustering of tested agent profiles based on pairwise correlation analysis and clustering of most similar profiles. Profiles that are similar with a Pearson's correlation coefficient ($r \geq 0.7$) are connected by lines. Agents that do not cluster with one another are interpreted as mechanistically distinct. Cytotoxic concentrations are excluded from cluster analysis. Functional clustering of the agent profiles generated during this analysis uses Pearson's correlation values for pairwise comparisons of the profiles for each agent at each concentration, and then subjects the pairwise correlation data to multidimensional scaling. SNA-120 clusters internally at two concentrations. Internal clustering suggests the phenotypic signature of this compound is maintained across a range of concentrations, a characteristic commonly observed in marketed drugs.

Conclusions

[662] In this study SNA-120 was characterized by profiling in the BioMAP Diversity PLUS panel of human primary cell based assays modeling complex tissue and disease biology of organs (vasculature, immune system, skin, lung) and general tissue biology. The Diversity

PLUS panel evaluates the biological impact of test agents in conditions that preserve the complex crosstalk and feedback mechanisms that are relevant to in vivo outcomes.

[663] SNA-120 was broadly active and non-cytotoxic in the Diversity PLUS panel. Antiproliferative effects to multiple cell types were observed, a feature that is appropriate for compounds developed for oncology, but not autoimmune indications. Inhibition of several inflammation-related readouts were observed with a sharp dose response noted between the two highest and two lowest concentrations. This suggests that the compound may have additional targets at the higher concentrations ($> 9.2 \mu\text{M}$). Robust activity was also observed in systems containing epithelial cells, indicating target expression on this cell type and potentially utility for pulmonary indications. At the top tested concentration, SNA-120 shared 12 common activities with the requested benchmark compound SR221 1, a RORy inhibitor, predominantly in the BT system. SNA-120 shared 15 common activities, also predominantly in the BT system, with the pan-AKT inhibitor GSK690693. Other top database matches with a Pearson's correlation coefficient $r > 0.7$ include BSK_4925, a Lck inhibitor, and filgotinib, a JAK inhibitor. It is noted that several activities of this test agent, particularly in the BE3C, BF4T and HDF3CGF systems, are consistent with the EGFR inhibitor mechanism class (see Figure 56). An EGFR inhibitor was among the top reference database matches for this compound, but the overall Pearson's correlation was less than $r = 0.7$, thus the relevance of the similarity is unknown. Acneiform rash is a commonly associated adverse effect of EGFR inhibitors.

Example 14: BioMAP Platform Analysis of SNA-125

Aim of Study

[664] The goal of this study was to characterize SNA-125 in the BioMAP Diversity PLUS panel of 12 human primary cell-based systems that was described in Example 13.

Materials and Methods

Test Agent

[665] SNA-125 was profiled in the BioMAP Diversity PLUS panel at concentrations of 3900 nM, 1300 nM, 430 nM, and 140 nM. Tofacitinib was employed as the benchmark compound.

Methods and Analysis

[666] BioMAP Platform Analysis was performed as described in Example 13.

Results

BioMAP Profile

[667] Figure 58 depicts the BioMAP profile of SNA-125 in the Diversity PLUS Panel. SNA-125 was found to be modestly active with 4 annotated readouts, mediating changes in key biomarker activities listed by biological and disease classifications in Table 18 below. SNA-125 mediated changes in key biomarker activities are listed by biological and disease classifications. SNA-125 impacted inflammation-related activities (decreased sTNFa) and

tissue remodeling activities (modulated MMP-1). There are no cytotoxic or antiproliferative impacts detected at the concentration range tested.

TABLE 18 - Key Biomarker Activities Impacted by SNA-125

Biological and Disease Relevance Category	Decreased activity	Modulated activity
Inflammation-related activities	sTNFa	
Tissue remodeling activities		MMP1

Reference Benchmark Overlay

[668] Figure 59 depicts an overlay of SNA-125 at 3.9 μ M and the selected reference benchmark tofacitinib at 3.3 pM. Tofacitinib is a JAK1/3 kinase inhibitor approved in 2012 for the treatment of rheumatoid arthritis. There are 5 common activities that are annotated within the following systems: BT (sIL-17A, sIL-17F, IgG, sTNFa), and IMphg (sIL-10).

[669] Differentiating biomarkers (not shown) are defined when one profile has a readout outside of the significance envelope with an effect size > 20% ($|\log_{10} \text{ratio}| > 0.1$), and the readout for the other profile is either inside the envelope or in the opposite direction. There are 39 differentiating activities between the two compounds: 3C (HLA-DR, MIG, uPAR), 4H (Eotaxin 3, P-selectin, VCAM-1, VEGFR2), LPS (CD69, IL-1a, sTNFa), SAg (CD38, CD40, CD69, E-selectin, IL-8, MCP-1, MIG, Prolif), BT (Prolif, sIL-2, sIL-6), BF4T (MMP-3, MMP-9), BE3C (I-TAC, IP-10, MIG, uPA), CASM3C (HLA-DR, LDLR, M-CSF, MCP-1, MIG, VCAM-1), HDF3CGF (IP-10, M-CSF, VCAM-1), and KF3CT (ICAM-1, IP-10, MIG).

BT System Secretion Profiles

[670] Figure 54 depicts changes in the secretion of IL-17F, IgG, IL-17A, and TNFa in the BioMAP BT system mediated by SNA-125 (3.9 pM), Tofacitinib (3.3 pM), Apremilast (3.3 pM), SR221 1 (3.3 pM), and Cyclosporin A (3.3 pM). Tofacitinib was found to be more active than SNA-125 in decreasing IL-17F secretion, displaying an activity similar to SR221 1 (Figure 54A). SNA-125 and Tofacitinib were both very active in decreasing secreted IgG, with SNA-125 as active as Cyclosporin A (Figure 54B). Surprisingly, SNA-125 was found to be as active as tofacitinib in decreasing IL-17A secretion (Figure 54C). SR221 1 decreased IL-17A secretion as expected, while Apremilast increased IL-17A secretion. SNA-125 was found to have activity with regards to reducing TNFa secretion (Figure 54D).

Top Database Search Result for SNA-125

[671] In a search for mathematically similar compound profiles from the BioMAP reference database, SNA-125 (3.9 pM) was most similar to SB203580 (10 pM) (Pearson's correlation, $r = 0.649$). The Pearson's correlation coefficient between these two profiles is below our determined threshold ($r < 0.7$) indicating that the relevance of the similarity is unknown. SB203580 is a p38 MAPK Inhibitor. Figure 60 depicts an overlay of SNA-125 (3.9 pM) and SB203580 (10 pM).

[672] There are 6 common activities that are annotated within the following systems: LPS (sTNFa), BT (SIL-17A, SIL-17F, slgG), BE3C (MMP-1), and IMphg (sIL-10).

Top BioSeek Reference Database Matches for SNA-125

[673] Table 19 depicts the top 3 similarity matches from a search of the BioMAP Reference Database of > 4,000 agents for each concentration of SNA-125. The similarity between agents is determined using a combinatorial approach that accounts for the characteristics of BioMAP profiles by filtering (Tanimoto metric) and ranking (BioMAP Z-Standard) the Pearson's correlation coefficient between two profiles. Profiles are identified as having mechanistically relevant similarity if the Pearson's correlation coefficient is ≥ 0.7 . For profiles with a Pearson's correlation coefficient below our determined threshold ($r < 0.7$), the relevance of the similarity is unknown.

TABLE 19 - Top BioMAP Reference Database Matches for SNA-125

Conc. SNA-125	Database Match	BioMAP Z-Standard	Pearson's Score	# of Common Readouts	Database Match (Mechanism Class)
0.5 μ M	SB273503, 15 μ M	0.517	0.848	148	p38 MAPK inhibitor
	SB13-729, 3.2 μ M	0.218	0.844	148	p38 MAPK inhibitor
	SB20-790, 1.5 μ M	0.167	0.841	148	p38 MAPK inhibitor
1.5 μ M	SB415286, 10 μ M	0.550	0.838	148	PI3K inhibitor
	Tekovantolol A, 41 nM	0.471	0.838	148	H2602 inhibitor
	Quercetin-TNF α , 370 μ M	0.406	0.821	148	TNF α inhibitor
400 nM	SB20-790, 3.2 μ M	0.472	0.851	148	p38 MAPK inhibitor
	S-13, 230 μ M	0.415	0.848	148	Cytokine
	Bicalutamide, 120 nM	0.347	0.844	148	See Family inhibitor
140 nM	Fenretinone, 5.15 μ M	0.286	0.838	148	PI3K inhibitor
	SAV-039, 14 nM	0.260	0.478	148	Tamoxifen 12 inhibitor
	Taxagomol, 1.1 μ M	0.577	0.450	148	EGFR inhibitor

Mechanism HeatMAP Analysis of SNA-125

[674] Figure 6.1 depicts Mechanism HeatMAP Analysis of SNA-125, with the 148 biomarker readouts within the Diversity PLUS panel compared to 19 consensus mechanism class profiles. This analysis informs on the regulatory mechanisms controlling increases or decreases in each of the biomarker readouts.

Conclusions

[675] In this study SNA-125 was characterized by profiling in the BioMAP Diversity PLUS panel of human primary cell based assays modeling complex tissue and disease biology of organs (vasculature, immune system, skin, lung) and general tissue biology. The Diversity PLUS panel evaluates the biological impact of test agents in conditions that preserve the complex crosstalk and feedback mechanisms that are relevant to in vivo outcomes. SNA-125 was selectively active in the Diversity PLUS panel, with specific inhibition of sTNFa activity in the LPS and BT systems. No cytotoxic or antiproliferative effects were observed. Inhibition of TNFa activity or production is consistent with drugs currently approved for the

treatment of psoriasis, such as anti-TNF antibodies (e.g. adalimumab and infliximab) and JAK inhibitors (e.g. tofacitinib).

Example 15: Additional BioMAP Platform Analysis of SNA-125

Aim of Study

[676] The goal of this study was to repeat the characterization of SNA-125 in the BioMAP Diversity PLUS panel of 12 human primary cell-based systems using higher concentrations than employed in Example 13.

Materials and Methods

Test Agent

[677] SNA-125 was profiled in the BioMAP Diversity PLUS panel at concentrations of 30 μ M, 10 μ M, 3.3 μ M, and 1.1 μ M. K252a was employed as the benchmark compound.

Methods and Analysis

[678] BioMAP Platform Analysis was performed as described in Example 13.

Results

BioMAP Profile

[679] Figure 62 depicts the BioMAP profile of SNA-125 in the Diversity PLUS Panel. SNA-125 was found to be active with 52 annotated readouts, mediating changes in key biomarker activities listed by biological and disease classifications in Table 20 below. SNA-125 mediated changes in key biomarker activities are listed by biological and disease classifications. SNA-125 impacted inflammation-related activities (decreased Eotaxin 3, E-selectin, MCP-1, VCAM-1, sTNFa, MIP-1a, IL-8, IL-1a, P-selectin, sPGE2, IL-6), immunomodulatory activities (decreased CD40, sIL-6, M-CSF, sIgG, sIL-10, sIL-6, sIL-17A, sIL-17F, sIL-2), tissue remodeling activities (decreased Collagen I, TIMP-2, Decorin, MMP-1, uPA, PAI-1, Collagen III, uPAR, MMP-9), and hemostasis-related activities (decreased TF; increased TM). SNA-125 is antiproliferative to B cells, endothelial cells, fibroblasts, and T cells (as indicated grey arrows of Figure 62). SNA-125 had no cytotoxic effects in the concentration range tested.

TABLE 20 - Key Biomarker Activities Impacted by SNA-125

Biological and Disease Relevance Category	Decreased activity	Increased activity
Inflammation-related activities	MCP-1, Eot3, sEot-1, VCAM-1, E-selectin, P-selectin, IL-8, IL-1a, IL-6, sTNFa, sPGE2	
Immunomodulatory activities	CD40, sIL-6, M-CSF, sIgG, sIL-10, sIL-6, sIL-17A, sIL-17F	
Tissue remodeling activities	uPAI, Col-I, Col-III, MMP-1, MMP-9, PAI-1, TIMP-2, uPA, Decorin	
Hemostasis-related activities	TF	TM

Reference Benchmark Overlay

[680] Figure 63 depicts an overlay of SNA-125 at 30 μ M and the selected reference benchmark K252a at 30 nM. K252a is a non-selective protein kinase inhibitor that inhibits

PKC, Ca²⁺/calmodulin-stimulated phosphodiesterases, MLCK, and receptor tyrosine kinases. K252a is an analog of staurosporine.

[681] There are 37 common activities that are annotated within the following systems: 3C (IL-8, Prolif, TF, uPAR), 4H (Eotaxin 3, MCP-1, P-selectin), LPS (CD40, E-selectin, IL-1a, IL-8, M-CSF, MCP-1, TF, VCAM-1, sTNFa), SAg (Prolif), BT (SIL-17A, SIL-17F, sIL-6, slgG, sTNFa), BF4T (Eotaxin 3, MMP-1), BE3C (IL-8, MMP-1, MMP-9, PAI-1, uPA, uPAR), HDF3CGF (MMP-1, Prolif 72, TIMP-2), KF3CT (MCP-1), and IMphg (E-selectin, MIP-1a, sIL-10).

[682] Differentiating biomarkers (not shown) are defined when one profile has a readout outside of the significance envelope with an effect size > 20% ($|\log_{10} \text{ratio}| > 0.1$), and the readout for the other profile is either inside the envelope or in the opposite direction. There are 21 differentiating activities within the following systems: 3C (TM), LPS (CD69, sPGE2), SAg (IL-8), BT (sIL-2), BF4T (VCAM-1), BE3C (tPA), CASM3C (IL-6), HDF3CGF (Collagen I, Collagen III, EGFR, I-TAC, IL-8, MCP-1), KF3CT (IL-8), MyoF (Decorin, MMP-1, VCAM-1), and IMphg (IL-1a, IL-8, MCP-1).

Top Database Search Result for SNA-125

[683] In a search for mathematically similar compound profiles from the BioMAP reference database, SNA-125 (30 μM) was most similar to IKK 16 (370 nM) (Pearson's correlation, $r = 0.826$). The Pearson's correlation coefficient between these two profiles is above our determined threshold ($r \geq 0.7$) indicating these compounds share mechanistically relevant similarity. IKK 16 (IKK Inhibitor VII) is selective I-kappa-b kinase (IKK) inhibitor for IKK-2, IKK complex, and IKK-1. Figure 64 depicts an overlay of SNA-125 (30 pM) and IKK 16 (370 nM).

[684] There are 24 common activities that are annotated within the following systems: 3C (IL-8, TF), LPS (E-selectin, IL-1a, IL-8, MCP-1, TF, TM, VCAM-1, sPGE2, sTNFa), SAg (IL-8, Prolif), BT (Prolif, SIL-17A, sIL-2, sIL-6, slgG, sTNFa), CASM3C (IL-6), and IMphg (E-selectin, IL-1a, IL-8, MIP-1a).

Top BioSeek Reference Database Matches for SNA-125

[685] Table 21 depicts the top 3 similarity matches from a search of the BioMAP Reference Database of > 4,000 agents for each concentration of SNA-125. The similarity between agents is determined using a combinatorial approach that accounts for the characteristics of BioMAP profiles by filtering (Tanimoto metric) and ranking (BioMAP Z-Standard) the Pearson's correlation coefficient between two profiles. Profiles are identified as having mechanistically relevant similarity if the Pearson's correlation coefficient is ≥ 0.7 .

TABLE 21 - Top BioMAP Reference Database Matches for SNA-125

REF_SNA-125	Database Match	BioMAP Z-Score	Pearson's Score	# of Common Readouts	Database Match (Mechanism Class)
30 μ M	IKK 18, 370 nM	14.022	0.820	145	IKK2 Inhibitor
	Fingolimod, 10 μ M	10.815	0.817	145	JAK1 Inhibitor
	3i-101, 120 nM	10.731	0.815	145	TK Inhibitor
10 μ M	AMG510, 1 μ M	13.700	0.813	145	p38 MAPK Inhibitor
	IKK 18, 370 nM	10.111	0.801	145	IKK2 Inhibitor
	PHA-403, 1.1 μ M	12.731	0.789	145	IKK2 Inhibitor
3.3 μ M	Dexamethasone, 110 nM	7.579	0.563	144	p38 MAPK Inhibitor
	AMG510, 17 μ M	7.449	0.555	145	p38 MAPK Inhibitor
	Dexamethasone, 330 nM	7.449	0.555	145	p38 MAPK Inhibitor
1.1 μ M	CG48506920, 19 μ M	8.370	0.601	145	Ad Inhibitor
	Docetaxel, 10 μ M	7.858	0.573	145	Sec Family Inhibitor
	Sotaterceptine Disproportionate, 10 μ M	7.732	0.569	145	OP Agonist

[686] The Pearson's correlation coefficient between profiles that is above our determined threshold ($r \geq 0.7$) indicates these compounds share mechanistically relevant similarity. For profiles with a Pearson's correlation coefficient below our determined threshold ($r < 0.7$), the relevance of the similarity is unknown.

Mechanism HeatMAP Analysis of SNA-125

[687] Figure 65 depicts Mechanism HeatMAP Analysis of SNA-125, with the 148 biomarker readouts within the Diversity PLUS panel compared to 19 consensus mechanism class profiles. This analysis informs on the regulatory mechanisms controlling increases or decreases in each of the biomarker readouts.

SNA-125 BioMAP Profile Overlay with Methotrexate and Tofacitinib

[688] Figure 66 depicts the BioMAP profile overlay of SNA-125 (10 μ M) with Methotrexate (10 μ M) and Tofacitinib (10 μ M). Differences in the LPS system and /Mphg system were observed. A similar impact on T-cell proliferation can be seen in the Figure 66 overlay of the three compounds. It is noted that Tofacitinib activities in 3C consistent with systemic side effects, not present with SNA-125

Conclusions

[689] In this study SNA-125 was characterized by profiling in the BioMAP Diversity PLUS panel of human primary cell based assays modeling complex tissue and disease biology of organs (vasculature, immune system, skin, lung) and general tissue biology. The Diversity PLUS panel evaluates the biological impact of test agents in conditions that preserve the complex crosstalk and feedback mechanisms that are relevant to in vivo outcomes.

[690] SNA-125 was broadly active across the Diversity PLUS panel with 52 annotated readouts affecting biomarkers involved with inflammation, immunomodulation, tissue remodeling, and hemostasis. SNA-125 was antiproliferative to B cells, endothelial cells, fibroblasts, and T cells and had no effects on cytotoxicity at the concentration range tested.

SNA-125 was previously profiled in the Diversity PLUS panel at a lower concentration range (140 nM - 3900 nM, refer to project #SNE001-01-b), where only inhibition of TNF α and modulation of MMP-1 were observed. SNA-125 and its reference benchmark K252a, a non-selective protein kinase inhibitor, had 37 common activities and 21 differentiating activities; in general, SNE-SNA-125 was more active at the top tested concentration (30 μ M) than the top tested concentration of K252a (30 nM). The top database search match for SNA-125 was IKK 16, an IKK2 inhibitor, and that match was above our threshold for significance ($r \geq 0.7$). There were 24 common activities between SNA-125 and IKK2, most of which were in the LPS system modeling Th1 type inflammation and monocyte activation and the BT system modeling T cell dependent B cell activation. Inhibitors of IKK2 and p38 MAPK were among the top matches for this compound, indicating that SNA125 may have phenotypic impacts on the NF κ B and/or MAPK signaling pathways.

Example 16: Testing SNA-125 in an IMQ-induced Psoriasis Mouse Model

Background

[691] Imiquimod (IMQ) is a TLR7/8 ligand and a potent immune activator that is used topically for genital and perianal warts, as well as for superficial basal cell carcinomas and actinic keratosis. An acute model of psoriasis has been developed in mice by topical application of a 5% IMQ cream (Aldara™), based on the clinical observation that IMQ exacerbates psoriasis. Similar to human psoriasis, IL-23/IL-17 axis plays a pivotal role in IMQ-induced psoriasis in mice.

[692] In a study by Ma et al. (J Clin Cell Immunol 2013, 4:6), oral Tofacitinib was tested in the IMQ-induced psoriasis model. Topical daily dose of IMQ was administered on the back and right ear for 3 consecutive days followed by an additional application on Day 5. 30 mg/kg of tofacitinib was given orally as a prophylactic treatment, twice daily starting on Day 0. There, ear thickness was measured on Day 5 and cytokines transcripts from mouse ears at study termination. Here, different concentrations of SNA-125 were tested in the clinically relevant IMQ-induced psoriasis mouse model.

Materials and Methods

[693] The IMQ-induced psoriasis mouse study design is depicted in Figure 67 and the test groups are shown in Table 22 below. Animals were monitored daily for clinical symptoms, body weight, and psoriasis clinical score. Ear thickness was measured on days 0, 4, 6, 8 and 10. Cytokines levels were measured on day 4 on ear punch biopsies. Spleen weight and histology (H&E) analysis of back skin were performed on day 10.

TABLE 22 - Test Groups

Group Number	Group Size	Group Description	Disease Induction	TI/vehicle Route	TI/vehicle Dose Level	Dosing Route/Regimen
1	N=5	Naïve	NA	NA	NA	NA
2	N=10	IMQ Only	5% IMQ on back and on both ears daily	NA	NA	NA
3	N=10	Clobetasol		Topical	50mg applied to backs, 5 mg applied to each ear	1×daily, 2 hrs post IMQ application
4	N=10	Vehicle*		Topical	100 µl applied to back, 20 µl applied to each ear	2×daily, 2 and 8 hrs post IMQ application
5	N=10	0.5% SNA-125				
6	N=10	1.0% SNA-125				
7	N=10	5.0% SNA-125				

* 25% Transcutol P, 75% Propylene Glycol

Results

Body weight

[694] As shown in Figure 68, one-time daily administration of IMQ onto the shaved back and right ear of the animals resulted in body weight loss as compared to naïve animals. Two times daily treatment with 3 different doses of SNA-125 modestly increased the body weight over IMQ only animals at the beginning of the study as compared to vehicle-treated mice. The observed dramatic body weight loss in clobetasol-treated animals is a known side effect of the steroid and was expected.

Psoriasis clinical score

[695] Figure 69 depicts the changes in the total psoriasis score throughout the study, which was calculated by summing the plaque score, the erythema score and the punctate redness/scabbing score. The difference between SNA-125 at 5% and the vehicle is statistically significant from day 7. The differences between SNA-125 at 0.5% and 1% and the vehicle are statistically significant on day 10 only.

Erythema score

[696] Figure 70 depicts the changes in the erythema score throughout the IMQ-induced psoriasis mouse study. SNA-125 at 5% is statistically significant from the vehicle from day 7. SNA-125 at 0.5% and 1% are statistically significant on day 10 only.

Plaque Scores

[697] Figure 71 depicts the changes in the plaque score throughout the IMQ-induced psoriasis mouse study. SNA-125 at 5% is statistically significant from the vehicle on day 10.

Punctate Redness/scabbing Scores

[698] Figure 72 depicts the changes in the punctate redness/scabbing score throughout the IMQ-induced psoriasis mouse study. As expected, IMQ only animals exhibited increased punctate redness and scabbing, which was diminished by SNA-125 application.

Spleen Weight and Ear Thickness

[699] Figures 73 and 74 depict the changes in spleen thickness and ear thickness throughout the IMQ-induced psoriasis mouse study, respectively. Topical application of the IMQ cream causes the enlargement of spleen and lymph nodes, and increased ear thickness. The commonly used antipsoriatic agent clobetasol almost completely attenuated these IMQ-induced changes. Neither treatments with vehicle or test compound significantly modulated spleen weight. There was no difference on ear thickness found between the vehicle and 3 different doses of SNA-125.

[700] Daily application of IMQ induced significant splenomegaly in IMQ only animals on day 10, which is indicative of a systemic inflammatory response. Daily treatment with clobetasol significantly decreased splenomegaly as seen by a reduction in spleen weight. Neither treatments with vehicle or test compounds significantly modulated the spleen weight on day 10. However, there was a slight non-significant reduction in spleen weight between the vehicle (mean 0.180 ± 0.014) and 5% SNA-125 treatment group (0.155 ± 0.007).

[701] Daily application of IMQ significantly increased ear thickness and positive control clobetasol showed significant reduction on the ear thickness induced by IMQ. All treatments including vehicle showed significant ear thickness reduction. However, there was no difference on ear thickness found between the vehicle and 3 different doses of SNA-125.

Cytokine Levels in Ear Samples

[702] Figure 75 depicts the levels of cytokines IL-22, IL-17A, IL17F, and TNFa in ear samples taken at Day 4. Note that while the cytokines were measured on day 4, different cytokines can have different pharmacodynamics behavior (i.e. peak at different days post IMQ application). Daily application of IMQ induced an increase (significant in IL-22, IL-17F and TNFa) in inflammation/psoriasis-associated cytokines in the ears of diseased animals on day 4. Daily treatment with clobetasol decreased the cytokine production to baseline levels. As compared to vehicle group, twice daily treatment with 5% SNA-125 had no significant effects on cytokine production, though a moderate decrease in two pathogenic cytokines, TNFa (vehicle, mean 9.331 ± 2.267 ; 5% SNA-125, mean 4.860 ± 0.973) and IL-22 (vehicle, mean 9.650 ± 2.339 ; 5% SNA-125, mean 5.243 ± 1.759) was observed.

Conclusions

[703] Different concentrations of SNA-125 were found to have an ameliorative effect when tested in the clinically relevant IMQ-induced psoriasis mouse model.

Example 17: Plasma Pharmacokinetic Profile of CT327 (SNA-120) Following a Single IV Administration to Rats

Aim of the Study

[704] The purpose of the study was to investigate the pharmacokinetic profile of CT327 (SNA-120) when given by intravenous route to female rats with a single IV administration at 18 mg/kg body weight. This Example reports the analyses of CT327 plasma levels in the blood samples collected from 5 minutes to 8 hours after drug administration.

Materials and methods

IV administration to rats and collection of blood samples

Study Design

[705] A single administration of CT327 was given to rats and blood samples were collected at scheduled times in order to verify the kinetic profile. One group of 9 female rats received the test item by the intravenous injection, at 18 mg/kg dose level.

[706] The animals were monitored for body weight and clinical signs during the *in vivo* phase of the study. On the day of dosing, bleeding was carried out at the following time points: before dosing, 5, 10, 20 and 30 minutes, 1, 2, 4 and 8 hours after the administration. Plasma samples were stored for subsequent analysis.

Vehicle and Formulation Procedure

[707] The vehicle was physiological saline solution (NaCl 0.9%). The required amount of CT327 was dissolved in the vehicle at the concentration of 3.6 mg/ mL. Concentration of the test item was calculated in terms of active ingredient according to the assay results (97.7%), as reported in the certificate of analysis. The formulation was prepared on the day of dosing. Since the formulation had to be administered within 30 minutes after preparation and on the basis of the bleeding scheme, three formulations of the test item were prepared at the scheduled times.

Treatment

[708] The study consisted of one group comprising 9 female rats. The test item was administered by intravenous bolus injection into the tail vein over a period of approximately 1 mL/minute at a dose volume of 5 mL/kg body weight. The dose was administered to each animal on the basis of the most recently recorded body weight and the volume administered was recorded for each animal. Each animal received a single dose.

Blood collection

[709] Blood samples were collected from the animals at the following sampling times: 0 (predose), 5, 10, 20 and 30 minutes, 1, 2, 4 and 8 hours after the single dose administration. Blood samples were collected from each animal at 3 alternating time points. The theoretical and actual times of collection were recorded. At each sampling time, blood samples of approximately 0.8 mL each were collected under light isoflurane anaesthesia from the

retroorbital sinus of 3 animals and transferred into light-protected tubes containing EDTA anticoagulant, immediately centrifuged at 4°C and 3000g for 10 minutes and the plasma frozen at -80°C. Two different aliquots were prepared: the first contained 250 µL of plasma and the second (100 µL). In addition, about 10 mL of plasma were obtained from 5 untreated female rats of the same batch of the study for analytical (calibration curve) purposes.

Animal Observation

[710] Examination of individual animals for signs of reaction to treatment was carried out immediately after dosing, 15-30 minutes, 1 and 4 hours after dosing. No signs were recorded at clinical examination. All animals did not show reactions to treatment.

[711] Each animal was weighed on the day of treatment. Body weights were within the expected range for this strain and age of animals.

Analysis of CT327 plasma levels in obtained blood samples

Materials

CT327

H₂O MilliQ

Rat plasma (pool obtained from 5 untreated female rats of the same batch "stock order 661" used for the experiment, i.e. Hsd: Sprague Dawley SD rats, 45-53 days old and with body weight of approximately 150-174 g)

Acetonitrile (ACN) HPLC grade

Methanol HPLC grade

Solid Phase Extraction (SPE) cartridges, Oasis® HLB 1 cc/10mg, particle size 30 µm
HPLC column XTerra® RP18 particle size 3.5 µm, 3.0x50 mm

Methods

[712] The analysis of CT327 plasma levels was performed by HPLC using a Beckman System Gold® chromatograph 126 solvent module, 168 UV-Vis Detector, 508 autosampler, equipped with a XTerra® RP18 column 3.0 x 50 mm.

[713] Plasma samples were purified before HPLC analysis, in order to eliminate any possible source of interference, by SPE technique using Waters Oasis® HLB (Hydrophilic-Lipophilic Balance reverse-phase sorbent) cartridges. The purification of the biological samples consisted in the following consecutive steps: (i) conditioning of the cartridge with 1 mL of methanol; (ii) equilibration with 1 mL of MilliQ water; (iii) loading of the sample properly diluted in water (i.e. 0.1 mL of plasma sample in 0.9 mL of water, to give a total volume of 1.0 mL); (iv) washing of the unbound components with 5% methanol in water; and (v) elution with 1 mL of methanol.

[714] The purification was performed using an extraction manifold (supplied by Waters S.p.A., Vimodrone, Italy) able to accommodate up to 20 cartridges and connected to a dual-headed vacuum pump (supplied by VWR International Srl, Milano, Italy).

[715] The eluate from each biological sample was collected in polypropylene vials, evaporated under vacuum for about 4 hours by a Speed Vac system (Eppendorf concentrator 5301) and the residues were dissolved in 100 μ L of MilliQ H_2O /ACN 160/40, in order to proceed to test item quantitation. To get complete dissolution each sample was sonicated in an ultrasound bath for 10 sec., vortexed for 10-20 sec. and then briefly centrifuged by a microcentrifuge (MiniFuge VWR International Sri., max. RCF 2000g).

[716] The quantitative analysis was achieved by HPLC by means of an appropriate calibration curve obtained in the same processing conditions of the unknown samples. CT327 standard samples in plasma were prepared with the above described procedure by purifying blank plasma samples (rat plasma recovered from untreated animals) spiked with a solution of test item in MilliQ H_2O up to a volume of 0.1 ml, as depicted below in Tables 23 and 24.

TABLE 23 - Calibration curve at low CT327 concentrations

CT327 concentration (nM)	Spiking of rat plasma
50	5.0 μ L CT327 1 μ M + 10.0 μ L MilliQ water + 85.0 μ L rat plasma
250	2.5 μ L CT327 10 μ M + 12.5 μ L MilliQ water + 85.0 μ L rat plasma
450	4.5 μ L CT327 10 μ M + 10.5 μ L MilliQ water + 85.0 μ L rat plasma
650	6.5 μ L CT327 10 μ M + 8.5 μ L MilliQ water + 85.0 μ L rat plasma
850	8.5 μ L CT327 10 μ M + 6.5 μ L MilliQ water + 85.0 μ L rat plasma
1050	10.5 μ L CT327 10 μ M + 4.5 μ L MilliQ water + 85.0 μ L rat plasma
1250	12.5 μ L CT327 10 μ M + 2.5 μ L MilliQ water + 85.0 μ L rat plasma
1450	14.5 μ L CT327 10 μ M + 0.5 μ L MilliQ water + 85.0 μ L rat plasma

TABLE 24 – Calibration curve at high CT327 concentrations

CT327 concentration (μ M)	Spiking of rat plasma
1	2.0 μ L CT327 50 μ M + 13.0 μ L MilliQ water + 85.0 μ L rat plasma
5	10.0 μ L CT327 50 μ M + 5.0 μ L MilliQ water + 85.0 μ L rat plasma
10	2.0 μ L CT327 500 μ M + 13.0 μ L MilliQ water + 85.0 μ L rat plasma
15	3.0 μ L CT327 500 μ M + 12.0 μ L MilliQ water + 85.0 μ L rat plasma
20	4.0 μ L CT327 500 μ M + 11.0 μ L MilliQ water + 85.0 μ L rat plasma
25	5.0 μ L CT327 500 μ M + 10.0 μ L MilliQ water + 85.0 μ L rat plasma
30	6.0 μ L CT327 500 μ M + 9.0 μ L MilliQ water + 85.0 μ L rat plasma
35	7.0 μ L CT327 500 μ M + 8.0 μ L MilliQ water + 85.0 μ L rat plasma

[717] HPLC separation and analysis were performed at a flow rate of 1 mL/min, with detection by measurement of the absorbance at 280 and 294 nm (the latter wavelength representing the maximum UV/Vis absorption peak of CT327 and therefore used for quantitative analysis). The injection volume was 25 μ L and each sample was analysed in triplicate, keeping the autosampler at 4°C and with the elution gradient shown in Table 25 (eluent A= MilliQ water, eluent B = acetonitrile).

TABLE 25 - Elution Gradient

Time (min)	Eluent A (%)	Eluent B (%)
0	95	5
2	95	5
8	29	71
9.1	0	100
11.1	0	100
12.1	95	5
14.1	95	5

Results

[718] Using the above described conditions the peak corresponding to CT327 shows a retention time of about 8.5 minutes. The quantitation of CT327 in the chromatograms was performed by integration of the corresponding peak with the following parameters: peak threshold 200 and peak width 0.2. Blank plasma samples were also analysed and included in the sample analysis run in order to control the specificity of the analytical procedure.

[719] The calibration curve (peak area *versus* test item concentration) is shown in Figure 76 for plasma samples spiked with CT327 and was obtained by plotting the detected response versus the nominal sample concentration. The values of correlation coefficient (r^2) suggest a good linearity over the range investigated, with a LOD (Limit of Detection) of 35.5 nM and a LOQ (Limit of Quantification) of 69.8 nM for the calibration curve at low concentration and with LOD 0.9 μ M/LOQ 1.7 μ M for the calibration curve at high concentration.

[720] Mean plasma concentrations of CT327 were calculated for each time point on the above reported calibration curves (low range curve for ≤ 1 h) using a standard spreadsheet software (Microsoft® EXCEL®) and are reported in Table 26. The results are plotted as mean values $\pm$ error (95% CI, confidence interval) in Figure 77. Each point represents the average of 9 analyses, corresponding to 3 mice for each time point and HPLC injection in triplicate.

TABLE 26 - Detailed Results of Pharmacokinetic Analysis

Time	Average Area	CT327 Concentration (μM)	CI (95%)
5 min	2585775	33.36	1.09
10 min	1666038	21.52	0.89
20 min	883565	11.45	0.92
30 min	633148	8.22	0.96
1 h	173618	2.31	1.08
2 h	24904	0.3948	0.03834
4 h	5769	0.08102	0.04443
8 h	3116	0.03751	-

[721] As expected, CT327 reached the maximum peak concentration at the first sampling time point (5 minutes) and quantifiable levels were observed until 4 hours post-administration in all the animals.

[722] Pharmacokinetic evaluation of the mean data was performed according to a noncompartmental analysis using NCOMP software version 3.1 (Paul B. Laub, Fox Chase Cancer Center, Philadelphia, USA). Using Lagrange polynomials method, an interpolating curve between each pair of adjacent data points was constructed and the corresponding partial area computed.

[723] The following parameters were determined:

CL (clearance, in L/h), expressed as the volume of blood cleared of drug per unit time;

MRT (mean residence time, in h);

V_{ss} (apparent volume of distribution at steady state, in L);

$t_{1/2}$ (terminal half-life, in h), determined by one-phase exponential decay fitting (GraphPad Prism software analysis). The correlation coefficient (r^2) for the goodness of the fit of the regression line through the data points was high enough (0.991) for the value to be considered reliable; the 95% confidence interval for the $t_{1/2}$ estimate was 0.13-0.23 h;

C_{max} (initial concentration at $t = 0$ hours, in μM), extrapolated by log-linear regression of the first two measured data points;

[724] $\text{AUC}_{0-\infty}$ (area under the curve from time 0 to infinity, in $\mu\text{M}\cdot\text{h}$), calculated by extrapolation of area from the time of the last measurement to time infinity and computed by estimating the terminal disposition rate by least squares fitting to slope of the logarithm of

concentration vs. time. $AUC_{0-\infty}$ was considered reliable since the extrapolation from the last data point to infinity represented only the 0.1 % of the total $AUC_{0-\infty}$.

[725] The mean pharmacokinetic parameters are summarized in Table 27 below.

TABLE 27 - PK parameters of CT327 after intravenous administration in rat

PK parameters	Dose 18 mg/kg, IV bolus
CL (L/h)	0.11
MRT (h)	0.51
V_{ss} (L/kg)	0.055
Terminal $t_{1/2}$ (h)	0.17
C_{max} (μ M)	51.88
$AUC_{0-\infty}$ (μ M*h)	14.01

Discussion and Conclusion

[726] The pharmacokinetics of CT327 was investigated after intravenous administration to rats in order to assess the systemic exposure, distribution and elimination of the drug candidate. All the analyzed samples (3 animals/timepoint) showed detectable and consistent CT327 plasma levels up to 8 hours after administration.

[727] CT327 elimination from the circulation system was extremely rapid, as plasma concentrations of the administered compound declined monoexponentially with a half-life of 10 minutes.

[728] Most interestingly, the calculated apparent volume of distribution V_{ss} was 55 mL/kg, i.e. 10.78 mL, corresponding to rat blood volume (BV), considering an average animal body weight (BW) of 196 g and the generally accepted figure of BV as 7% BW. These data suggest a full drug compartmentalization within the vascular compartment with no or very low distribution into tissues.

[729] In conclusion, the observed time course of CT327 plasma levels confirmed its surprising and unique chemico-physical properties conferred by its mini-PEG moiety, resulting in high systemic exposure upon IV bolus (thanks to high water solubility), very rapid elimination from the blood stream (low molecular weight, probable fast kidney filtration with urinary elimination) and large confinement to the circulatory system (the compound undergoes no or very limited distribution from blood into tissues). These overall features are in line with the optimal safety profile shown by CT327 from a toxicological point of view: even if absorbed upon topical administration, one can exclude drug distribution and accumulation thanks to an extremely rapid clearance.

Example 18: Efficacy Study of SNA-125 and SNA-352 in the Treatment of Oxazolone-Induced Colitis

Aim of Study

[730] The goal of this study was to characterize the efficacy of SNA-125 and SNA-352 delivered by oral and intracecal routes for the treatment of colitis with the use of an oxazolone-challenged mouse model of colitis. For comparison, oxazolone-challenged mice were also treated with Tofacitinib and Prednisolone by oral and intracecal routes.

Study Design

[731] Two hours following the Day 0 AM Treatment dose, colitis was induced in 114 male BALB/C mice by intrarectal administration of 100 μ L of 2% Oxazolone (OXZ) under isoflurane anesthesia on day 0. One additional group of eight animals served as no-disease controls (Group 1). Animals were dosed with test article twice daily (BID) via oral gavage (PO) or intracecal (IC) as indicated in Table 28. All animals were weighed daily and assessed visually for the presence of diarrhea and/or bloody stool at the time of dosing. Mice had video endoscopy on Days 2 & 4 to assess colitis severity. Additionally, stool consistency was scored during endoscopy. Following endoscopy on day 4, all animals from each treatment group were sacrificed and blood collected. Following euthanasia, the colon was excised, rinsed, measured, weighed, and then trimmed to 6.0 cm in length and divided into 2 pieces as outlined in Figure 78A; the most distal 5.0 cm section was swiss rolled and placed in formalin for subsequent histological evaluation. The details of the study design are shown in Table 28.

TABLE 28 - Study Design

Group	Number of Animals	OXA Day 0	Treatment	Dose	Route	Dose Schedule (12 Hours Apart)
1	8	---	---	---	---	---
2	15	2%	Vehicle	---	PO	BID Days -1 to 4
3	15		Vehicle	---	IC	BID Days -1 to 4
4	12		Tofacitinib	15mg/kg	PO	BID Days -1 to 4
5	12		Tofacitinib	1mg/kg	IC	BID Days -1 to 4

6	12		Prednisolone	1mg/kg	PO	BID Days -1 to 4
7	12		Treatment A (SNA-125)	400mg/kg	PO	BID Days -1 to 4
8	12		Treatment B (SNA-352)	400mg/kg	PO	BID Days -1 to 4
9	12		Treatment A (SNA-125)	400mg/kg	IC	BID Days -1 to 4
10	12		Treatment B (SNA-352)	400mg/kg	IC	BID Days -1 to 4

Experimental Procedures

Disease Induction

[732] Two hours following the Day 0 AM treatments, colitis was induced in 114 male BALB/C mice by intrarectal administration of 100 μ L of 2% OXZ under isoflurane anesthesia on day 0. Mice were maintained in vertical position for 1 minute after intra-rectal administration to ensure the complete distribution of OXZ/vehicle throughout the colon.

Cecal/Colon Cannulation

[733] All animals were allowed a minimum of 7 days to recover from surgery. Animals were placed under isoflurane anesthesia, and the cecum was exposed via a mid-line incision in the abdomen. A small point incision was made in the distal cecum through which 1-2 cm of the cannula (Norfolk Medical MMP-3S mouseport with a 3 french silicone catheter and 2 moveable beads for securing suture) was inserted and directed into the proximal colon.

Dosing

[734] Animals were dosed with test article twice daily (BID) via oral gavage (PO) or intracecal (IC) as indicated in Table 28.

Endoscopy

[735] Each mouse underwent video endoscopy on Days 2 & 4 using a small animal endoscope (Karl Storz Endoskope, Germany), under isoflurane anesthesia. During each endoscopic procedure still images as well as video were recorded to evaluate the extent of colitis and the response to treatment. Additionally, an image was captured from each animal at the most severe region of disease identified during endoscopy. Colitis severity was scored using a 0-4 scale as defined in Table 29. Additionally, stool consistency was scored during endoscopy using the parameters defined in Table 30.

TABLE 29 - Endoscopy Colitis Severity Scoring Criteria

Score	Description
0	Normal

1	Loss of vascularity
2	Loss of vascularity and friability
3	Friability and erosions;
4	Ulcerations and bleeding

TABLE 30 - Stool Consistency Scoring Criteria

Score	Description
0	Normal, well-formed pellet
1	Loose stool, soft, staying in shape
2	Loose stool, abnormal form with excess moisture
3	Watery or diarrhea
4	Bloody diarrhea

Sample Collection

[736] Peripheral blood and colon tissue were collected at sacrifice on day 4 as follows. Blood was collected via cardiac puncture into KiEDTA-coated tubes and centrifuged at 4000x g for 10 minutes. Plasma was collected, flash frozen, and stored at -80°C. The colon was excised, rinsed, measured, weighed, and then trimmed to 6.0 cm in length and divided into 2 pieces; the most distal 5.0 cm section was swiss rolled and placed in formalin for subsequent histological evaluation (see Figure 78A). The proximal 1.0 cm portion was weighed, snap frozen, and stored at -80°C.

Histopathology

[737] Each colon sample was rolled into a swiss roll. Tissues were embedded in paraffin and sectioned at approximately 5 microns. One slide for each colon was stained with hematoxylin and eosin and examined by a board-certified veterinary pathologist. The pathologist was blinded to the treatment that each group received at the time of assessment. Each slide containing one rolled colon was split into four approximately equal quarters. Each quarter was evaluated and scored for inflammation, edema, and mucosal necrosis, according to the scoring criteria listed below in Tables 31 to 33. As depicted in Figure 78B, with the slide label to the left, quarters were evaluated starting at the top left and moving clockwise.

TABLE 31 -Inflammation Histopathologic Scoring Criteria

Score	Description
0	None present
1	Minimal change: Focal aggregate of a few cells of minimal diffuse inflammation
2	Mild change: Larger focal aggregates, multifocal small aggregates, or diffuse mild inflammation

3	Moderate change: Multifocal aggregates sometimes coalescing with one another or moderate diffuse change
4	Severe change: Marked diffuse inflammation

TABLE 32 - Edema Histopathologic Scoring Criteria

Score	Description
0	None present
1	Minimal change: Focal edema or minimal diffuse edema
2	Mild change: Larger focal areas of edema or diffuse mild edema
3	Moderate change: Multifocal areas of edema coalescing with one another or moderate diffuse edema
4	Severe change: Marked diffuse edema

TABLE 33 - Mucosal Necrosis/Loss Histopathologic Scoring Criteria

Score	Description
0	None present
1	Minimal change: Focal epithelial necrosis
2	Mild change: Larger focal areas of necrosis or multifocal areas of necrosis
3	Moderate change: Multifocal necrosis coalescing into larger areas
4	Severe change: Areas of erosion through the mucosa to the submucosa

Multiplex Analysis of Colon Tissue Homogenate Samples

[738] Colon tissue homogenate supernatants were analyzed for protein levels of a panel of mouse inflammatory mediators: IFN- γ , IL-10, IL-6, & TNF- α using a multiplex system (MAGPIX, EMD MNipore).

ResultsIn-life Observations

[739] Figure 79 depicts the effect of oral and intracecal administration of SNA-125, SNA-352, tofacitinib, and prednisolone on the body weight of animals challenged with oxazolone. Figure 80 depicts this data according to last observation carried forward analysis. A reduction of the body weight of animals treated with oxazolone was observed. A trend towards a decrease of the body weight loss was observed in the oral SNA-125 administration group.

Endoscopy Results

[740] Figures 81 and 83 depict the effect of oral and intracecal administration of SNA-125, SNA-352, tofacitinib, and prednisolone on the Day 2 and 4 endoscopy scores, respectively, of animals challenged with oxazolone. At Day 2, oral and intracecal Tofacitinib administration yielded a 10-15% improvement in endoscopy scores. Surprisingly, orally administered SNA-125 demonstrated a 22% reduction of the Day 2 endoscopy score. At Day 4, oral and

intracecal Tofacitinib administration yielded a 10-15% improvement in endoscopy scores. Surprisingly, orally administered SNA-125 yielded a 15% reduction of the endoscopy score. Figures 108 to 113 depict representative Day 2 and Day 4 endoscopy images.

[741] Figures 82 and 84 depict the effect of oral and intracecal administration of SNA-125, SNA-352, tofacitinib, and prednisolone on the Day 2 and 4 stool consistency scores, respectively, of animals challenged with oxazolone. Oral SNA-125 showed a significant reduction (65%) in the stool consistency score on Day 2.

Disease Activity Index

[742] Figure 85 depicts the effect of oral and intracecal administration of SNA-125, SNA-352, tofacitinib, and prednisolone on the disease activity index (DAI) score of animals at Days 2 and 4 following challenge with oxazolone.

Colon Weight/Length Ratio

[743] Figure 86 depicts the effect of oral and intracecal administration of SNA-125, SNA-352, tofacitinib, and prednisolone on the colon weight/length ratio of animals challenged with oxazolone.

Histopathology Scoring Results

[744] Oxazolone produced mild to moderate colitis characterized by multifocal inflammation, edema, and necrosis.

[745] Figures 87-89 depict the histopathology scoring results for inflammation, edema, and mucosal necrosis/loss, respectively, while Figure 90 depicts the summation of these scores. Note that control animals not given oxazolone were essentially normal and were not included in the statistical analysis.

Inflammation Scoring

[746] As shown in Figure 87, untreated control animals that did not receive oxazolone had minimal scattered background inflammation, while all other animals were given oxazolone and had varying degrees of inflammation. Inflammation tended to be mild to moderate with some regions more severely affected than others. When including all groups in the analysis, the treatment effect approached significance (one-way ANOVA, $p = 0.0656$). When oral and intracecal groups were analyzed independently treatment did significantly influence inflammation for oral groups (one-way ANOVA, $p = 0.0199$) but not for intracecal groups (one-way ANOVA, $p = 0.5970$).

[747] In the oral groups, both SNA-125 and SNA-352 tended to reduce inflammation compared to vehicle, and this improvement was more noticeable than for either prednisolone or tofacitinib.

Edema Scoring

[748] As shown in Figure 88, untreated control animals that did not receive oxazolone had minimal, random edema, while all other animals were given oxazolone and had varying

degrees of edema that was generally associated with inflammation. Edema tended to be mild to moderate with some regions more severely affected than others. When including all groups in the analysis, treatment did not have a significant effect on edema (one-way ANOVA, $p = 0.2845$). When oral and intracecal groups were analyzed independently, the oral groups approached significance (one-way ANOVA, $p = 0.0855$) but not the intracecal groups (one-way ANOVA, $p = 0.4033$). In the oral groups, both SNA-125 and SNA-352 tended to reduce edema compared to vehicle and this improvement was more noticeable than for either prednisolone or tofacitinib.

Mucosal Necrosis Results

[749] As depicted in Figure 89, untreated control animals that did not receive oxazolone did not have any mucosal necrosis, while all other animals were given oxazolone and had varying degrees of multifocal mucosal erosion and necrosis. Necrosis tended to be regional with some areas more severely affected than others and some animals more severely affected than others.

[750] In the oral groups, both SNA-125 and SNA-352 tended to reduce mucosal necrosis compared to vehicle and this improvement was more noticeable than for either prednisolone or tofacitinib.

Sum Score Results

[751] As shown in Figure 90, summed histopathology scores (Inflammation + Edema + Mucosal Necrosis) were moderate in all animals administered oxazolone.

[752] In the orally treated groups, both SNA-125 and SNA-352 tended to reduce the sum score compared to vehicle, and this improvement was more noticeable than for either prednisolone or tofacitinib. For inflammation, this treatment effect was statistically significant while for edema and the sum score, this effect approached significance.

[753] Overall, intracecal treatment was found to be less effective than an oral route of administration.

Analysis of Colon Histopathology Micrographs

[754] Sections of colon were often thickened by inflammation and edema that variably extended into the lamina propria, submucosa and muscular wall. The inflammation was pyogranulomatous - composed of a mixture of neutrophils, macrophages, lymphocytes, and plasma cells. Mucosal necrosis was also variably present and characterized by partial or complete loss of the surface epithelium with erosion to underlying lamina propria or submucosa. There was multifocal peritonitis suggesting that there was multifocal full thickness erosion. Due to the multifocal distribution of these changes, the inflammation, edema, and mucosal necrosis were variable along the swiss rolled section. Representative photomicrographs are shown in Figures 91-93.

Control Animals

[755] As seen in Figure 91, control animals in this study had basically normal colons without significant inflammation, mucosal necrosis, or edema.

Orally-treated Animals

[756] Administration of oxazolone was associated with the development of multifocal mucosal ulceration, inflammation, and edema which was seen in all groups to varying extent. Vehicle-treated animals had moderate inflammation (unfilled arrows) with edema (filled arrows) and multifocal ulceration (bracket).

[757] Treatment with prednisolone or tofacitinib did not demonstrably reduce colitis. Animals still had a diffuse increase in background inflammation with multifocal pockets of more severe inflammation and multifocal mucosal ulceration (Figure 92).

[758] Treatment with both SNA-125 and SNA-352 orally tended to reduce the severity of colitis. These animals tended to have a more mild increase in background inflammation without significant ulceration.

Intracecal-treated Animals

[759] The intracecal vehicle group had relatively mild colitis compared to other groups. While there were some areas in some animals with inflammation, edema, and mild necrosis (inset), most of the colons had mild inflammation with mild edema and minimal to no necrosis (Figure 93). Animals treated with intracecal tofacitinib had diffuse inflammation with pockets of more severe inflammation and mucosal necrosis. Animals treated intracecal with SNA-125 were divided between mild colitis and severe colitis. Animals treated intracecally with SNA-352 had reduced colitis compared to tofacitinib treated animals, but since the vehicle group had such mild colitis there was no noticeable change compared to vehicle.

Multiplex Analysis of Colon Homogenates

[760] Figures 94-97 depicts the effect of oral and intracecal administration of SNA-125, SNA-352, tofacitinib, and prednisolone on the levels of IFN γ , IL-10, IL-6 and TNF α in colon homogenates. Oral and intracecal SNA-352 administration decreased IFN γ . Intracecal SNA-125 significantly increased TNF α as compared to the intracecal vehicle control (with the intracecal control outlier removed). Oral tofacitinib significantly increased IL-10 levels, and a strong trend was also observed for the intracecal Tofacitinib group.

Conclusions

[761] Oxazolone produced mild to moderate colitis characterized by multifocal inflammation, edema, and necrosis. Changes tended to be multifocal in nature with some areas affected more severely than others. It appeared that the distal colon was more severely affected than the proximal colon. Clear signs of efficacy for both SNA-125 and SNA-352 were observed. Overall, there was a clear trend for both SNA-125 and SNA-352 to improve colitis in this model. Both compounds showed comparable or even better results

than tofacitinib and prednisolone. SNA-125 seems to work differently if administered orally or intracecally (intracecal group data affected by animal loss).

[762] Oral treatment with both SNA-352 and SNA-125 tended to reduce inflammation, edema, and necrosis compared to oral treatment with vehicle, tofacitinib, or prednisolone. For inflammation, this treatment effect was statistically significant while for edema and the sum score, this effect approached significance. In contrast, treatment with tofacitinib and prednisolone orally were ineffective at treating oxazolone-induced colitis in this study.

[763] Intracecal treatment was less effective. Treatment with intracecal SNA-352 may have mildly reduced colitis compared to tofacitinib treatment and SNA-125; however any change was mild and not statistically significant.

Conclusions

[764] Different concentrations of SNA-125 were found to have an ameliorative effect when tested in the clinically relevant IMQ-induced psoriasis mouse model.

EXAMPLE 19: MOUSE MODEL OF IMQ-INDUCED PSORIASIS

Aim of Study

[765] The objective of this study was to determine the efficacy of SNA-101, SNA-125, and SNA-352 as a therapeutic in the mouse model of IMQ-induced psoriasis.

Methodology

Test groups and experimental timing

[766] Table 34 depicts the test groups and Figure 98 depicts the timing of the experiments performed in this study.

TABLE 34: TEST GROUPS

Group Number	Group Size	Group Description	Disease Induction	TI/vehicle Route	TI/vehicle Dose Level	Dosing Route/Regimen
1	N=5	Naïve	NA	NA	NA	NA
2	N=10	IMQ Only	5% IMQ on back and on both ears daily	NA	NA	NA
3	N=10	Clobetasol		Topical	50mg applied to backs, 5 mg applied to each ear	1×daily, 2 hrs post IMQ application
4	N=10	Vehicle		Topical	100 µl applied to	2×daily, 2 and 8 hrs post IMQ
5	N=10	1.0% SNA-352				

6	<i>N=10</i>	5.0% SNA-352			back, 10 μ l applied to each ear	application
7	<i>N=10</i>	10.0% SNA-352				
8	<i>N=10</i>	5.0% SNA-125				
9	<i>N=10</i>	10.0% SNA-125				
10	<i>N=10</i>	20.0% SNA-101				

Psoriasis Clinical Scoring

[767] The animals were examined for signs of psoriasis on study day 0. These scores served as a baseline for the psoriasis clinical score parameter. Starting from IMQ cream application on day 0, psoriasis responses were examined daily until termination of the study.

[768] Psoriasis reactions (erythema and plaques) were scored based on the parameters shown in Table 35 and recorded according to a 0-12 scale. The clinical score is determined by summing the score of each section.

TABLE 35: PSORIASIS CLINICAL SCORING PARAMETERS

<u>Psoriasis Score</u>					
Plaques	<u>Grade</u>	Erythema	<u>Grade</u>	<u>Punctate redness/scabbing</u>	<u>Grade</u>
Normal	0	Normal	0	No red dots	0
Very few plaques (cover about 5-10% of the back area).	1	Slight	1	Very few (1-10) red dots present on the entire back	1
Several plaques (cover about 20%-30% of the back area).	2	Moderate	2	Red dots are diffuse and cover ~50% of the back (10-25 red dots)	2
Moderate plaques (cover about 50% of the back area).	3	Marked	3	Red dots cover the entire back and are more concentrated (>25 red dots)	3
Spread plaques (cover over 90% of the back area).	4	Extreme	4		

Plaques are no longer detected, however the skin is irritated and no fur is growing on the skin	5	
---	---	--

Results

Psoriasis clinical score

[769] The total psoriasis score was determined by summing the plaque score, the erythema score and the punctate redness/scabbing score. As seen in Figure 99, the difference between SNA-125 at 5% and the vehicle is statistically significant on day 8 and 10, while for SNA-125 at 10% is significant from day 8 to day 10. Further, a statistically significant difference between SNA-352 at 5% and the vehicle on day 10 was found, as well as for SNA-125 at 10% from day 8 to day 10. Further, the difference between SNA-101 at 20% and the vehicle is statistically significant from day 8 to day 10.

Erythema scores

[770] As shown in Figure 100, the differences between SNA-125 at 5% and 10% and the vehicle are statistically significant from day 8 to day 10. Further, statistically significant differences were found between SNA-352 at 5% and 10% and the vehicle from day 8 to day 10. Additionally, the difference between SNA-101 at 20% and the vehicle is statistically significant from day 8 to day 10.

Plaque scores

[771] As shown in Figure 101, the difference between SNA-125 at 5% and the vehicle is statistically significant from day 5 to day 8, while for SNA-125 at 10% it is significant at day 8. Additionally, a statistically significant difference between SNA-352 at 10% and the vehicle was observed from day 5 to day 8. Additionally, the difference between SNA-101 at 20% and the vehicle is statistically significant at day 8.

Punctate redness/scabbing scores

[772] As shown in Figure 102, the difference between SNA-352 at 10% and the vehicle is statistically significant on day 9. Additionally, a statistically significant difference between SNA-101 at 20% and the vehicle was observed on day 9 and day 10.

Spleen weight and ear thickness

[773] Topical application of the IMQ cream causes the enlargement of spleen and lymph nodes, and increased ear thickness. The commonly used antipsoriatic agent clobetasol almost completely attenuated these IMQ-induced changes. Neither treatments with vehicle or SNA-125, SNA-352, SNA-101 significantly modulated spleen weight (Figure 103A). There was no difference on ear thickness found between the vehicle and the different doses of

SNA-125, SNA-352 and SNA-101 (Figure 103B). Figure 103C depicts the daily weight of mice throughout the study.

Cytokine analysis

[774] Left ears were biopunched on day 4 and after tissue homogenization, the levels of cytokines IL-17F, TNF- α , IL-22, and IL-17A in the tissue lysates were measured via multiplex and then normalized with total protein amounts. Mean values for each group are displayed in Figure 104.

EXAMPLE 20: IL-23-INDUCED PSORIASIS MOUSE MODEL

Aim of Study

[775] The objective of this study was to determine the efficacy of SNA-120 and SNA-352 as a therapeutic in the mouse model of IL-23-induced psoriasis. The IL-23/Th17 pathway has been shown to play a major role in psoriasis, and injection of IL-23 into mice produces clinical features associated with psoriasis such as hyperproliferation of keratinocytes and thickened epidermis with infiltration of mononuclear cells. It has been found that an IL-23 mouse model simulates human AD (i.e. 37% homology with human AD transcriptome). In this model, tofacitinib administration has found to reduce ear swelling and inflammatory infiltrates in mouse skin in a dose-dependent manner.

Methodology

Test groups

[776] Table 36 depicts the test groups and Figure 105 depicts a schematic of the timing of the experiments performed.

TABLE 36: TEST GROUPS

Group Number	Group Size	Description	Disease Induction	Route	Dose Level (mg/kg)	Volume Dosage	Dosing Regime
1	n=5	Sham	10 μ l of PBS on right ear 3 $\times$ weekly	NA	NA	NA	NA
2	n=5	IL-23 Only	500 ng of IL-23 on right ear 3 $\times$ weekly	NA	NA	NA	NA
3	n=10	Clobetasol		Topical	5 mg on both ears	NA	1 $\times$ daily
4	n=10	Vehicle		Topical	NA	20 μ l to right ear	BID
5	n=10	1.0% SNA-352		Topical	NA	20 μ l to right ear	BID
6	n=10	5.0% SNA-352		Topical	NA	20 μ l to right ear	BID

7	<i>n</i> =10	10.0% SNA-352		Topical	NA	20 µl to right ear	BID
8	<i>n</i> =10	10.0% SNA-120		Topical	NA	20 µl to right ear	BID

Psoriasis clinical scoring

[777] The animals were examined for signs of psoriasis on study day 0. These scores served as a baseline for the psoriasis clinical score parameter. Starting from IL-23 injection on day 0, psoriasis responses were examined 3 times weekly until termination of the study. Psoriasis reactions (erythema and plaques) were scored using the parameters depicted in table 37 and recorded according to a 0-6 scale. The clinical score is determined by summing the score of each section.

TABLE 37: PSORIASIS CLINICAL SCORING PARAMETERS

Psoriasis Score			
Erythema	Grade	Plaques	Grade
Normal	0	Normal	0
Slight	1	Very few plaques (cover about 5-10% of the ear area).	1
Moderate	2	Moderate plaques (cover about 50% of the ear area).	2
Severe	3	Spread plaques (cover about 90% of the ear area).	3

Results

[778] Figure 106A depicts the total psoriasis clinical scores for each group over time. Figure 106B depicts right ear thickness for each group (measured with a caliper 3 times weekly) while Figure 106C depicts changes in body weight throughout the study.

EXAMPLE 21: PROPHETIC STUDY - ACETONE-DIETHYL-ETHER-WATER MODEL OF DRY SKIN PRURITUS

[779] Dry skin pruritus is common in the elderly, and the Acetone-diethyl-Ether-Water (AEW) model has become a recognized animal model of chronic itch. Studies have shown that AEW treatment causes dryness (measured as increased transepidermal water loss and

decreased stratum corneum hydration). Further, studies indicate that increased secretion of NGF may induce dry skin itch. Old mice scratch more than young mice and AEW treatment induces more scratching compared to saline treatment both in young and old mice. It has further been reported that AEW treatment increases NGF levels in skin biopsies, with old mice having higher levels of NGF than young mice.

[780] The efficacy of SNA-120, SNA-125, SNA-352 will be tested in the AEW model. AEW or 0.9% saline was administered topically BID. Additionally, SNA-120, SNA-125, SNA-352, or vehicle will be administered topically BID. Scratching measurements will be performed 14 hour after the last AEW treatment each day for one hour. Non-specific clinical signs and treatment site assessments will be performed from Day 1 to Day 6. All animals will be weighted on Day 1 and Day 6. On day 6 animals will be terminated and skin biopsies will be performed. Additionally, histology (H&E) and skin biopsies for NGF analysis were performed at study termination.

[781] It is predicted that NGF and TrkA levels will be increased in old mice after AEW treatment, and that SNA-120, SNA-125, and SNA-352 administration will reverse this trend in a dose-dependent manner. It is predicted that SNA-120, SNA-125, and SNA-352 administration will reverse the AEW-induced skin thickening and skin dryness of young and old mice in a dose-dependent manner.

[782] Parakeratosis is a mode of keratinization characterized by the retention of nuclei in the stratum corneum. In the skin, this process leads to the abnormal replacement of annular squames with nucleated cells. Parakeratosis is associated with the thinning or loss of the granular layer and is usually seen in diseases of increased cell turnover, whether inflammatory or neoplastic. Parakeratosis also is seen in the plaques of psoriasis and in dandruff. It is predicted that in young mice treated with saline, a normal epidermis with keratinocytes organized as a single line will be observed. It is predicted that in old mice treated with saline, normal epidermis with keratinocytes frequently organized as a single line and a few signs of parakeratosis will be observed. In young mice treated with AEW, it is predicted that histology assessments will show normal epidermis with some increase in stratification. In old mice treated with AEW, it is predicted that histology assessments will demonstrate normal epidermis with increase in stratification and signs of parakeratosis. It is predicted that that SNA-120, SNA-125, and SNA-352 administration will reverse AEW-induced stratification and parakeratosis in a dose-dependent manner.

EXAMPLE 22: EFFICACY ANALYSIS OF SNA-125, SNA-352 AND SNA-103 IN A VEGF-INDUCED PROLIFERATION ASSAY USING HRMVEC CELLS

Aim of Study

[783] The aim of this study was to compare the efficacy of SNA-125, SNA-352 and SNA-103 in a VEGF-induced proliferation assay using Human Retinal Microvascular Endothelial

(HRMVEC) cells. SNA-125 and SNA-352 have been observed to inhibit kinases in VEGF signaling pathway: ERK and RAF for SNA-125, and PCKa, PCKb2 and PKg for SNA-352. It is contemplated that these compounds could have an anti-angiogenic effect in addition to their anti-inflammatory effect.

Methodology

Experimental outline

[784] Primary HRMVEC cells were seeded onto collagen-coated 96 well plates at a concentration of 2 or 5×10^3 and treated with VEGF at 10 or 50 ng/ml. As a control, cells without VEGF were included to give background levels of non-VEGF driven proliferation. Cells were incubated for 72 hours at 37°C with 5% CO_2 . Proliferation was measured by pulsing cells for the last 24 hours with tritiated thymidine. Plates were then harvested and assayed for tritiated thymidine incorporation. The ability of the lead compounds to inhibit proliferation was assessed by pre-incubation of cells with the lead compounds for 18 hours prior to stimulation with VEGF. Each lead compound was tested at eight concentrations. Motesanib Diphosphate (AMG-706) was included as a positive control. Four concentrations were tested. Each condition was tested in sextuplicate. To readout cell proliferation, cells were pulsed with ^3H -Thymidine and harvested 24 hours later. Radiation was then quantified. IC_{50} values were calculated (where possible) for each compound.

Treatment Groups and Dosages

[785] The doses of the test SNA compounds used were 300 , 100 , 33.3 , 11.1 , 3.7 , 1.2 , 0.41 , and 0.14 μM . The doses of motesanib diphosphate used were 30 , 10 , 3.33 , and 1.11 nM. The pre-incubation time was 18 hours. Table 38 depicts the treatment groups and dosages employed in this study.

TABLE 38: TREATMENT GROUPS AND DOSAGES

Condition	Dose	Cell density	VEGF	Readout
Unstimulated	n/a	2 x 10 ³	n/a	Cell proliferation ³ H-Thymidine incorporation
SNA-125	Doses 1-8		10 ng/mL 72 hours	
SNA-352	Doses 1-8			
SNA-103	Doses 1-8			
Motesanib Diphosphate	Doses 1-4			
SNA-125	Doses 1-8		50 ng/mL 72 hours	
SNA-352	Doses 1-8			
SNA-103	Doses 1-8			
Motesanib	Doses 1-4			

Diphosphate				
Unstimulated	n/a		n/a	
SNA-125	Doses 1-8			
SNA-352	Doses 1-8			
SNA-103	Doses 1-8			
Motesanib Diphosphate	Doses 1-4	5 x 10 ³	10 ng/mL 72 hours	
SNA-125	Doses 1-8			
SNA-352	Doses 1-8			
SNA-103	Doses 1-8			
Motesanib Diphosphate	Doses 1-4		50 ng/mL 72 hours	

Results

[786] Figure 107 depicts the inhibition of VEGF-induced proliferation following treatment with SNA-125, SNA-352, SNA-103, and motesanib diphosphate. The IC₅₀ values calculated based on this analysis are shown in Table 39.

TABLE 39: IC₅₀ VALUES FOR SNA-103, SNA-125, SNA-352

Cell density (cells/well)	VEGF concentration (ng/ml)	SNA-125 IC ₅₀ (μM)	SNA-352 IC ₅₀ (μM)	SNA-103 IC ₅₀ (μM)
2 x 10 ³	10	7.638	4.405	122.2
	50	9.838	4.184	213.3
5 x 10 ³	10	34.34	4.208	~103
	50	26.59	5.031	111.5

Conclusions

[787] SNA-125 showed inhibition of VEGF-induced proliferation. Lower IC₅₀ values were seen when treating the lower cell density (values between 7 and 9 μM). SNA-352 also showed consistent inhibition of VEGF-induced proliferation at both concentrations and cell densities tested, with IC₅₀ values between 4 and 5 pM. SNA-103 appeared to have an effect on proliferation at the top concentration, with IC₅₀ values between 100 and 200 pM. Motesanib diphosphate did not appear to inhibit proliferation with this cell line.

EXAMPLE 23: PROPHETIC STUDY - RABBIT MODEL OF DRY EYE

Study 1: Evaluate the Tolerability and Ocular Distribution of SNA-125 and SNA-352 after Topical Application in New Zealand White Rabbits

[788] Rabbits will receive BID topical dose in both eyes in accordance with the treatment assignment and study schedule depicted in table 40.

TABLE 40: STUDY SCHEDULE

Group	n	Treatment	Dose Route (OU)	Exams	Sample collection at termination
1	2	SNA-125	Topical: BID	Days 1-3: once per day	Tears and conjunctiva
2	2	SNA-352	Topical: BID	Days 1-3: once per day	Tears and conjunctiva

Study 1a: Evaluate the Efficacy of SNA-125 and SNA-352 for Treatment of keratoconjunctivitis sicca (dry eye) in New Zealand White rabbits

[789] Rabbits will be housed at ~ 20% humidity with increased airflow and administered daily with 50 ml of 1% atropine topically into both eyes until study conclusion. After disease induction and baseline exam, topical dose of test articles will be administered in both eyes for 21 days in accordance with the study schedule depicted in Table 41. In-life assessments to be performed are depicted in Table 42.

TABLE 41: STUDY SCHEDULE

Group	n	Treatment	Dose Route (OU)	Euthanasia	Matrices collected
1	3	Vehicle (BSS)	Topical: BID	Day 21	AH, iris/ciliary body, cornea, conjunctiva, VH and the main lacrimal gland
2	3	Xalatan (latanoprost ophthalmic solution)	Topical: BID	Day 21	
3	3	SNA-125	Topical: BID	Day 21	
4	3	SNA-352	Topical: BID	Day 21	

TABLE 42: IN-LIFE ASSESSMENTS

Assessments	Frequency
Body weight	Baseline (prior to dosing) Treatment period: days 0 and 21 (all ± 2)
Tear collection	Treatment period: days 14 and 21 (all ± 2)
Blood collection	First day of dosing: pre-dose, 2, 4, 8, 24, 48 and 72 hours after treatment
Clinical ophthalmic exams (slit lamp only) with slit lamp photographs	Baseline (prior to dosing) Induction period: days 5, 8, 14 (all ± 1) Treatment period: days 0, 7, 14 and 21 (all ± 2)
Schirmer tear test and fluorescein tear breakup tests (TBTU)	
Fluorescein staining	
Lissamine green staining	

EXAMPLE 24: TNBS-INDUCED COLITIS MOUSE MODEL

[790] Intra-rectal administration of 4 mg of TNBS in C57Bl/6 mice will provide a model of colitis in mice. Both oral (PO) and intracecal (IC) administration of the vehicle and test agents are planned. Contemplated treatment groups are SNA-125 (400mg/kg), SNA-352 (400 mg/kg), vehicle (water), prednisolone (2 mg/kg PO; positive control), and tofacitinib (15 mg/kg PO and 1 mg/kg IC). The vehicle, SNA-125, SNA-352 and tofacitinib will be administered BID Days 0-4 while prednisolone will be administered QD Days 0-4. In-life observations (body weight, morbidity, presence of diarrhea and/or bloody stool) will be conducted. The disease activity index DAI (weight loss, diarrhea and blood stool) will be determined for all study groups. Endoscopy will be performed on days 2 and 4, and colitis severity will be scored using a 0-4 scale. In addition, colon histopathology (inflammation, edema & mucosal necrosis scoring) will be undertaken. Finally, multiplex analysis on colon homogenates will be performed for the following cytokines: IFN- γ , IL-10, IL-12(p40), IL12(p70), IL-13, IL-1 b, IL-2, IL-6 and TNFa.

EXAMPLE 25: PROPHETIC STUDY - DSS-INDUCED COLITIS ACUTE MODEL

[791] Colitis will be induced by administration of 3% DSS on Days 0-5 in C57Bl/6 male mice. Both oral (PO) and intracecal (IC) administration of the vehicle and test agents are planned. Contemplated treatment groups are SNA-125 (400 mg/kg), SNA-352 (400 mg/kg), vehicle (water), Anti-p40 (10 mg/kg IP; positive control), and tofacitinib (15mg/kg PO and 1 mg/kg IC). The vehicle, SNA-125, SNA-352 and tofacitinib will be administered BID Days 0-19 while Anti-p40 will be administered Q3D Days 0-18.

[792] In-life observations (body weight, morbidity, presence of diarrhea and/or bloody stool) will be conducted. The disease activity index DAI (weight loss, diarrhea and blood stool) will be determined for all study groups. Endoscopy will be performed on days 10, 14 and 19, and colitis severity will be scored using a 0-4 scale. In addition, colon histopathology (inflammation, edema & mucosal necrosis scoring) will be undertaken. Finally, multiplex analysis on colon homogenates will be performed for the following cytokines: IFN-g, IL-10, IL-12(p40), IL12(p70), IL-13, IL-1 b, IL-2, IL-6 and TNFa.

EXAMPLE 26: EFFICACY OF SNA-125 IN THE TREATMENT OF (CHRONIC) DSS-INDUCED COLITIS IN C57BL/6 MALE MICE

[793] Objective: The objective of this study was to determine the efficacy of SNA-125 in the treatment of DSS-induced (chronic) colitis, in male C57Bl/6 mice.

[794] Results & Conclusions: Animals in which chronic colitis was induced demonstrated decreased mean body weight gain as compared to naive animals. The mean body weight change observed in animals dosed therapeutically with either tofacitinib, high-dose SNA-352 or high-dose SNA-125 was non-significantly reduced as compared to animals dosed therapeutically with vehicle. Additionally, mean body weight change observed in animals

dosed preventatively with SNA-125 was non-significantly reduced as compared to animals dosed preventatively with vehicle. All animals in which colitis was induced demonstrated mean increases in overall composite DAI score as compared to naive animals. The overall mean composite DAI score was significantly reduced in animals treated therapeutically with high-dose SNA-125 as compared to animals treated therapeutically with vehicle if body weight change is calculated relative to Day 0. The mean composite DAI scores were generally reduced in the Tofacitinib treated group, as well as in groups treated therapeutically with either high-dose SNA-352 or low- and high-dose SNA-125 as compared to animals treated therapeutically with vehicle. Increased mean colitis severity scores, mean stool consistency scores, and mean endoscopy DAI scores were observed for all groups in which colitis was induced as compared to naive groups. No significant differences in any endoscopy parameters were observed when comparing treatment groups to respective vehicle control groups. All groups in which colitis was induced demonstrated significantly increased mean colon weight:length ratios as compared to naive animals. No other significant differences in colon weight:length ratio were observed between groups. Mean colon cytokines generally increased in all groups in which colitis was induced as compared to naive animals, with the notable exception of mean colon IFN γ in animals dosed therapeutically with SNA-125. Mean colon levels of TNF α were also generally reduced in this group as compared to animals dosed therapeutically with vehicle. Mean plasma CRP generally increased in all groups in which colitis was induced as compared to naive animals. Mean plasma CRP decreased in the Tofacitinib dosed group as compared to the therapeutic control group. No other major differences in mean plasma CRP levels were observed between treatment groups and vehicle control groups. DSS caused moderate colitis characterized by multifocal inflammation, edema, and mucosal necrosis. Overall, the histopathological results revealed that under the conditions of the current study, both SNA-352 and SNA-125, but especially SNA-352 improved DSS-induced colitis. Improvement was especially prominent in animals treated from Days 0-34 but was also present in animals treated from Days 10-34, and treated with 800 mg/kg SNA-352.

[795] Study Design: Colitis was induced by exposure of mice to 2 % DSS in drinking water, following a five-days on, seven-days off cycle for a period of three cycles. One additional group of eight animals served as naive controls. Animals in Groups 2-4 and 6-11 were dosed with vehicle or test article once daily (QD) via oral gavage (PO) as indicated in Table 44. Animals in Group 5 were dosed by intraperitoneal injection (IP) every third day (Q3D) on Days 0-30. All animals were weighed daily and assessed visually for the presence of diarrhea and/or blood in stool at the time of dosing. The disease activity index was scored daily as described in Table 45. Mice underwent video endoscopy on Days 10, 21, and 34 to assess colitis severity (Table 46). Additionally, stool consistency was scored during

endoscopy using the parameters defined in Table 47. The colitis severity and stool consistency scores were used to calculate endoscopy DAI, as described in Table 48. Following endoscopy on Day 34, all animals from each treatment group were sacrificed, and blood was collected. Following euthanasia, blood was collected and prepared for plasma using K₂EDTA as an anti-coagulant. The colon was excised, rinsed, measured, weighed, trimmed to 6.0 cm in length and divided into two pieces. The most distal (rectum end) 5.0 cm section was swiss rolled and placed in formalin for subsequent histological evaluation. The proximal (cecum end) 1.0 cm portion was weighed and snap-frozen in liquid nitrogen. All plasma and frozen colon tissue was stored at -80 °C until use for selected endpoint analysis.

Table 44.

Group	Number of Animals	DSS Day 0-4, 12-16, &	Treatment	Dose	Route	Dose Schedule
1	8	---	---	---	---	---
2	15	2%	Vehicle	---	PO	QD Days 0-34
3	15		Vehicle	---	PO	QD Days 10-34
4	12		Tofacitinib	30 mg/kg	PO	QD Days 10-34
5	12		Anti-p40	10 mg/kg	IP	Q3D Days 0-30
6	12		SNA-352	800 mg/kg	PO	QD Days 0-34
7	12		SNA-352	400 mg/k	PO	QD Days 10-34
8	12		SNA-352	800 mg/kg	PO	QD Days 10-34
9	12		SNA-125	800 mg/kg	PO	QD Days 0-34
10	12		SNA-125	400 mg/kg	PO	QD Days 10-34
11	12		SNA-125	800 mg/kg	PO	QD Days 10-34

Table 44 (continued).

Group	Disease Activity Index	Endoscopy Schedule	Terminal Collection (Day 34)
-------	------------------------	--------------------	------------------------------

1	Days 0– 34	Days 10, 21, & 34	Blood: Plasma Colon: -Snap Frozen -Formalin
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			

[796] Disease Activity Index (DAI): Each mouse was observed for the presence and severity of diarrhea and/or blood in stool on a daily basis, and was assigned a score of 0, 2 or 4 for each parameter. Additionally, a score of 0-4 was assigned to each animal on a daily basis based on body weight lost. These three scores were combined to generate a daily composite DAI score. The details on individual score parameter assignments and the daily composite DAI score can be found in Table 45.

Table 45. Composite Disease Activity Index (DAI) Scoring Scale.

Score	Weight Loss	Diarrhea	Blood Stool
0	0.0-4.99%	Normal	Not Present
1	5.0-9.99%		
2	10.0-14.99%	Loose Stools	Blood Observe
3	15.0-19.99%		
4	>20.0%	Diarrhea	Gross Bleeding

[797] Endoscopy: Each mouse underwent video endoscopy on Days 10, 21, and 34 using a small animal endoscope (Karl Storz Endoskope, Germany), under isoflurane anesthesia. During each endoscopic procedure, still images as well as video were recorded to evaluate the extent of colitis and the response to treatment. Additionally, an attempt was made to capture an image from each animal at the most severe region of disease identified during endoscopy. Colitis severity was scored using a 0-4 scale, as described in Table 46.

Additionally, stool consistency was scored during endoscopy using the parameters defined in Table 47. Finally, the colitis severity and stool consistency scores were combined with the score assigned to each animal based on body weight loss (as described above: Disease Activity Index) to determine the endoscopy DAI score, as described in Table 48.

Table 46. Colitis Severity Scoring Scale.

Score	Description:
0	Normal
1	Loss of vascularity
2	Loss of vascularity and friability
3	Friability and erosions
4	Ulcerations and bleeding

Table 47. Stool Consistency Scoring Scale.

Score	Description:
0	Normal, well-formed pellet
1	Loose stool, soft, staying in shape
2	Loose stool, abnormal form with excess moisture
3	Watery or diarrhea
4	Bloody diarrhea

Table 48. Endoscopy Disease Activity Index (DAI) Scoring Scale.

Score	Weight Loss	Colitis Severity	Stool Consistency
0	0.0-4.99%	Normal	Normal, well-formed pellet
1	5.0-9.99%	Loss of vascularity	Loose stool, soft, staying in shape
2	10.0-14.99%	Loss of vascularity and friability	Loose stool, abnormal with excess
3	15.0-19.99%	Friability and erosions	Watery or diarrhea
4	>20.0%	Ulcerations and bleeding	Bloody diarrhea

[798] Survival: Animal deaths were evaluated during the course of the study. In this model, animal deaths are commonly attributable to severe weighed loss due to severe colitis, perforation of the colon during endoscopy, or euthanasia due to fight wounds.

[799] Multiplex Analysis: The snap-frozen proximal 1.0 cm colon portion was homogenized, and colon homogenate supernatants were analyzed for protein levels of a panel of mouse inflammatory mediators (IFN γ , IL-10, IL-6, and TNF α) using a multiplex system (MAGPIX,

EMD Millipore). The plasma aliquot reserved for C-Reactive Protein (CRP) analysis underwent such analysis using a multiplex system (MAGPIX, EMD Millipore). For each analyte on the panel, a standard curve was generated and evaluated for fitness. Two quality controls were analyzed for each plate, one high and one low, each of which was evaluated on the MAGPIX software (Luminex Corp). Sample values that were out-of-range (OOR) on the low end of the curve were entered at 50% of the lowest standard; if on the high end of the curve, they were entered as the highest standard value.

[800] Colon Histopathology: The formalin-fixed, swiss-rolled colon samples were embedded in paraffin and were sectioned at approximately 5 microns. One slide for each animal was stained with hematoxylin and eosin and were examined by a board-certified veterinary pathologist. Each slide containing one rolled colon was split into four approximately equal quarters. Each quarter was evaluated and scored for inflammation, edema, and mucosal necrosis, according to the scoring criteria listed in Table 49. With the slide label to the left, quarters were evaluated starting at the top left and moving clockwise.

Table 49. Histopathologic Scoring Criteria (Inflammation, Edema, and Mucosal Necrosis).

Inflammation Score	Description
0	None present
1	Minimal change: Focal aggregate of a few cells of minimal diffuse inflammation
2	Mild change: Larger focal aggregates, multifocal small aggregates, or diffuse mild inflammation
3	Moderate change: Multifocal aggregates sometimes coalescing with one another or moderate diffuse change
4	Severe change: Marked diffuse inflammation
Edema Score	Description
0	None present
1	Minimal change: Focal edema or minimal diffuse edema
2	Mild change: Larger focal areas of edema or diffuse mild edema
3	Moderate change: Multifocal areas of edema coalescing with one another or moderate diffuse edema
4	Severe change: Marked diffuse edema
Mucosal Necrosis/Loss	Description

0	None present
1	Minimal change: Focal epithelial necrosis
2	Mild change: Larger focal areas of necrosis or multifocal areas of necrosis
3	Moderate change: Multifocal necrosis coalescing into larger areas
4	Severe change: Areas of erosion through the mucosa to the submucosa

[801] Statistical Analyses: Statistically significant differences between groups were determined by either one-way ANOVA (parametric data) with either Tukey's multiple comparisons test used to compare all groups to one another, or Dunnett's multiple comparisons test used to compare treatment groups to their respective vehicle control; or Kruskal-Wallis test (non-parametric) with Dunn's multiple comparisons test used to compare all groups to one another. All statistical analyses were performed using GraphPad Prism 7 (La Jolla, CA).

[802] Outcome Evaluation: Study endpoints included body weight loss, composite DAI score, colitis severity and stool consistency scores, endoscopy DAI score, colon weightlength ratio, plasma CRP, colon cytokines, and colon histopathology.

[803] **Body Weight Change:** Animals were weighed daily for the duration of the study, and the percent body weight change relative to either Day 0 (Figure 114) or to the mean body weight change of the naive group (Figure 115) was calculated. In order to determine the statistical significance of overall differences in mean percent body weight change between groups, the area under the curve (AUC) was calculated using the trapezoidal rule transformation and is shown inset in Figure 114 and Figure 115.

[804] Animals in which chronic colitis was induced by exposure to 2% DSS-treated drinking water demonstrated decreased mean body weight gain as compared to naive animals, regardless of whether percent weight change was calculated relative to Day 0 or to naive animals. This decrease in body weight gain as compared to naive animals (Group 1) reached statistical significance for both vehicle control groups (Group 2 - preventative and Group 3 - therapeutic, respectively), as well as groups dosed with Tofacitinib (Group 4), SNA-352 preventatively (Group 6), low-dose SNA-352 therapeutically (Group 7 - 400 mg/kg), and low-dose SNA-125 therapeutically (Group 10 - 400 mg/kg). While the difference in body weight change between naive animals and those dosed preventatively with SNA-125 (Group 9) was statistically significant when body weight change was calculated relative to Day 0 (Figure 114), the difference failed to reach statistical significance when body weight change was calculated relative to naive animals (Figure 115). The difference in body weight change between naive animals and either those dosed therapeutically with high-dose SNA-

352 (Group 8 - 800 mg/kg) and those dosed therapeutically with high-dose SNA-125 (Group 11 - 800 mg/kg) was not statistically significance regardless of how body weight change was calculated.

[805] The overall mean body weight change observed in animals dosed therapeutically with either tofacitinib, high-dose SNA-352 or high-dose SNA-125 was non-significantly reduced as compared to animals dosed therapeutically with vehicle. Additionally, mean body weight change observed in animals dosed preventatively with SNA-125 was non-significantly reduced as compared to animals dosed preventatively with vehicle. No other major differences in body weight change as compared to the vehicle control groups were observed.

[806] Composite DAI Score: Animals were assigned a score of 0, 2, or 4 based on the presence and severity of diarrhea and/or blood in stool on a daily basis. A score of 0-4 was also assigned to each animal daily based on body weight loss. These three scores were combined to generate a daily composite DAI score. The details on individual score parameter assignments and the daily composite DAI score can be found in Table 45. The weight loss score was assigned based on body weight change relative to either Day 0 (Figure 116) or to the mean body weight change in naive animals (Figure 117). The individual assigned score for body weight loss relative to Day 0 is shown for all groups in Figure 118. The individual assigned score for body weight loss relative to naive animals is shown for all groups in Figure 119. Finally, the individually assigned scores for diarrhea and blood in stool for all groups are shown in Figure 120 and Figure 121, respectively. In order to determine the statistical significance of overall differences in mean composite DAI or individual scores between groups, the AUC was calculated using the trapezoidal rule transformation and is shown inset in Figures 116-117.

[807] All animals in which colitis was induced by exposure to DSS demonstrated mean increases in overall composite DAI score as compared to naive animals, regardless of how body weight change was calculated. These increases reached statistical significance for both vehicle control groups (Groups 2-3) as well as the anti-p40 dosed group (Group 5) if body weight change is calculated relative to Day 0 (Figure 116); these increases reached statistical significance for all groups in which colitis was induced, with the exception of the group treated therapeutically with high-dose SNA-125 (Group 11), if body weight change is calculated relative to naive animals (Figure 117). Notably, the overall mean composite DAI score was significantly reduced in animals treated therapeutically with high-dose SNA-125 (Group 11) as compared to animals treated therapeutically with vehicle (Group 3) if body weight change is calculated relative to Day 0 (Figure 116). The mean composite DAI scores were generally reduced in the Tofacitinib treated group (Group 4), as well as in Groups

treated therapeutically with either high-dose SNA-352 or low- and high-dose SNA-125 as compared to animals treated therapeutically with vehicle.

[808] Endoscopy: Animals underwent video endoscopy on Days 10, 21, and 34 in order to assess colonic inflammation. Colitis was scored visually on a five-point scale that ranges from 0 for normal to 4 for severe ulceration (Table 46). Stool consistency was likewise scored visually on a five-point scale that ranges from 0 for normal to 4 for bloody diarrhea (Table 47). Finally, the colitis severity and stool consistency scores were combined with the score assigned to each animal based on body weight loss (as described in Section 5.6.4: Disease Activity Index) to determine the endoscopy DAI score (Table 48). The mean colitis severity (Figure 122), stool consistency (Figure 123), and endoscopy DAI (Figure 124) at each timepoint are shown in Figures 122-124. Mean endoscopy scores from each timepoint are shown in Table 50.

Table 50. Mean endoscopy scores

Group	Mean endoscopy score		
	Day 10	Day 21	Day 34
1 Naïve	0	0	0
2 Vehicle control (Days 0–34)	1.43	1.93	2.14
3 Vehicle control (Days 10–34)	1.60	1.71	2.21
4 Tofacitinib (Days 10–34)	1.33	1.73	2.09
5 Anti-p40 (Days 0–34)	1.33	1.55	1.82
6 SNA-352 (800 mg/kg; Days 0–34)	1.50	2.18	2.36
7 SNA-352 (400 mg/kg; Days 10–34)	1.25	1.92	2.00
8 SNA-352 (800 mg/kg; Days 10–34)	1.42	2.00	1.92
9 SNA-125 (800 mg/kg; Days 0–34)	1.60	1.60	2.00
10 SNA-125 (400 mg/kg; Days 10–34)	1.33	1.42	2.25
11 SNA-125 (800 mg/kg; Days 10–34)	1.27	1.64	2.27

[809] Increased mean colitis severity scores (Figure 122), mean stool consistency scores (Figure 123), and mean endoscopy DAI scores (Figure 124) were observed for all groups in which colitis was induced as compared to naive groups. Mean increases in colitis severity scores reached statistical significance for all groups in which colitis was induced at the Day 10 timepoint, for all groups with the exception of animals treated therapeutically with low-dose SNA-125 (Group 10) at the Day 21 timepoint, and for all groups with the exception of animals treated with anti-p40 (Group 5) at the Day 21 timepoint. Mean increases in stool consistency scores reached statistical significance for both vehicle control groups (Groups 2-3), the tofacitinib treated group (Group 4), and the preventative SNA-125 treated group (Group 9) at the Day 10 timepoint; for the therapeutically dosed vehicle group, the anti-p40

group, all SNA-352 groups (Groups 6-8), the preventatively dosed SNA-125 group (Group 9), and the therapeutically dosed low-dose SNA-125 group at the Day 21 timepoint; and for the therapeutically dosed vehicle group, the anti-p40 dosed group, the preventatively dosed SNA-352 group, the high-dose therapeutically dosed SNA-352 group, the preventatively dosed SNA-125 group, and the therapeutically dosed low-dose SNA-125 group at the Day 34 timepoint. Mean increases in endoscopy DAI scores reached statistical significance for all groups in which colitis was induced with the exception of the anti-p40 treated group at the Day 10 timepoint, for all groups with the exception of the preventatively dosed vehicle group (Group 2) and the therapeutically dosed high-dose SNA-125 group (Group 11) at the Day 21 timepoint, and for all groups with the exception of the preventatively dosed vehicle group, the tofacitinib dosed group, and the preventatively dosed SNA-125 group at the Day 34 timepoint. No significant differences in any endoscopy parameters were observed when comparing treatment groups to respective vehicle control groups.

[810] Colon Weight:Length Ratio at Euthanasia: All surviving animals were euthanized following endoscopy on Day 34. The colon was excised, weighed, and measured at this time; and the colon weight:length ratio was calculated (Figure 125). All groups in which colitis was induced demonstrated significantly increased mean colon weight:length ratios as compared to naive animals. No other significant differences in colon weight:length ratio were observed between groups.

[811] Colon Cytokine Analysis: Colon samples were collected for histopathology and cytokine analysis upon euthanasia; the most proximal 1.0 cm of colon was weighed and snap-frozen for downstream analysis of IFN γ , IL-10, IL-6, and TNF α by multiplex (MAGPIX, EMD Millipore). Ahead of analysis, the snap-frozen portion was homogenized in PBS with protease inhibitor and was centrifuged; the homogenate supernatant was used for analysis. Colon cytokines from all groups are shown in Figures 126-129.

[812] Mean colon cytokines generally increased in all groups in which colitis was induced as compared to naive animals, with the notable exception of mean colon IFN γ in animals dosed therapeutically with SNA-125 (Group 11; Figure 126). Mean colon levels of TNF α were also generally reduced in this group as compared to animals dosed therapeutically with vehicle (Group 3; Figure 129), however this reduction did not reach statistical significance. Mean colon IFN γ was significantly elevated in animals dosed preventatively with SNA-352 as compared to those dosed preventatively with vehicle (Figure 126), and mean colon IL-10 was significantly reduced in naive animals (Group 1) as compared to those dosed preventatively with vehicle (Figure 128). Mean colon IL-6 was significantly increased in animals dosed therapeutically with low-dose SNA-352 (Group 7) as compared to those dosed therapeutically with vehicle (Group 3; Figure 127). Though no other significant differences in colon cytokines were observed between groups, the following general trends

were observed. Mean colon IFN γ (Figure 126) was generally lowest in groups dosed with anti-p40 (Group 5) and therapeutic high-dose SNA-125 (Group 11); mean colon IL-6 (Figure 127) was generally lowest in groups dosed therapeutically with high-dose SNA-352 (Group 8) or high-dose SNA-125 (Group 11); mean colon IL-10 was generally highest in animals dosed preventatively with SNA-125 (Group 9); and mean colon TNF α (Figure 129) was generally lowest in animals dosed therapeutically with high-dose SNA-352, and both low (Group 10) and high doses of SNA-125.

[813] Plasma CRP Analysis: Blood was collected following euthanasia and was processed for plasma by centrifugation; an aliquot of plasma was analyzed for levels of C Reactive Protein (CRP). The results for plasma CRP for all groups are shown in Figure 130.

[814] Mean plasma CRP generally increased in all groups in which colitis was induced as compared to naive animals; while mean plasma CRP increased 60% and 86% in the preventative and therapeutic vehicle controls, respectively, as compared to naive animals, these increases did not reach statistical significance. Mean plasma CRP decreased 18% in the Tofacitinib dosed group as compared to the therapeutic control group, however this decrease did not reach statistical significance. No other major differences in mean plasma CRP levels were observed between treatment groups and vehicle control groups.

[815] Colon Histopathology: DSS caused moderate colitis characterized by multifocal inflammation (see Figure 131), edema (see Figure 132), and mucosal necrosis (see Figure 133). Analysis was separated into two groups: 1) groups treated from days 0-34; and 2) groups treated from Days 10-34.

[816] For groups with animals treated from Days 0-34, anti-p40 treatment resulted in more, not less, severe colitis compared to the vehicle control. In contrast, both 800 mg/kg SNA-352 and 800 mg/kg SNA-125 significantly improved colitis compared to both vehicle and anti-p40 treatment. This was true for all measured parameters except for necrosis, for which only SNA-125 significantly improved compared to vehicle.

[817] For groups with animals treated from Days 10-34, tofacitinib, 400 mg/kg SNA-352 and 800 mg/kg SNA-352 all improved colitis compared to vehicle, but this change was only significant for 800 mg/kg SNA-352. In contrast, neither 400 mg/kg for 800 mg/kg SNA-125 improved colitis compared to the vehicle control. Animals treated with 800 mg/kg SNA-125 showed mild improvement compared to animals treated with 400 mg/kg but neither was significantly improved compared to vehicle.

[818] Overall, the histopathological results revealed that under the conditions of the current study, both SNA-352 and SNA-125, but especially SNA-352 improved DSS-induced colitis (see Figure 134). Improvement was especially prominent in animals treated from Days 0-34 but was also present in animals treated from Days 10-34, and treated with 800 mg/kg SNA-352.

[819] Results & Conclusions: Animals in which chronic colitis was induced demonstrated decreased mean body weight gain as compared to naive animals, regardless of whether percent weight change was calculated relative to Day 0 or to naive animals. Body weight loss in diseased animals as compared to naive animals was as expected. The mean body weight change observed in animals dosed therapeutically with either tofacitinib, high-dose SNA-352 or high-dose SNA-125 was non- significantly reduced as compared to animals dosed therapeutically with vehicle. Additionally, mean body weight change observed in animals dosed preventatively with SNA-125 was non- significantly reduced as compared to animals dosed preventatively with vehicle. No other major differences in body weight change as compared to the vehicle control groups were observed.

[820] All animals in which colitis was induced demonstrated mean increases in overall composite DAI score as compared to naive animals, regardless of how body weight change was calculated. Overall increases in DAI scores in diseased animals as compared to naive animals were as expected. Notably, the overall mean composite DAI score was significantly reduced in animals treated therapeutically with high-dose SNA-125 (Group 11) as compared to animals treated therapeutically with vehicle (Group 3) if body weight change is calculated relative to Day 0. The mean composite DAI scores were generally reduced in the Tofacitinib treated group (Group 4), as well as in groups treated therapeutically with either high-dose SNA-352 or low- and high-dose SNA-125 as compared to animals treated therapeutically with vehicle.

[821] Increased mean colitis severity scores, mean stool consistency scores, and mean endoscopy DAI scores were observed for all groups in which colitis was induced as compared to naive groups. Increases in endoscopy scores in diseased animals as compared to naive animals were as expected. No significant differences in any endoscopy parameters were observed when comparing treatment groups to respective vehicle control groups.

[822] All groups in which colitis was induced demonstrated significantly increased mean colon weight:length ratios as compared to naive animals. Increases in colon weight:length ratio in diseased animals as compared to naive animals were as expected. No other significant differences in colon weight:length ratio were observed between groups.

[823] Mean colon cytokines generally increased in all groups in which colitis was induced as compared to naive animals, with the notable exception of mean colon IFN γ in animals dosed therapeutically with SNA-125. Mean colon levels of TNF α were also generally reduced in this group as compared to animals dosed therapeutically with vehicle. However this reduction did not reach statistical significance. Mean colon IFN γ was significantly elevated in animals dosed preventatively with SNA-352 as compared to those dosed preventatively with vehicle, and mean colon IL-10 was significantly reduced in naive animals as compared to those dosed preventatively with vehicle. Mean colon IL-6 was significantly

increased in animals dosed therapeutically with low-dose SNA-352 as compared to those dosed therapeutically with vehicle. No other significant differences in colon cytokines were observed between groups.

[824] Mean plasma CRP generally increased in all groups in which colitis was induced as compared to naive animals; while mean plasma CRP increased 60% and 86% in the preventative and therapeutic vehicle controls, respectively, as compared to naive animals, these increases did not reach statistical significance. Mean plasma CRP decreased 18% in the Tofacitinib dosed group as compared to the therapeutic control group, however this decrease did not reach statistical significance. No other major differences in mean plasma CRP levels were observed between treatment groups and vehicle control groups.

[825] DSS caused moderate colitis characterized by multifocal inflammation, edema, and mucosal necrosis. For groups with animals treated from Days 0-34, anti-p40 treatment resulted in more, not less, severe colitis compared to the vehicle control. In contrast, both 800 mg/kg SNA- 352 and 800 mg/kg SNA-125 significantly improved colitis compared to both vehicle and anti-p40 treatment. This was true for all measured parameters except for necrosis, for which only SNA-125 significantly improved compared to vehicle. For groups with animals treated from Days 10- 34, tofacitinib, 400 mg/kg SNA-352 and 800 mg/kg SNA-352 all improved colitis compared to vehicle, but this change was only significant for 800 mg/kg SNA-352. In contrast, neither 400 mg/kg for 800 mg/kg SNA-125 improved colitis compared to the vehicle control. Animals treated with 800 mg/kg SNA-125 showed mild improvement compared to animals treated with 400 mg/kg but neither was significantly improved compared to vehicle. Overall, the histopathological results revealed that under the conditions of the current study, both SNA-352 and SNA-125, but especially SNA-352 improved DSS-induced colitis. Improvement was especially prominent in animals treated from Days 0-34 but was also present in animals treated from Days 10-34, and treated with 800 mg/kg SNA-352.

**EXAMPLE 27: PHARMACOKINETICS AND SAFETY ASSOCIATED WITH MAXIMAL USE
OF 0.5 % W/W SNA-120 (PEGCANTRATINIB1 OINTMENT ADMINISTERED TWICE
DAILY IN SUBJECTS WITH PRURITUS ASSOCIATED WITH PSORIASIS VULGARIS**

[826] This study, using an assay that is five times more sensitive than previously available, demonstrated minimal to no systemic exposure after twice daily topical application of SNA-120 under maximal use conditions, with no accumulation of SNA-120 in the plasma. There were no associated clinically relevant changes in the overall safety parameters evaluated, including no systemic safety signals (i.e. laboratory assessments, electrocardiograms (ECG), QTc duration, and adverse events (AEs)). A total of 9 subjects reported 12 adverse events (AEs), only one of which was deemed related to study drug and which was categorized as mild pruritus. There were also no dermal tolerability events reported, no

subject discontinuations due to an AE, and no severe AEs or serious AEs (SAEs). The majority of subjects had no detectable levels of SNA-120 in the plasma (66.7% and 67.9% of subjects, respectively, on Day 1 and 29). Low (less than 2.5 ng/mL) detectable levels of SNA-120 in the plasma were observed in 30.0% and 28.6% of subjects, respectively, on Day 1 and 29. No accumulation of SNA-120 in the plasma was seen following repeated twice-daily topical application. SNA-120 was well tolerated with no subjects discontinuing the study due to Adverse Events (AE). Improvements in pruritus and psoriasis were observed in exploratory efficacy analyses. For example, in a post hoc analysis, approximately 57 percent of subjects had at least a 4-grade improvement in the Itch Numeric Rating Scale (I-NRS).

[827] Chronic itch affects roughly 80 percent, or 6.3 million (Global Data (2016). PharmaPoint: Psoriasis - Global Drug Forecast), of the 7.9 million patients suffering from psoriasis in the United States (Mentor et al. Guidelines of care for the management of psoriasis and psoriatic arthritis. *J Am Acad Dermatol.* 2008;58(5):826-850). It is an important—and often overlooked—symptom in patients with psoriasis. One study found that 50% of patients said they had difficulty sleeping due to itch and 75% reported having to scratch until they bleed (Prignano F, Ricceri F, Pescitelli L, Lotti T. Itch in psoriasis: epidemiology, clinical aspects and treatment options. *Clin Cosmet Investig Dermatol*). The itch that affects the majority of psoriasis patients can be as debilitating and harmful to their quality of life as the plaques themselves, yet there are no FDA-approved, targeted, topical medications available that are addressing this bothersome itch that could extend beyond the plaque.

[828] This is a multicenter, open-label, Phase 1b study designed to evaluate the PK with maximal use of 0.5% w/w SNA-120 ointment, as measured by circulating plasma levels of SNA-120 in men and women aged ≥ 18 years who have at least moderate (e.g., moderate to severe) pruritus associated with at least moderate (e.g., moderate to severe) psoriasis vulgaris (PV) covering at least 20% body surface area (BSA). SNA-120 was evaluated as a topical, non-steroidal ointment to treat itch (pruritus) associated with psoriasis. SNA-120 was designed to achieve high local drug concentration in the target tissue while minimizing systemic exposure for patients. SNA-120 directly targets the peripheral itch pathway in the psoriatic plaque.

[829] One objective of the study is to evaluate the pharmacokinetics (PK) with maximal use of 0.5 % weight-to-weight ratio (w/w) SNA-120 ointment as measured by circulating plasma levels of SNA-120 in subjects with chronic pruritus associated with psoriasis vulgaris (plaque psoriasis). Another objective is to evaluate the effect of maximum therapeutic dose of SNA-120 treatment on Fridericia's corrected QT interval (QTcF).

[830] Approximately 30 eligible subjects will receive 0.5% w/w SNA-120 ointment (2 mg/cm²) proportional to approximately 35 mg active drug at each application for a subject

with at least 20% BSA affected, administered twice daily over 4 weeks to the indicated areas in the Treatment Area. The "Treatment Area" is defined as the BSA (including the face, scalp, intertriginous, and genital areas) exclusive of the mucosa, palms, and soles. The "Affected Areas" to be treated within the Treatment Area shall include: 1) all psoriasis (e.g., plaques) present in the Treatment Area at Baseline; 2) any new or recurrent areas of psoriasis that may develop during the study; and 3) the original plaque area, even in the event that the psoriasis (erythema, induration, scale) resolves and the lesions are no longer clearly visible (aside from hyper/hypo pigmentation). Subjects should continue to apply study drug to current active plaques, resolved/resolving plaques (residual hyper/hypopigmentation), or newly appearing plaques for the full 4 weeks of treatment, always covering a minimum of 20% BSA.

[831] On Study Days 1 and 29 subjects will be asked to assume the supine position and remain quiet but awake for at least 20 minutes around each nominal electrocardiogram (ECG) extraction point (15 minutes before the time point until 5 minutes after each time point). Once the ECG extraction is started (time: 0 h), the subject should remain at rest in the supine position for the next 5 minutes, with the exception of PK draws. The extraction time points from the continuous ECG monitoring of the QT interval corrected for heart rate (QTc) analysis are as follows: predose (within 1 hour of the start of dosing), and 0.5, 0.75, 1, 2, 4, 8, and 12 hours after the end of the SNA-120 application.

[832] The average of triplicate ECG measurements from each time point will serve as each subject's QT value. Electrocardiograms will be measured using semi-automated methods for the ECG intervals. Annotations will be made on the global superimposed median beat. The Mortara eScribe software (or equivalent) will be used for the measurement. Interpretations for each ECG will be made by a board-certified cardiologist.

[833] Serial blood draws for PK analysis will be obtained approximately the same time of day on Study Days 1 and 29. On those days, blood draws will be collected after each nominal ECG extraction time point. Predose blood samples on Days 1, 8, 15, and 29 should be taken within 1 hour prior to treatment administration start time. Administration may be performed by the study staff so that the subject can remain at rest.

[834] When multiple activities are concurrent, the preferred collection order will be vital signs, safety ECGs, continuous 12-lead monitoring ECG, then PK sample.

[835] A fasting schedule will apply for Study Days 1 and 29. Treatment administration will be under fasting conditions. A fast of at least 4 hours prior to dosing will begin before treatment on Days 1 and 29. Subjects should remain in a fasted state until completion of the 4-hour ECG and PK activities, after which a snack or light meal may be provided.

[836] After signing consent, subjects must complete all Screening and Baseline procedures and meet all eligibility requirements to qualify for treatment. Approximately 30 eligible

subjects will administer 2 mg/cm² of 0.5% w/w SNA-120 ointment to the Treatment Areas twice daily over 4 weeks to allow for evaluation of the PK with maximal use of 0.5% w/w SNA-120 ointment (proportional to approximately 35 mg active drug at each application for a subject with at least 20% BSA affected); safety and tolerability will also be assessed.

[837] On Days 1 and 29, study assessments will be performed first, followed by the administration of study drug, which may be applied by the study staff. On Days 8 and 15, 12-lead ECGs, PK sample collection, and the study assessments will be performed first, followed by the administration of study drug, which will be applied according to instructions. Subjects will return to the research facility at Days 8, 15, and 29 for protocol-defined assessments and procedures, assessment of adverse events (AEs), and confirmation of compliance with study drug usage. A further follow-up visit is scheduled at Day 43, approximately 2 weeks after the last study drug treatment, for final pruritus, psoriasis and safety assessments.

[838] All visit assessments of the subjects' psoriasis and associated pruritus will be made before application of study drug. Subjects should continue application of study drug for the full 4 weeks. Any subject who discontinues treatment prior to Day 29 will be asked to return to the clinic at the time of stopping study drug to undergo end-of-study assessments.

[839] Investigational Product, Dosage and Mode of Administration: Study drug is 0.5% w/w SNA-120 ointment for topical administration.

[840] The Affected Areas within the Treatment Area shall be treated twice daily during the entire study treatment period (Visit 2 [Study Day 1] to the end-of-treatment visit [Week 4]).

[841] Subjects will be required to apply a thin layer (approximately 2 mg/cm²) of SNA-120 ointment to their Affected Areas twice daily during the conduct of the study covering at least 20% affected BSA. All applications should be at approximately the same time each day, approximately 12 hours apart. Prior to application, the area(s) to be treated should be clean and dry.

[842] The investigative staff will educate subjects on the measurement and application of study ointment. The first application of ointment will be done under the supervision of the investigative site staff, at Study Day 1 (baseline), using a spatula to extract the ointment and a finger(s) to apply the ointment to the plaque. Subsequent applications will be applied at home, using the same technique as taught at the clinic.

[843] The prescribed amount of ointment is based on the BSA affected by psoriasis (proportional to approximately 7.0 g of ointment [containing approximately 35 mg active drug] each morning and evening for subjects with 20% affected BSA). The ointment should be evenly distributed with a thin coating over all the psoriatic plaques. Before covering with clothing, the subject should wait until areas of application are dry and will not soil clothing. The investigative staff will weigh each jar of study drug before dispensing the study drug and

again upon return by the subjects. On each study visit, treatment will take place after all assessments have been completed.

[844] Duration of Participation: Up to 4 weeks of screening and 4 weeks treatment with study drug followed by a 2-week follow-up period; maximum total duration per subject of 10 weeks.

[845] Pharmacokinetic Evaluations: Area under the plasma concentration versus time curve from time 0 to the last measurable time point (AUC_{0-t}); Area under the plasma concentration versus time curve from time 0 to 12 hours (AUC_{0-12}); Plasma concentration at 12 hours post the morning application (C_{12h}); Pre-morning dose plasma concentration on days after treatment initiation ($C_{t_{rough}}$); Maximum plasma concentration (C_{max}); Time of maximum concentration (t_{max}); Amount excreted in urine; and Renal clearance.

[846] Safety Evaluations: Changes in ECGs, as well as incidence of QTcF prolongation; Changes in vital signs (blood pressure and pulse); Incidence and severity of all spontaneously reported AEs, including local site reactions; Changes in clinical laboratory results; Physical examinations; Concomitant medications; and Concurrent procedures.

[847] Efficacy Evaluations: Change from baseline at each study visit in the I-NRS; Change from baseline at each study visit in the Psoriasis Area and Severity Index (PASI) score; PASI-75: response defined as a reduction of $\geq 75\%$ from baseline at each study visit on the PASI; PASI-50: response defined as a reduction of $\geq 50\%$ from baseline at each study visit on the PASI; Change from baseline at each study visit in the Investigator Global Assessment (IGA); Proportion of subjects with a ≥ 1 -grade decrease on the IGA at each study visit; Proportion of subjects with a ≥ 2 -grade decrease on the IGA at each study visit; and Change from baseline at each study visit in BSA affected by psoriasis.

[848] Dosing Schedule: Subjects should continue to apply study drug to current active plaques, resolved/resolving plaques (residual hyper/hypopigmentation), or newly appearing plaques for the full 4 weeks of treatment, always covering a minimum of 20% BSA.

[849] SNA-120 0.5% w/w ointment will be applied in a thin and complete layer to the Affected Areas twice daily over 4 weeks.

[850] Approximately 2 mg/cm² SNA-120 will be applied to their psoriatic plaques twice daily during the conduct of the study. All applications should be at approximately the same time each day, approximately 12 hours apart.

[851] The investigative staff will educate subjects on the measurement and application of study drug. Administration at the study site may be performed by the study staff so that the subject can remain at rest. All other applications will be applied at home, using the same technique as demonstrated at the study site.

[852] Study staff should note administration start time (for predose activities) and stop time (for postdose activities).

[853] Prior to application of study drug, the plaque area should be clean and dry. The prescribed amount of ointment, based on the BSA affected by psoriasis, should be evenly distributed over the psoriatic plaques in a thin and complete layer. The subject should not dress until areas of application are dry and will not soil clothing. The technique should be identical for each application throughout the study.

[854] For a subject with psoriasis affecting 20% of BSA, approximately 14 g per day of ointment should be used (7 g in the morning and 7 g in the evening). Spatulas will be provided to measure ointment. One spatula measures out approximately 1 g of ointment. Each gram of the ointment should be sufficient to cover approximately 4% BSA. However, subjects should be instructed that more or less may be required and it is important that they ensure that all psoriatic lesions are completely covered with a thin, uniform, visible layer of the study drug.

[855] The ointment contains SNA-120 (pegcantratinib [active ingredient]), butylated hydroxytoluene, benzyl alcohol, propylene glycol, white soft paraffin (white petrolatum), white wax, stearyl alcohol, and cholesterol.

[856] Visits - There are a total of 6 scheduled visits: screening (Visit 1); treatments Visits 2 through 5 at Days 1 (baseline, 8, 15 and 29, and then, post-treatment Visit 6 at Day 43, 2 weeks after stopping treatment.

[857] Pharmacokinetics - Blood: Serial blood draws for PK analysis will be obtained approximately the same time of day for Study Days 1 and 29. The predose blood samples on Days 1, 8, 15, and 29 should be taken within 1 h prior to treatment administration start time. On Days 1 and 29, blood draws will be collected after each nominal ECG extraction time point; the actual times of collection will be recorded in the eCRF. Administration may be performed by the study staff so that the subject can remain at rest. Single PK sample blood draws will occur on Days 8 and 15 and should be within 1 hour prior to treatment administration start time.

[858] For all subjects, blood sampling will be performed under controlled conditions to determine the PK profile of SNA-120 from 0 to 12 hours postdose (after the end of the SNA-120 application). Blood samples will be collected at Days 1 and 29 for PK determination of SNA-120 plasma level at time = predose, and 0.5, 0.75, 1, 2, 4, 8, and 12 hours after the end of the SNA-120 application. The predose and 0.5, 0.75, and 1-hour blood samples for PK determination should be collected within 5 minutes after SNA-120 administration. The 2, 4, and 8-hour PK blood samples should be collected within 15 minutes of the nominal collection time and the 12-hour PK blood sample should be collected within 30 minutes of the nominal collection time.

[859] Pharmacokinetics - Urine: A predose sample and a 0 to 12-hour total urine postdose sample will be collected from all subjects on Study Days 1 and 29 for PK determination of SNA-120, SNA-125, and K252b levels.

[860] Subject Assessments - Itch Numeric Rating Scale: The I-NRS is a 1 item daily PRO measure that the subjects use to assess pruritus severity at its worst in the 24 hours prior to the assessment based on an 11-point numeric rating scale ranging from 0 ("no itching at all") to 10 ("worst possible itching"). The I-NRS assesses overall pruritus severity. An I-NRS score of at least 4 is required to be eligible for the study. The investigator will evaluate subject eligibility based on itch associated with PV plaques at Screening. The Baseline measurement will be collected on Study Day 1 prior to study drug administration. The change from Baseline at each study visit will be determined.

[861] Investigator Assessments: Investigator assessments include: BSA affected by psoriasis; IGA of overall psoriasis (Apremilast (Otezla) CDER Medical Review of Celgene NDA, 2014. [Accessdata.fda.gov](https://www.accessdata.fda.gov/drugsatfda_docs/nda/2014/012507Orig1s000.pdf). Accessed August 8, 2017.); and PASI (Fredriksson T, Pettersson U. Severe psoriasis-oral therapy with a new retinoid. *Dermatologica*. 1978;157(4):238-244.).

[862] Body Surface Area Affected by Psoriasis: Assessment of the percentage of a subject's BSA affected by psoriasis will be made by best estimates of the investigator, using the "handprint" method where 1% = 1 subject handprint. At screening and baseline this should be $\geq 20\%$ of BSA. The change from baseline BSA affected by psoriasis will be determined.

[863] Investigator Global Assessment: The IGA is a site-based clinician-reported outcome measure that assesses the overall severity of psoriasis by evaluating induration (plaque elevation), erythema, and desquamation (scaling) on a 5-point scale from 0 to 4, where 0 = Clear to 4 = Severe. The investigator will make this assessment to calculate the overall improvement in psoriasis (the proportion of subjects achieving a rating of "Clear" or "Almost Clear" and at least a 2-point decrease [improvement] on the 5-point scale) using the IGA. The proportion of subjects achieving at least a 1-grade and 2-grade decrease from baseline will also be calculated.

[864] Psoriasis Area and Severity Index: The PASI score is a site-based ClinRO by which a weighted sum of erythema, scaling, and induration/thickness scores over different regions of the body is determined, using the algorithm given in Fredriksson T, Pettersson U., *Dermatologica*. 1978;157(4):238-244.). Each symptom will be scored on a 5-point scale from 0 to 4, where 0 = Clear and 4 = Very marked. The change from Baseline in the PASI score, and the percentages of subjects who have achieved a $\geq 75\%$ or $\geq 50\%$ reduction from baseline (PASI-75 and PASI-50, respectively), will be determined.

Summary of Results

[865] Study subject disposition is presented in Table 51. Demographic and baseline characteristics are summarized in Table 52. The amount and duration of study drug exposure is summarized in Table 53.

Table 51. Subject Disposition

Category	N (%)
Enrolled	30
Treated	30 (100)
Completed Study (Visit 6)	27 (90)
Discontinued	3 (10)
Discontinued due to AE	0 (0)

Table 52. Subject Demographics and Baseline Characteristics

Characteristic	N =30
Age (years) mean (SD)	45.8 (10.1)
Female n (%)	11 (36.7)
Race – White n (%)	28 (93.3)
BMI (kg/m ²) mean (SD)	34.3 (8.1)
Percent BSA mean (SD)	30.1 (10.5)
PASI mean (SD)	18.72 (6.86)
IGA	
• Moderate (3) n (%)	23 (76.7)
• Severe (4) n (%)	7 (23.3)
NRS mean (SD)	8.3 (1.3)

Table 53. Study Drug Exposure

SNA-120 (N=30)	Mean (SD)
Duration of exposure (days)	28.0 (3.9)
Total amount of study drug applied (g)	830.43 (585.36)

[866] Pharmacokinetic Results: On Days 1 and 29, the majority of subjects had no detectable levels of SNA-120 in the plasma (66.7% and 67.9% of subjects, respectively). 10 and 9 out of 30 and 28 subjects (33.3% and 32.1%, respectively on Days 1 and 29) had detectable levels of SNA-120 in the plasma. Nearly all were lower than 2.5 ng/mL (i.e., <1 nM), except for 1 subject who had a C_{max} of 5.04 ng/mL (i.e., 2.05 nM) on Day 1 and 4.64 ng/mL (i.e., 1.89 nM) on Day 29, respectively. Overall, there was no accumulation of SNA-120 in the plasma following repeated BID topical application. In all post-baseline PK sampling timepoints, the maximum number of subjects with a detectable level of SNA-120 was 6 (out of 30). Of 489 post-baseline blood PK samples, only 65 (13.3%) had detectable

levels of SNA-120 (>0.500 ng/mL). In addition, there were no detectable plasma levels of the amide form of SNA-120 (<1.00 ng/mL) or K252b (<0.250 ng/mL), the two potential metabolites of SNA-120, at any post-baseline timepoint. PK results are summarized in Table 54.

Table 54. SA-120 Plasma Concentrations at Days 1 and 29

	Peak Plasma SNA-120 Concentrations (ng/mL)		
	Below Quantitation Limit (BQL; i.e., 0.500 ng/mL plasma)	<2.50 (2.5 ng/mL is approximately 1 nM)	≥2.50
Day 1 (N=30)	<0.500 (n=20; 66.7 %)	0.500–1.51 (n=9; 30.0 %)	5.04 (n=1; 3.3 %)
Day 29 (N=28)	<0.500 (n=19; 67.9 %)	0.583–0.902 (n=8; 28.6 %)	4.64 (n=1; 3.6 %)

[867] ECG Including QTcF Assessment: The number of subjects undergoing standard ECG recordings at each pre-specified post-dosing time point varied from 27 to 29, and the number of subjects with Holter-extracted ECGs ranged from 22 to 29. The standard ECG recording results are summarized in Table 55. The observed mean changes from baseline were small. No trends over time were present. No individual changes were clinically meaningful. Results from the Holter-extracted ECGs are shown in Table 56. Changes from baseline during Days 1 and 29 were generally small and consistent with circadian and spontaneous variation. No dose-response pattern was present. No individual changes were clinically meaningful. Overall, no subjects in this study demonstrated a QTcF duration >500 msec or an increase from baseline of >60 msec. In this study of 30 patients with on-treatment ECG recordings at up to 15 pre-specified time points on multiple days and within-day sampling, there was no evidence of an ECG effect of 0.5% (w/w) SNA-120.

Table 55. Standard ECG Results: Minimum and Maximum Mean Change

ECG	Minimum mean (SD)	Day	Maximum Mean (SD)	Day
HR, bpm	-0.1 (9.9)	43	3.1 (11.3)	8
PR, msec	-1.5 (12.8)	15	2.4 (13.5)	8
QRS, msec	-0.5 (6.0)	15	4.0 (6.6)	43
QTcF, msec	1.7 (23.6)	15	9.1 (16.4)	43

Table 56. Holter ECG Results - Minimum and Maximum Mean Change from Baseline Assessed Over 12 Hours on Days 1 and 29

ECG	Minimum mean (SD)	Day/Hour	Maximum Mean (SD)	Day/Hour
HR, bpm	-5.12 (6.23)	1/1	8.14 (5.68)	1/12
	-4.25 (7.80)	29/1	9.50 (9.73)	29/8

PR, msec	-1.31 (8.08)	1/12	2.33 (6.66)	1/0.75
	0.39 (10.84)	29/12	3.90 (8.61)	29/0.5
QRS, msec	-1.32 (4.95)	1/8	1.13 (3.85)	1/0.75
	-1.90 (4.75)	29/8	1.46 (5.23)	29/1
QTcF, msec	-5.12 (11.32)	1/12	2.93 (9.63)	1/2
	-4.97 (13.96)	29/12	5.98 (10.45)	29/0.75

[868] Adverse Events: A total of 9 subjects reported 12 AEs, one of which (i.e., mild pruritus) was deemed related to study drug. No subject reported a severe AE. There were no serious AEs (SAEs) and no deaths reported during the study. No subject discontinued the study due to an AE. Table 57 summarizes AEs.

Table 57. Adverse Events

Type of Event	N=30 n (%) [events]
AE	9 (30) [12]
Severe AE	0 (0)
SAE	0 (0)
AE related to study drug	1 (3.3) [1]
AE resulting in discontinuation	0 (0)

[869] Exploratory Efficacy: This study enrolled moderate to severe psoriasis patients with >20% BSA and at least moderate pruritus (NRS >4) (e.g., moderate to severe). At baseline, subjects had a mean PASI score of 18.72 and a mean itch-NRS of 8.3. Subjects were treated twice daily with 0.5% SNA-120 (w/w) ointment in an open label protocol. After 4 weeks of treatment, subjects experienced a mean improvement from baseline in itch-NRS of 3.4 (or 41.35%). Sixty percent of subjects had at least a 3-grade improvement in itch-NRS. After 4 weeks of treatment, subjects experienced a mean improvement from baseline in PASI of 6.44. Ten (33.3%) and 3 (10%) of the subjects achieved PASI 50 and PASI 75, respectively. With respect to IGA, 30% and 13.3% of subjects experienced at least a 1-grade and 2-grade improvement, respectively, from baseline to 4 weeks. Efficacy results are summarized in Table 58.

Table 58. Exploratory Efficacy Results

Endpoint		Week 4
NRS (change from baseline)	mean (SD)	-3.4 (2.4 %)
NRS (% change from baseline)	mean (SD)	-41.35 (29.30 %)
NRS 50	n (%)	14 (46.7 %)

NRS 3-grade	n (%)	18 (60.0 %)
PASI (change from baseline)	mean (SD)	-6.44 (7.35 %)
PASI 50	n (%)	10 (33.3 %)
PASI 75	n (%)	3 (10.0 %)
IGA 1-grade	n (%)	9 (30 %)
IGA 2-grade	n (%)	4 (13.3 %)

[870] Conclusions: These results support the hypotheses described in the study protocol regarding the PK and safety profiles of SNA-120. There was minimal to no systemic exposure after topical application of SNA-120 under maximal use conditions and no associated clinically significant ECG changes, including no evidence of QTc prolongation. There were no systemic safety signals and no dermal tolerability issues. Additionally, improvements in pruritus and psoriasis were observed in exploratory efficacy analyses.

**EXAMPLE 28: PHASE 1 RANDOMIZED. VEHICLE- AND COMPARATOR-
CONTROLLED. DOUBLE-BLIND TRIAL TO ASSESS EFFICACY AND SAFETY OF SNA-
125 IN A PSORIASIS PLAQUE TEST**

[871] Psoriasis is a common and often chronic skin disease that affects approximately 2 % of the population. Chronic plaque psoriasis is the most common form, affecting 85 to 95 % of all patients with the disease. The rate of psoriasis varies according to age, gender, region and ethnicity. Chronic plaque psoriasis is characterized by raised, well-demarcated, erythematous oval plaques covered with adherent silvery scales and is most commonly found on the scalp, nails, lower back and the extensor sides of the joints. The scales result from a hyperproliferative epidermis with premature maturation of keratinocytes and incomplete cornification, with retention of nuclei in the stratum corneum. This thickening of the epidermis and an inflammatory infiltrate result in thickening of the skin and the raised nature of the skin lesions observed clinically. The inflammatory infiltrate consists of dendritic cells, macrophages and T-cells in the dermis, with neutrophils and some T-cells in the epidermis. Abnormalities of the innate immune system are also involved.

[872] While systemic biologics have greatly improved the therapeutic options for moderate to severe psoriasis, the majority of psoriasis patients have mild-to-moderate disease. Topical corticosteroids and vitamin D analogs are first-line for treating mild-to-moderate disease and almost 90 % of all psoriasis patients across all disease severities utilize topical drug products as their sole treatment or as an adjunct to their systemic treatment. However, 75 % of all patients treated with a topical agent fail to achieve significant, long-lasting improvement, and as a result, 40 % of diagnosed patients choose to forgo treatment. A 2013 survey analyzing the patient treatment landscape highlighted under treatment and non-treatment as the most

significant issues in psoriasis; widespread patient dissatisfaction with current treatment options was cited as the primary driver.

[873] SNA-125 represents one approach to the treatment (e.g., topical) of atopic dermatitis, psoriasis vulgaris, or both, as well as the substantial and undertreated condition of associated pruritus. Two positive controls will also be tested in this study: SNA-120; and DAIVONEX® Ointment 50 µg (calcipotriol)—a topical treatment for psoriasis vulgaris.

[874] In this example SNA-125 has been formulated for topical administration as an aqueous gel containing dimethyl sulfoxide (DMSO), propylene glycol, hydroxyethyl cellulose, benzyl alcohol, and trolamine at 2% w/w and 0.2% w/w strengths. A matching placebo vehicle with the same quantitative/qualitative composition with the exclusion of the active component has also been developed. Of note, the placebo vehicle was found to possess mild irritation potential in skin of subjects with a history of atopic dermatitis in a 14-day cumulative irritation assay.

[875] The following adverse reactions have been identified for the treatment with DAIVONEX® 50 µg/g calcipotriol ointment 0.005 % (18):

Diseases of the skin and the subcutaneous cell tissue:

Frequent ($\geq 1\%$ - $< 10\%$): pruritus, burning, stinging, skin dryness, erythema, skin irritation/rash (incl. erythematous, maculopapulous or pustulous irritations),

Occasionally ($\geq 0.1\%$ - $< 1\%$): eczema, contact dermatitis, worsening of psoriasis, and

Very rarely ($< 0.01\%$): temporary change of the skin pigmentation (hyperpigmentation, depigmentation), temporary photosensitivity, hypersensitivity (including urticaria, periorbital or facial edema, angioedema), perioral or facial dermatitis; and

Metabolism and nutritional disturbances:

Very rarely ($< 0.01\%$): hypercalcemia, hypercalciuria.

[876] One objective of this trial is to evaluate antipsoriatic efficacy and safety of SNA-125 in topical formulations compared to topically applied reference formulations and vehicle in subjects with psoriasis vulgaris.

[877] This is a 2-center, randomized, vehicle- and comparator-controlled, double-blind trial. Treatments will be randomly assigned to the test fields. All subjects will receive all treatments, with intra-individual comparison of treatments. Fifteen (15) male or female subjects of non- childbearing potential aged 18 years or older with chronic psoriasis vulgaris will be randomized to produce at least 12 evaluable cases at the end of the trial.

[878] A total of 5 test fields located on the torso or the extremities will be examined per subject. The test fields will be treated occlusively for a 12-day treatment period (no IMP application on Day 7 and 12).

[879] Treatment will be topical application of approximately 100 μL per test field (1.1 cm^2) of the respective IMP once daily during a 12-day treatment period (10 treatments, no application on Days 7 and 12).

[880] Investigational medicinal products to be administered are as follows:

IMP 1: 2% w/w SNA-125, daily dosage: approx. 2 mg SNA-125, total dosage for the treatment period: approx. 20 mg SNA-125;

IMP 2: 0.2% w/w SNA-125, daily dosage: approx. 0.2 mg SNA-125, total dosage for the treatment period: approx. 2 mg SNA-125;

IMP 1 and IMP 2, total combined dosage of SNA-125: daily dosage: approx. 2.2 mg SNA-125, total dosage for the treatment period: approx. 22 mg SNA-125;

IMP 3: 0.5% w/w SNA-120 (comparator), daily dosage: approx. 0.5 mg SNA-120, total dosage for the treatment period: approx. 5 mg SNA-120;

IMP 4 (vehicle): active ingredient-free vehicle to IMP 1 and IMP 2;

IMP 5 (marketed comparator): DAIVONEX® 50 pg/g ointment (calcipotriol 0.005%), daily dosage: approx. 0.005 mg calcipotriol, total dosage for the treatment period: approx. 0.05 mg calcipotriol.

[881] Details of the IMPs are given in Table 59 and Table 60.

Table 59.

Generic name/brand name/INN	IMP 1: SNA-125 Gel 2%	IMP 2: SNA-125 Gel 0.2%	IMP 3: (comparator) SNA-120
Formulation	Gel	Gel	Ointment
Active ingredient	SNA-125	SNA-125	SNA-120
Amount per unit	20 mg/g (2%)	2 mg/g (0.2%)	5 mg/g (0.5%)
Additional ingredients	<ul style="list-style-type: none"> • purified water • benzyl alcohol • propylene glycol • dimethyl sulfoxide (DMSO) • hydroxyethyl cellulose (HEC) • trolamine 	<ul style="list-style-type: none"> • purified water • benzyl alcohol • propylene glycol • dimethyl sulfoxide (DMSO) • hydroxyethyl cellulose (HEC) • trolamine 	<ul style="list-style-type: none"> • butylated hydroxytoluene (BHT) • benzyl alcohol • propylene glycol • white soft paraffin • white wax • stearyl alcohol • cholesterol

Table 60.

Generic name/brand	IMP 4 (vehicle to IMP 1 and IMP 2):	IMP 5 (marketed comparator):
--------------------	-------------------------------------	------------------------------

name/INN		DAIVONEX [®] 50 µg/g (calcipotriol 0.005%)
Formulation	Gel	Ointment
Active ingredient	-	calcipotriol
Amount per unit	-	50 µg/g
Additional ingredients	<ul style="list-style-type: none"> • purified water • benzyl alcohol • propylene glycol • DMSO • Trolamine • hydroxyethyl cellulose (HEC) 	<ul style="list-style-type: none"> • disodium edetate • sodium monohydrogen-phosphate-dihydrate • viscous paraffin • white petrolatum • macrogol (2) stearyl ether • propylene glycol • all-rac-α-tocopherole • purified water

[882] All subjects will receive the same treatments. There will be no subdivision into treatment groups. A subject's test fields will be numbered with 1 to 5. Figure 135 shows representatives of 2 of a number of possible scenarios. The actual distribution of plaques and test fields may vary individually. Randomization of the IMPs 1 to 5 will be performed using a Williams design. For each subject, a treatment sequence will be randomly assigned.

[883] Approximately 100 µl of each IMP will be applied to a total of 5 test fields using special test chambers (Finn chambers, 12 mm inside Ø, 14 mm outside Ø). This is the amount required to completely fill the test chamber. The IMPs will be applied in the Finn chamber and seated in holes punched in a hydrocolloid dressing. The hydrocolloid dressing will be fixed before on the skin with adhesive patches containing the same holes for the chambers like the hydrocolloid dressing.

[884] The chambers themselves will be fixed in place with LEUKOSILK[®] or the like and will be removed before each new application. The distance between the chambers must be at least 1.5 cm to exclude interactions with neighboring fields. The fields will be treated occlusively for a treatment period of 12 days. On 10 of the trial days treatments will be performed (Days 1 to 6 and 8 to 11). Before each new application, remaining preparation residues will be removed by gently cleansing each test field with a separate soft tissue. The hydrocolloid dressing will be renewed on Days 4 and 8.

[885] In a subgroup of at least 5 subjects, a total of 12 punch biopsies per subject will be taken under local anesthesia for hematoxylin and eosin stain (H&E), immunohistochemistry (IHC) and biomarker analyses. At Baseline, 4 biopsies will be obtained from lesional untreated and non-lesional skin (two 4-mm biopsies from each skin type). On Day 12 (EoT)

8 biopsies will be obtained from test fields treated with IMP 1 (SNA-125 high dose), IMP 2 (SNA-125 low dose), IMP 3 (SNA-120) and IMP 4 (vehicle control) (two 4-mm biopsies from each test field). All biopsies will be excised after photographic documentation, sonography and clinical assessments. All skin biopsies will be processed and analyzed using established methods and protocols.

[886] Blood pressure and pulse rate will be measured at screening, baseline and Day 12 (EoT). A physical examination will be performed at screening, Day 1 and on Day 12.

[887] The trial is aimed to detect a treatment effect as compared to DAIVONEX® 50 µg/g ointment (calcipotriol 0.005%) within the planned trial design. A former in-house trial revealed a psoriatic infiltrate thickness reduction on Day 12 of about 51 % of the baseline level with a coefficient of variance (CV) below 0.97 for the difference to vehicle in change from baseline.

Summary of Results

[888] Study subject disposition is presented in Table 62.

Table 62. Subject Disposition Category

	N (%)
Enrolled	15 (100)
Treated	15 (100)
Completed Study (Visit 14)	15 (100)
Discontinued	0 (0)
Discontinued due to AE	0 (0)

[889] The amount and duration of study drug exposure is summarized in Table 63.

Table 63. Study Drug Exposure SNA-125 2%

SNA-125 2%	N = 15
No. treatments	10
Duration of exposure (days)	12
Total amount of study drug applied (mg)	20
SNA-125 0.2%	N = 15
No. treatments	10
Duration of exposure (days)	12
Total amount of study drug applied (mg)	2
SNA-120 0.5%	N = 15
No. treatments	10
Duration of exposure (days)	12
Total amount of study drug applied (mg)	5
Calcipotriol 0.005%	N = 15

No. treatments	10
Duration of exposure (days)	12
Total amount of study drug applied (mg)	0.05

[890] Analyses of histology and biomarkers were conducted on biopsy samples obtained from lesional (LS) and non-lesional skin (NLS) from a subset of 11 subjects.

[891] A total of 12, 4 mm punch biopsies were taken per subject. Four biopsies were taken at baseline from lesional and non-lesional skin. Eight biopsies were taken at Day 12 (EoT) from treated lesional skin including 2 from each test field for 2% and 0.2% SNA-125, gel vehicle, and 0.5% SNA-120.

[892] H&E and immunohistochemistry (IHC) results from one subject are shown in panel display in Figure 136. In aggregate, these data demonstrate an improvement on epidermal thickness and staining for various biomarkers. Specifically, 0.2 % SNA-125 had a reduction in epidermal thickness and K16 expression. Also, 0.5 % SNA-120 showed a reduction in K16 staining.

[893] Figure 137 shows the aggregate epidermal thickness results for treated lesional skin at Day 12. There was a statistically significant improvement in epidermal thickness for 0.2 % SNA-125 compared to Baseline (day 1) lesional skin.

[894] The normalization and expression controls for lesional and non-lesional skin in this study indicated the expected molecular signatures of psoriasis based on historical experience. Aggregate microarray (MA) expression data are presented in Figure 138 for each test article showing global up- and down-regulation of genes.

[895] Improvement in gene expression results was attained with 0.2 % SNA-125, where an approximate 11 % mean improvement in gene expression was observed. This level of response is consistent with very early (i.e., one week) treatment responses seen with some biologics (e.g., etanercept and ustekinumab) in separate experiments prior to their full clinical effect.

[896] Figure 139 shows the results of disease (i.e. psoriasis) transcriptome (i.e., discrete set of co-modulated genes) analysis, quantification of improvement of different disease pathways. The best improvements were seen with 0.2 % SNA-125 and 0.5 % SNA-120.

[897] Subjects with 50 % improvement with any test article for keratin 16 (K16) (MA gene) and K16 (RTPCR marker) were considered “responders” to treatment. There were 3 subjects out of 11 subjects with biopsies who met this definition. The responder analysis of global gene expression data is shown in Figure 140. Responders showed higher overall % improvement than non-responders for all test articles (with evident increased variability - SD).

[898] Conclusions: this exploratory Phase 1/2, double-blind, within-subject vehicle-controlled study in 15 subjects, using a psoriasis microplaque model, measured an effect on

local tolerability, and histology and biomarkers with two doses of SNA-125 prototype gel applied once daily for 10 days. In this model, SNA-125 was well-tolerated and showed no safety signals; there were no application site treatment emergent adverse events (TEAEs) in any of the test fields. Histological and biomarker analysis showed a statistically significant reduction with SNA-125 0.2 % in epidermal thickness from baseline (-17 %, $p < 0.05$), as well as modulation of certain psoriasis-relevant biomarkers and gene expression profiles. Histological and biomarker analyses indicate there was a signal for a drug effect with 0.2 % SNA-125 that was seen when using pathway- or whole transcriptome-related approaches, an effect that was statistically different from vehicle.

[899] Overall, these data support impact of SNA-125 (i.e. the 0.2 % dose) on the histological characteristics and molecular signature of lesional psoriasis skin.

EXAMPLE 29: TNBS-INDUCED COLITIS MOUSE MODEL

[900] TNBS-induced colitis model is a widely used model of Crohn's Disease. Ethanol disrupts the intestinal barrier and enables the interaction of TNBS with colon tissue protein. Intrarectal administration of TNBS renders colonic proteins immunogenic to the host immune system. Mice develop a transmural colitis mainly driven by a polarized T_H1 -immune response. Thus, colitis was induced in C57Bl/6 mice by intra-rectal administration of 5 mg of TNBS in 50 % ethanol/water. Vehicle and test agents were administered orally (PO). Treatment groups were SNA-125 (400 mg/kg and 600 mg/kg), SNA-352 (400 mg/kg and 600 mg/kg), vehicle (water), prednisolone (1 mg/kg PO; positive control), and tofacitinib (15 mg/kg PO). The vehicle, SNA-125, SNA-352, prednisolone, and tofacitinib were administered BID days -1-4. In-life observations (body weight, morbidity, presence of diarrhea and/or bloody stool) were conducted. The disease activity index DAI (weight loss, diarrhea and blood stool) was determined for all study groups and the area under the curve (AUC) was calculated using trapezoidal rule transformation (see Table 66). Endoscopy was performed on days 2 and 4, and colitis severity was scored using a 0-4 scale (see Example 26). The study design is summarized in Table 64.

Table 64. TNBS-induced colitis study design.

Group	Number of Animals	TNBS Day 0	Treatment	Dose	Route	Dose Schedule	Disease Activity Index	Endoscopy Schedule	Terminal Collection (Day 4)
1	8	---	---	---	PO	BID Days -1 to 4	Days -1 to 4	Days 2 & 4	Blood: Plasma Colon: Snap
2	18	5 mg	Vehicle	---	PO				
3	15		Tofacitinib	15 mg/kg	PO				
4	15		Prednisolone	1	PO				

				mg/kg						frozen; Formalin
5	15		SNA-125	400 mg/kg	PO					
6	15		SNA-125	600 mg/kg	PO					Record time of final dose and time of sacrifice
7	15		SNA-352	400 mg/kg	PO					
8	15		SNA-352	600 mg/kg	PO					

[901] TNBS induced substantial colitis. No signs of efficacy were observed for the positive control (prednisolone). The tofacitinib group showed a significant reduction in the colon weight/length ratio. SNA-352 and SNA-125 showed signs of efficacy. A trend towards significance, as compared to vehicle, was present in the composite DAI score for SNA-125 (24 % DAI reduction for both 400 mg/kg BID and 600 mg/kg BID). Statistical significance compared to vehicle was present in the scores for Colitis Severity by Endoscopy Day 2, Stool Consistency Day 4, Colitis Severity by Endoscopy Day 4, and Composite Endoscopy DAI Day 4 for SNA-352 (600 mg/kg BID), as well as a trend towards significance for Endoscopy DAI Day 2 and colon weight/length ratio for SNA-352 (600 mg/kg BID). Results are shown in Figures 141-149 (ANOVA, Fisher post test; as compared to vehicle). Statistical results (compared to vehicle) are summarized in Table 65. In Table 65, "X" indicates statistical significance, and "+" indicates a trend toward significance.

Table 65. TNBS-induced colitis study statistical results.

	Stool Cons. Day 2	Colitis Sev. Day 2	Endo DAI Day 2	Stool Cons. Day 4	Colitis Sev. Day 4	Endo DAI Day 4	BW Change	W/L Ratio	DAI
Naïve	X	X	X	X	X	X	X	X	X
Tofacitinib		+						X	
Prednisolone								+	
SNA-125 (800 mg/kg)				+		+	+	+	+
SNA-125 (1200 mg/kg)				+				+	+
SNA-352 (800 mg/kg)							+		
SNA-352 (1200 mg/kg)		X	+	X	X	X		+	

Table 66. DAI scoring.

	Weight Loss	Diarrhea	Blood Stool
0	0.0–4.99 %	Normal	Normal
1	5.0–9.99 %		
2	10.0–14.99 %	Loose stools	Loss of vascularity and friability
3	15.0–19.99 %		
4	>20.0 %	Diarrhea	Gross bleeding

**EXAMPLE 30: EVALUATION OF THE SAFETY AND EFFICACY OF TOPICALLY
APPLIED SNA-125 GEL IN HEALTHY VOLUNTEERS AND SUBJECTS WITH ATOPIC
DERMATITIS IN A DOUBLE-BLIND. RANDOMIZED. INTRAINDIVIDUAL. VEHICLE-
CONTROLLED STUDY**

[902] The study subjects consisted of 2 sequential cohorts of adult male or female subjects: (1) healthy volunteers (HV) and (2) subjects with atopic dermatitis (AD). In Cohort 1, 4 HV were evaluated for the safety of topically applied SNA-125 gel and vehicle following 7 days of treatment. The gel contains SNA-125 (active ingredient), benzyl alcohol, propylene glycol, dimethylsulfoxide (DMSO), hydroxyethylcellulose (HEC), trolamine, and water (as needed) (the vehicle formulation was the same as the SNA-125 formulation without the active). In Cohort 2, 30 subjects with AD were included in a double-blind, randomized, intraindividual, 4-arm, vehicle-controlled design to evaluate the safety and efficacy of topically applied SNA-125 gel, applied once-daily for 14 days. Subjects with AD were required to have at least 4 application areas with AD lesions of moderate severity defined by a Total Signs Score (TSS) of >5. Four study products were applied in the clinic without occlusion at approximately 4 mg/cm² on individual application areas of 3 cm in diameter (approximately 7cm²). Application areas were randomized (1:1:1:1) to SNA-125 0.2 %, SNA-125 2 %, vehicle, and mometasone 0.1 % cream. Six skin biopsies were collected from all AD subjects for immunohistochemistry (IHC) and gene expression analysis using RT-PCR and genomic profiling. Two biopsies were taken at baseline (Day 1), 1 from non-lesional and 1 from lesional skin, and 4 biopsies were taken at Day 15 within application areas (1 biopsy per application area).

[903] TSS was determined as follows. Six items (erythema, edema/papulation, oozing/crusting, excoriation, lichenification, and dryness) were selected to evaluate the AD severity. The intensity of each item was graded using the following 4-point scale: 0 = absence; 1 = mild; 2 = moderate; and 3 = severe (half steps not allowed). The area chosen for grading was required to have been representative (average intensity) for each item. The individual intensity ratings for each item were then added (ranging from 0-18). The sum of the measures above represented the lesional TSS, which can vary from 0 to 18.

[904] [000191] This study showed that topical application of SNA-125 (0.2 % and 2 %) was well tolerated and showed no safety signals in both healthy subjects and patients with atopic dermatitis, with no serious adverse events reported and demonstrated acceptable safety. Similar modest reductions in clinical scores of target lesions were observed for both SNA-125 and vehicle.

[905] Analyses of histology and biomarkers were conducted on biopsy samples obtained from lesional and non-lesional skin from all subjects.

[906] Effects on various biomarkers related to AD were observed in the SNA-125 0.2 % dose. For example, a reduction in epidermal thickness was observed, as well as changes in the expression levels of certain relevant disease genes. The following genes showed statistically significant expression differences compared to baseline: NGFR (p75), NGF, NTRK1, IL-23/p19, IL23A, IL5, IL4R, CCL5, cGRP, CxCL2, CxCL1, DEFB4B, S100A9, S100A7, CCL26, CCL13, IL32, CxCL9, CxCL10. As compared to vehicle, expression levels of the following genes were statistically significantly different for SNA-125 0.2 %: NGFR (p75), TPSB2, IL5, and CCL26.

[907] For SNA-125 0.2 %, there was a statistically significant correlation between positive improvement in the expression of some relevant disease genes and improvement in clinical TSS score.

EXAMPLE 31: RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, PHASE 2B, MULTICENTER STUDY TO EVALUATE THE SAFETY, EFFICACY, AND TOLERABILITY OF SNA-120 (PEGCANTRATINIB1 OINTMENT IN MALE AND FEMALE SUBJECTS WITH PRURITUS ASSOCIATED WITH PSORIASIS VULGARIS

[908] The study subjects (mild-to-moderate psoriasis patients with at least moderate pruritus (itch)) consisted of 208 adult subjects that were ≥ 18 years of age, randomized 1:1:1 to SNA-120 0.05 %, SNA-120 0.5 % and vehicle. Subjects self-administered the assigned study drug (SNA-120 ointment or vehicle; the vehicle formulation was the same as the SNA-120 formulation without the active) to the indicated areas twice-daily over 12 weeks. The ointment contains SNA-120 (pegcantratinib [active ingredient]), butylated hydroxytoluene, benzyl alcohol, propylene glycol, white soft paraffin (white petrolatum), white wax, stearyl alcohol, and cholesterol. Assessments were performed to determine the treatment effect on pruritus, as well as the underlying visible plaque psoriasis, and safety and local tolerability of SNA-120. Pruritus was assessed by the subject-administered I-NRS and recorded on their daily diaries. Plaque psoriasis was assessed by the investigator by Investigator Global Assessment (IGA) and Psoriasis Area Severity Index (PASI). At baseline, subjects were required to have a weekly mean I-NRS score of at least 5 on the 11 point I-NRS scale, and mild to moderate plaque psoriasis (IGA 2 or 3).

[909] Investigator Global Assessment: The IGA is a site-based (clinician-reported outcome measure) that assesses the overall severity of psoriasis by evaluating induration (plaque elevation), erythema, and desquamation (scaling) on a 5-point scale from 0 to 4, where 0 = Clear to 4 = Severe. The investigator will make this assessment to calculate the overall improvement in psoriasis (the proportion of subjects achieving a rating of "Clear" or "Almost Clear" and at least a 2-point decrease [improvement] on the 5-point scale) using the IGA. The proportion of subjects achieving at least a 1-grade and 2-grade decrease from baseline will also be calculated.

[910] Psoriasis Area and Severity Index: The PASI score is a site-based investigator assessed (clinician reported outcome measure) by which a weighted sum of erythema, scaling, and induration/thickness scores over different regions of the body is determined, using the algorithm given in Fredriksson T, Pettersson U., *Dermatologica*, 1978, 157(4):238-244). Each symptom will be scored on a 5-point scale from 0 to 4, where 0 = Clear and 4 = Very marked. The change from Baseline in the PASI score, and the percentages of subjects who have achieved a $\geq 75\%$ or $\geq 50\%$ reduction from baseline (PASI-75 and PASI-50, respectively), will be determined.

[911] As seen in Table 67, SNA-120 0.05 % demonstrated a statistically significant effect on two key measures of psoriasis, PASI-75 and IGA >2-grade improvement with clear (grade 0) or almost clear (grade 1). SNA-120 ointment was well tolerated and demonstrated an acceptable safety profile based on the treatment-emergent adverse events, the majority of which were mild to moderate.

[912] As seen in Table 68, there was a reduction from baseline to the study Week 8 (primary endpoint) in the week's mean (+SD) daily pruritus score (Itch Numeric Rating Scale [I-NRS]; the I-NRS is an 11-point scale ranging from 'no itch' (0) to the 'worst imaginable itch' (10) that study participants used daily to report the intensity of their worst itch in the previous 24 hours) of -4.3 ± 2.41 ($p = 0.244$) for SNA-120 ointment 0.05 %, -3.7 ± 2.34 ($p = 0.499$) for SNA-120 ointment 0.5 %, and -4.0 ± 2.57 for vehicle. The I-NRS reduction of ≥ 3 point and >4 point were observed for $45 \pm 72.6\%$ ($p = 0.251$) and $33 \pm 53.2\%$ ($p = 0.557$), $37 \pm 57.8\%$ ($p = 0.545$) and $28 \pm 43.8\%$ ($p = 0.097$), and $41 \pm 63.1\%$ and $38 \pm 58.5\%$ of subjects for SNA-120 0.05 %, SNA-120 0.5 %, and vehicle, respectively, at Week 8.

[913] SNA-120 was well-tolerated with no serious treatment-related adverse events. In this study, subjects treated with SNA-120 (0.05%) achieved statistical significance, compared to vehicle, on endpoints of psoriasis disease severity. Patients treated with SNA-120 (0.05%) experienced a mean 4.3 point (58%) reduction from baseline on the I-NRS, compared to a mean 4.0 point (55%) reduction with vehicle (not statistically significant, $p=0.244$). SNA-120 (0.5%) showed similar results. Subjects treated with SNA-120 also experienced a

meaningful reduction in itch; the result did not reach statistical significance against placebo (i.e., subjects treated with vehicle lacking SNA-120 experienced a meaningful reduction in itch).

[914] On pre-specified, secondary endpoints related to the clearance of plaques, SNA-120 (0.05%) demonstrated statistically significant and clinically meaningful improvement. Specifically, 27% of subjects experienced a 75% reduction in their Psoriasis Area and Severity Index (PASI 75) score from baseline, compared to 13% of subjects treated with vehicle ($p=0.045$). The study also included an Investigator Global Assessment (IGA), in which 29% of patients achieved a two-grade improvement and 'clear' or 'almost clear' skin, compared to 13% of subjects treated with vehicle ($p=0.036$). Both the PASI 75 and IGA results remained statistically significant at 14 weeks, two weeks after discontinuation of treatment.

Table 67.

Treatment Group	Week 8	Week 12
IGA $\geq$ 2 grade and 0/1 score (proportion of subjects) (p value)		
SNA-120 0.05 %	10 (16.4 %) $p = 0.518$	17 (28.8 %) $p = 0.036$
Vehicle	8 (12.5 %)	8 (13.3 %)
PASI-75 (proportion of subjects) (p value)		
SNA-120 0.05 %	15 (24.6 %) $p = 0.063$	16 (27.1 %) $p = 0.045$
Vehicle	8 (12.5 %)	8 (13.3 %)

Table 68.

I-NRS Measure	Baseline	Week 1	Week 8 (Primary)	Week 12(EoT)
Mean (SD); CFB (p value)				
SNA-120 0.05%	7.3 (1.31)	5.7 (1.53) -1.6 (1.51) $P = 0.212$	3.0 (1.96) -4.3 (2.41) $P = 0.244$	2.6 (2.10) -4.6 (2.44) $P = 0.231$
SNA-120 0.5%	7.4 (1.24)	5.8 (1.63) -1.6 (1.43) $P = 0.423$	3.8 (2.24) -3.7 (2.34) $P = 0.499$	3.5 (2.5) -3.9 (2.61) $P = 0.384$
Vehicle	7.4 (1.39)	6.0 (1.78) -1.3 (1.53)	3.4 (2.63) -4.0 (2.57)	3.0 (2.56) -4.3 (2.60)
% of subjects with ≥ 3 point improvement (p value)				

SNA-120 0.05%	NA	9 (12.9) P = 0.602	45 (72.6) P = 0.251	47 (79.7) P = 0.297
SNA-120 0.5%	NA	10 (14.5) P = 0.811	37 (57.8) P = 0.545	36 (63.2) P = 0.300
Vehicle	NA	11 (15.9)	41 (63.1)	44 (72.1)
% of subjects with ≥ 4 point improvement (p value)				
SNA-120 0.05%	NA	5 (7.1) P = 0.256	33 (53.2) P = 0.557	34 (57.6) P = 0.890
SNA-120 0.5%	NA	5 (7.2) P = 0.244	28 (43.8) P = 0.097	32 (56.1) P = 0.750
Vehicle	NA	2 (2.9)	38 (58.5)	36 (59.0)

[915] Additionally, biopsies were taken from 22 of the adult subjects (SNA-120 0.5% (7), SNA-120 0.05% (9), and vehicle (6)) at 0, 4, and 12 weeks. Biopsy analyses were conducted, which included immunohistochemistry (IHC), microarray, and quantitative reverse transcription polymerase chain reaction (qRT-PCR).

[916] The Immunohistochemistry analysis of the SNA-120 0.05% cohort showed meaningful and statistically significant improvement in the epidermal thickness at Week 12 (epidermal thickness reduction of about 94 micrometers) supporting the effect of SNA-120 (0.05%) on keratinocyte hyperproliferation and inflammation. Unexpectedly, the higher dose cohort (SNA-120 0.5%) did not further improve the epidermal thickness, but instead no statistically significant improvement in epidermal thickness was observed at week 12. Surprisingly, the lower dose treatment showed better reduction in epidermal thickness than the higher dose treatment. No statistically significant reduction in epidermal thickness was observed at week 4 or week 12 for the vehicle alone. The data also demonstrated a statistically significant reduction in T cell and dendritic cell counts, confirming an immune response to treatment with SNA-120 0.05%.

[917] In the microarray analyses, SNA-120 (0.05%) was also observed to affect most of the key inflammatory pathways in psoriasis (40 of 45) in a statistically significant manner when compared to vehicle at Week 12. Multiple genes contribute to the pathophysiology of plaque psoriasis, including signs and symptoms of the disease condition. The microarray analysis showed expression of 3,251 genes that were altered, either upregulated or down regulated, between the lesional and non-lesional skin biopsies taken at baseline before drug treatment. SNA-120 (0.05%) normalized the expression of a significant number of genes from lesional to non-lesional state. The strongest impact of SNA-120 (0.05%) was seen on the downregulated genes (279 genes) vs the upregulated genes (127 genes). By comparison

SNA-120, 0.5% impacted on 20 genes and vehicle had no significant effect on the gene expression profile.

[918] In the qRT-PCR analysis, SNA-120 (0.05%) demonstrated substantial, statistically significant gene expression improvements in key cytokines the overexpression of which play an important role in psoriasis pathogenesis, including IL-23, IL-12, IL-17A and IFN γ , among other cytokines, in PASI 75 responders and even led to restoration to non-lesional expression levels, further validating the strong pharmacological effect of SNA-120.

[919] In summary of the SNA-120 Phase 2b trial results, SNA-120 (0.05%) demonstrated statistically significant improvement compared to vehicle on important pre-specified endpoints of psoriasis disease severity, including the Investigator's Global Assessment (IGA) 2-grade composite, comprising a 2-grade improvement from baseline and clear (0) or almost clear (1) skin, which has been the Phase 3 primary endpoint for topical psoriasis drugs approved by the U.S. Food and Drug Administration (FDA). Specifically, 29% of patients achieved success on the IGA 2-grade composite, compared to 13% of subjects treated with vehicle. Similarly, 27% of subjects also experienced a 75% reduction from baseline in their Psoriasis Area and Severity Index score (PASI 75), compared to 13% of subjects treated with vehicle. Subjects also experienced an approximately 60% reduction from baseline in the associated pruritus (itch), although the pruritus result did not reach statistical significance compared to vehicle. SNA-120 was well-tolerated with no serious treatment-related adverse events. Treatment-related adverse events were observed in two patients and included dermatitis (0.5% group) and pain and pruritus (vehicle group). SNA-120 has been administered to more than 500 subjects for up to 12 weeks and has been well tolerated across all trials, with minimal to no demonstrated systemic bioavailability.

Summary of experiments on properties and activities of CT340/SNA-125

[920] CT340 has the following chemical formula: (9S, 10R, 12R)-2,3,9, 10,11,12-hexahydro-10-hydroxy-N-mPEG2000-9-methyl-1-oxo-9,12-epoxy-1H-diindolo[1,2,3-fg:3',2',T-k,l]pyrrolo[3,4-i][1,6]benzodiazocine-10-carboxamide.

[921] CT340 proves to be a narrow selective kinase inhibitor, whose main kinase targets are TrkA, MAP2Ks (involved in the TRPV1-PAR2 axis, playing a major role in itch in AD) and JAK3 (whose inhibition demonstrated to be effective in reduction of pruritus due to AD in clinical settings).

[922] The activity of SNA-125 against a large panel of kinases showed strong inhibitory activity (>70%) against all the Trk neurotrophin receptors, some kinases of the p38 mitogen-activated kinase pathway, JAK3, CGK2, FLT3, NuaK1, ROS and MST1. Additional profiling demonstrated anti-proliferative activity, anti-inflammatory activity and inhibition of neuronal (itch) signalling in multiple nonclinical experiments, in the absence of toxicity.

[923] SNA-125 was found to be active in an *in vitro* (*ex vivo*) Human Epidermal Keratinocytes (HEKn cell line) assay. SNA-125 was observed to penetrate the keratinocyte cellular membrane and interact with the target kinases intracellularly within the cytoplasm, leading to inhibition of proliferation of keratinocytes in a non-toxic manner.

[924] SNA-125 is a potent inhibitor of NGF/TrkA-dependent capsaicin responses in cultured sensory neurons, indicating that this small molecule TrkA/JAK3 inhibitor, similar but more potent to SNA-120, is a promising candidate for also targeting neuronal hypersensitivity (itch). The morphological findings of this study also indicate that SNA-125 did not affect neurite length, suggesting lack of a toxic effect.

[925] SNA-125 has also shown anti-inflammatory activity in a rabbit hypertrophic scar model, where daily topical dosing of scars with a 5% formulation confirmed the ability of SNA-125 to penetrate the epithelial barrier and led to a reduction in the level of scar inflammation.

[926] While penetration of the epithelial barrier and permeation of cell membranes has been demonstrated in several assays, demonstration of "LSE" and lack of Systemic absorption has been investigated with the use of single topical administration of SNA-125 in rats and minipigs. The results have shown that no or negligible amounts of SNA-125 are systemically absorbed through the dermal route, even after daily repeated administration and by using vehicle excipients with penetration enhancer properties. PK studies have shown that negligible systemic absorption following epicutaneous administration both in rodent and non-rodent species and rapid systemic elimination after IV administration (half-life less than 20 minutes).

[927] Single dose toxicological studies have been undertaken at doses of up to 100 mg/kg when administered both topically (epicutaneously) and intravenously in rats and dermally in minipigs. No signs of clinical or behavioural toxicity were observed.

[928] Repeat dose toxicology studies have been carried out via daily topical administration of SNA-125 at doses of up to 2.2 mg/cm²/day (SNA-125 20% formulation) in minipigs for 14 consecutive days, and at doses of up to 100 mg/kg administered intravenously daily in rats for 14 consecutive days. With topical administration, there were no clinical signs of toxicity, no treatment-related alterations were noted following ophthalmic examination, haematological and clinical chemistry examination and there were no noteworthy histological or immunohistochemical findings. No SNA-125 systemic absorption through the dermal route was observed.

[929] After the repeated IV administration, no treatment-related mortality was observed, and the main findings were treatment and dose related changes at mid and high dose levels (mainly inflammation of some organs and tissues) with complete recovery after a 14-day recovery period, suggesting a NOAEL of 10 mg/kg/day.

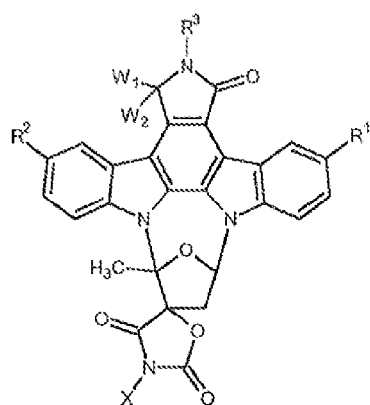
[930] PCT/EP2014/058584 is hereby incorporated by reference..

CLAIMS

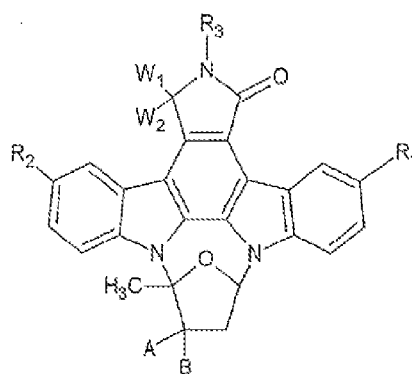
What is claimed is:

1. A method of treating a gastrointestinal system condition in a subject in need thereof, comprising administering to the subject a compound, or a pharmaceutically acceptable salt thereof, comprising an active entity linked to at least one polymer.
2. A method of treating multifocal inflammation caused by a gastrointestinal system condition in a subject in need thereof, comprising administering to the subject a compound, or a pharmaceutically acceptable salt thereof, comprising an active entity linked to at least one polymer.
3. A method of treating edema caused by a gastrointestinal system condition in a subject in need thereof, comprising administering to the subject a compound, or a pharmaceutically acceptable salt thereof, comprising an active entity linked to at least one polymer.
4. A method of treating mucosal necrosis caused by a gastrointestinal system condition in a subject in need thereof, comprising administering to the subject a compound, or a pharmaceutically acceptable salt thereof, comprising an active entity linked to at least one polymer.
5. A method of treating edema caused by a gastrointestinal system condition in a subject in need thereof, comprising administering to the subject a compound, or a pharmaceutically acceptable salt thereof, comprising an active entity linked to at least one polymer.
6. A method of treating blood in the stool caused by a gastrointestinal system condition in a subject in need thereof, comprising administering to the subject a compound, or a pharmaceutically acceptable salt thereof, comprising an active entity linked to at least one polymer.
7. A method of treating diarrhea caused by a gastrointestinal system condition in a subject in need thereof, comprising administering to the subject a compound, or a pharmaceutically acceptable salt thereof, comprising an active entity linked to at least one polymer.
8. A method of treating body weight loss caused by a gastrointestinal system condition in a subject in need thereof, comprising administering to the subject a compound, or a pharmaceutically acceptable salt thereof, comprising an active entity linked to at least one polymer.
9. A method of treating a dermal condition in a subject in need thereof, comprising administering to the subject a compound, or a pharmaceutically acceptable salt thereof, comprising an active entity linked to at least one polymer.

10. A method of reducing the psoriasis area and severity index (PASI) score of a subject suffering from a dermal condition, comprising administering to the subject a compound, or a pharmaceutically acceptable salt thereof, comprising an active entity linked to at least one polymer.
11. A method of reducing the body surface area affected by a dermal condition in a subject affected thereby, comprising administering to the subject a compound, or a pharmaceutically acceptable salt thereof, comprising an active entity linked to at least one polymer.
12. A method of reducing the investigator global assessment (IGA) score of a subject suffering from a dermal condition, comprising administering to the subject a compound, or a pharmaceutically acceptable salt thereof, comprising an active entity linked to at least one polymer.
13. A method of maintaining a plasma concentration of a compound or a metabolite thereof at or below a threshold level in a subject suffering from a dermal condition, comprising administering to the subject the compound, or a pharmaceutically acceptable salt thereof, wherein:
- the compound comprises an active entity linked to at least one polymer;
 - the compound, or a pharmaceutically acceptable salt thereof, treats the dermal condition; and
 - the threshold level is about 5.0 ng/ml.
14. The method of one of claims 1-13, wherein the compound is a compound of Formula (I) or of Formula (II):



formula (I)



formula (II)

or a pharmaceutically acceptable salt thereof,

wherein in formula (I) and (II)

R¹ and R² are the same or a different residue and are each independently selected from the group consisting of:

(a) hydrogen, halogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, hydroxy, lower alkoxy, carboxy, lower alkoxycarbonyl, acyl, nitro, carbamoyl, lower alkylaminocarbonyl, -NR⁵R⁶, wherein R⁵ and R⁶ are each independently selected from hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted aralkyl, substituted or unsubstituted lower alkylaminocarbonyl, substituted or unsubstituted lower arylaminocarbonyl, alkoxycarbonyl, carbamoyl, acyl or R⁵ and R⁶ are combined with a nitrogen atom to form a heterocyclic group,

(b) -CO(CH₂)_jR⁴, wherein j is 1 to 6, and R⁴ is selected from the group consisting of

(i) hydrogen, halogen, -N₃,

(ii) -NR⁵R⁶, wherein R⁵ and R⁶ are as defined above,

(iii) -SR⁷, wherein R⁷ is selected from the group consisting of hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted aralkyl, - (CH₂)_aCO₂R¹⁰ (wherein a is 1 or 2, and wherein R¹⁰ is selected from the group consisting of hydrogen and substituted or unsubstituted lower alkyl) and - (CH₂)_aCO₂NR⁵R⁶,

(iv) -OR⁸, -OCOR⁸, wherein R⁸ is selected from hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl

(c) -CH(OH)(CH₂)_jR⁴ wherein j and R⁴ are as defined above;

(d) -(CH₂)_dCHR¹¹CO₂R¹² or -(CH₂)_dCHR¹¹CONR⁵R⁶, wherein d is 0 to 5, R¹¹ is hydrogen, -CONR⁵R⁶, or -CO₂R¹³, wherein R¹³ is hydrogen or a wherein substituted or unsubstituted lower alkyl, and R¹² is hydrogen or a substituted or unsubstituted lower alkyl;

(e) -(CH₂)_kR¹⁴ wherein k is 2 to 6 and R¹⁴ is halogen, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, -COOR¹⁵, -OR¹⁵, (wherein R¹⁵ is hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl or acyl), -SR⁷ (wherein R⁷ is as defined above), -CONR⁵R⁶, -NR⁵R⁶ (wherein R⁵ and R⁶ are as defined above) or -N₃;

(f) -CH=CH(CH₂)_mR¹⁶, wherein m is 0 to 4, and R¹⁶ is hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted

heteroaryl, $-\text{COOR}^{15}$, $-\text{OR}^{15}$ (wherein R^{15} is as defined above) $-\text{CONR}^{5}\text{R}^6$ or $-\text{NR}^{5}\text{R}^6$ (wherein R^5 and R^6 are as defined above);

(g) $-\text{CH}=\text{C}(\text{CO}_2\text{R}^{12})_2$, wherein R^{12} is as defined above;

(h) $-\text{C}\equiv\text{C}(\text{CH}_2)_n\text{R}^{16}$, wherein n is 0 to 4 and R^{16} is as defined above;

(i) $-\text{CH}_2\text{OR}^{22}$, wherein R^{22} is tri-lower alkyl silyl in which the three lower alkyl groups are the same or different or wherein R^{22} has the same meaning as R^8

(j) $-\text{CH}(\text{SR}^{23})_2$ and $-\text{CH}_2-\text{SR}^7$ wherein R^{23} is lower alkyl, lower alkenyl or lower alkynyl and wherein R^7 is as defined above; and

R_3 is hydrogen, halogen, acyl, carbamoyl, substituted or unsubstituted lower alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted lower alkynyl or amino; and

W^1 and W^2 are independently hydrogen, hydroxy or W^1 and W^2 together represent oxygen;

and wherein in formula (I), X is a polymer moiety, either linear or branched,

and wherein in formula (II), A represents $-\text{L}^1-\text{X}'$ and B represents $-\text{L}^2-\text{Y}'$, wherein at least one of X' and Y' is a polymer moiety, either linear or branched, which is bound by L^1 and/or L^2 to the tetrahydrofuran ring of the compound of formula (II); L^1 and/or L^2 are a covalent chemical bond or a linker group;

when Y' is a polymer moiety, and X' is not a polymer, L^1 is a covalent chemical bond and X' is selected from the group consisting of

(a) hydrogen, lower hydroxyalkyl, acyl, carboxy, lower alkoxy, carbonyl,

(b) $-\text{CONR}^{17a}\text{R}^{17b}$, wherein R^{17a} and R^{17b} are each independently selected from

(i) hydrogen, lower alkyl, lower alkenyl, lower alkynyl,

(ii) $-\text{CH}_2\text{R}^{18}$; wherein R^{18} is hydroxy,

or (iii) $-\text{NR}^{19}\text{R}^{20}$, wherein R^{19} or R^{20} are each independently selected from hydrogen, lower alkyl, lower alkenyl, lower alkynyl or R^{19} or R^{20} are independently the residue of an α -amino acid in which the hydroxy group of the carboxyl group is excluded, or R^{19} or R^{20} are combined with a nitrogen atom to form a heterocyclic group; and

(c) $-\text{CH}=\text{N}-\text{R}^{21}$, wherein R^{21} is hydroxy, lower alkoxy, amino, guanidino, or imidazolylamino; and

when X' is a polymer moiety, and Y' is not a polymer, L^2 is a covalent chemical bond and Y' is selected from hydroxy, lower alkoxy, aralkyloxy, or acyloxy.

15. The method of claim 14, wherein the compound is a compound of Formula (I).

16. The method of claim 14, wherein the compound is a compound of Formula (II).

17. The method of claim 14, wherein:

R^1 is hydrogen, halogen, or lower alkyl;

R^2 is hydrogen, halogen, or lower alkyl;

R_3 is hydrogen, halogen, or lower alkyl; and

W^1 is hydrogen or hydroxy;

W^2 is hydrogen or hydroxy;

or W^1 and W^2 together represent oxygen;

X is a linear polymer moiety or a branched polymer moiety;

A represents $-L^1-X'$;

B represents $-L^2-Y'$;

L^1 is a covalent chemical bond or a linker group;

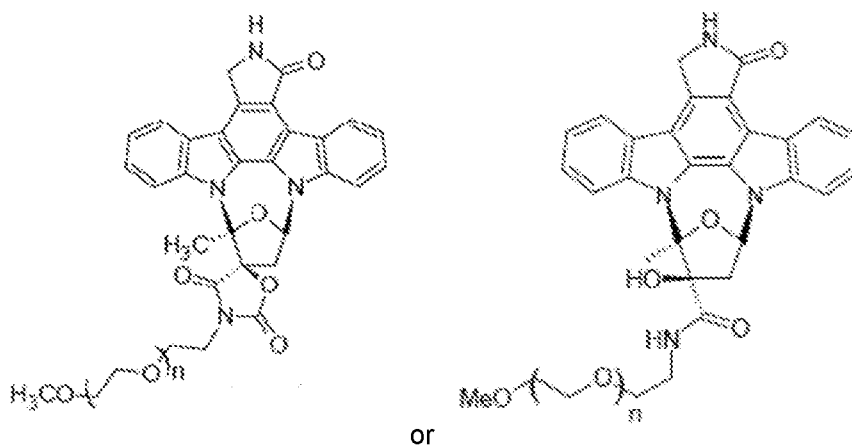
X is a polymer moiety;

L^2 is a covalent chemical bond; and

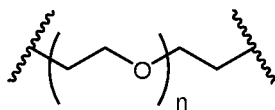
Y' is hydroxy, lower alkoxy, or aralkyloxy.

18. The method of claim 14, wherein the polymer moiety has a molecular weight of about 500 to about 10,000 Da.

19. The method of claim 14, wherein the compound is

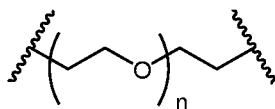


or a pharmaceutically acceptable salt thereof,
wherein



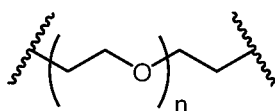
has a molecular weight of about 500 to about 10,000 Da.

20. The method of claim 19, wherein



has a molecular weight of about 1000-3000 Da.

21. The method of claim 19, wherein



has a molecular weight of about 2000 Da.

22. The method of claim 19, wherein the compound is SNA-120, or a pharmaceutically acceptable salt thereof.

23. The method of claim 19, wherein the compound is SNA-125, or a pharmaceutically acceptable salt thereof.

24. The method of one of claims 1-8, wherein the gastrointestinal system condition is inflammatory bowel disease, irritable bowel syndrome, small intestinal bacterial overgrowth, Crohn's disease, colitis, intestinal fibrosis, or colorectal cancer.

25. The method of claim 24, wherein the gastrointestinal system is colitis.

26. The method of claim 25, wherein the colitis is ulcerative colitis, collagenous colitis, lymphocytic colitis, ischemic colitis, diversion colitis, or indeterminate colitis.

27. The method of one of claims 1-8, wherein the gastrointestinal system condition is ulcerative colitis, Crohn's disease, intestinal fibrosis, or colorectal cancer.

28. The method of one of claims 9-13 and 15-23, wherein the dermal condition is pruritus, skin neoplasia, vascular tumor, inflammatory dermatosis, scarring, a bullous disease, a dermal condition caused by ultra-violet irradiation therapy, alopecia areata, or vitiligo.

29. The method of claim 28, wherein:

pruritus is pruritus due to psoriasis, pruritus due to atopic dermatitis, or pruritus due to prurigo nodularis;

skin neoplasia is squamous cell carcinoma, basal cell carcinoma, malignant melanoma, or malignant cutaneous lymphoma;

vascular tumor is angiosarcoma, kaposi's sarcoma, infantile hemangioma, or hemangioendothelioma;

inflammatory dermatosis is dermatitis, psoriasis, keloid, or rosacea; and

the bullous disease is bullous pemphigoid or erythema multiforme.

30. The method of claim 29, wherein dermatitis is atopic dermatitis or irritant contact dermatitis.

31. The method of one of claims 9-13 and 15-23, wherein the dermal condition comprises pruritic psoriatic plaques, pruritis associated with psoriasis vulgaris, psoriasis or dermatitis.

32. The method of one of claims 9-13 and 15-23, wherein the administration comprises topical administration.

33. The method of one of claims 9-13 and 15-23, wherein:

the subject is administered an ointment comprising about 0.5 % w/w of the compound, or a pharmaceutically acceptable salt thereof; and

the ointment is administered twice a day.

34. The method of one of claims 9-13 and 15-23, wherein:

the subject is administered an ointment comprising about 0.5 % w/w of the compound, or a pharmaceutically acceptable salt thereof; and

the ointment is administered at a frequency of about every 12 hours.

35. The method of claim 31, wherein:

the subject is administered an ointment comprising about 0.5 % w/w of the compound;

the compound is SNA-120, or a pharmaceutically acceptable salt thereof; and

the ointment is administered twice a day.

35. The method of claim 31, wherein:

the subject is administered an ointment comprising about 0.5 % w/w of the compound;

the compound is SNA-120, or a pharmaceutically acceptable salt thereof; and

the ointment is administered at a frequency of about every 12 hours.

36. The method of one of claims 9-12, wherein:

the subject is administered an ointment comprising about 0.5 % w/w of the compound;

the compound is SNA-120, or a pharmaceutically acceptable salt thereof;

a plasma concentration of the compound, or a metabolite thereof, is maintained at or below 5.0 ng/mL in the subject; and

the ointment is administered twice a day.

37. The method of one of claims 9-12, wherein:

the subject is administered an ointment comprising about 0.5 % w/w of the compound;

the compound is SNA-120, or a pharmaceutically acceptable salt thereof;

a plasma concentration of the compound, or a metabolite thereof, is maintained at or below 5.0 ng/mL in the subject; and

the ointment is administered at a frequency of about every 12 hours.

38. The method of claim 36, wherein the dermal condition affects at least about 20 % of the body surface area of the subject.

39. The method of claim 38, wherein the ointment is administered to at least about 20 % of the body surface area of the subject affected by the dermal condition.

40. The method of one of claims 9-13 and 15-23, wherein a dosage of the compound is topically administered to the subject twice in a day.

41. The method of one of claims 9-13 and 15-23, wherein a dosage of the compound is topically administered to the subject at a frequency of about every 12 hours.
42. The method of claim 40, wherein the daily dosage of the compound is up to about 100 mg.
43. The method of one of claims 9-13 and 15-23, wherein the compound is formulated in an ointment.
44. The method of claim 43, wherein the ointment comprises about 0.5 % w/w of the compound.
45. The method of one of claims 9-13 and 15-23, wherein about 7 g to about 14 g of an ointment is administered daily, and the ointment comprises about 0.5 % w/w of the compound.
46. The method of one of claims 9-13 and 15-23, wherein the compound is administered daily for about four weeks.
47. The method of one of claims 9-13 and 15-23, wherein the compound is administered daily for at least four weeks.
48. The method of claim 10, wherein the compound is administered once or twice daily for about four weeks, and the PASI score of the subject is reduced by about 50 % to about 75 % as compared to the PASI score of the subject without administration of the compound.
49. The method of one of claims 9-13 and 15-23, wherein about 30 mg to about 100 mg of the compound is administered.
50. The method of claim 13, wherein the threshold plasma concentration is about 2.5 ng/ml.
51. The method of claim 13, wherein the threshold plasma concentration is about 0.5 ng/ml.
52. The method of one of claims 1-13 and 15-23, wherein the compound is administered twice in a day.
53. The method of one of claims 1-13 and 15-23, wherein the compound is administered once in a day.
54. The method of one of claims 9-13, wherein:
the subject is administered an ointment comprising about 0.5 % w/w of the compound;
the compound is SNA-120, or a pharmaceutically acceptable salt thereof; and
the ointment is administered twice a day.
55. The method of one of claims 9-13, wherein:
the subject is administered an ointment comprising about 0.5 % w/w of the compound;
the compound is SNA-120, or a pharmaceutically acceptable salt thereof; and

the ointment is administered at a frequency of about every 12 hours.

56. A method of treating pruritus associated with psoriasis in a subject in need thereof, comprising topically administering twice daily to the subject an ointment comprising 0.5 % w/w of SNA-120.

57. The method of claim 56, wherein the ointment is administered at a frequency of about every 12 hours.

58. A method of improving the histological characteristics of lesional psoriasis skin in a subject in need thereof, comprising administering to the subject an effective amount of SNA-125.

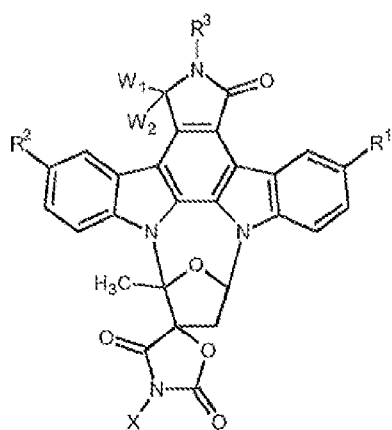
59. A method of improving the molecular signature of lesional psoriasis skin in a subject in need thereof, comprising administering to the subject an effective amount of SNA-125.

60. A method of treating psoriasis in a subject in need thereof, comprising administering to the subject an effective amount of SNA-125.

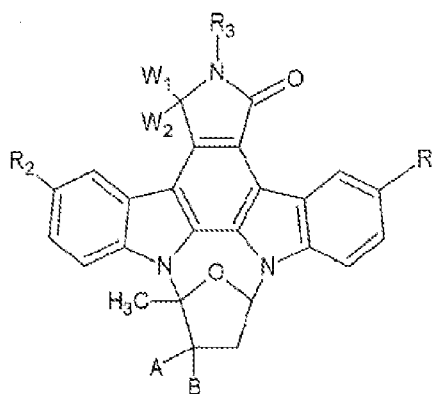
61. The method of one of claims 58-60, wherein the SNA-125 is formulated in a composition.

62. The method of claim 61, wherein the composition is administered topically and the composition is a pharmaceutical composition that is a cream, an ointment, a lotion, or a gel.

63. A method of treating a condition in a subject in need thereof, comprising administering to the subject an effective amount of a compound of Formula (I) or of Formula (II):



formula (i)



formula (II)

or a pharmaceutically acceptable salt thereof,

wherein in formula (I) and (II)

R¹ and R² are the same or a different residue and are each independently selected from the group consisting of:

(a) hydrogen, halogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, hydroxy, lower

alkoxy, carboxy, lower alkoxy, carbonyl, acyl, nitro, carbamoyl, lower alkylaminocarbonyl, -NR⁵R⁶, wherein R⁵ and R⁶ are each independently selected from hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted aralkyl, substituted or unsubstituted lower alkylaminocarbonyl, substituted or unsubstituted lower arylaminocarbonyl, alkoxy, carbonyl, carbamoyl, acyl or R⁵ and R⁶ are combined with a nitrogen atom to form a heterocyclic group,

(b) -CO(CH₂)_jR⁴, wherein j is 1 to 6, and R⁴ is selected from the group consisting of

(i) hydrogen, halogen, -N₃,

(ii) -NR⁵R⁶, wherein R⁵ and R⁶ are as defined above,

(iii) -SR⁷, wherein R⁷ is selected from the group consisting of hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, substituted or unsubstituted aralkyl, - (CH₂)_aCO₂R¹⁰ (wherein a is 1 or 2, and wherein R¹⁰ is selected from the group consisting of hydrogen and substituted or unsubstituted lower alkyl) and - (CH₂)_aCO₂NR⁵R⁶,

(iv) -OR⁸, -OCOR⁸, wherein R⁸ is selected from hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl

(c) -CH(OH)(CH₂)_jR⁴ wherein j and R⁴ are as defined above;

(d) -(CH₂)_dCHR¹¹CO₂R¹² or -(CH₂)_dCHR¹¹CONR⁵R⁶, wherein d is 0 to 5, R¹¹ is hydrogen, -CONR⁵R⁶, or -CO₂R¹³, wherein R¹³ is hydrogen or a wherein substituted or unsubstituted lower alkyl, and R¹² is hydrogen or a substituted or unsubstituted lower alkyl;

(e) -(CH₂)_kR¹⁴ wherein k is 2 to 6 and R¹⁴ is halogen, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, -COOR¹⁵, -OR¹⁵, (wherein R¹⁵ is hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl or acyl), -SR⁷ (wherein R⁷ is as defined above), -CONR⁵R⁶, -NR⁵R⁶ (wherein R⁵ and R⁶ are as defined above) or -N₃;

(f) -CH=CH(CH₂)_mR¹⁶, wherein m is 0 to 4, and R¹⁶ is hydrogen, substituted or unsubstituted lower alkyl, substituted or unsubstituted lower alkenyl, substituted or unsubstituted lower alkynyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, -COOR¹⁵, -OR¹⁵ (wherein R¹⁵ is as defined above) -CONR⁵R⁶ or -NR⁵R⁶ (wherein R⁵ and R⁶ are as defined above);

- (g) $-\text{CH}=\text{C}(\text{CO}_2\text{R}^{12})_2$, wherein R^{12} is as defined above;
- (h) $-\text{C}\equiv\text{C}(\text{CH}_2)_n\text{R}^{16}$, wherein n is 0 to 4 and R^{16} is as defined above;
- (i) $-\text{CH}_2\text{OR}^{22}$, wherein R^{22} is tri-lower alkyl silyl in which the three lower alkyl groups are the same or different or wherein R^{22} has the same meaning as R^8
- (j) $-\text{CH}(\text{SR}^{23})_2$ and $-\text{CH}_2-\text{SR}^7$ wherein R^{23} is lower alkyl, lower alkenyl or lower alkynyl and wherein R^7 is as defined above; and

R_3 is hydrogen, halogen, acyl, carbamoyl, substituted or unsubstituted lower alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted lower alkynyl or amino; and

W^1 and W^2 are independently hydrogen, hydroxy or W^1 and W^2 together represent oxygen;

and wherein in formula (I), X is a polymer moiety, either linear or branched,

and wherein in formula (II), A represents $-\text{L}^1-\text{X}'$ and B represents $-\text{L}^2-\text{Y}'$, wherein at least one of X' and Y' is a polymer moiety, either linear or branched, which is bound by L^1 and/or L^2 to the tetrahydrofuran ring of the compound of formula (II); L^1 and/or L^2 are a covalent chemical bond or a linker group;

when Y' is a polymer moiety, and X' is not a polymer, L^1 is a covalent chemical bond and X' is selected from the group consisting of

- (a) hydrogen, lower hydroxyalkyl, acyl, carboxy, lower alkoxy, carbonyl,
- (b) $-\text{CONR}^{17a}\text{R}^{17b}$, wherein R^{17a} and R^{17b} are each independently selected from
- (i) hydrogen, lower alkyl, lower alkenyl, lower alkynyl,
- (ii) $-\text{CH}_2\text{R}^{18}$; wherein R^{18} is hydroxy,
- or (iii) $-\text{NR}^{19}\text{R}^{20}$, wherein R^{19} or R^{20} are each independently selected from hydrogen, lower alkyl, lower alkenyl, lower alkynyl or R^{19} or R^{20} are independently the residue of an α -amino acid in which the hydroxy group of the carboxyl group is excluded, or R^{19} or R^{20} are combined with a nitrogen atom to form a heterocyclic group; and
- (c) $-\text{CH}=\text{N}-\text{R}^{21}$, wherein R^{21} is hydroxy, lower alkoxy, amino, guanidino, or imidazolylamino; and

when X' is a polymer moiety, and Y' is not a polymer, L^2 is a covalent chemical bond and Y' is selected from hydroxy, lower alkoxy, aralkyloxy, or acyloxy.

64. The method of claim 63, wherein the condition is a dermal condition, a gastrointestinal condition, an ophthalmological condition, a respiratory condition, or a combination thereof.

65. The method of claim 64, wherein the dermal condition is pruritus (e.g., pruritus due to psoriasis, pruritus due to atopic dermatitis, or prurigo nodularis), skin neoplasia (e.g., squamous cell carcinoma, basal cell carcinoma, malignant melanoma, or malignant cutaneous lymphoma), vascular tumor (e.g., angiosarcoma, Kaposi's sarcoma, infantile

hemangioma, or hemangioendothelioma), inflammatory dermatosis (e.g., dermatitis (e.g., atopic dermatitis or irritant contact dermatitis), psoriasis, keloid, or rosacea), scarring, a bullous disease (e.g., bullous pemphigoid or erythema multiforme), UV irradiation therapy, alopecia areata, vitiligo, dry skin pruritus in the elderly, warts, or a combination thereof.

66. The method of claim 64, wherein the dermal condition is angiosarcoma, Kaposi's sarcoma, infantile hemangioma, hemangioendothelioma, irritant contact dermatitis, rosacea, bullous pemphigoid, erythema multiforme, UV irradiation therapy, alopecia areata, vitiligo, or a combination thereof.

67. The method of claim 64, wherein the dermal condition is pruritus, prurigo nodularis, postherpetic neuralgia, seborrheic dermatitis, lichen simplex chronicus, Grover's disease, neurodermatitis, pemphigoid, bullous pemphigoid, epidermolysis bullosa, alopecia areata, atopic dermatitis, chronic actinic dermatitis, chronic mucocutaneous candidiasis, cutaneous t-cell lymphoma, dermatomyositis, erythema multiforme, graft-versus-host disease (cutaneous), hypereosinophilic syndrome, lupus erythematosus, mastocytosis and mast cell disease, STING vasculopathy, palmoplantar pustulosis, polyarteritis nodosa, psoriasis, vitiligo, dry skin pruritus in the elderly, warts, or a combination thereof.

68. The method of claim 64, wherein the dermal condition is pruritus due to intrahepatic cholestasis, posthepatic cholestasis, drug-induced cholestasis, chronic renal failure, dialysis treatment, diabetes mellitus, systemic parasitosis, Sjogren's syndrome, AIDS, drug eruption, pregnancy, a psychogenic condition, sclerosis multiplex, stroke, delusion of parasitosis, lymphoma, mastocytosis, polycythemia vera, iron-deficiency anemia, hyperthyroidism, hyperparathyroidism, brain tumor, myxoedema, acute eczema, chronic eczema, monetary eczema, sebaceous deficiency eczema, contact dermatitis, atopic dermatitis, seborrheic dermatitis, neurodermatitis, acute prurigo (strophus, urticaria, lichen urticatus), subacute prurigo, chronic prurigo (nodular prurigo), systemic skin pruritus, focal skin pruritus, or a combination thereof.

69. The method of claim 64, wherein the gastrointestinal condition is ulcerative colitis, Crohn's disease, intestinal fibrosis, colorectal cancer, irritable bowel syndrome, visceral pain, or a combination thereof.

70. The method of claim 64, wherein the ophthalmological condition is age-related macular degeneration (e.g., wet age-related macular degeneration or dry age-related macular degeneration), diabetic retinopathy, edema (e.g., macular edema (e.g., diabetic macular edema) or corneal edema), dry eye (e.g., spontaneous dry eye or aqueous deficiency dry eye), uveitis (e.g., juvenile idiopathic arthritis uveitis or posterior uveitis), pterygia, conjunctivitis (e.g., vernal keratoconjunctivitis (allergic conjunctivitis) or atopic keratoconjunctivitis), graft versus host disease (e.g., ophthalmic graft versus host disease),

glaucoma (e.g., primary open angle glaucoma), retinopathy of prematurity, or a combination thereof.

71. The method of claim 64, wherein the ophthalmological condition is age-related macular degeneration (e.g., wet age-related macular degeneration or dry age-related macular degeneration), edema (e.g., macular edema (e.g., diabetic macular edema) or corneal edema), dry eye (e.g., spontaneous dry eye or aqueous deficiency dry eye), juvenile idiopathic arthritis uveitis, posterior uveitis, pterygia, conjunctivitis (e.g., vernal keratoconjunctivitis (allergic conjunctivitis) or atopic keratoconjunctivitis), ophthalmic graft versus host disease, glaucoma (e.g., primary open angle glaucoma), retinopathy of prematurity, diabetic retinopathy, or a combination thereof.

72. The method of claim 64, wherein the respiratory condition is asthma (e.g., T_H2 asthma or non- T_H2 asthma), chronic obstructive pulmonary disease, pulmonary fibrosis (e.g., idiopathic pulmonary fibrosis), pulmonary hypertension, sinusitis with nasal polyposis, allergic rhinitis, cough (e.g., chronic cough), lymphangioleiomyomatosis, or a combination thereof.

73. The method of claim 63, wherein the condition is rheumatoid arthritis, osteoarthritis, neuropathic pain, neuropathic itch, or a combination thereof.

1/207

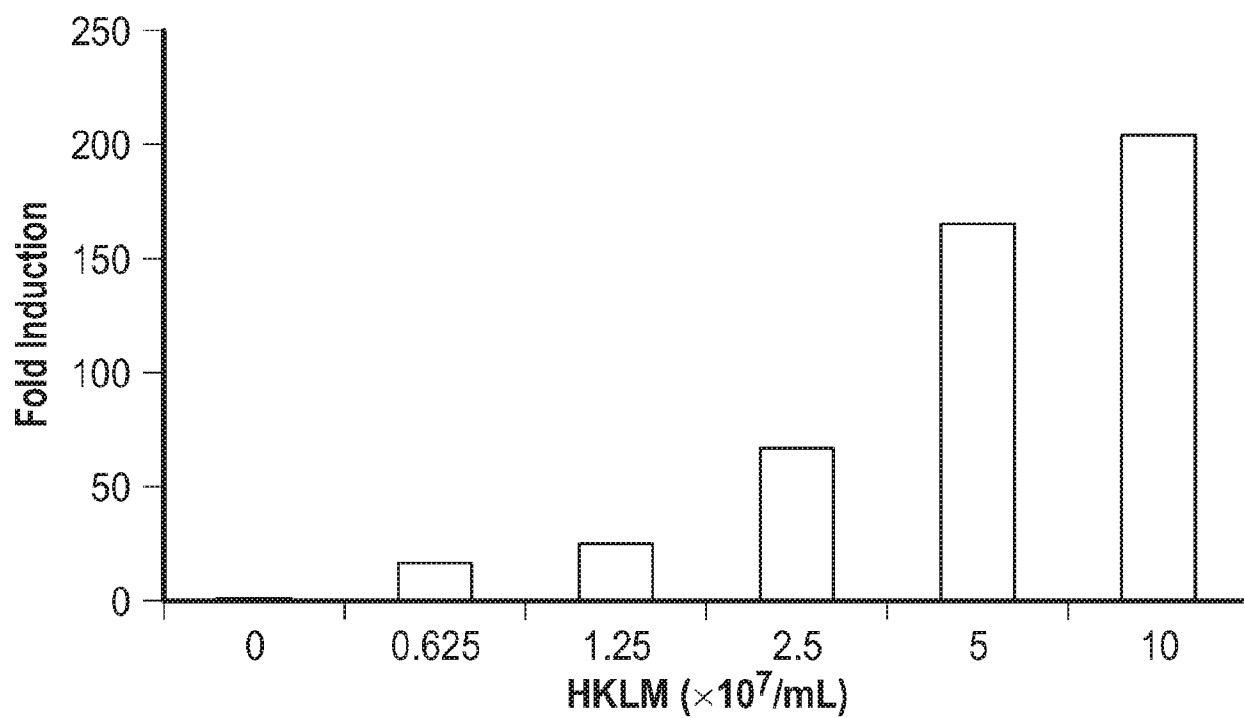
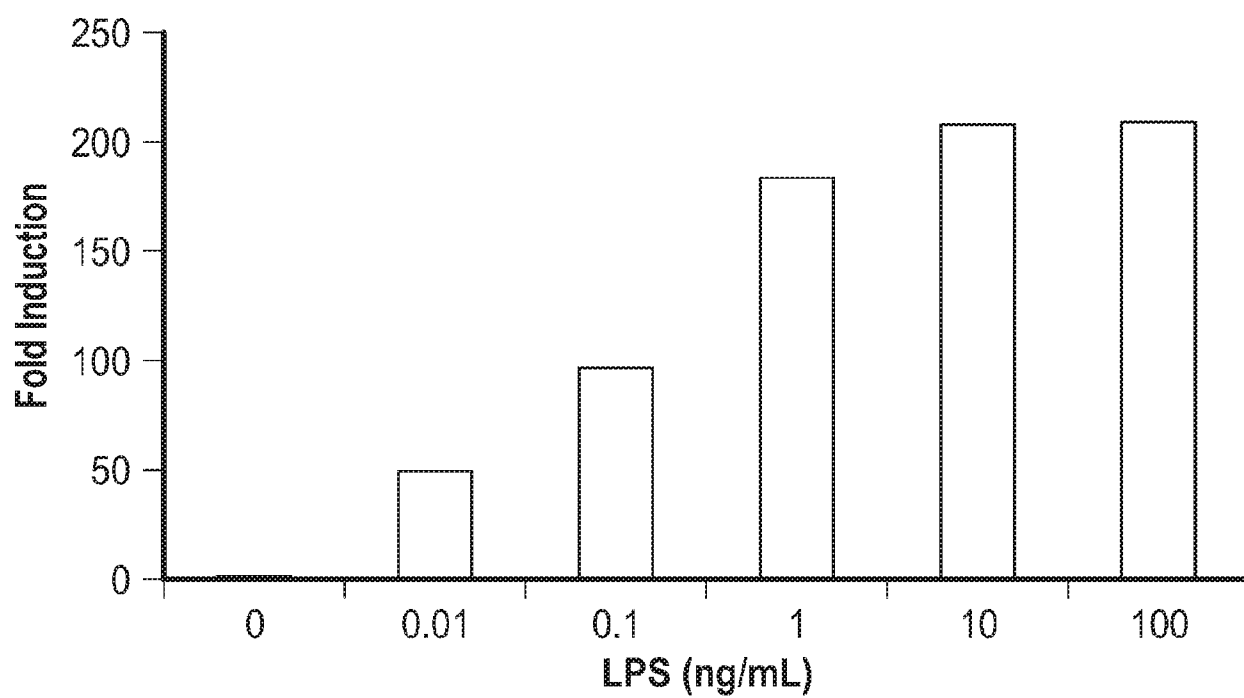
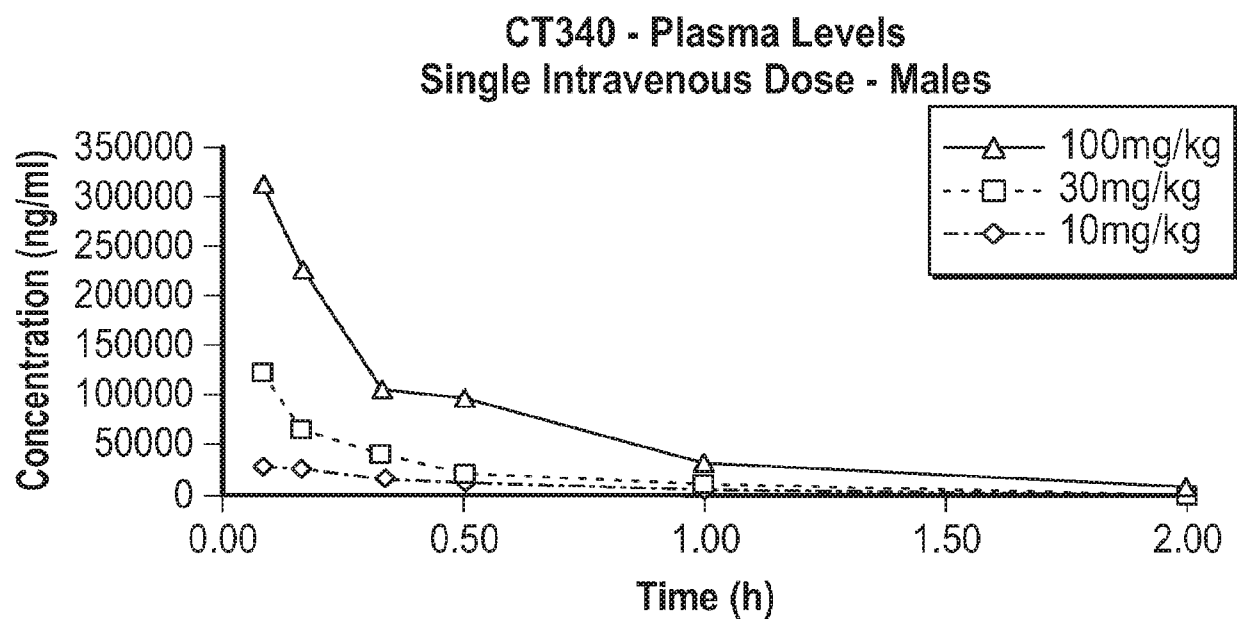
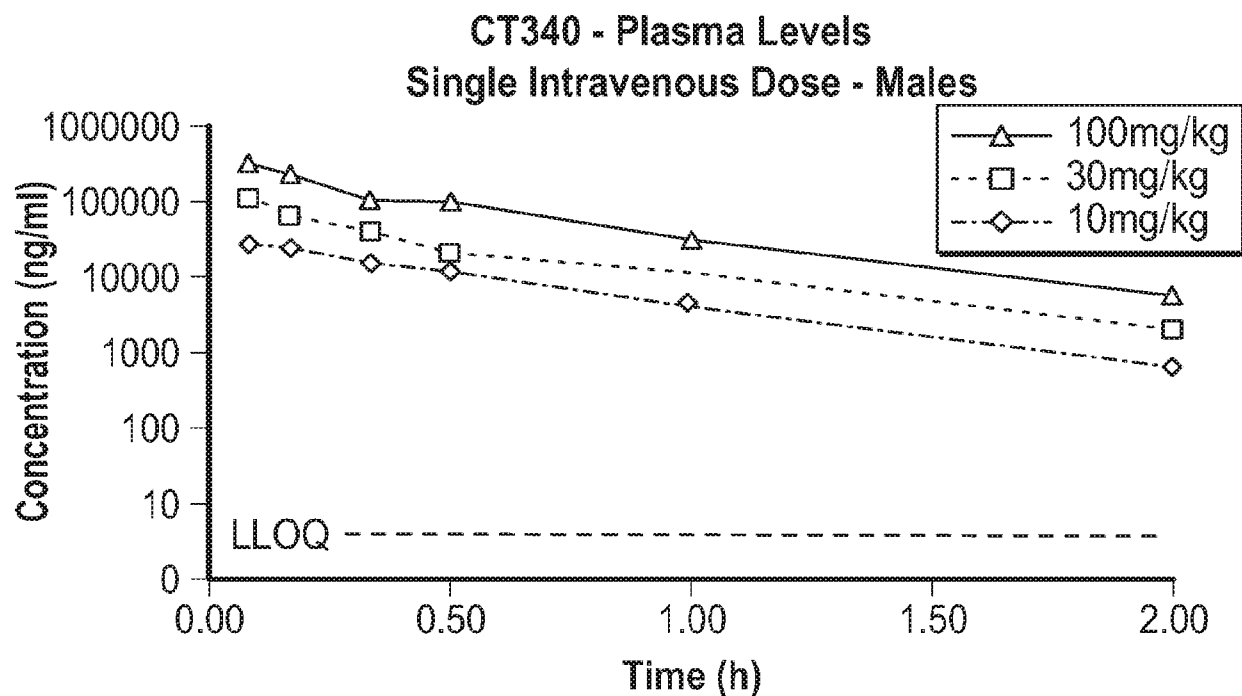


FIG. 1

2/207

**FIG. 2**

3/207

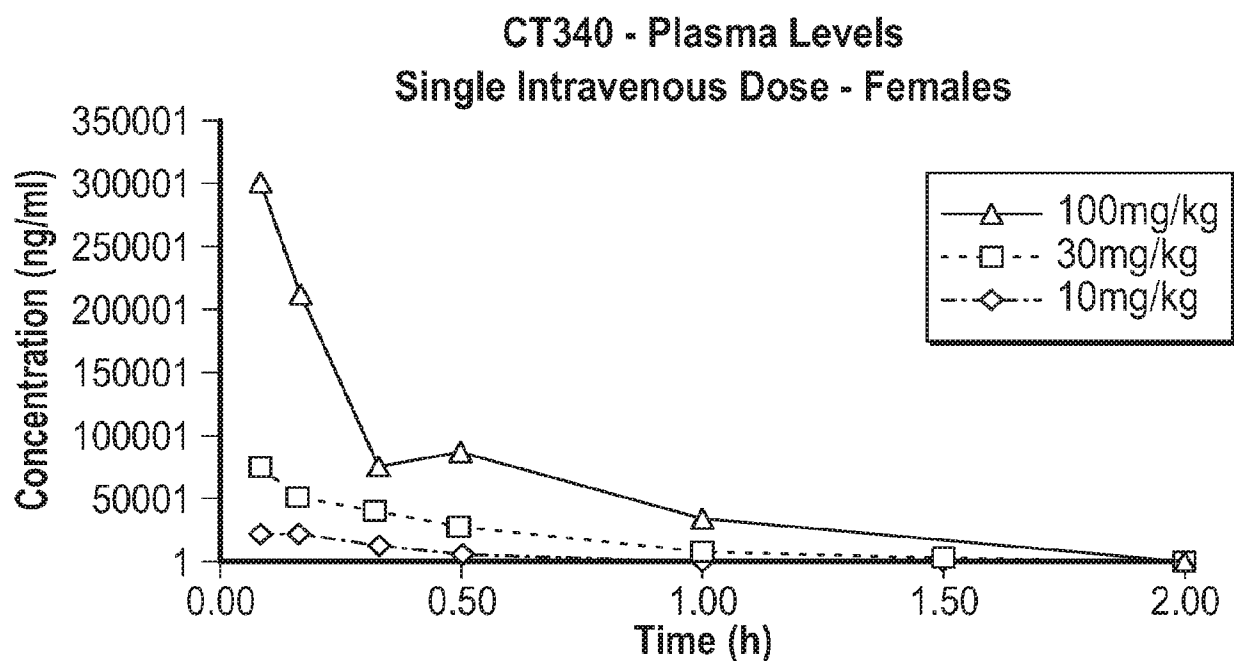
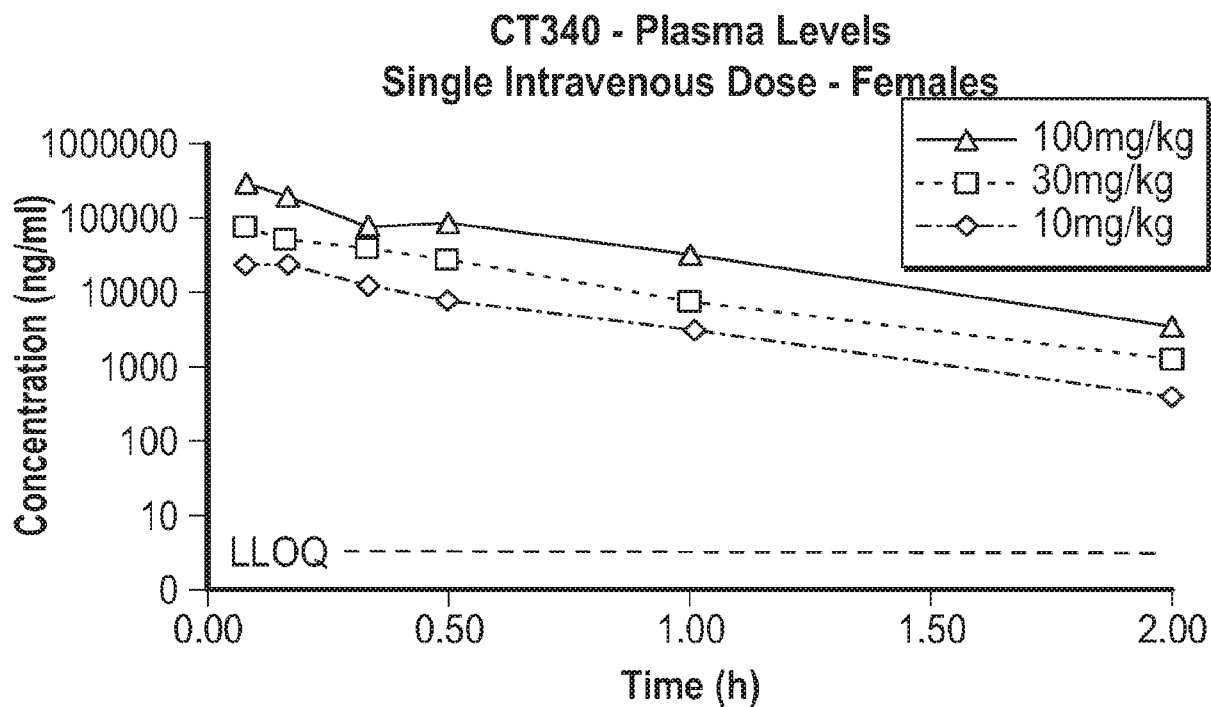


FIG. 2
(Continued)

4/207

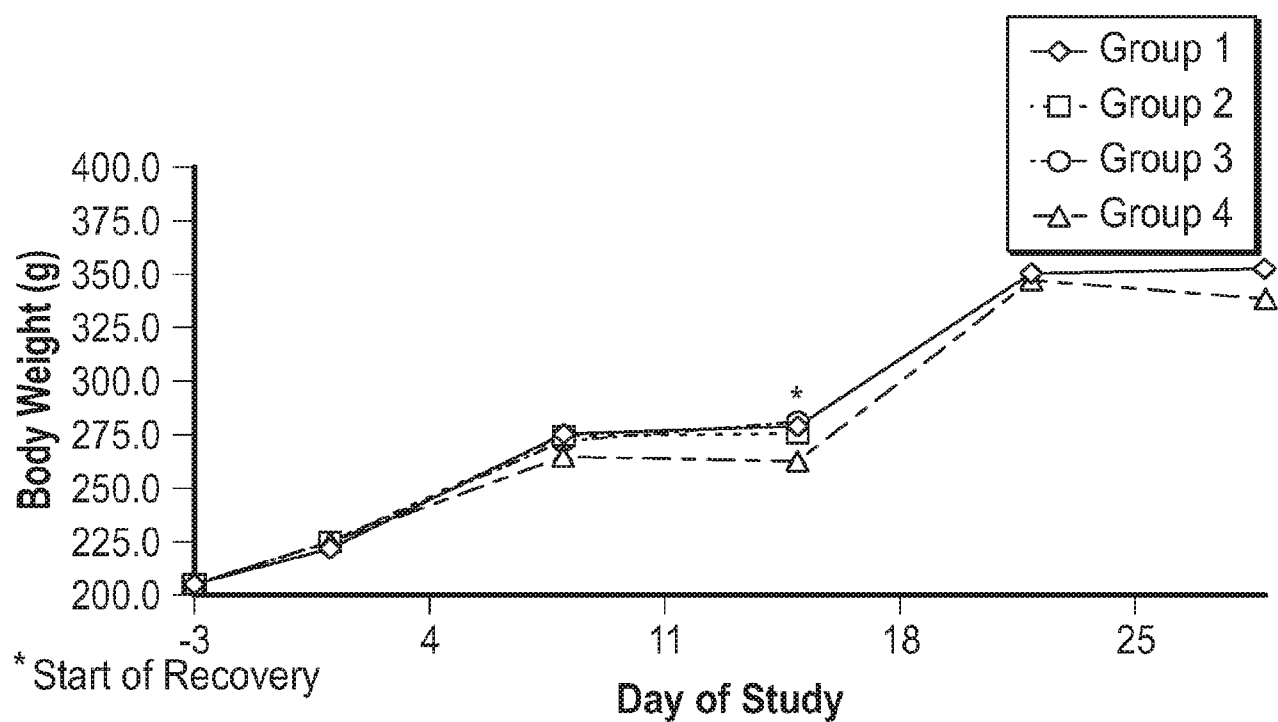


FIG. 3

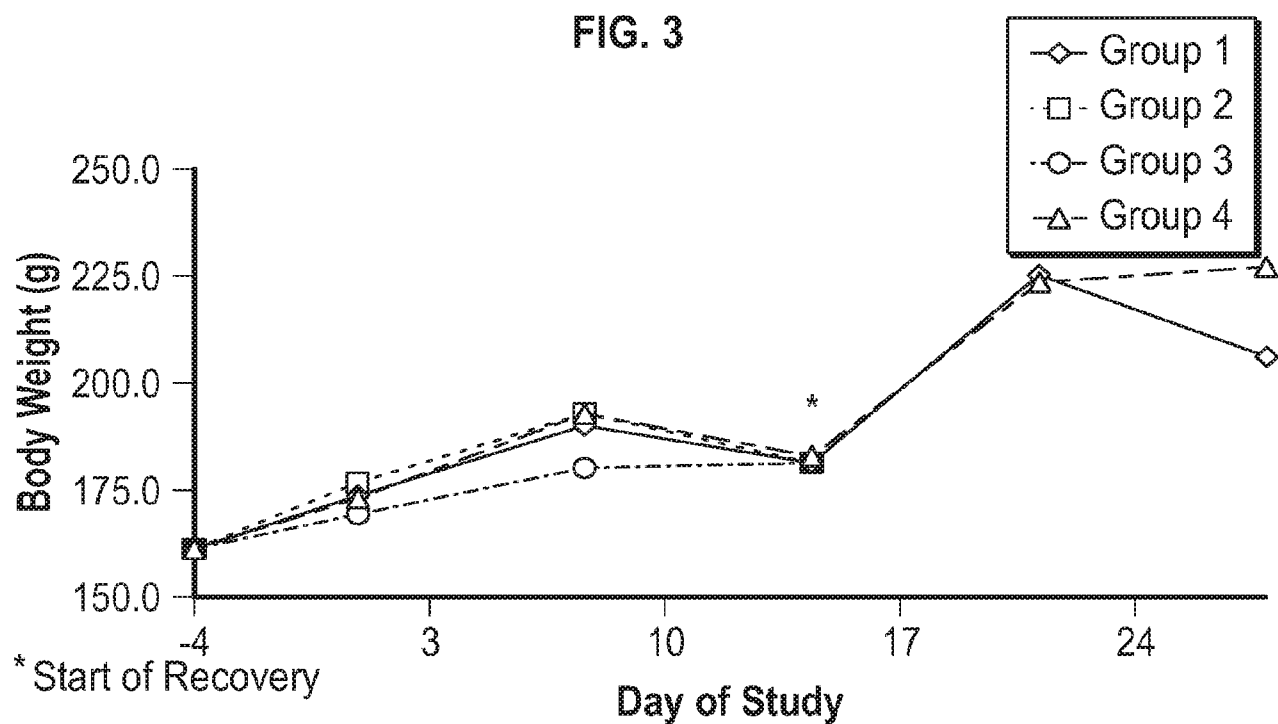
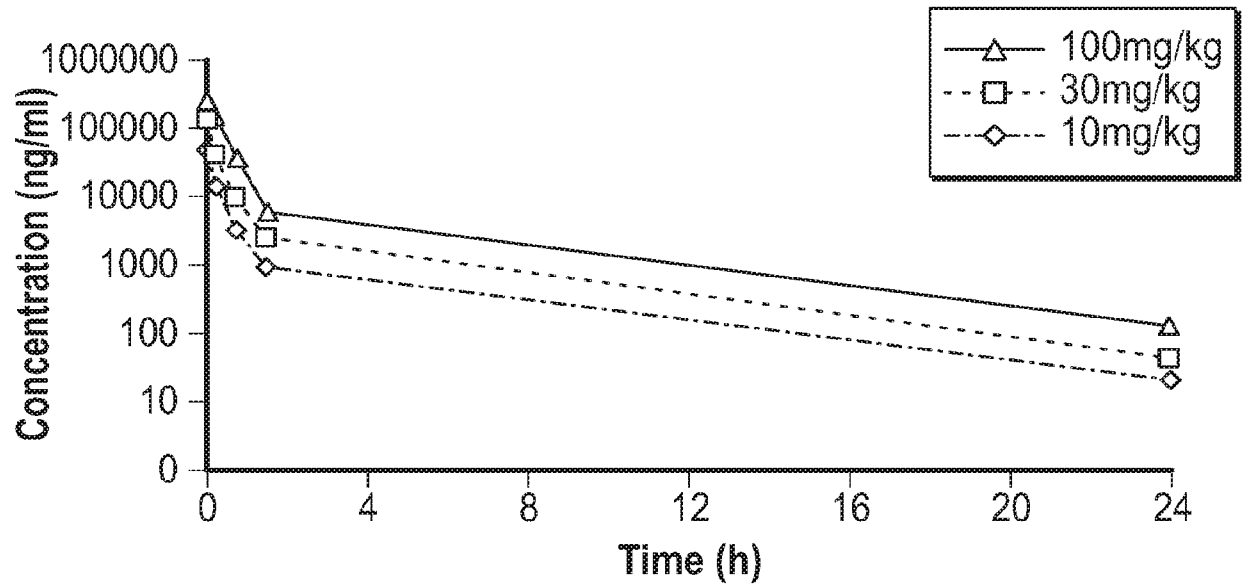


FIG. 4

5/207

CT340 - Plasma Levels - Day 1- Males



CT340 - Plasma Levels - Day 1- Females

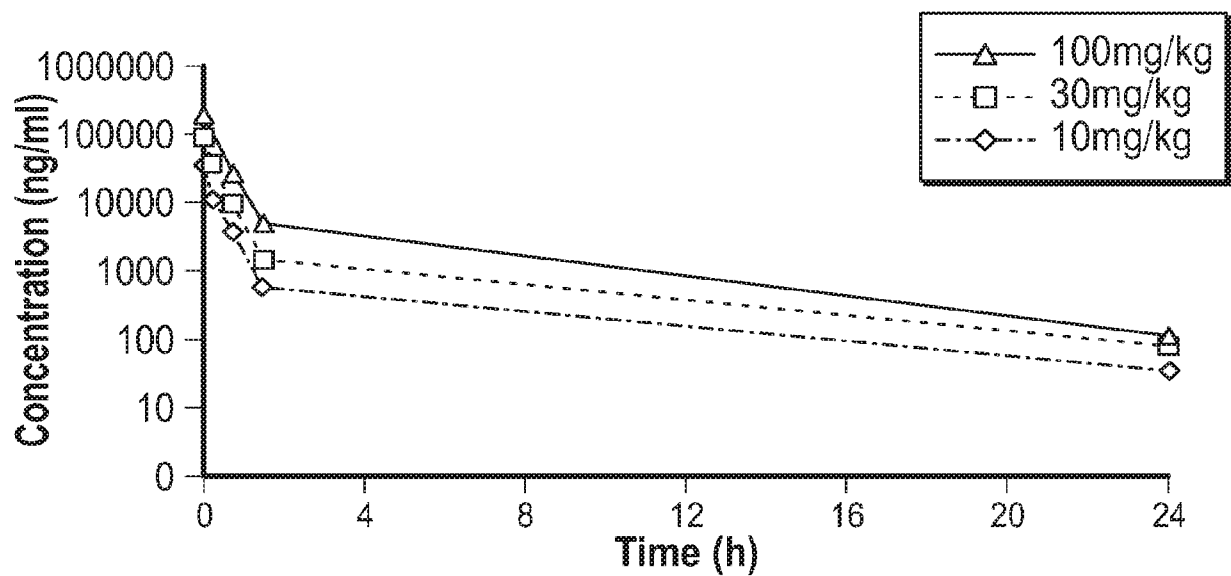
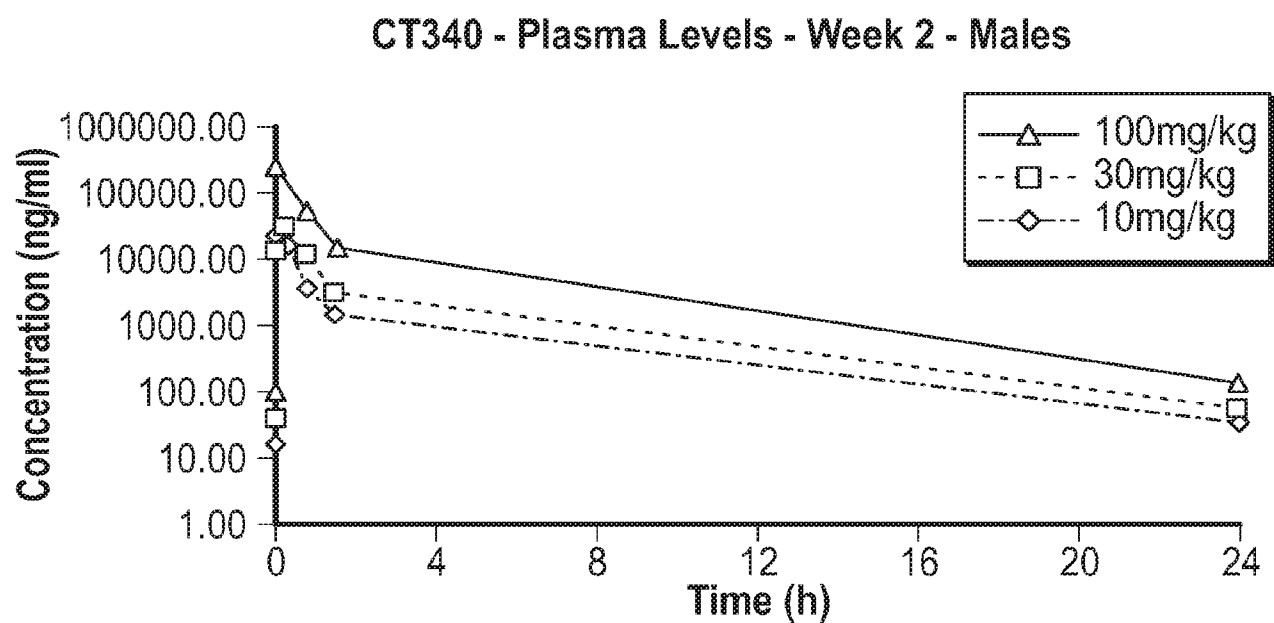
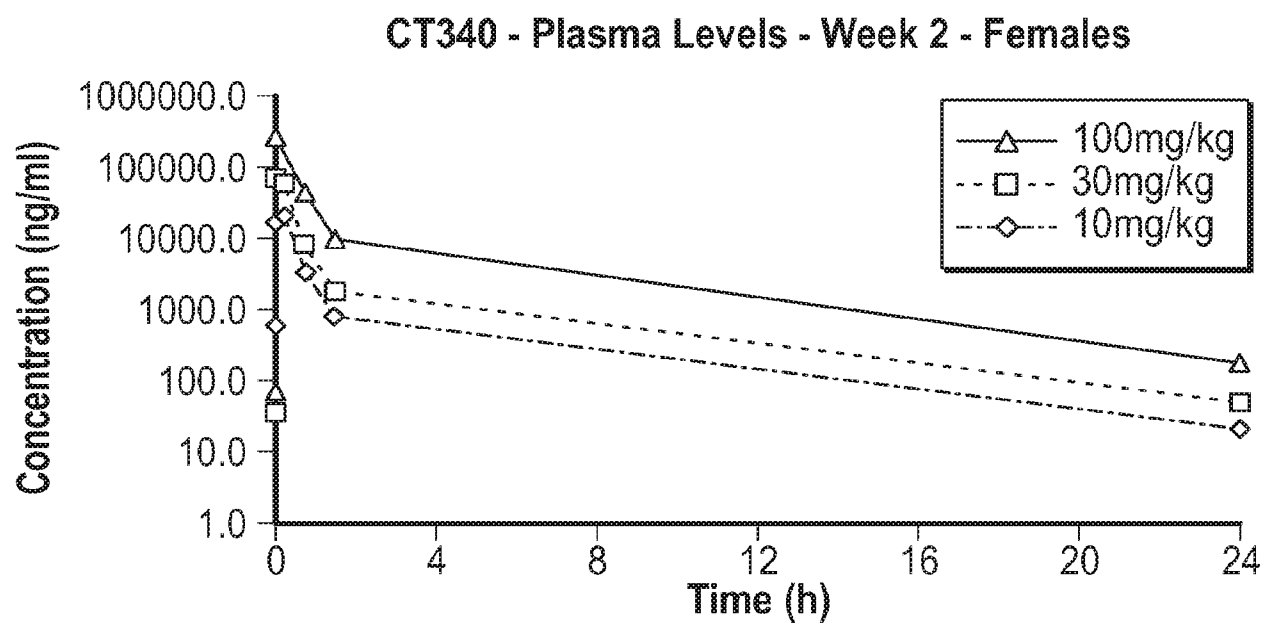


FIG. 5

6/207

**FIG. 6**

7/207

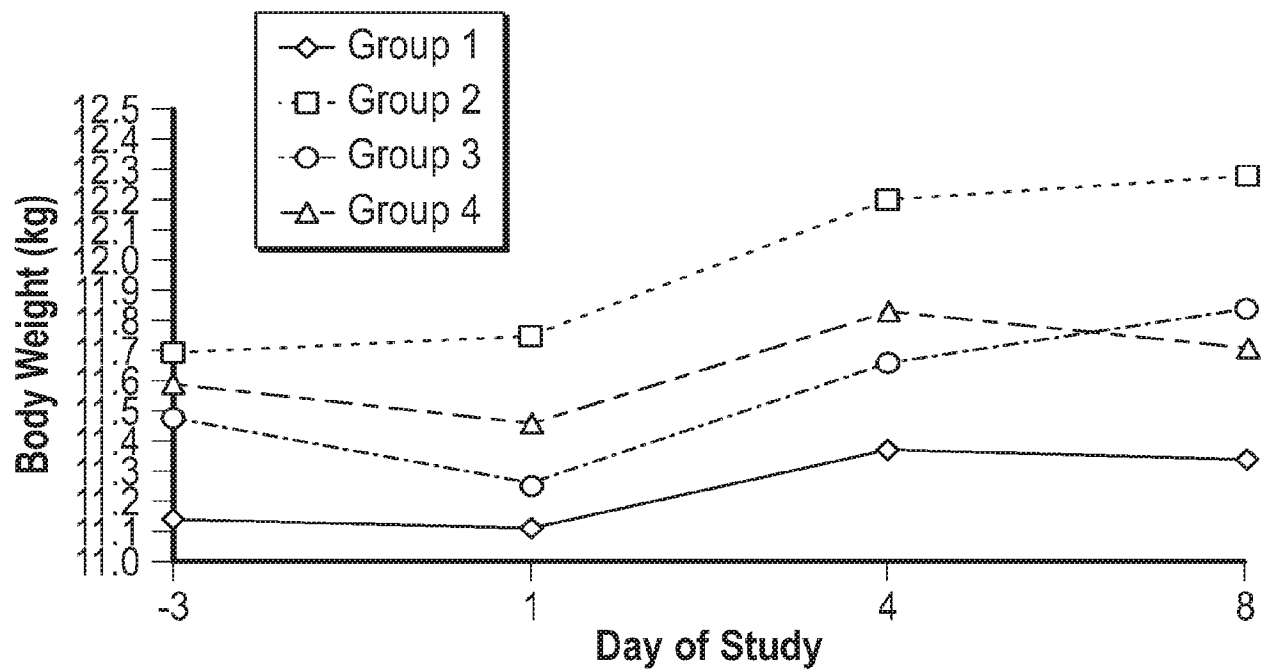


FIG. 7

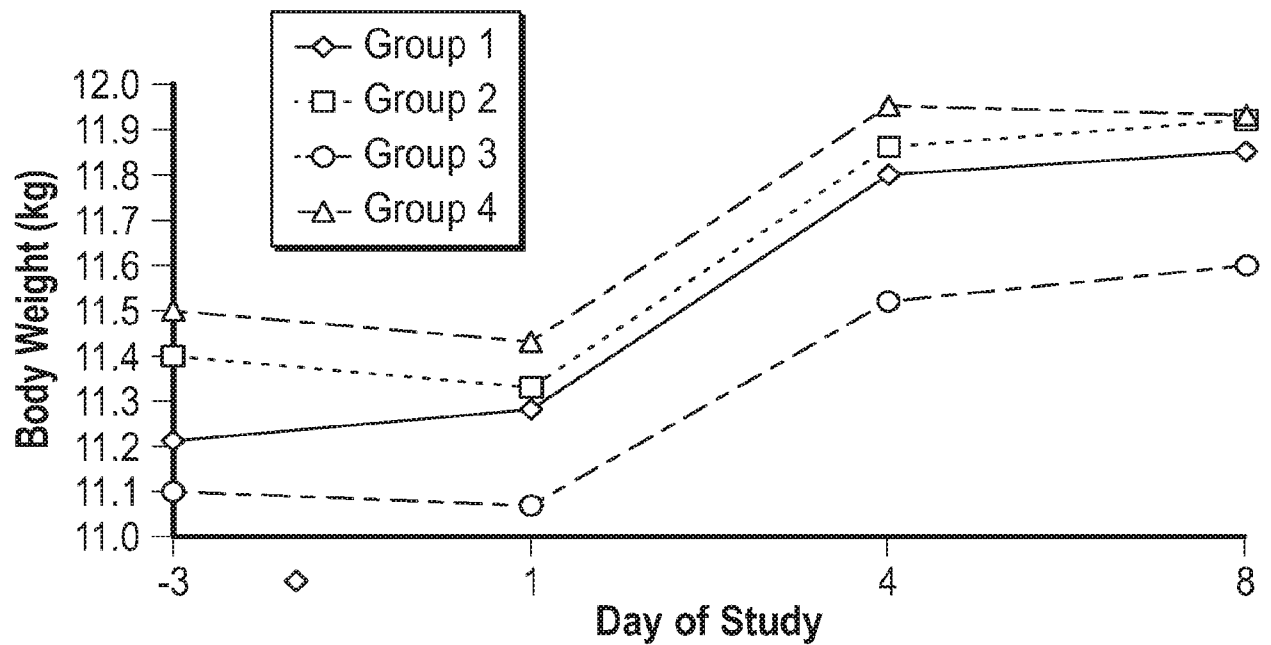
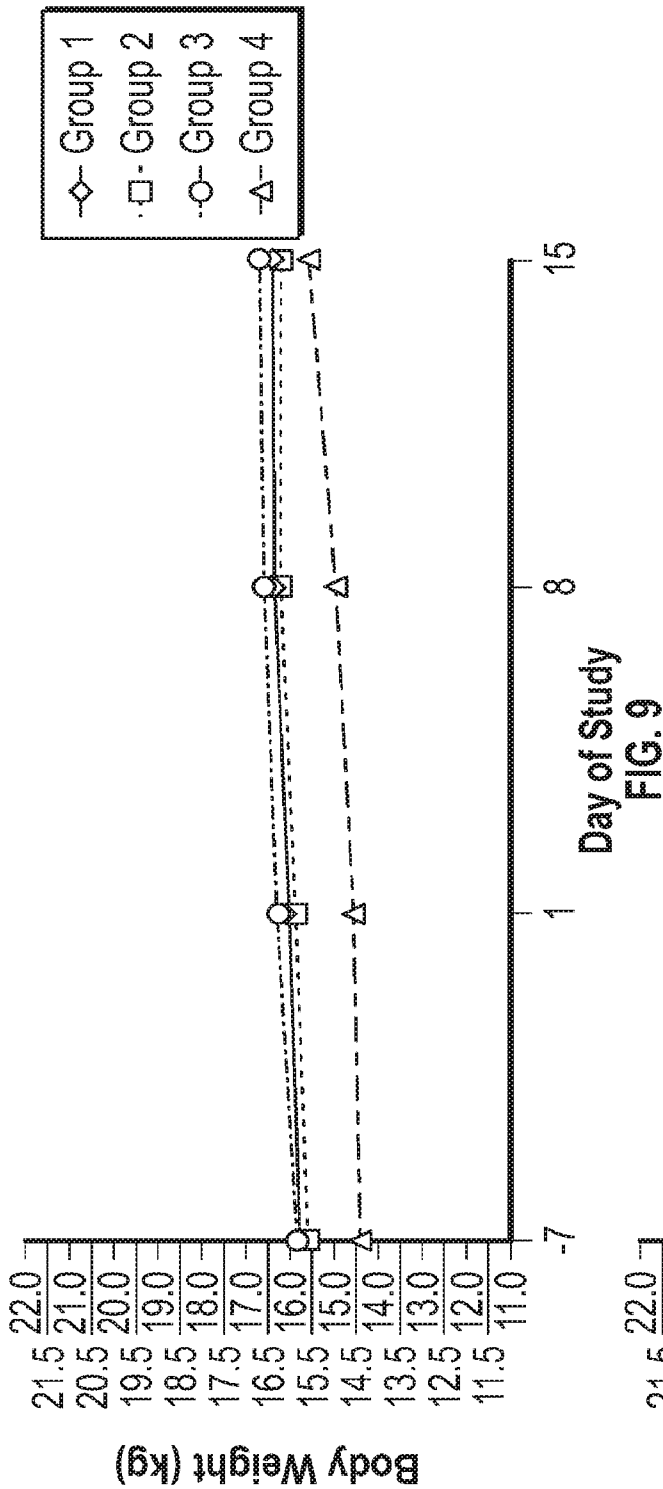
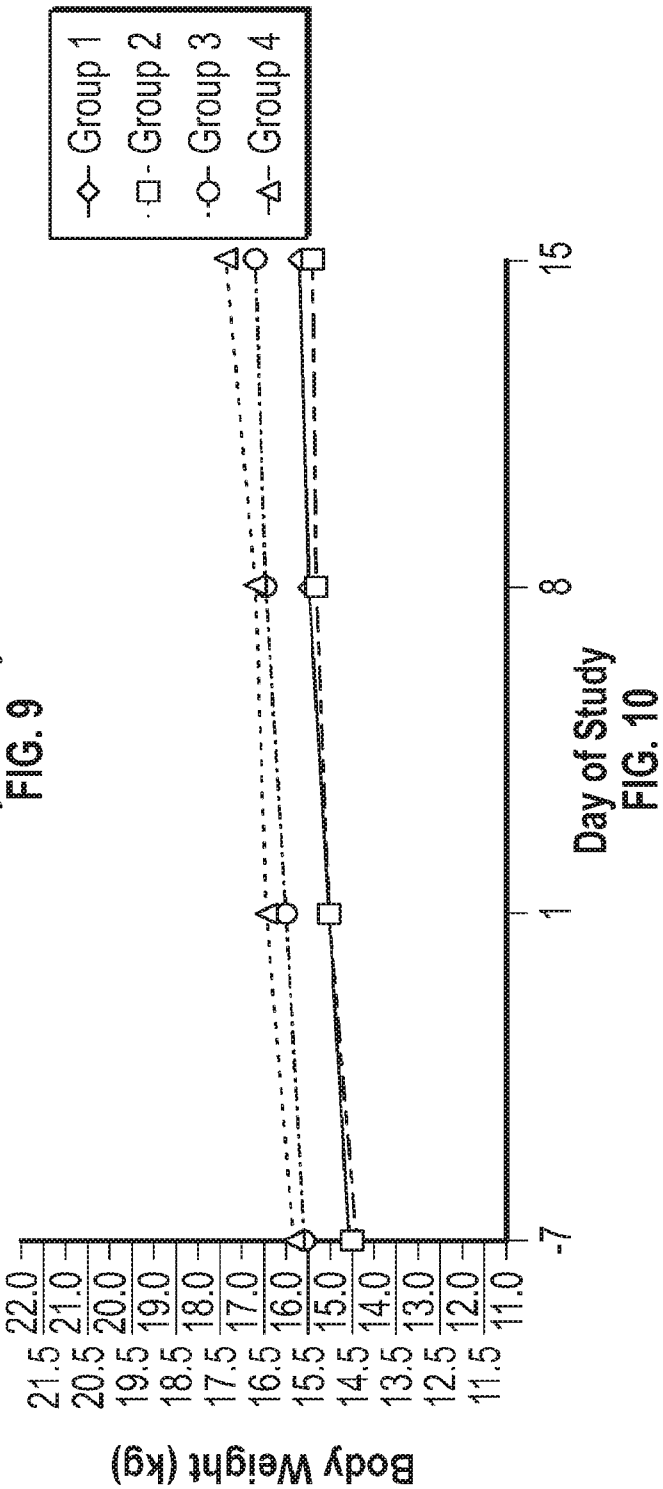


FIG. 8

8/207



9/207

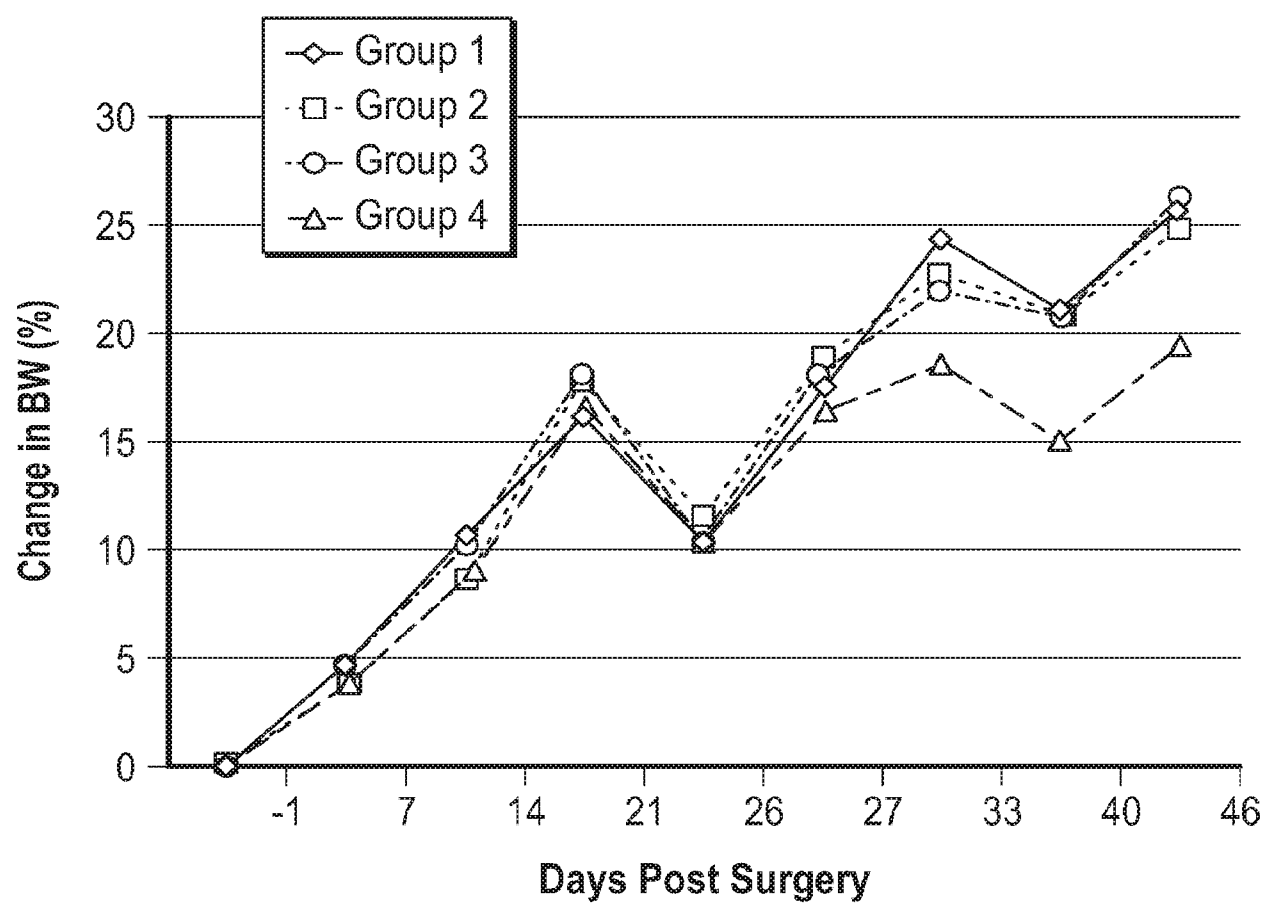


FIG. 11

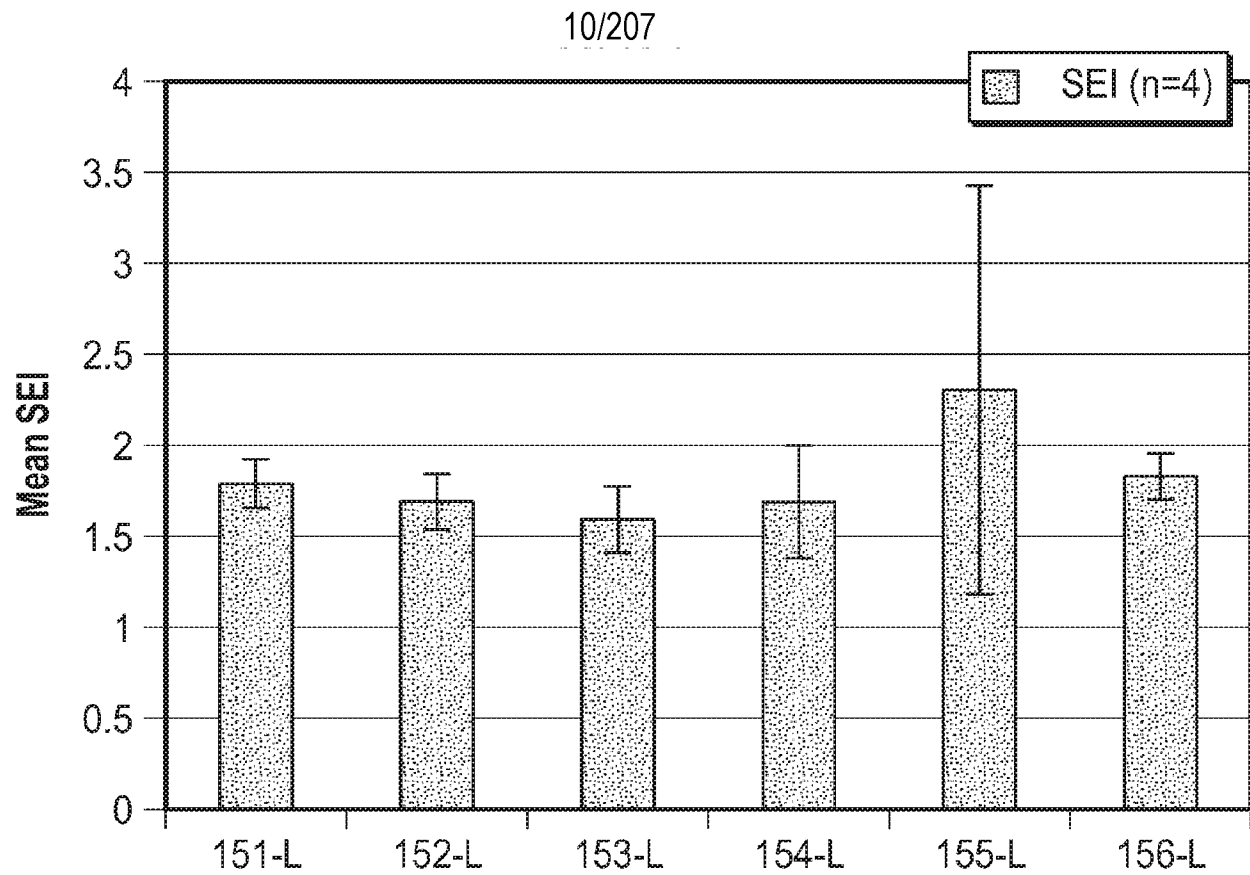


FIG. 12

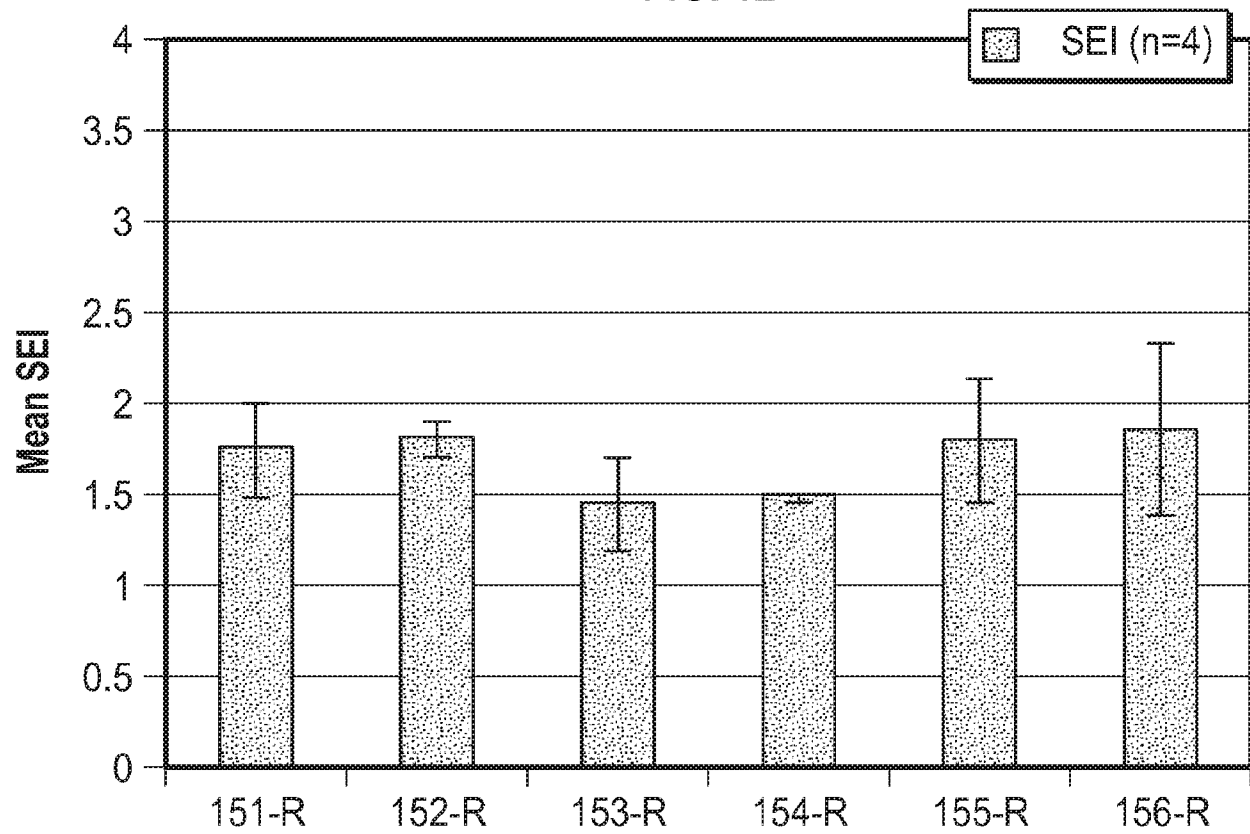


FIG. 13

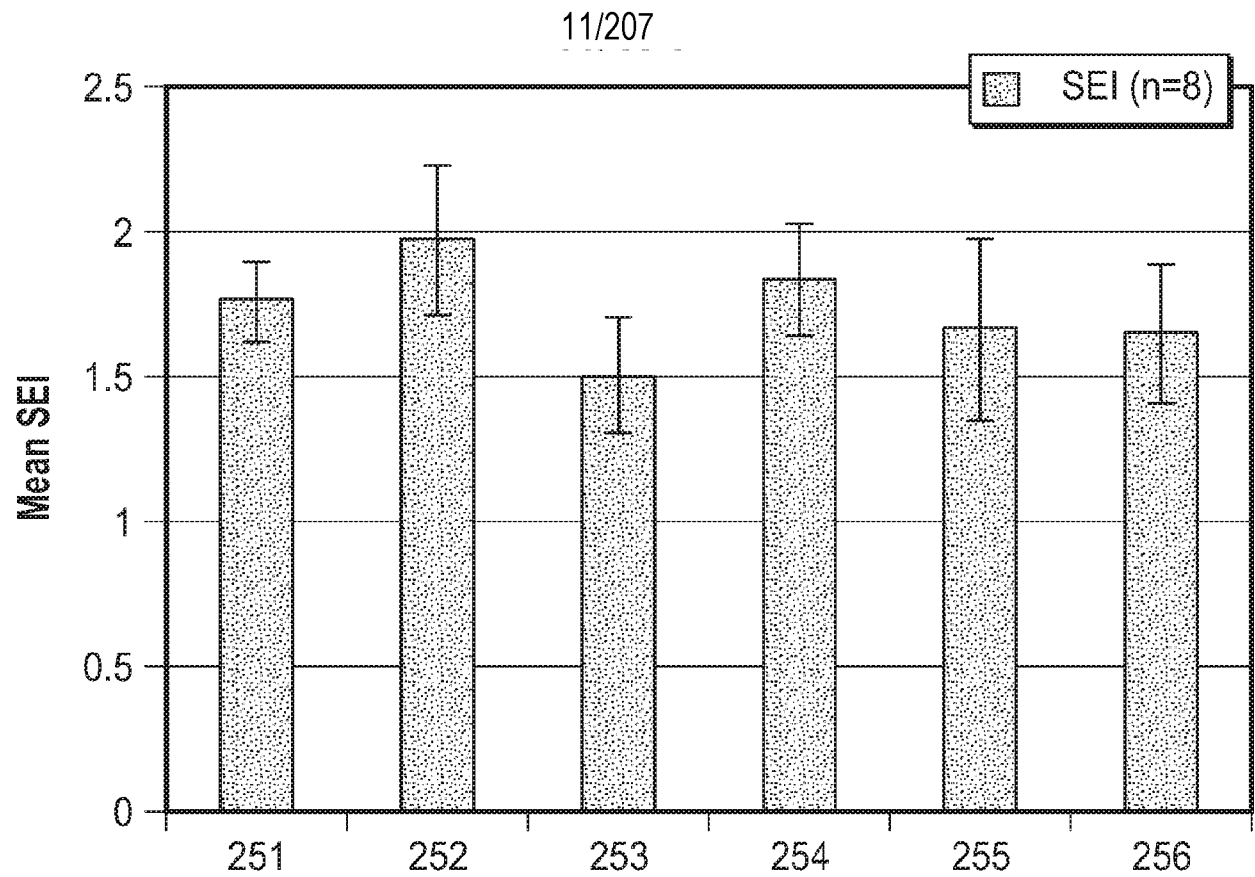


FIG. 14

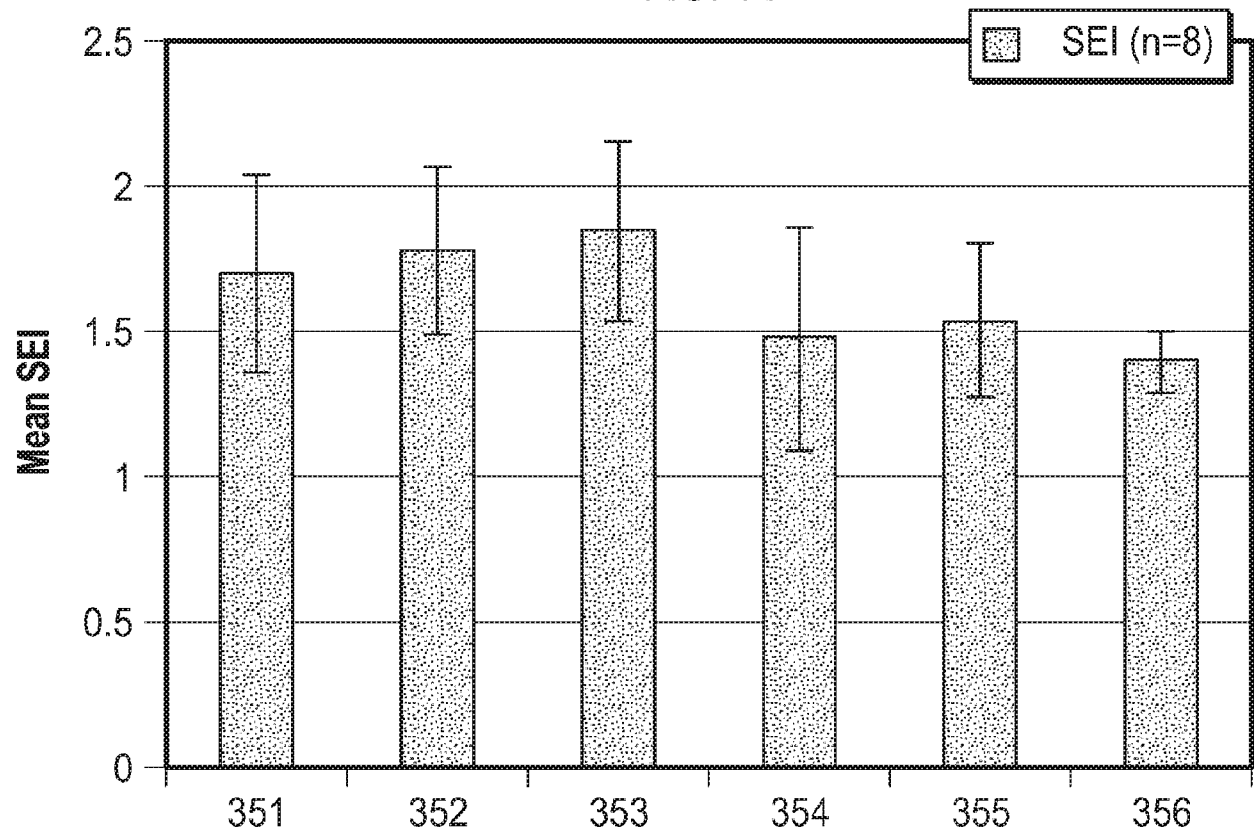


FIG. 15

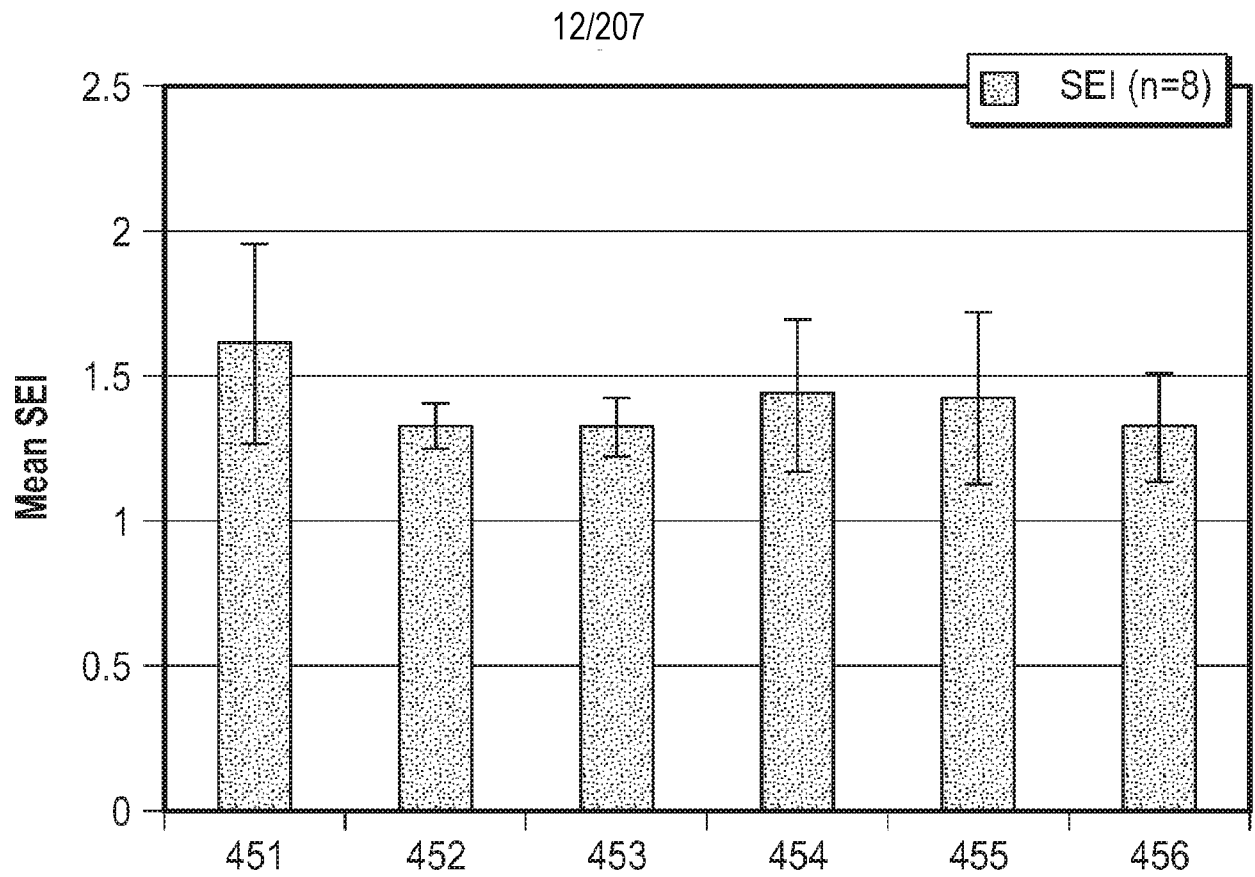


FIG. 16

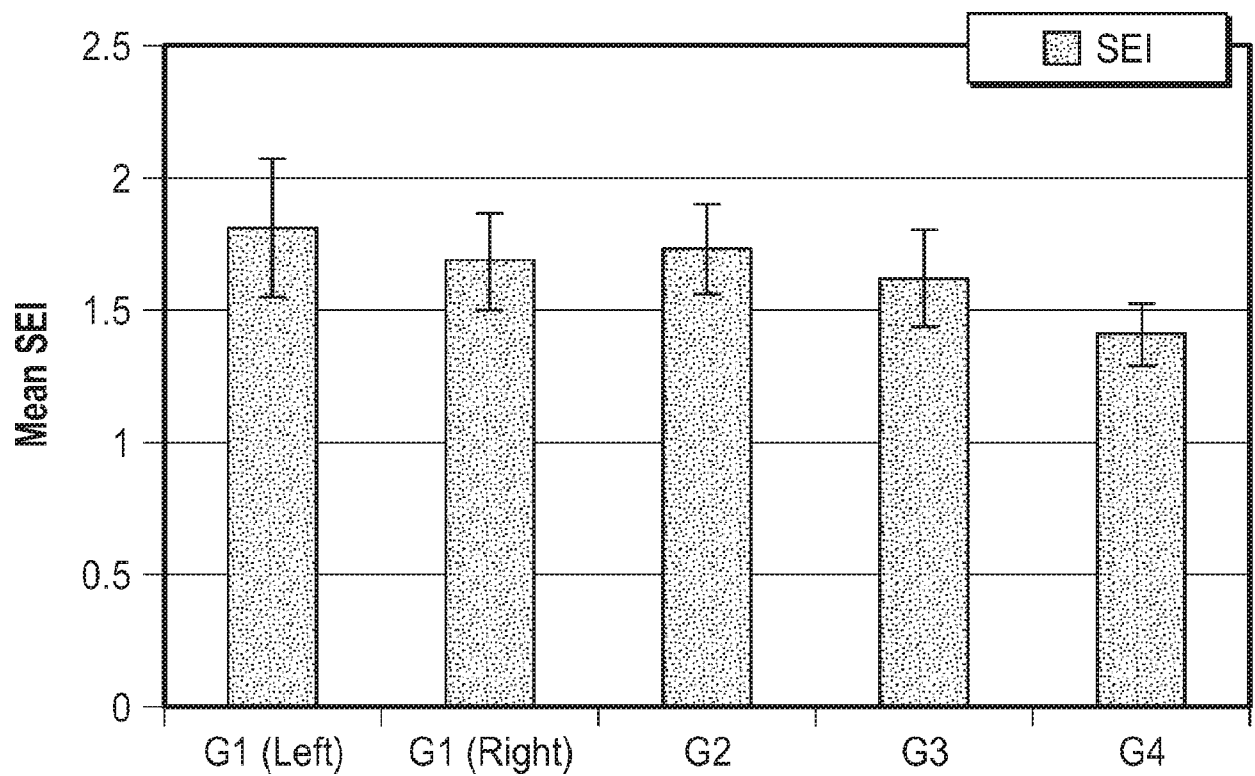


FIG. 17

13/207

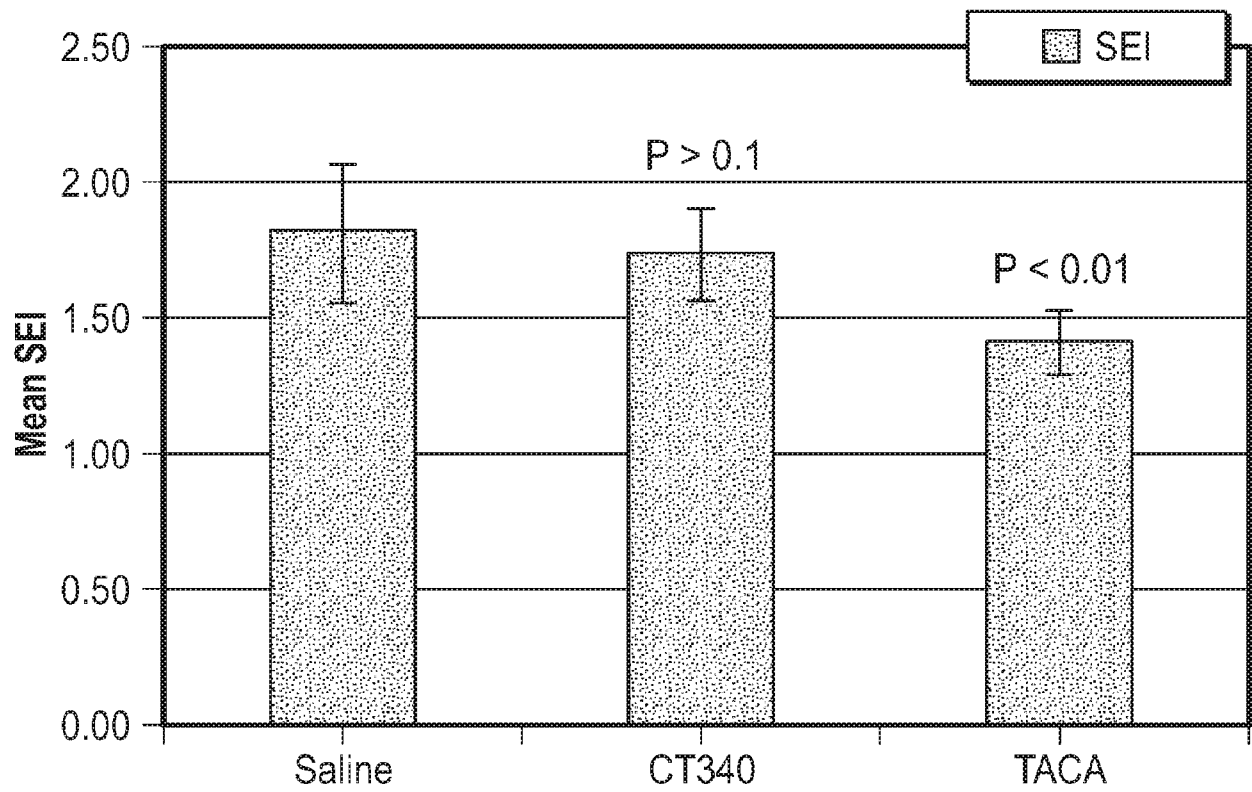


FIG. 18

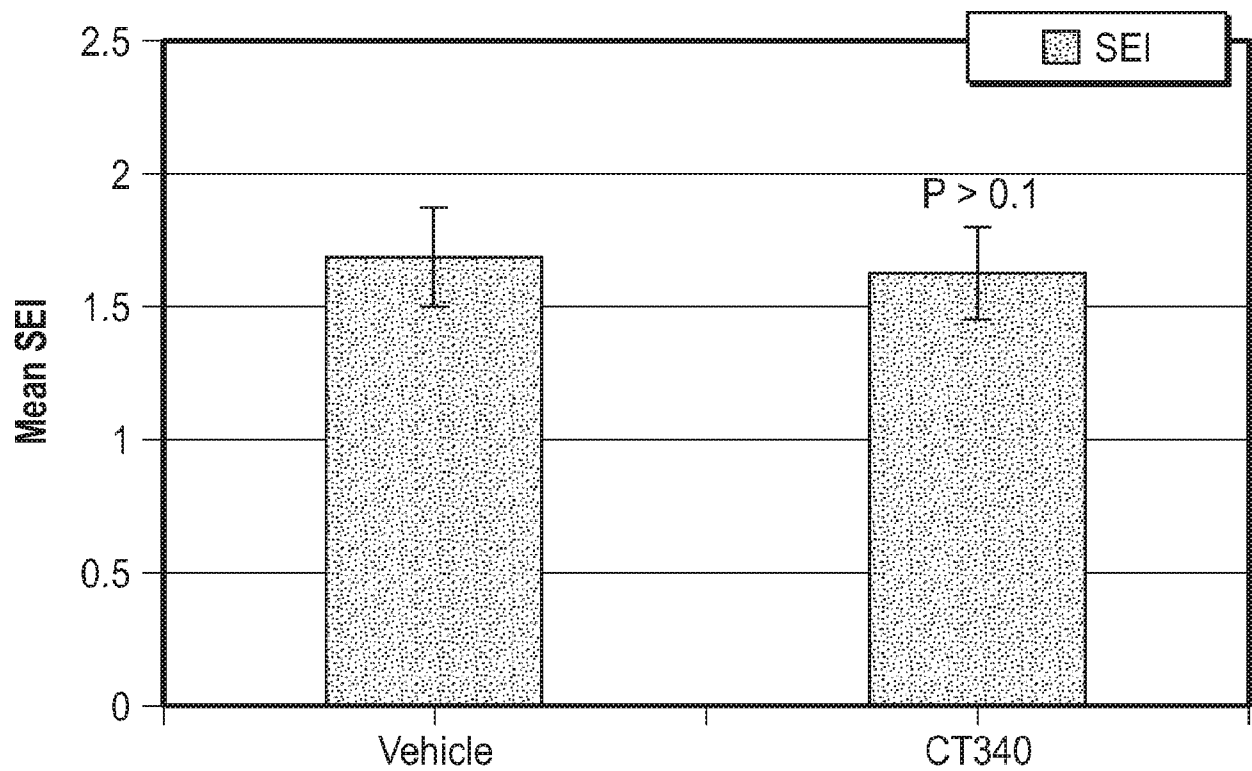


FIG. 19

14/207

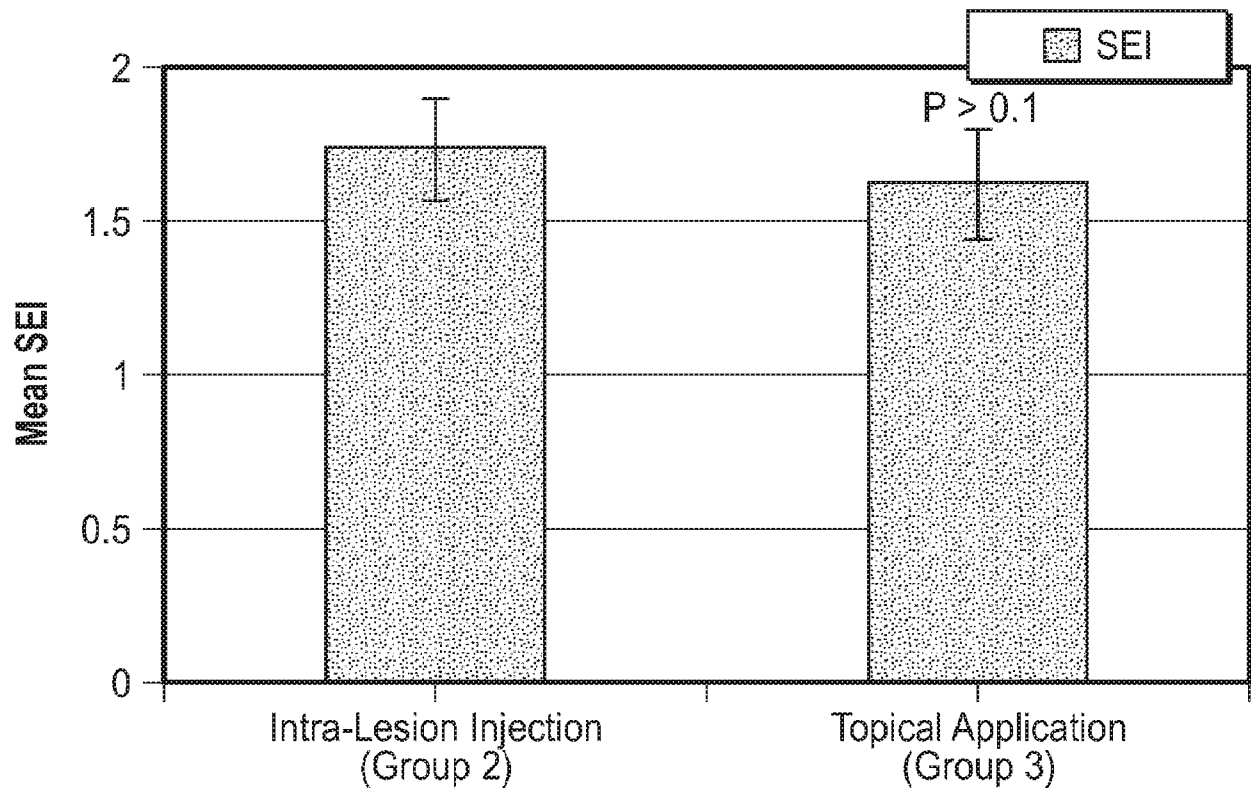


FIG. 20

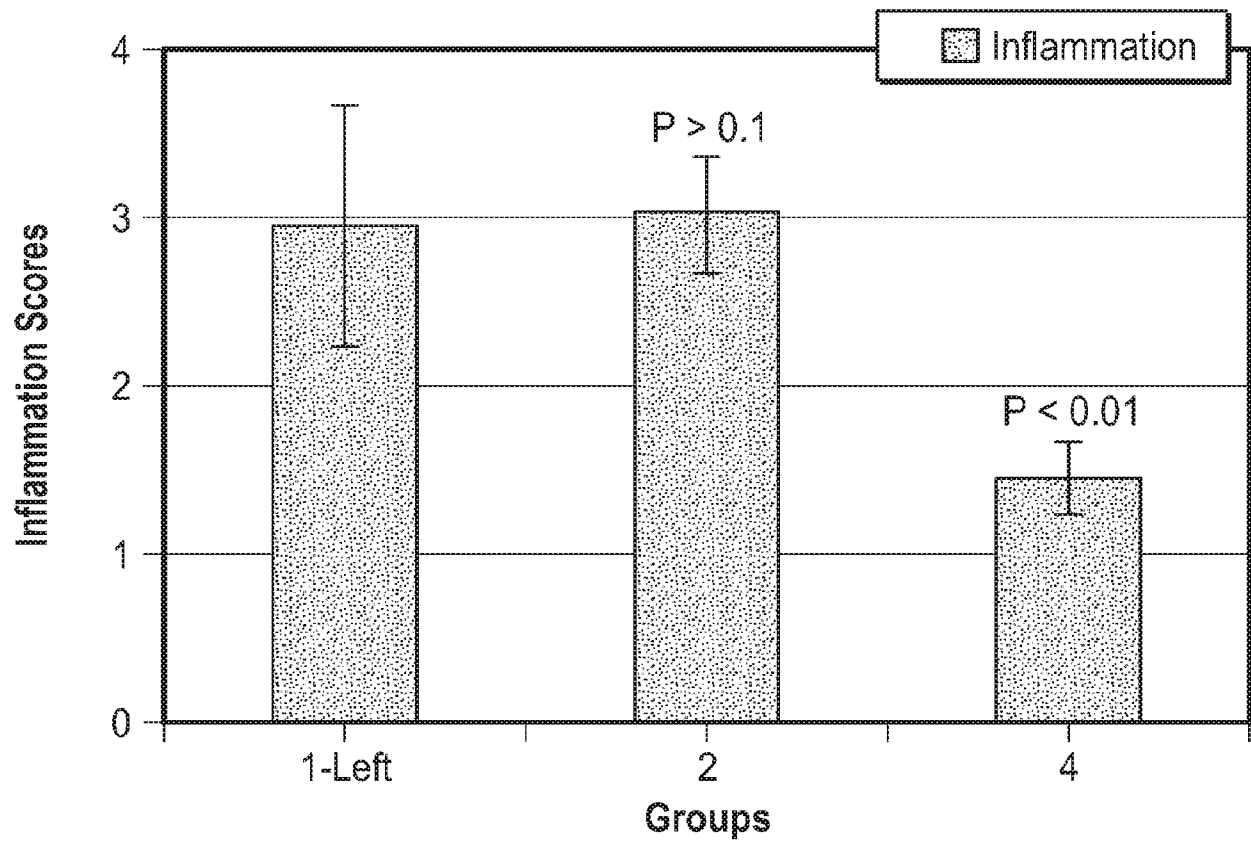


FIG. 21

15/207

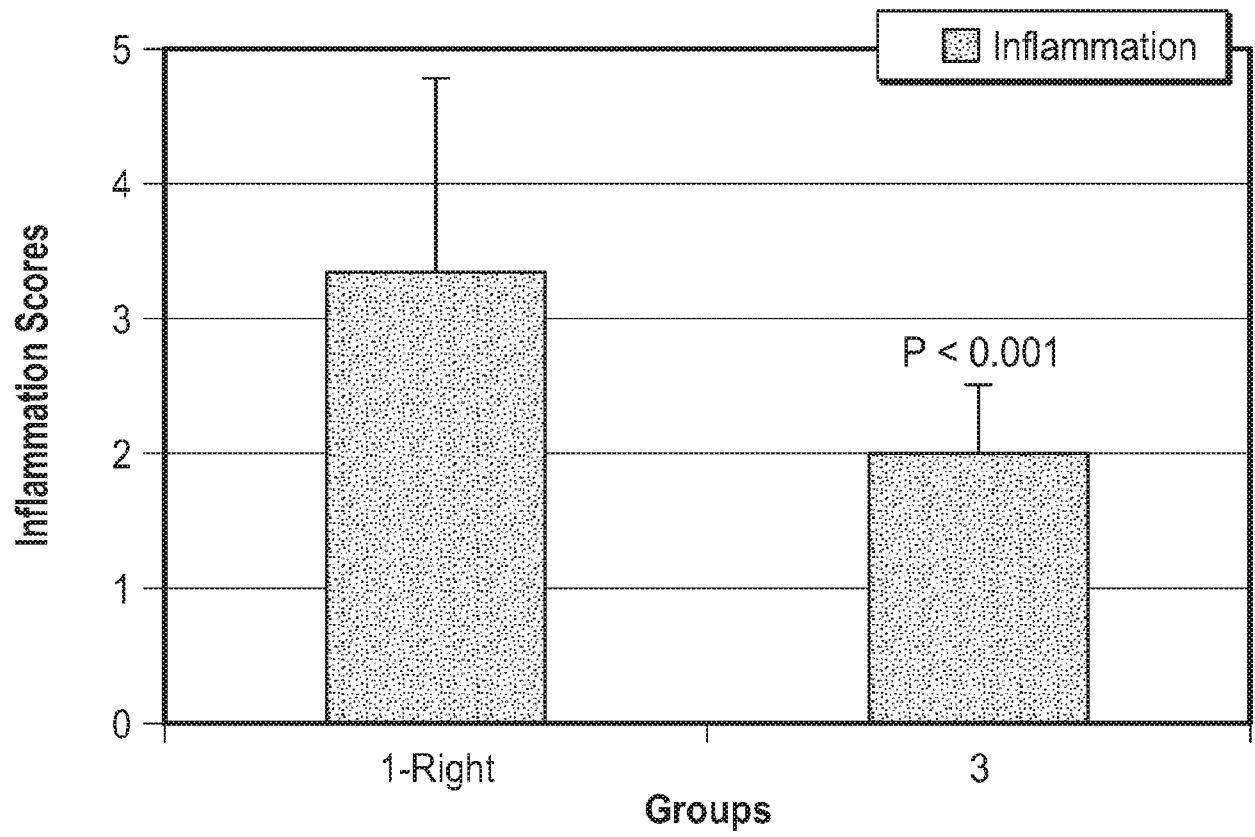
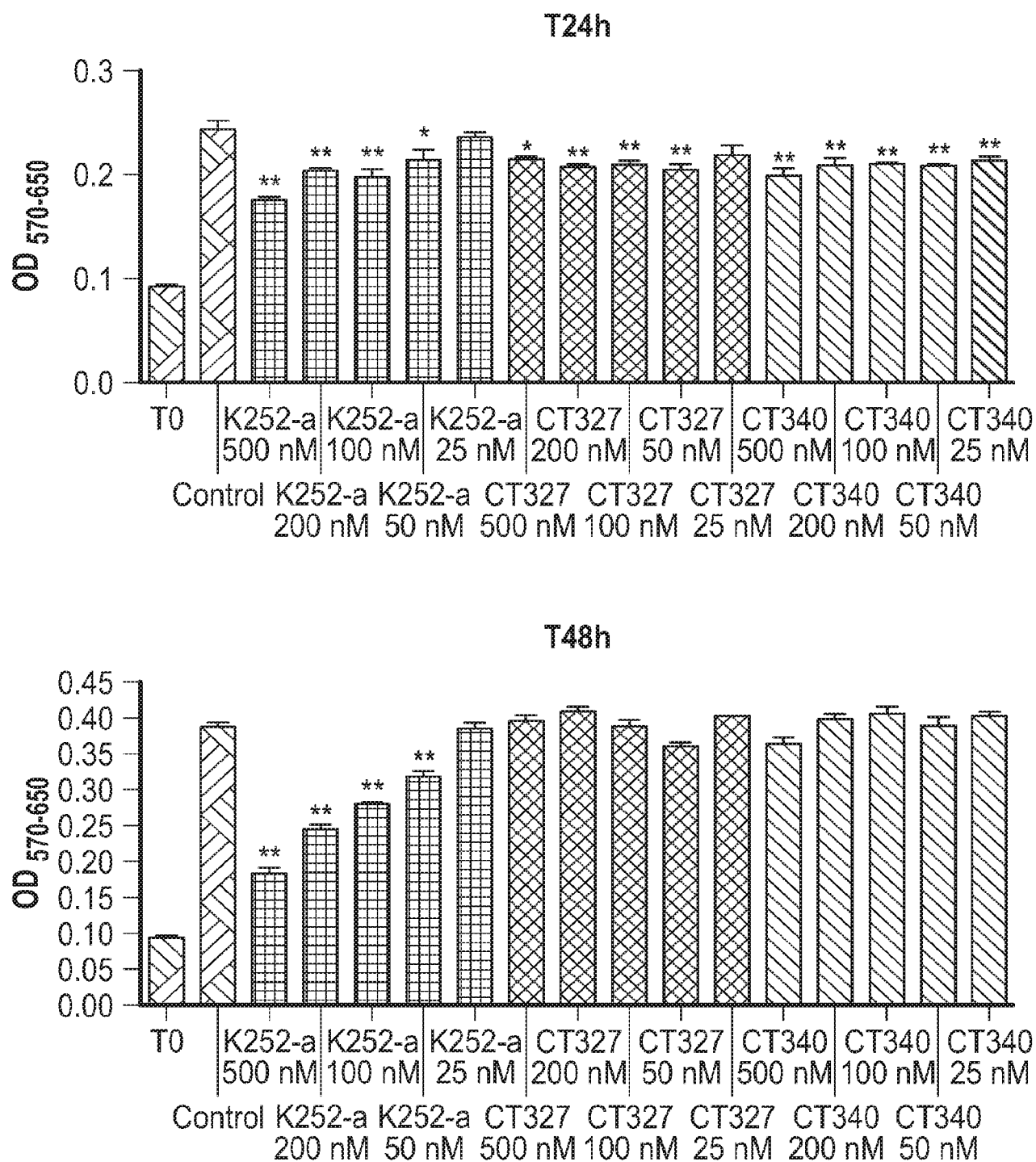


FIG. 22

16/207



17/207

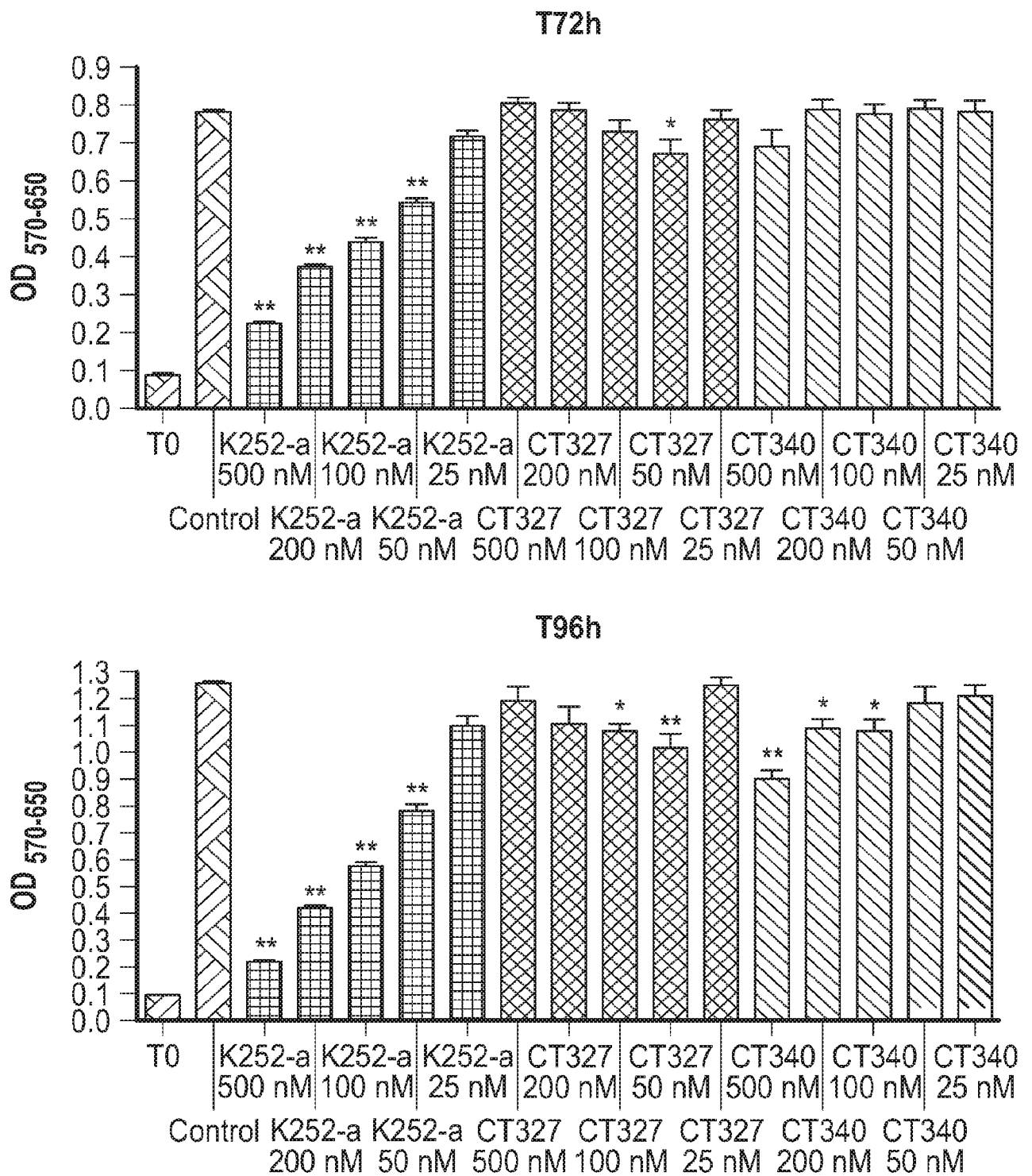
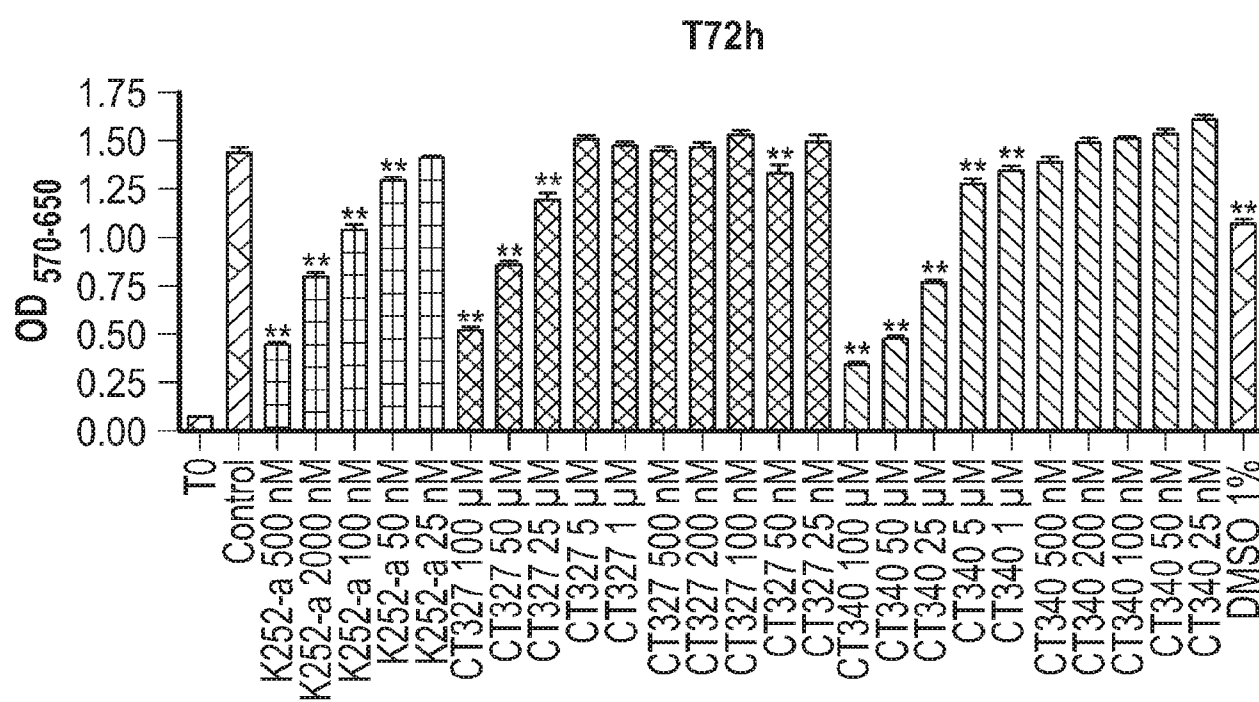


FIG. 23
(continued)

18/207

**FIG. 24**

19/207

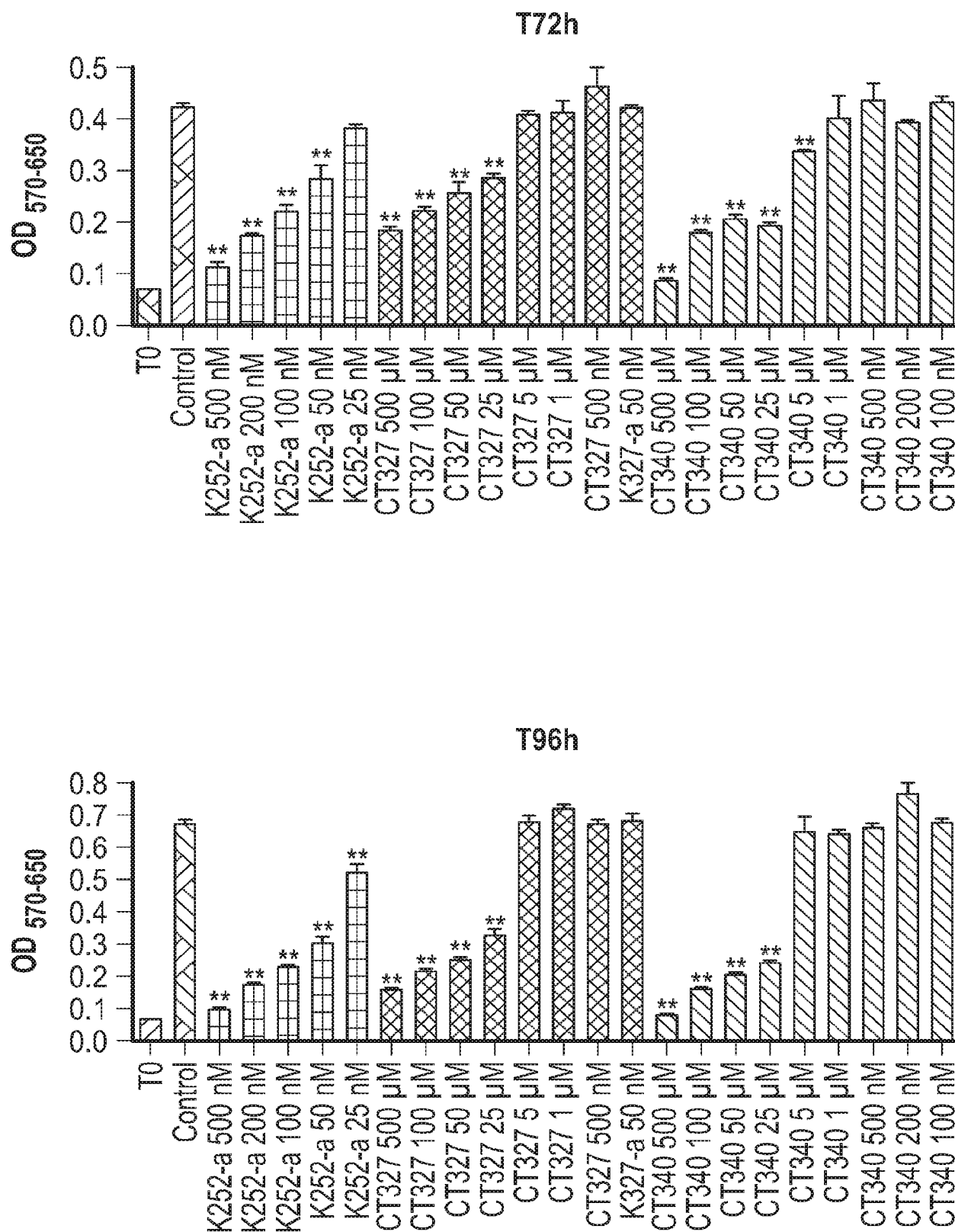


FIG. 25

20/207

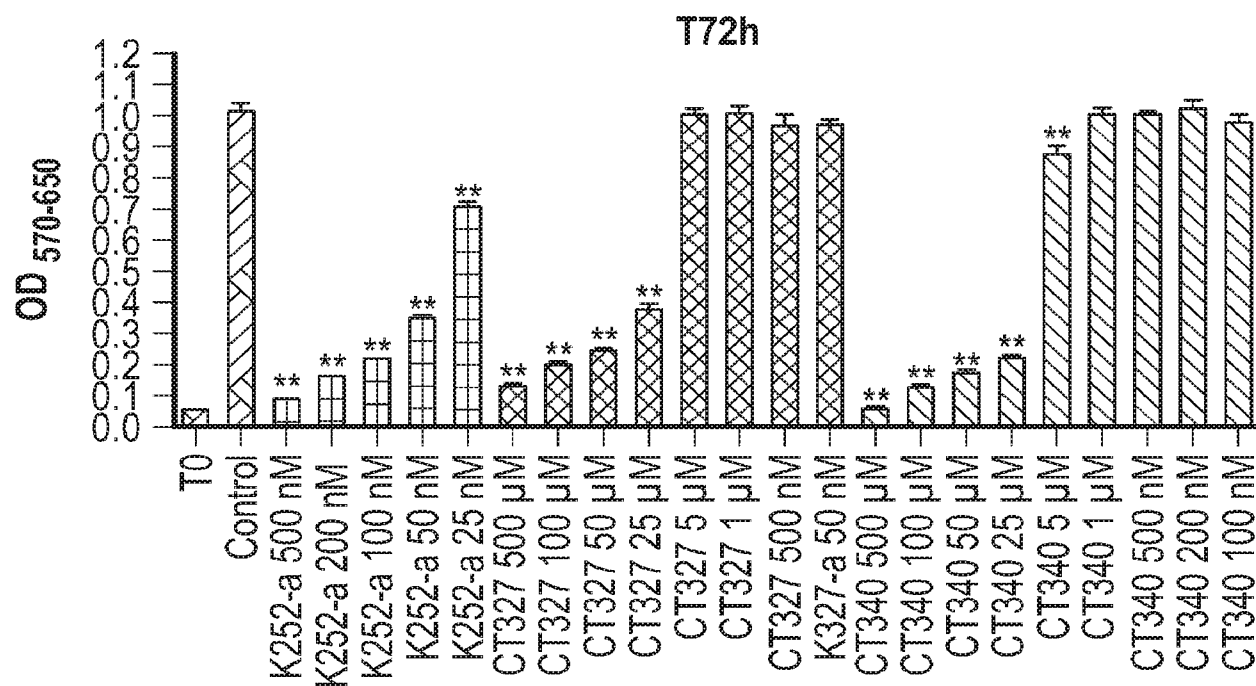


FIG. 25
(Continued)

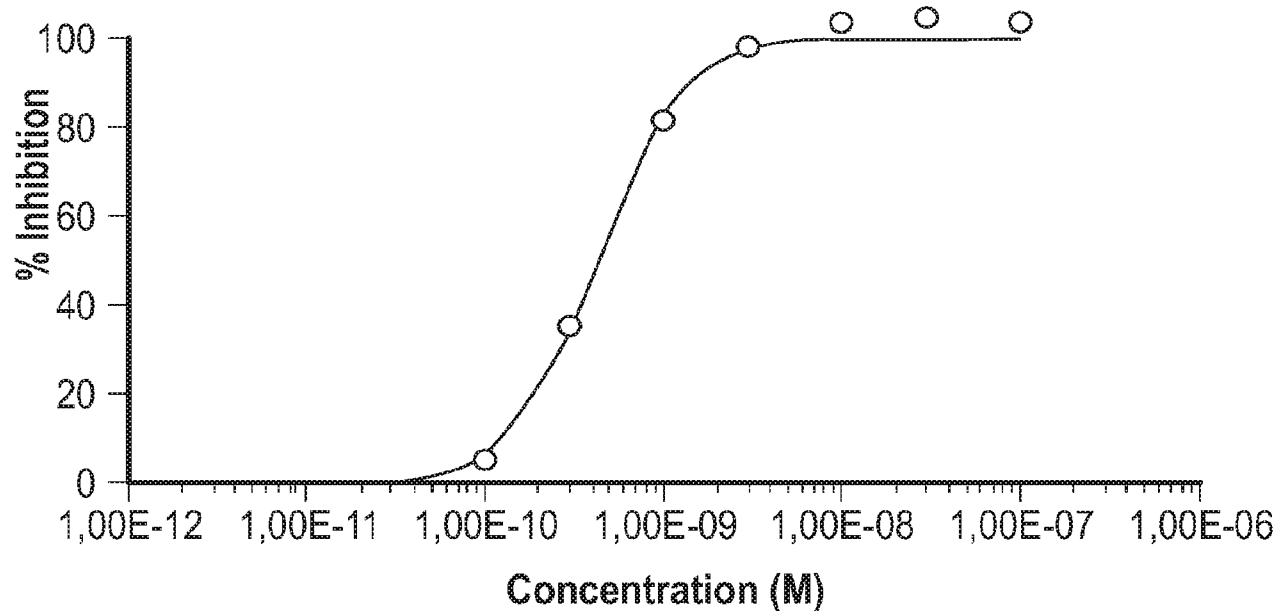


FIG. 26

21/207

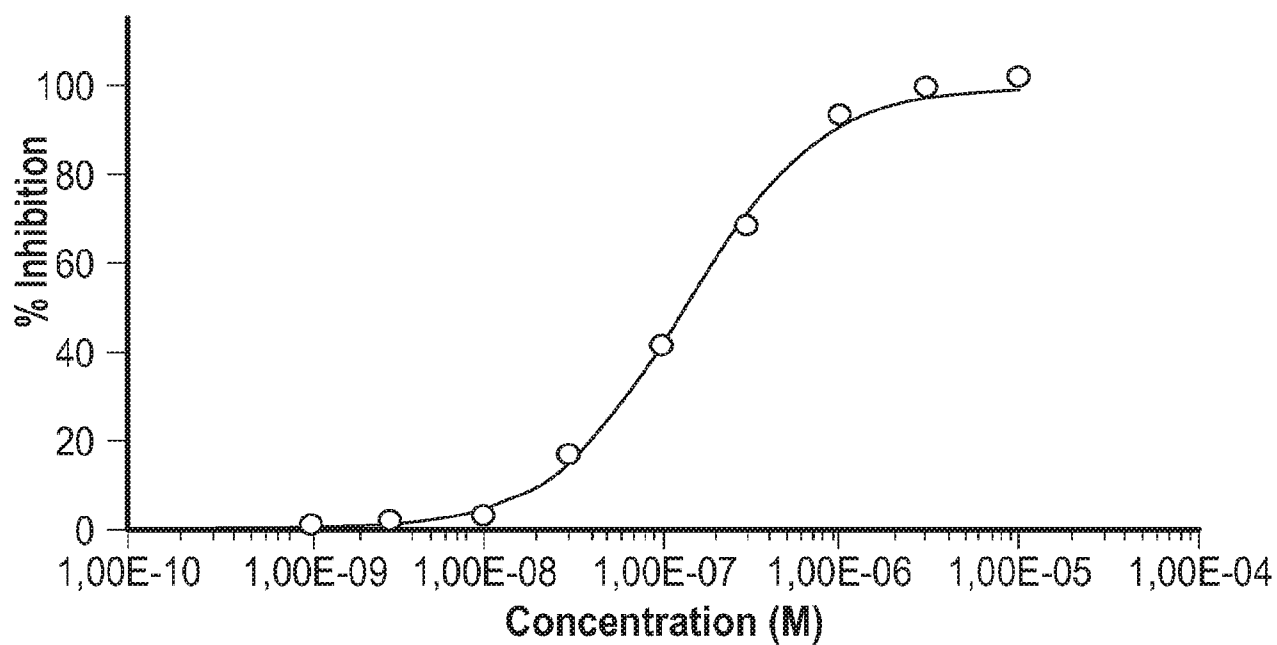


FIG. 27

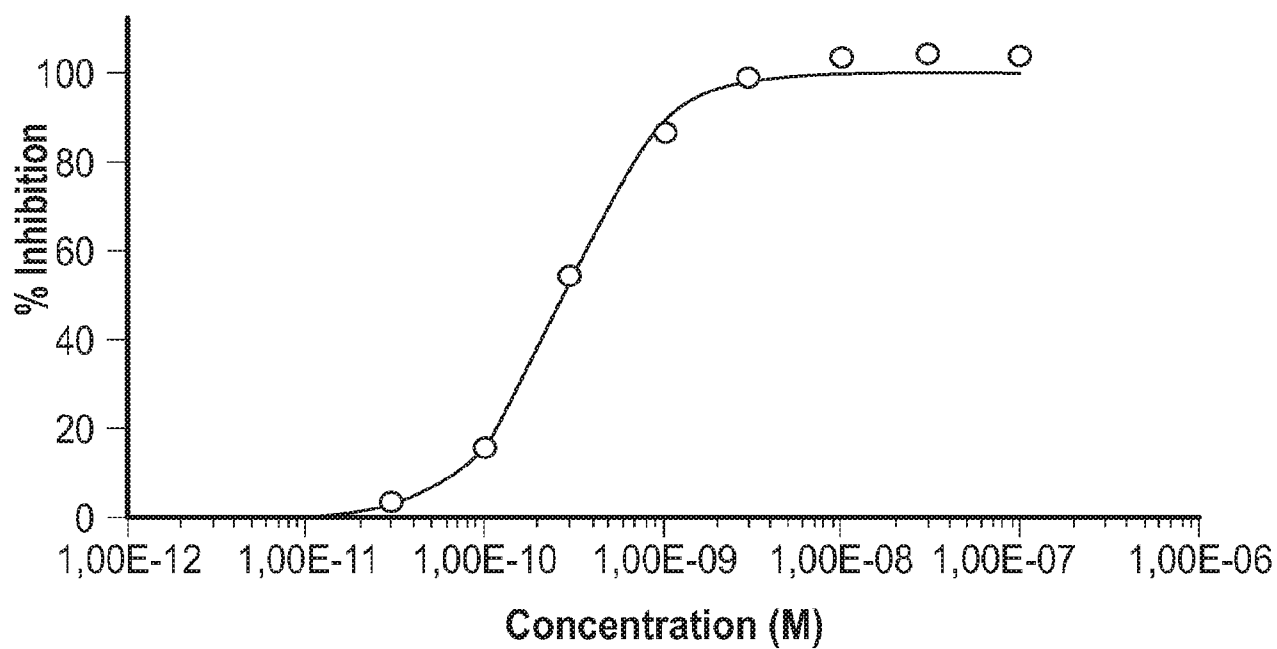


FIG. 28

22/207

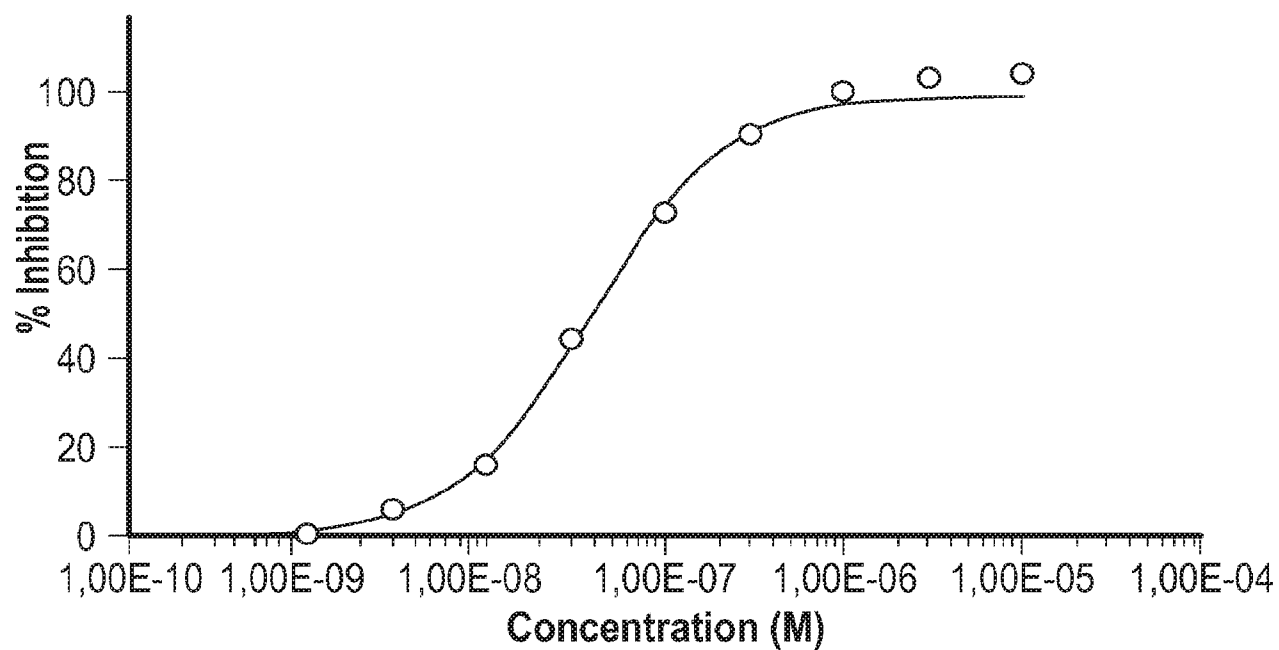


FIG. 29

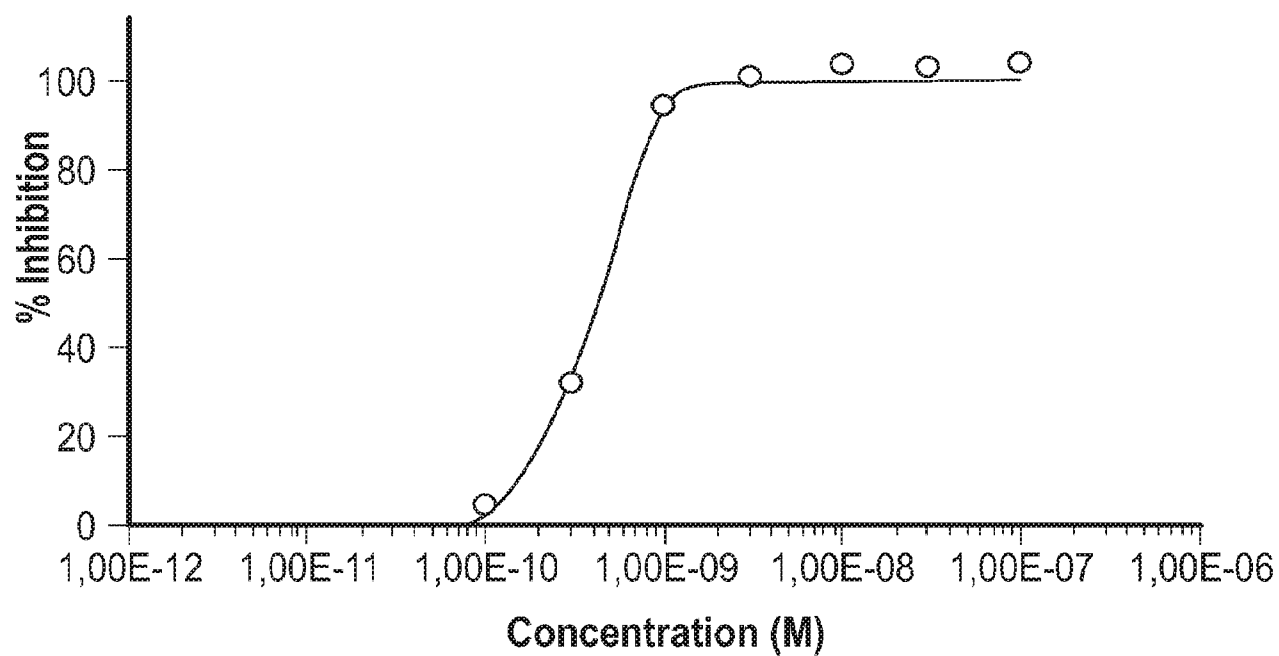


FIG. 30

23/207

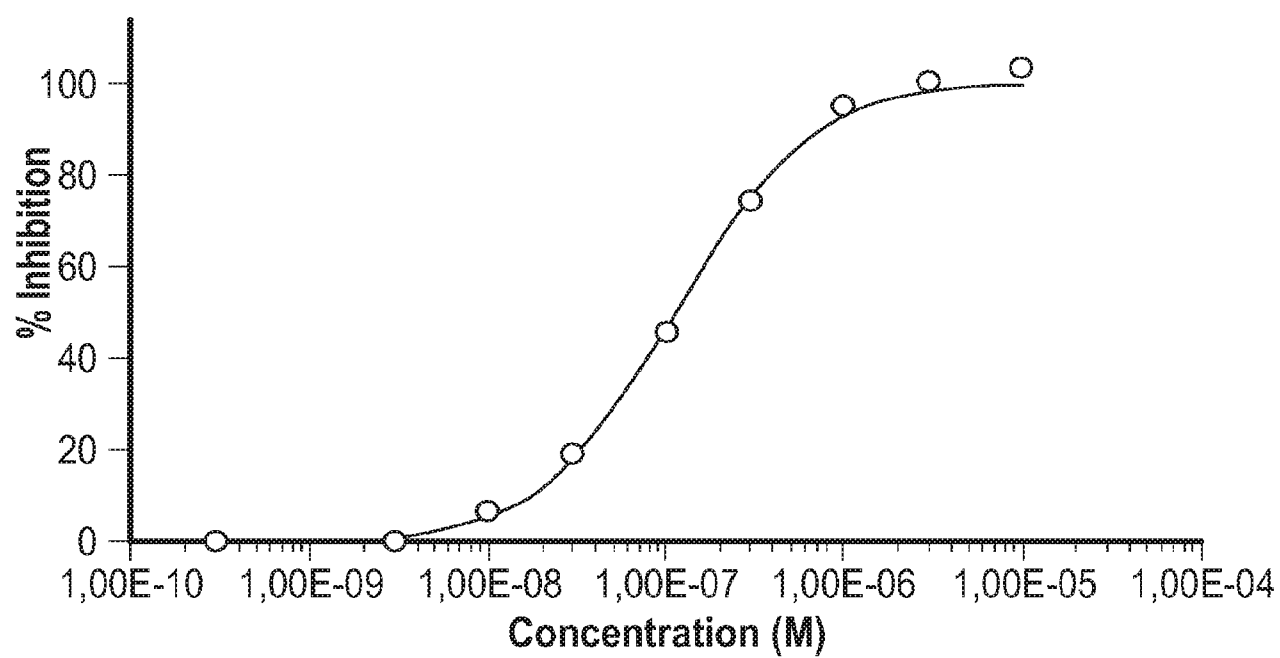


FIG. 31

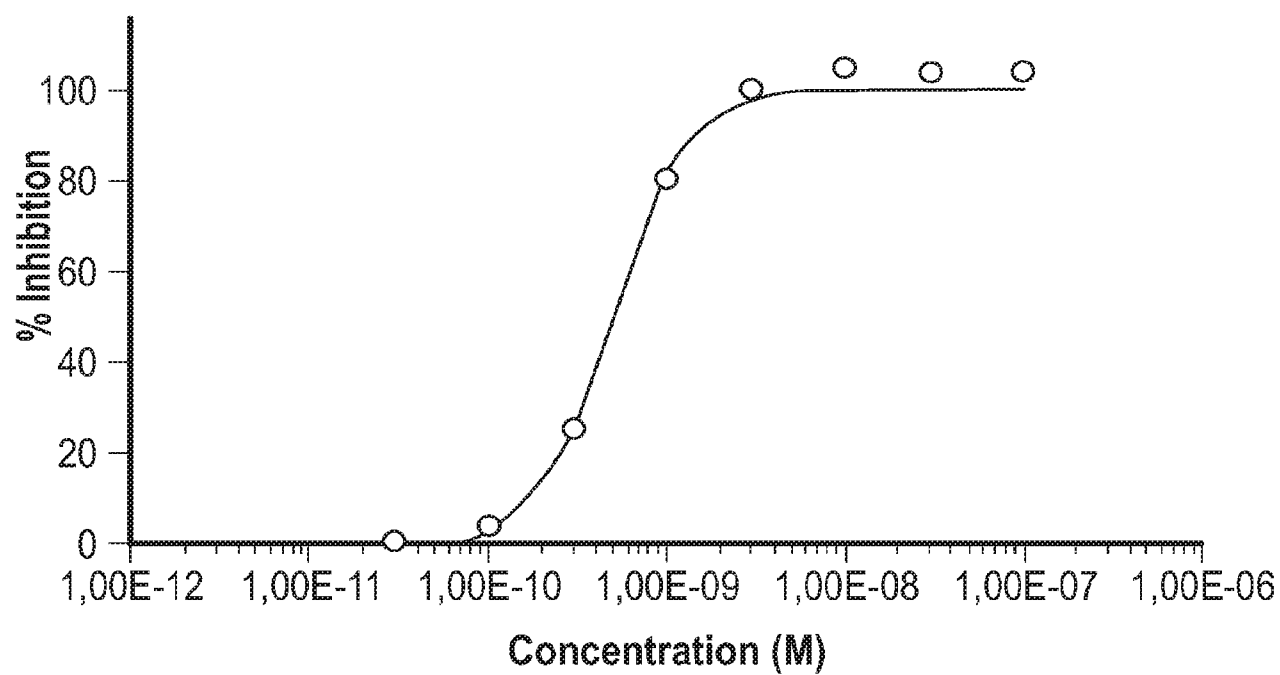


FIG. 32

24/207

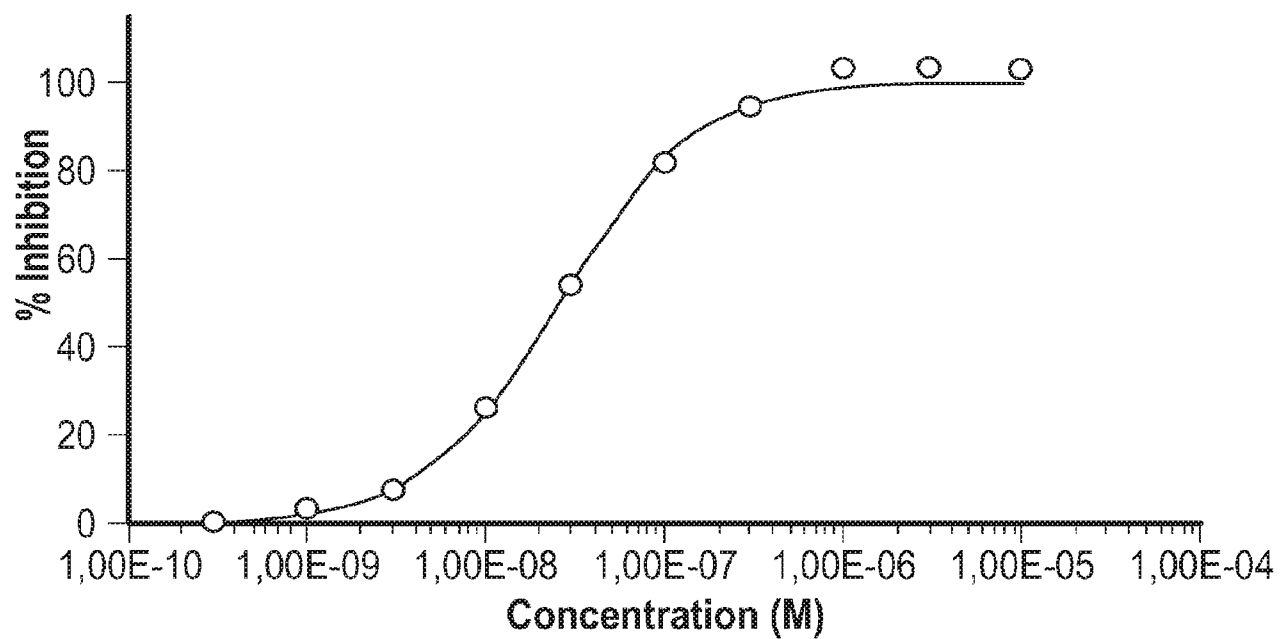


FIG. 33

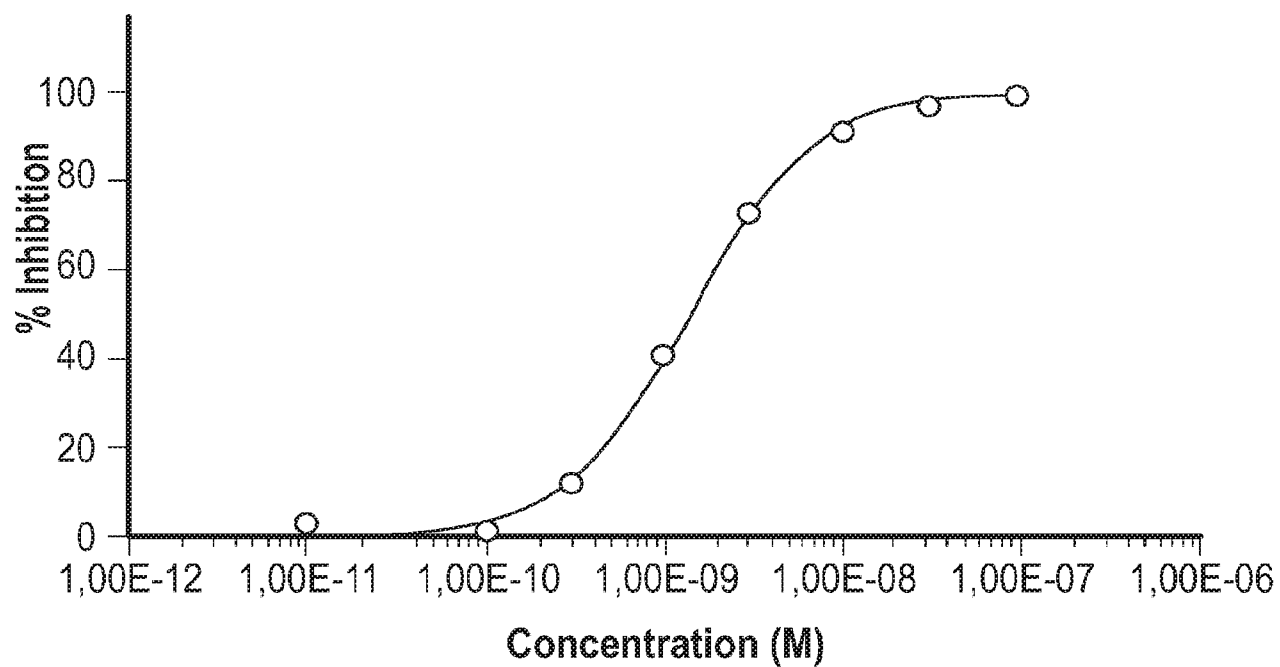


FIG. 34

25/207

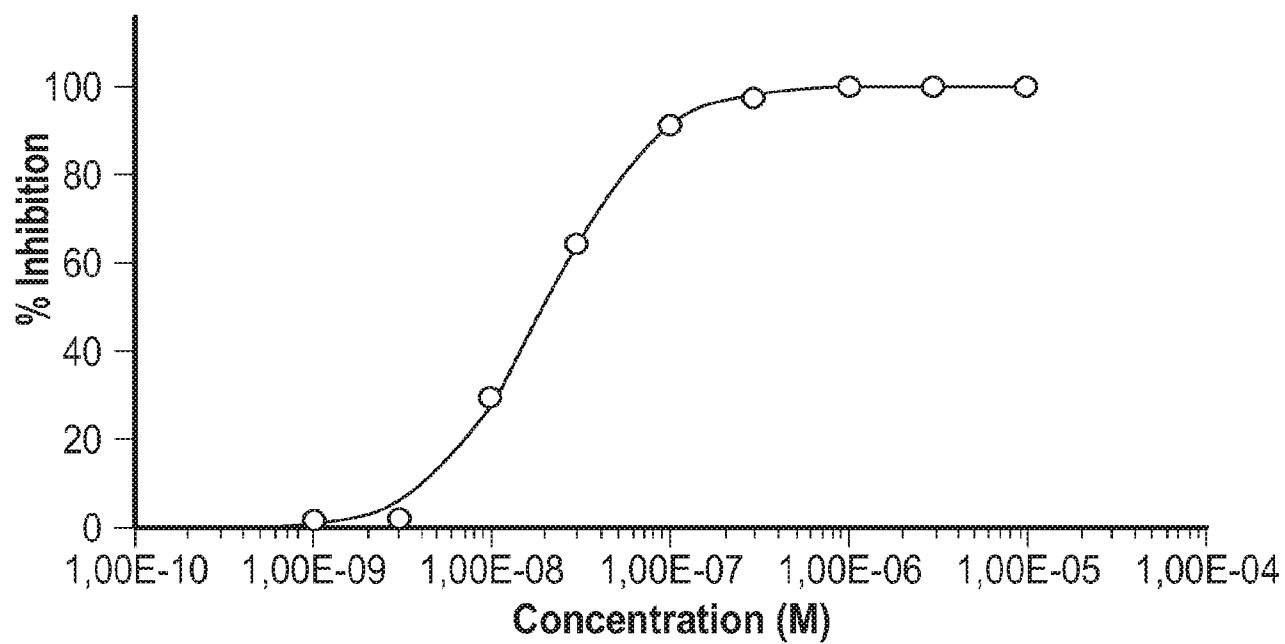


FIG. 35

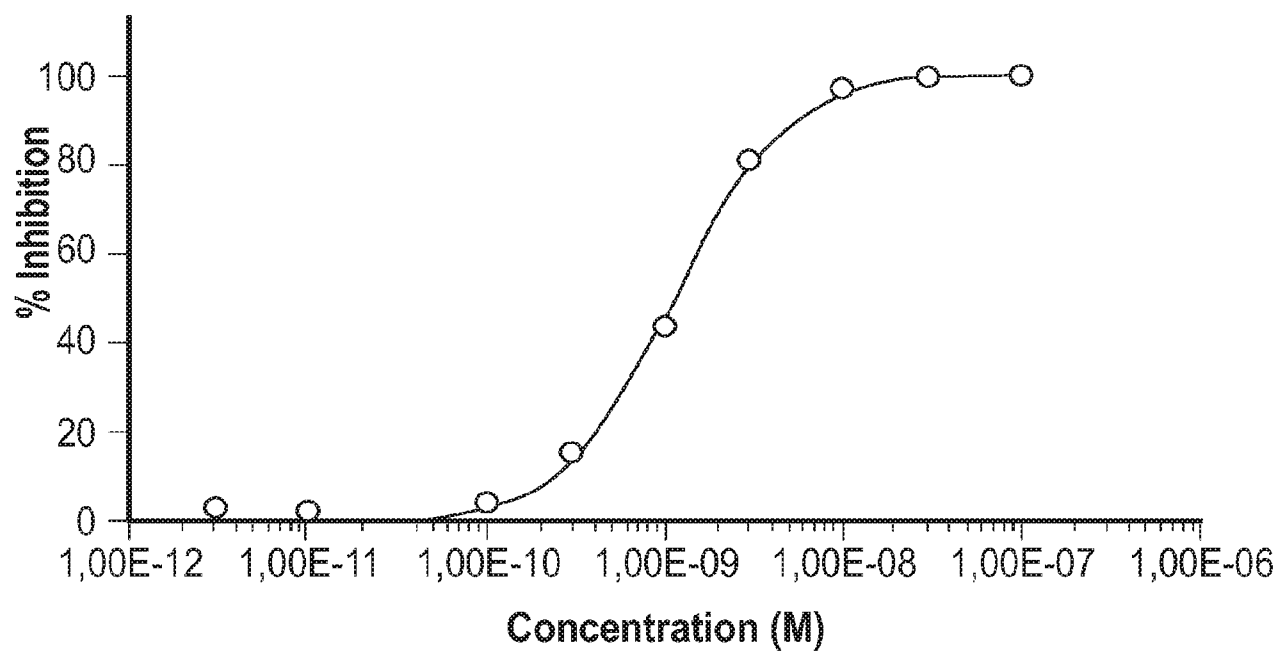


FIG. 36

26/207

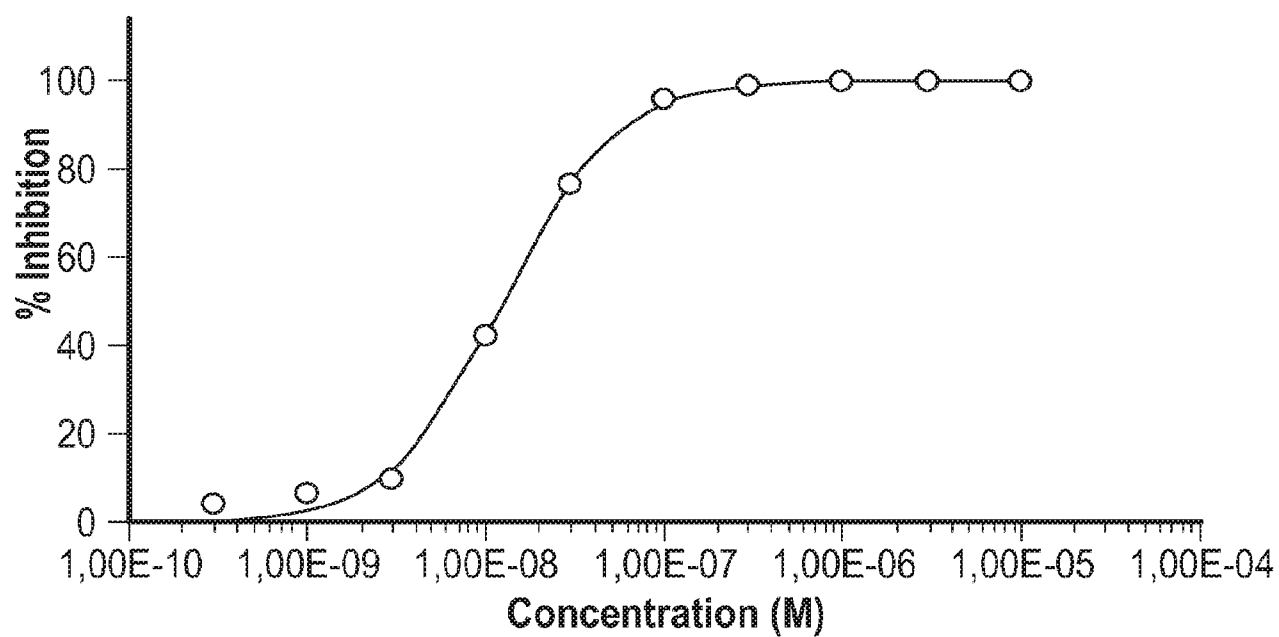


FIG. 37

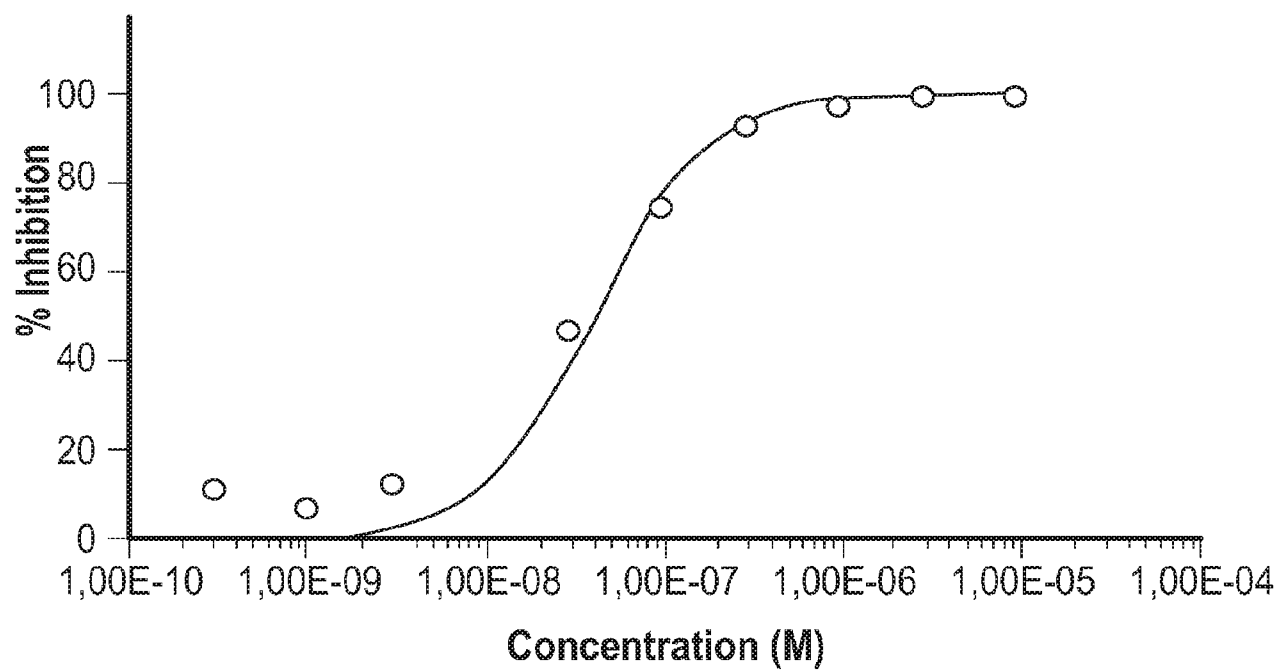


FIG. 38

27/207

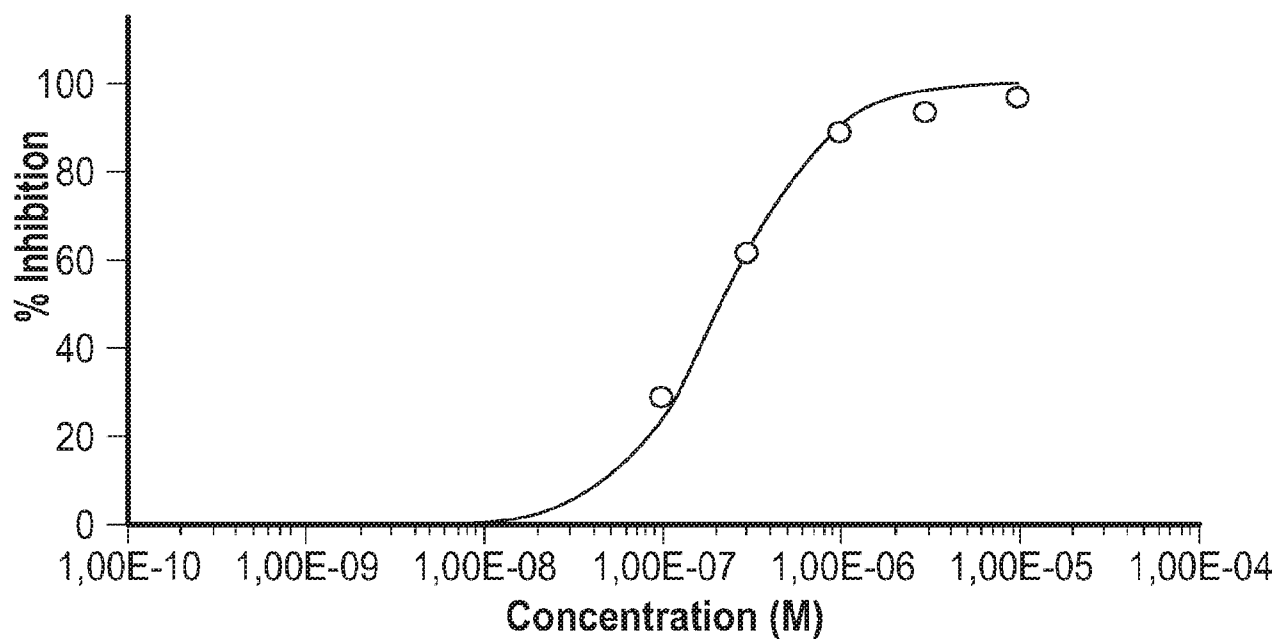


FIG. 39

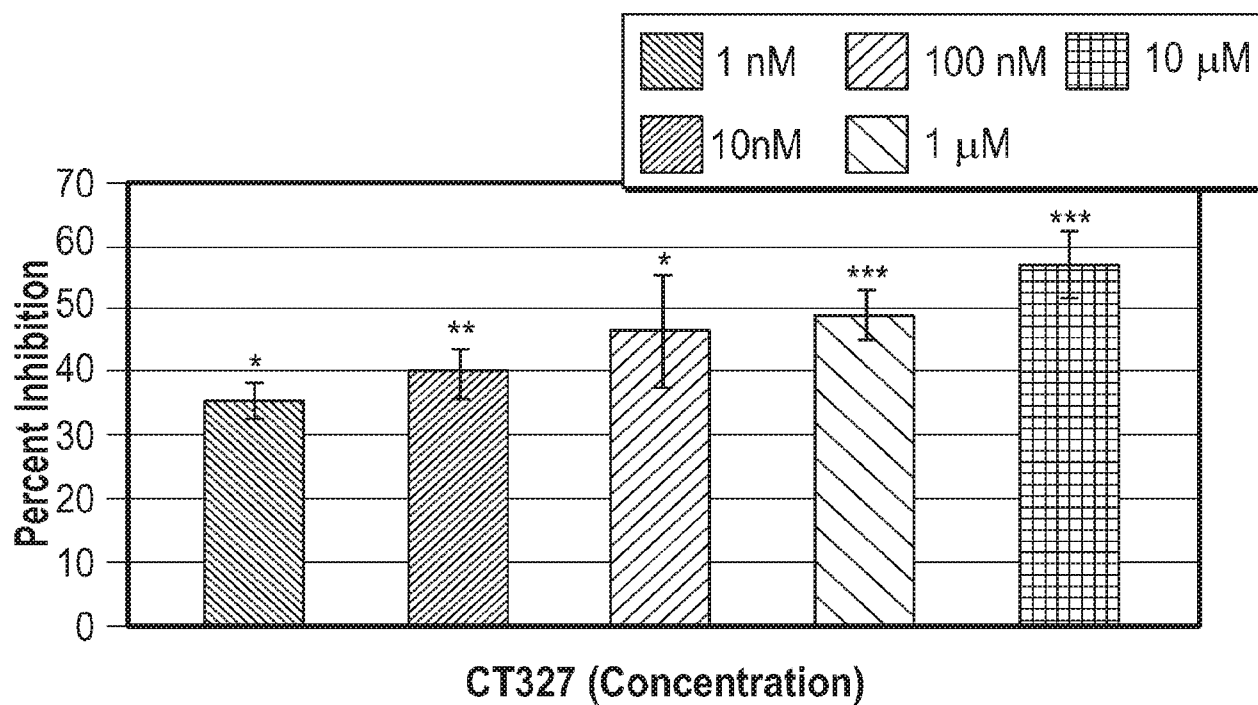


FIG. 40

28/207

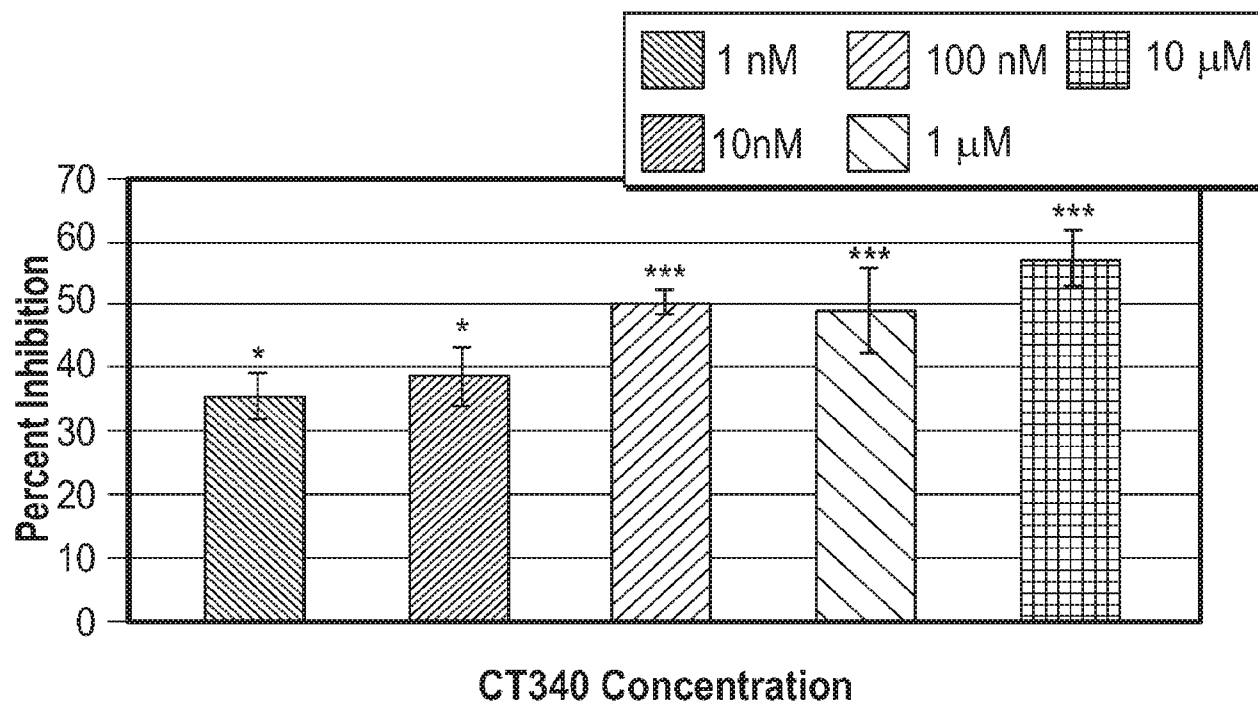


FIG. 41

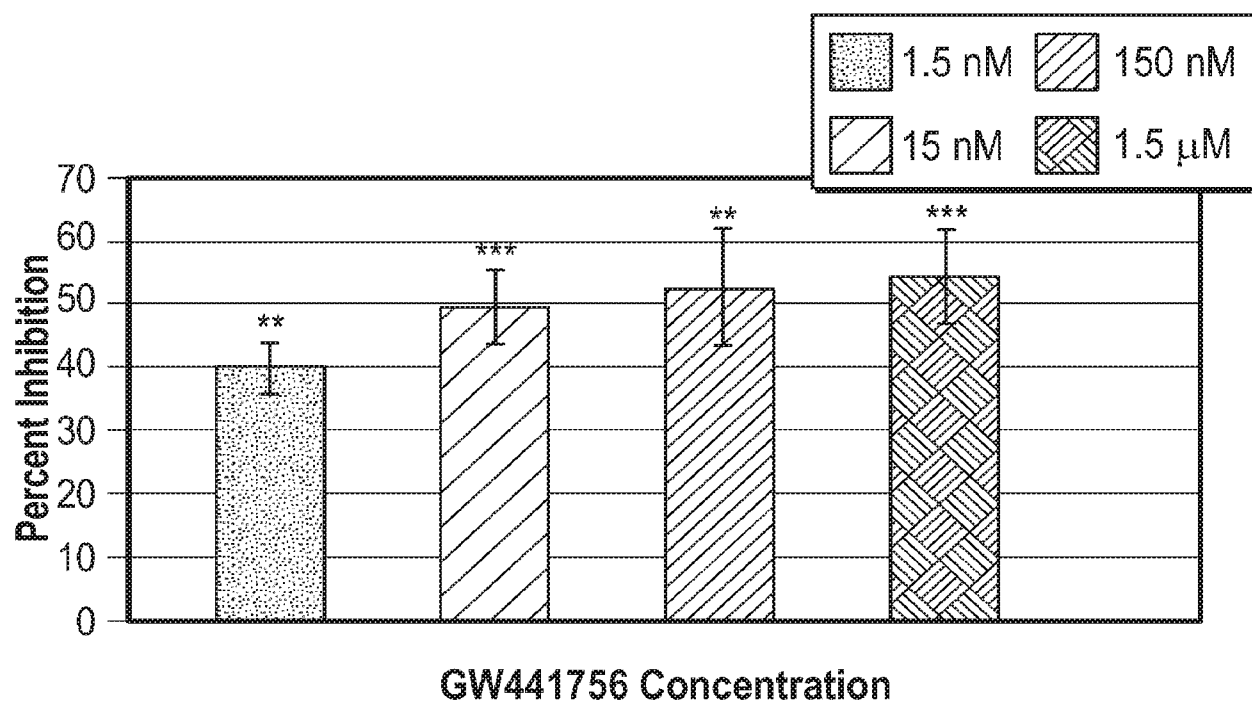


FIG. 42

29/207

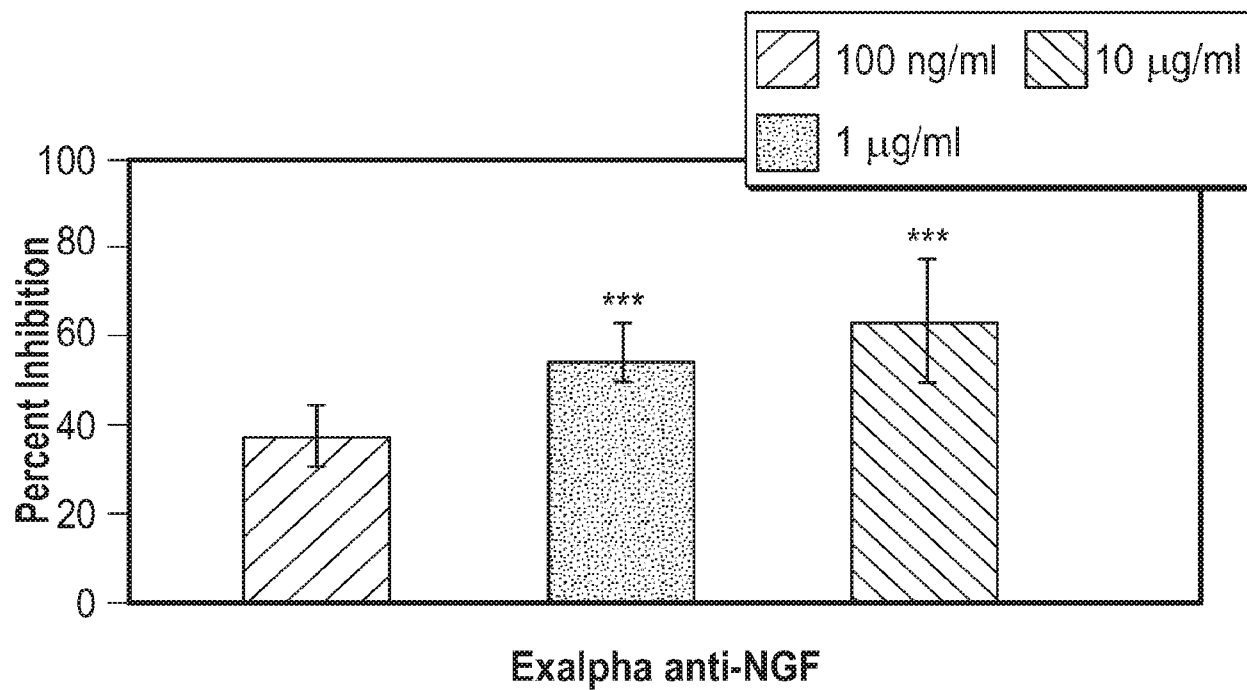


FIG. 43

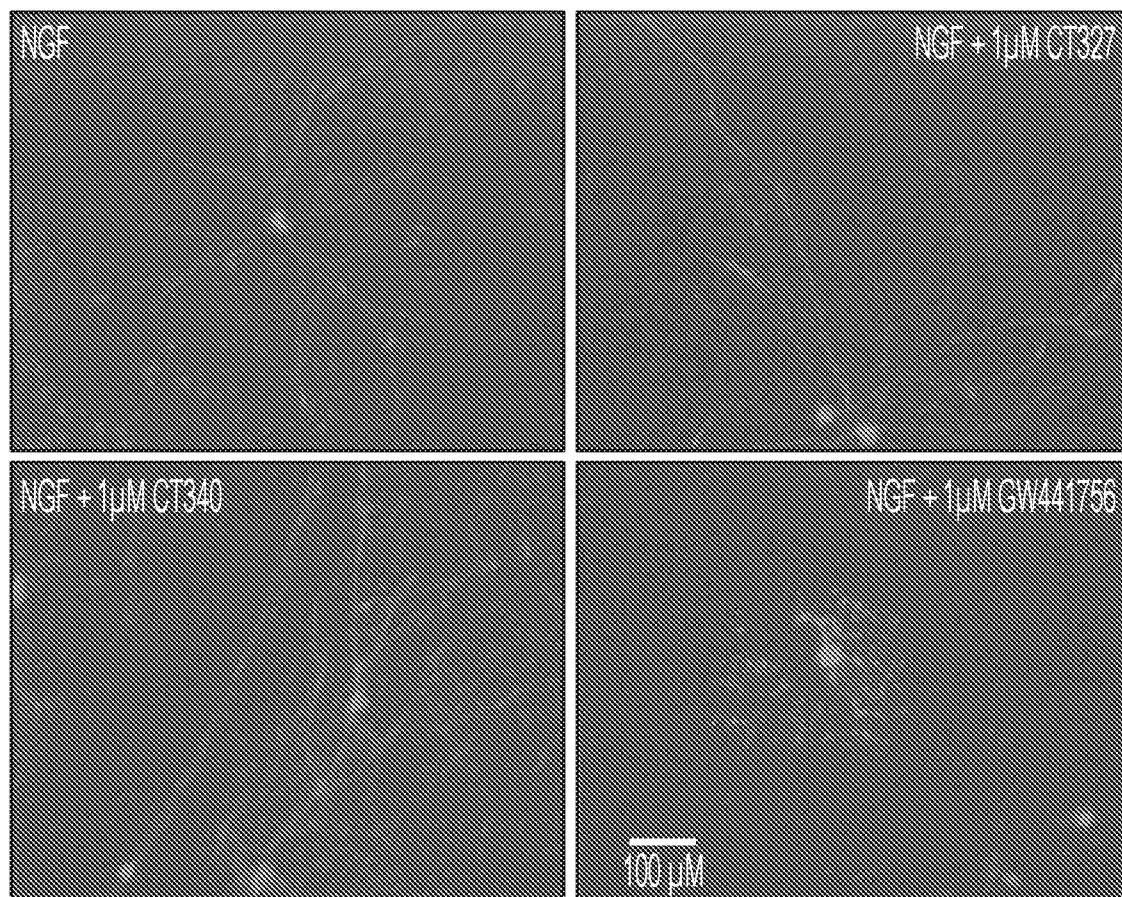


FIG. 44

SUBSTITUTE SHEET (RULE 26)

30/207

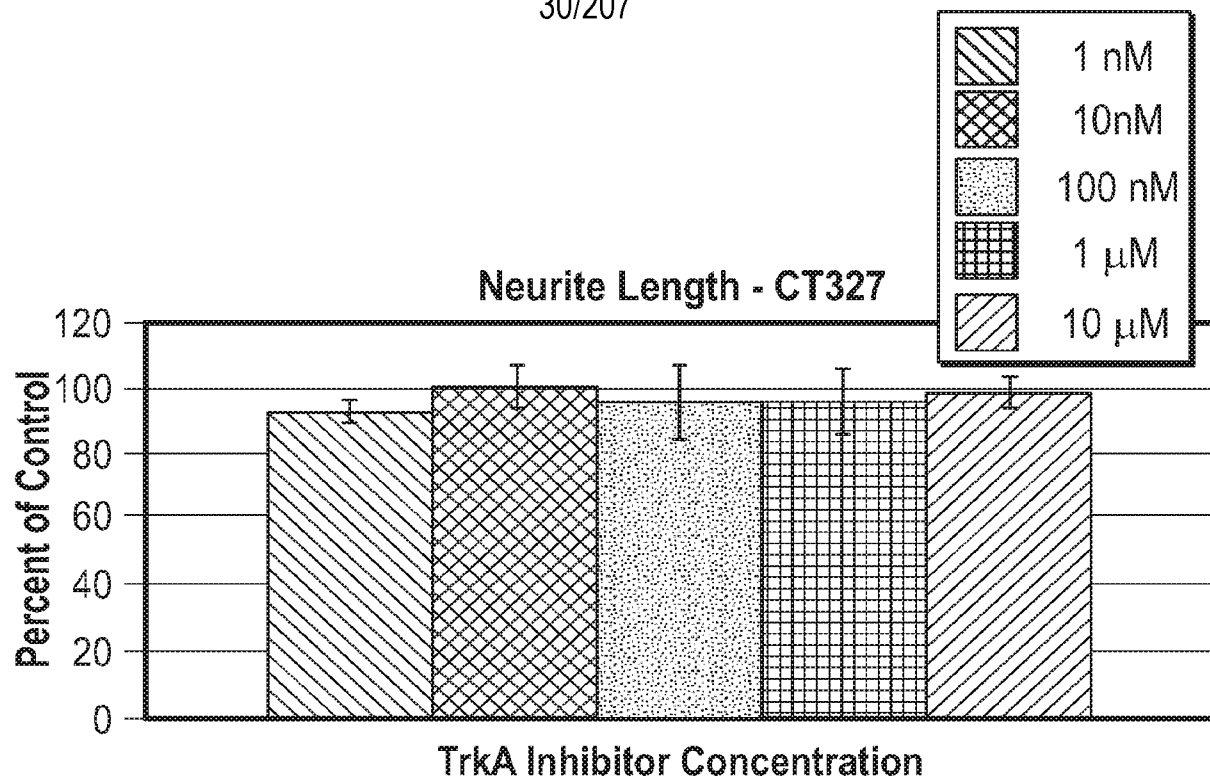


FIG. 45

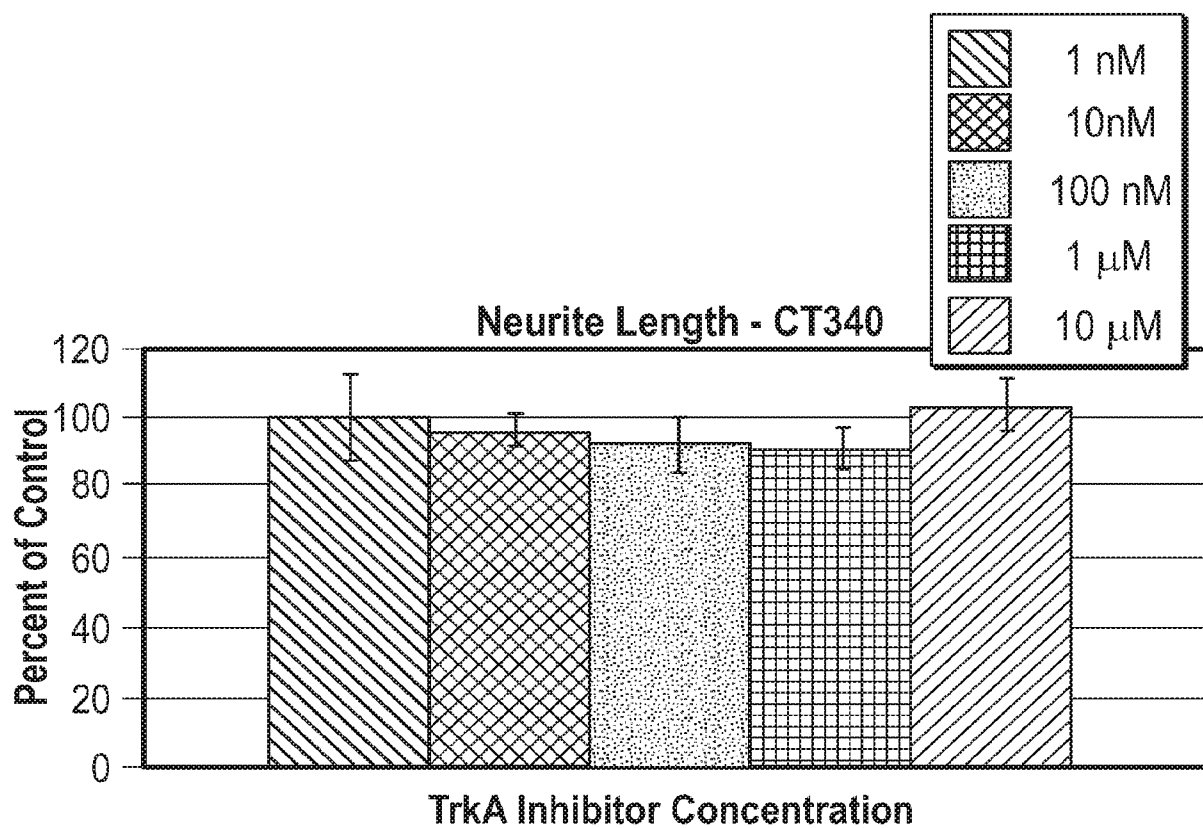


FIG. 46

31/207

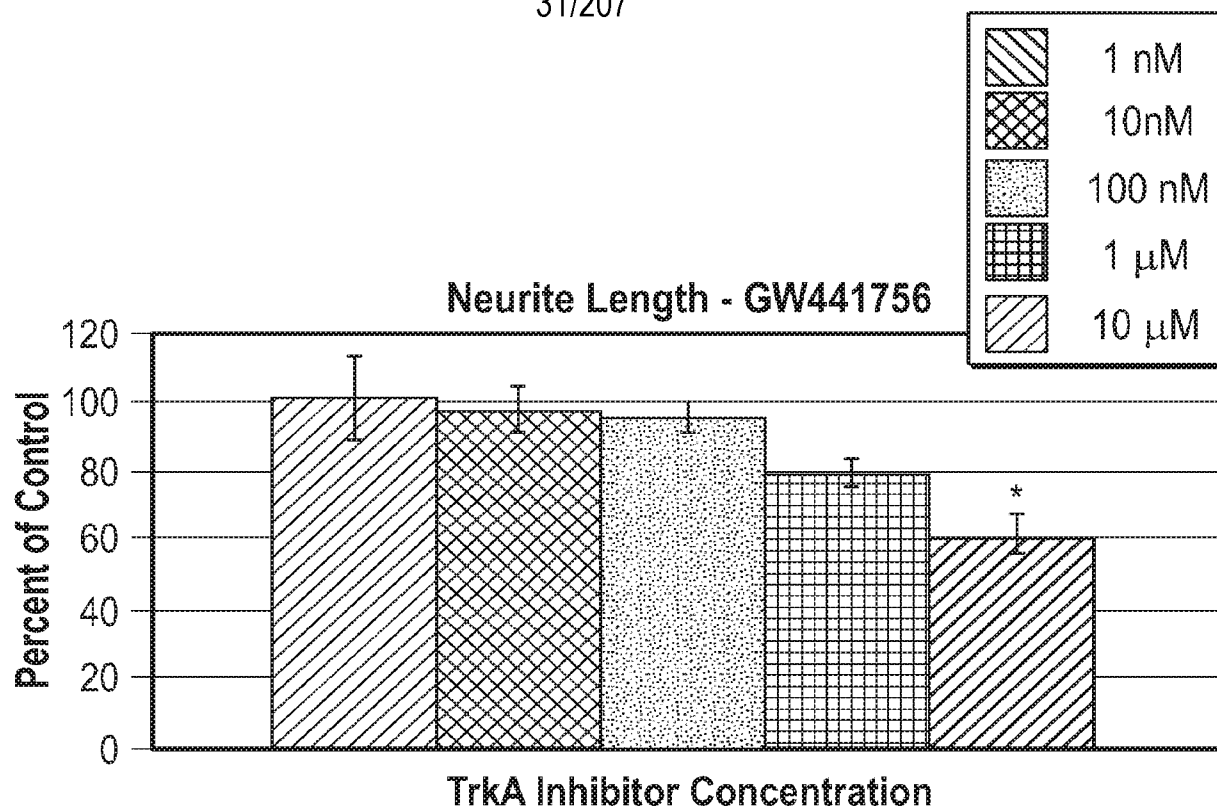


FIG. 47

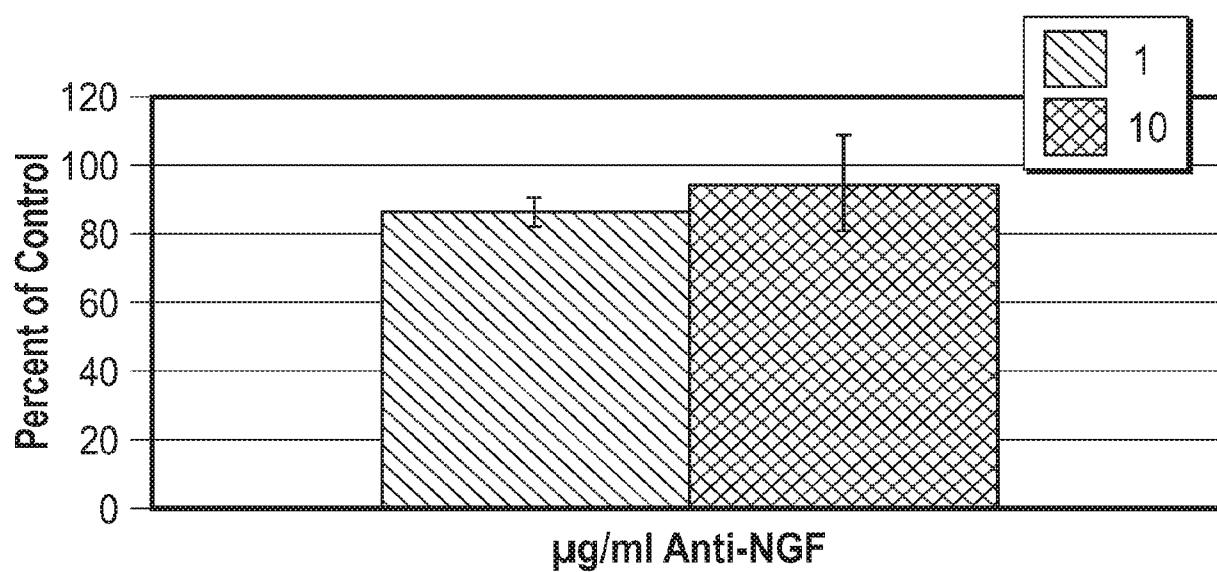


FIG. 48

32/207

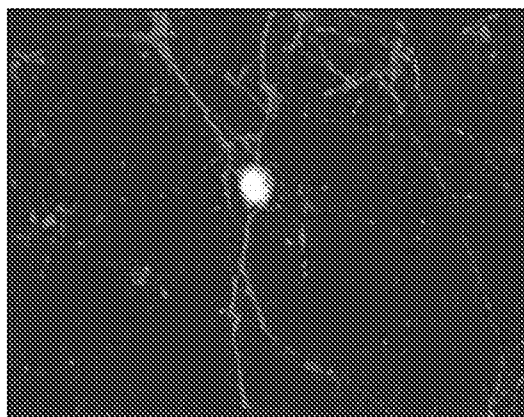


FIG. 49A

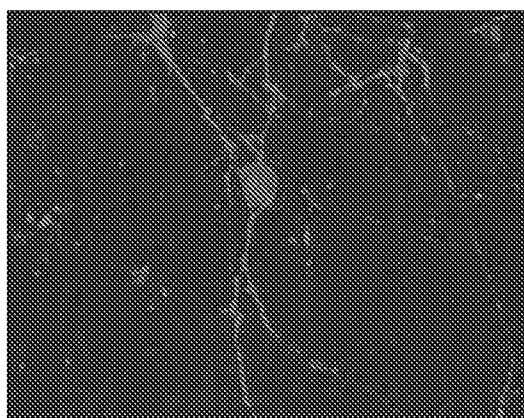


FIG. 49B

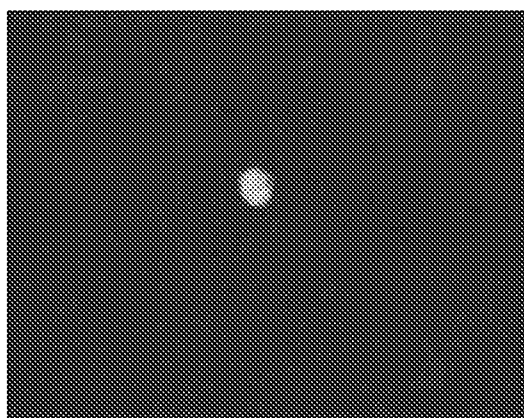


FIG. 49C

33/207

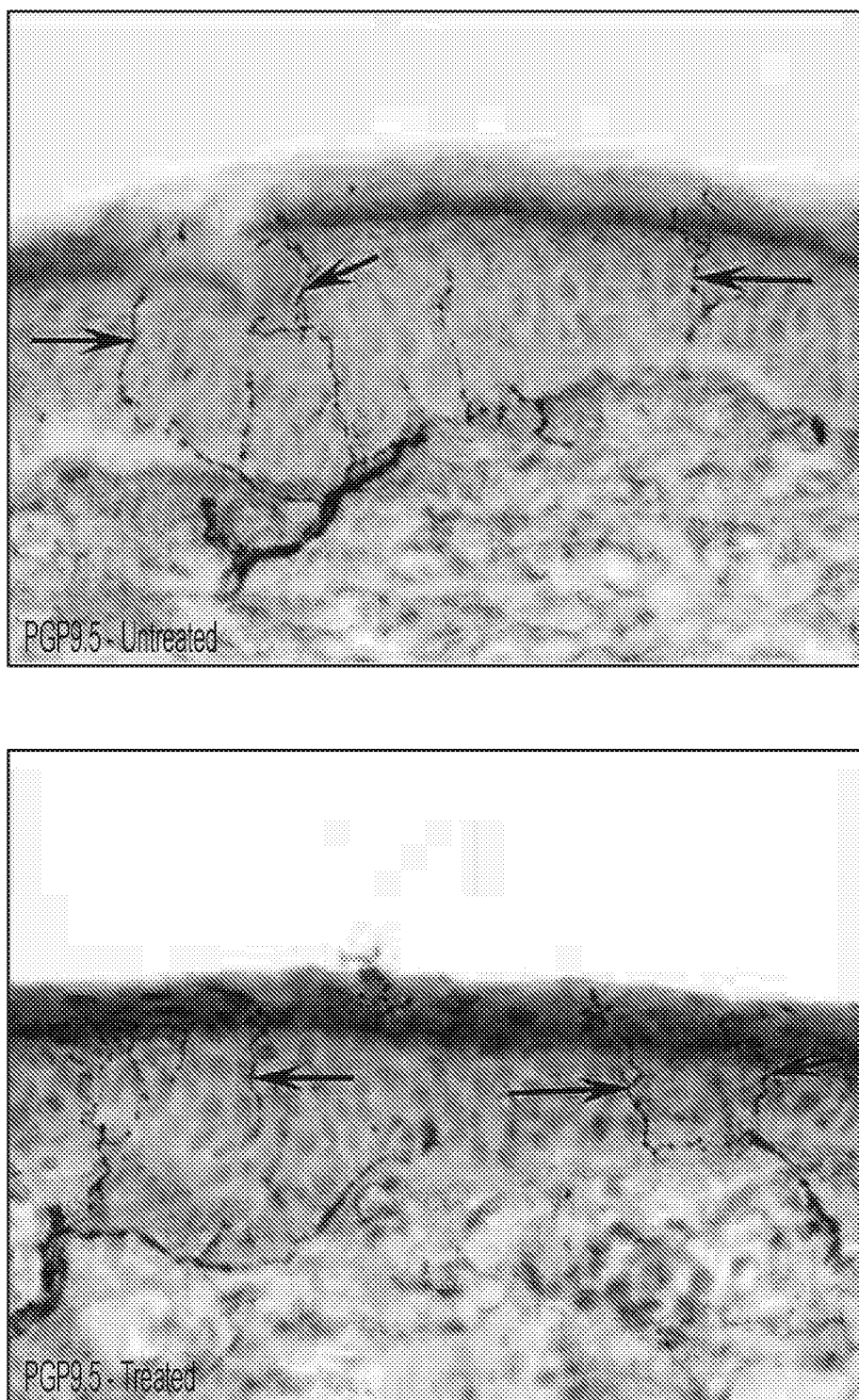


FIG. 50

34/207

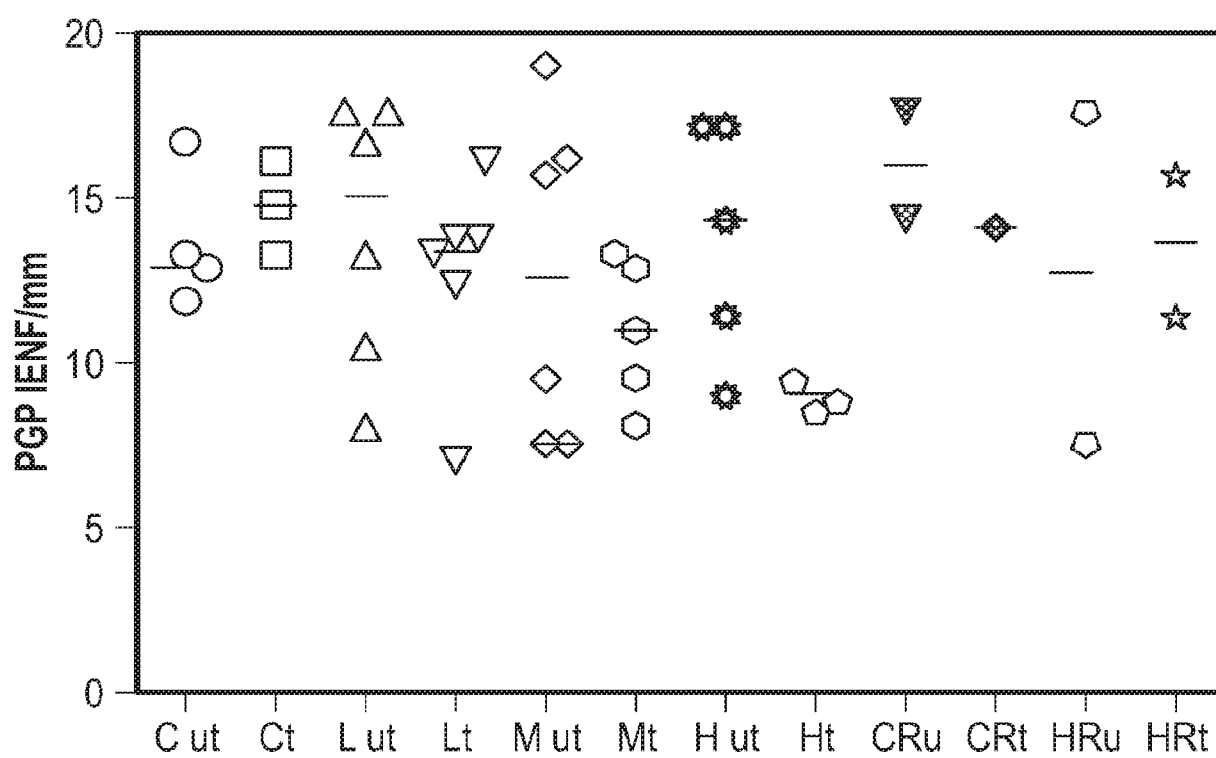


FIG. 51

35/207

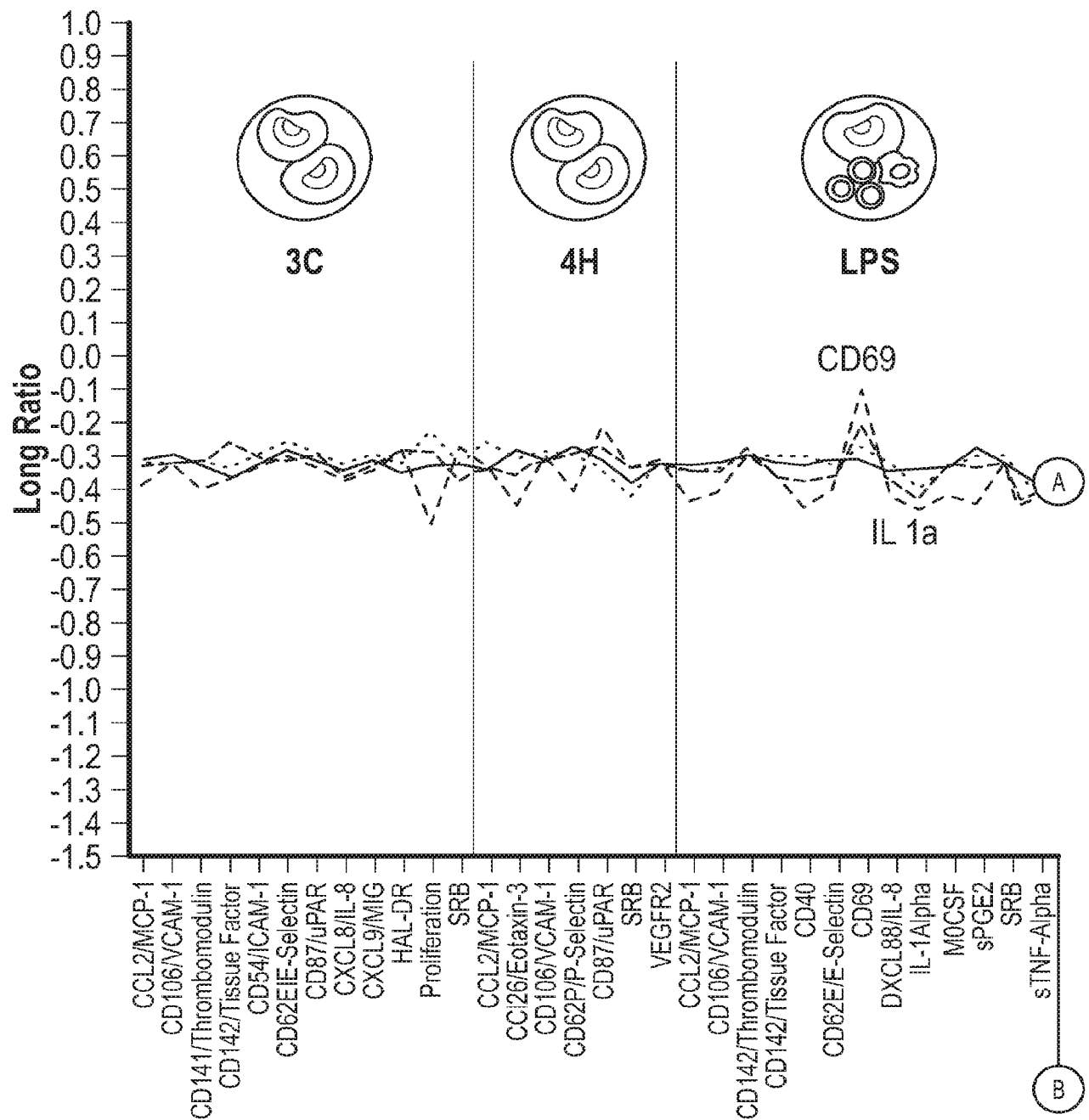


FIG. 52

36/207

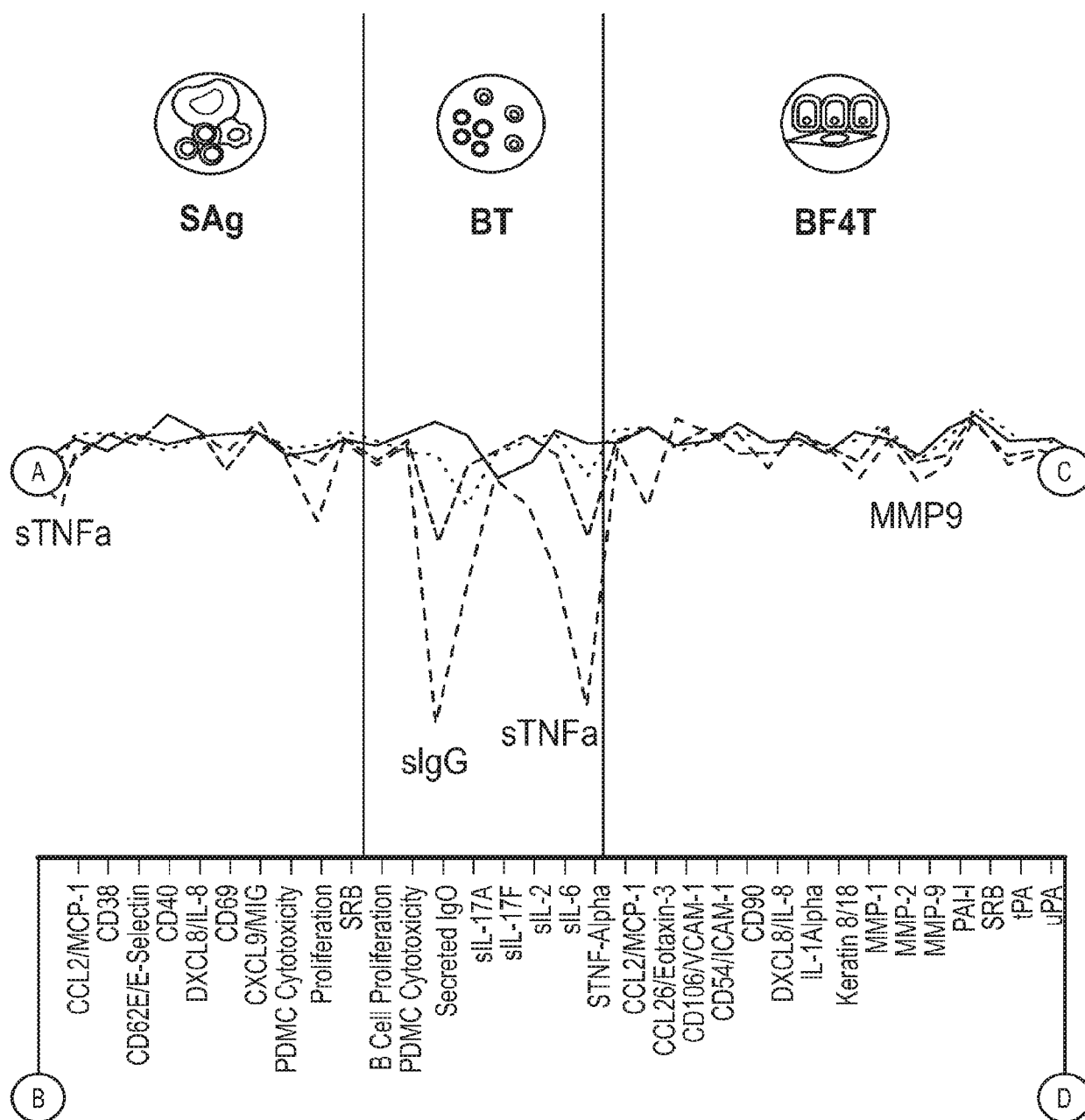


FIG. 52
(Continued)

37/207

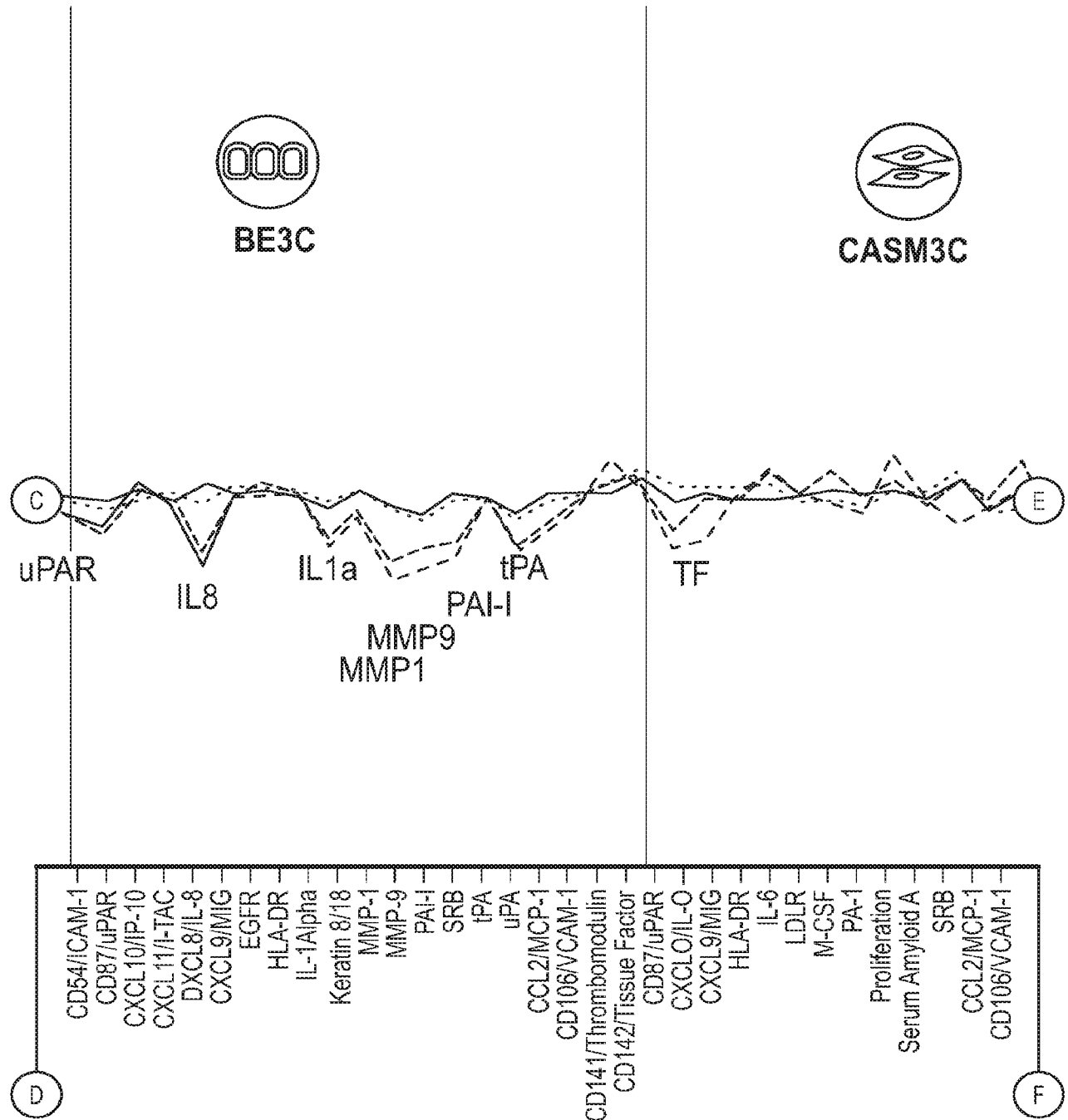


FIG. 52
(Continued)

38/207

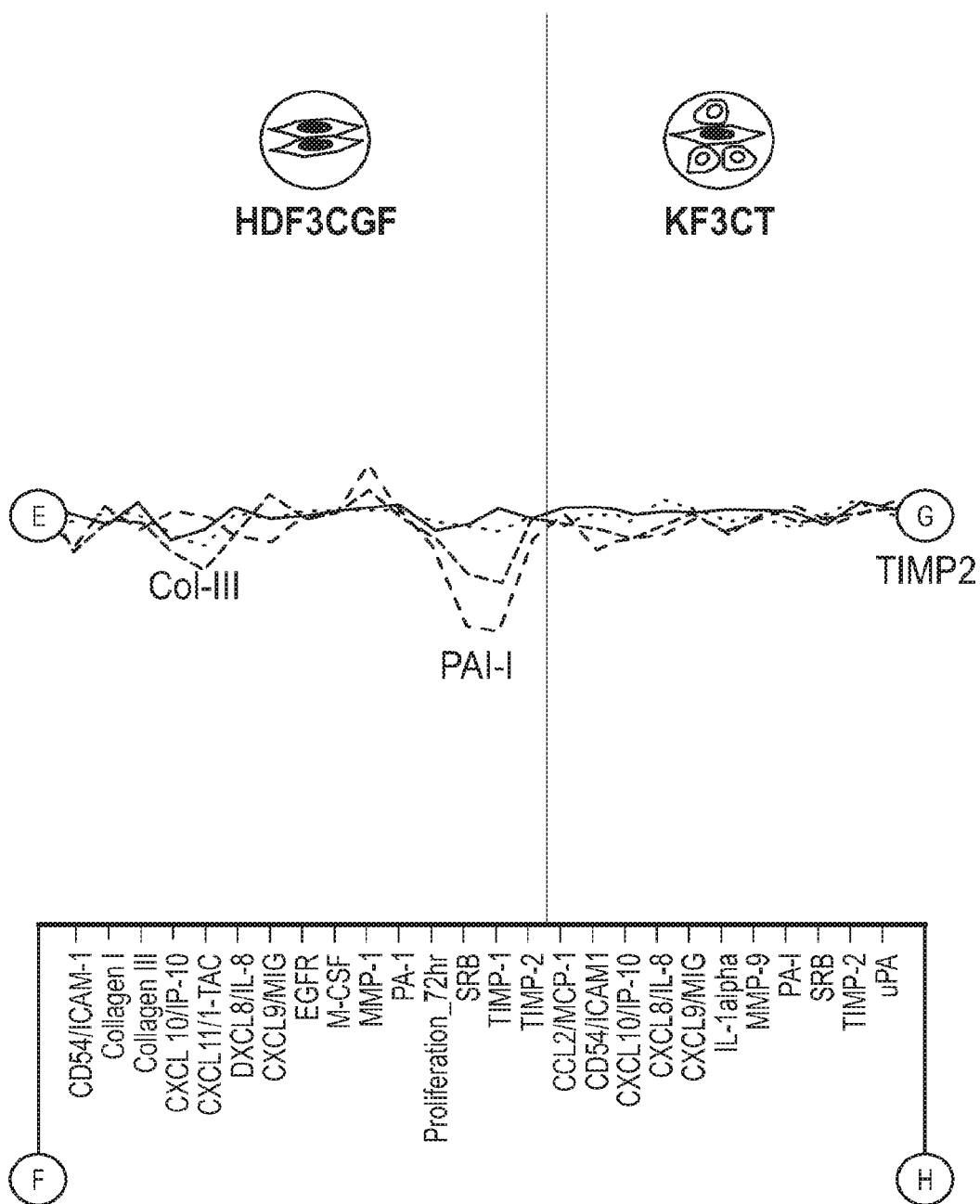


FIG. 52
(Continued)

39/207

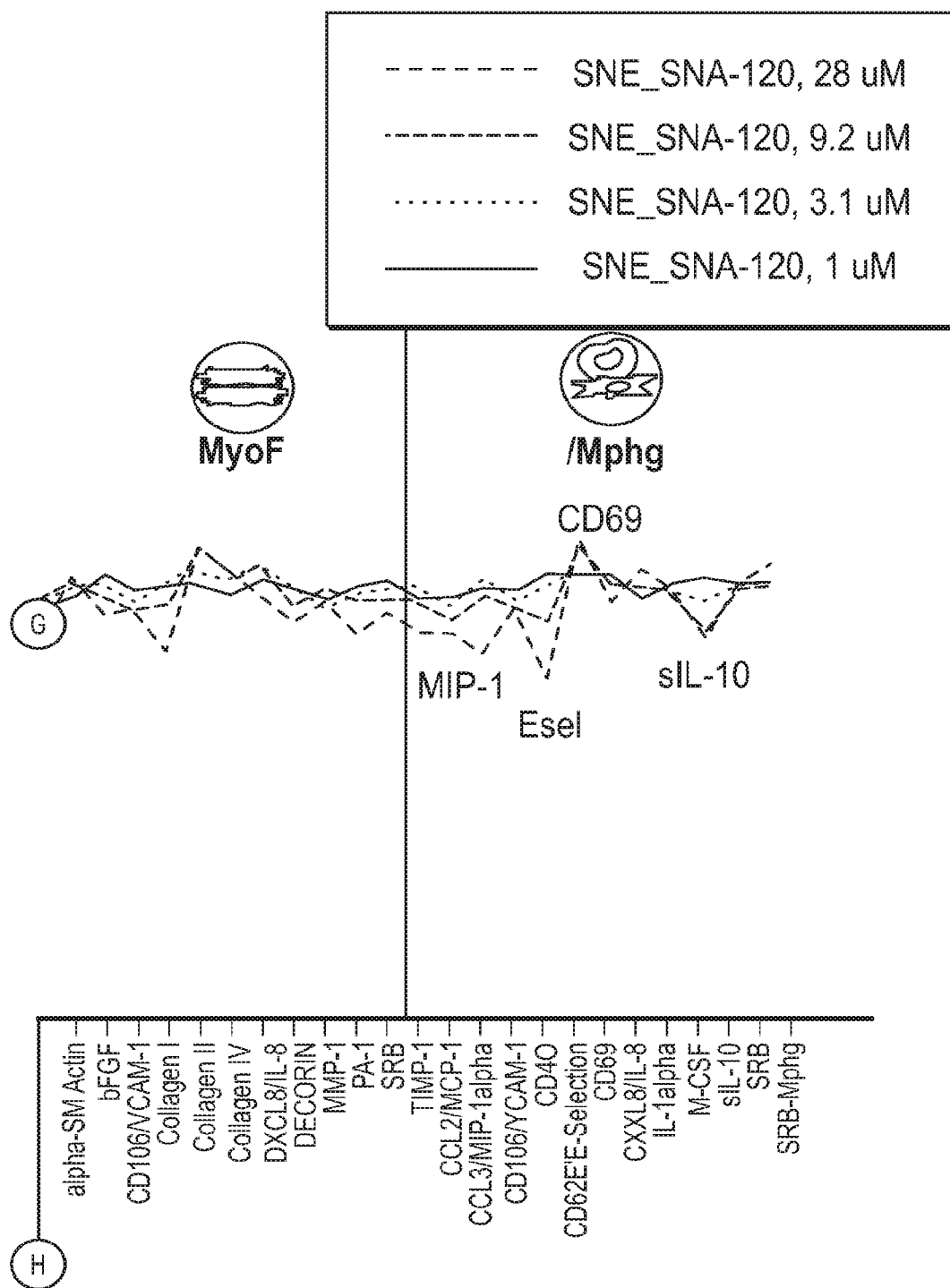


FIG. 52
(Continued)

40/207

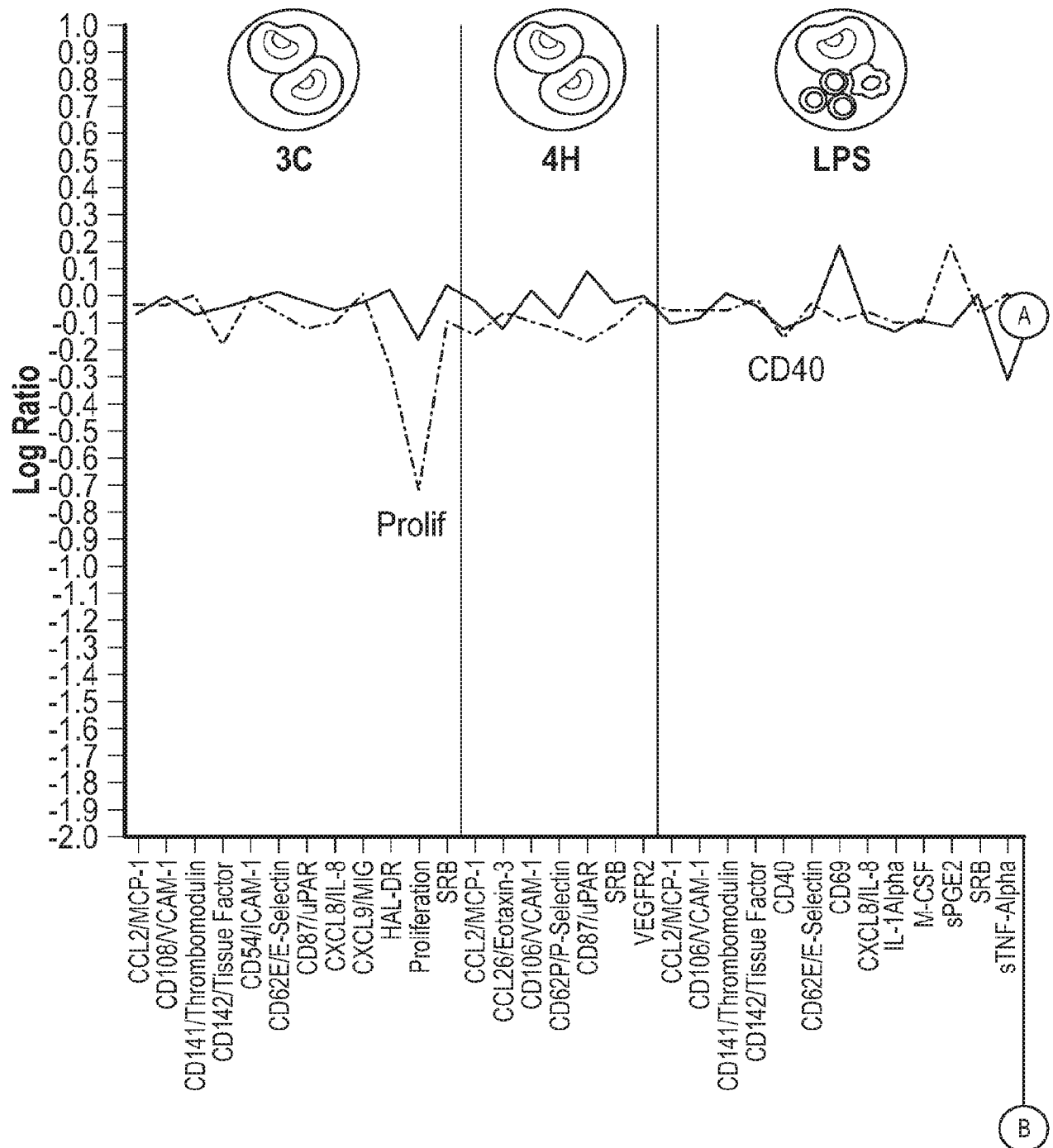


FIG. 53

41/207

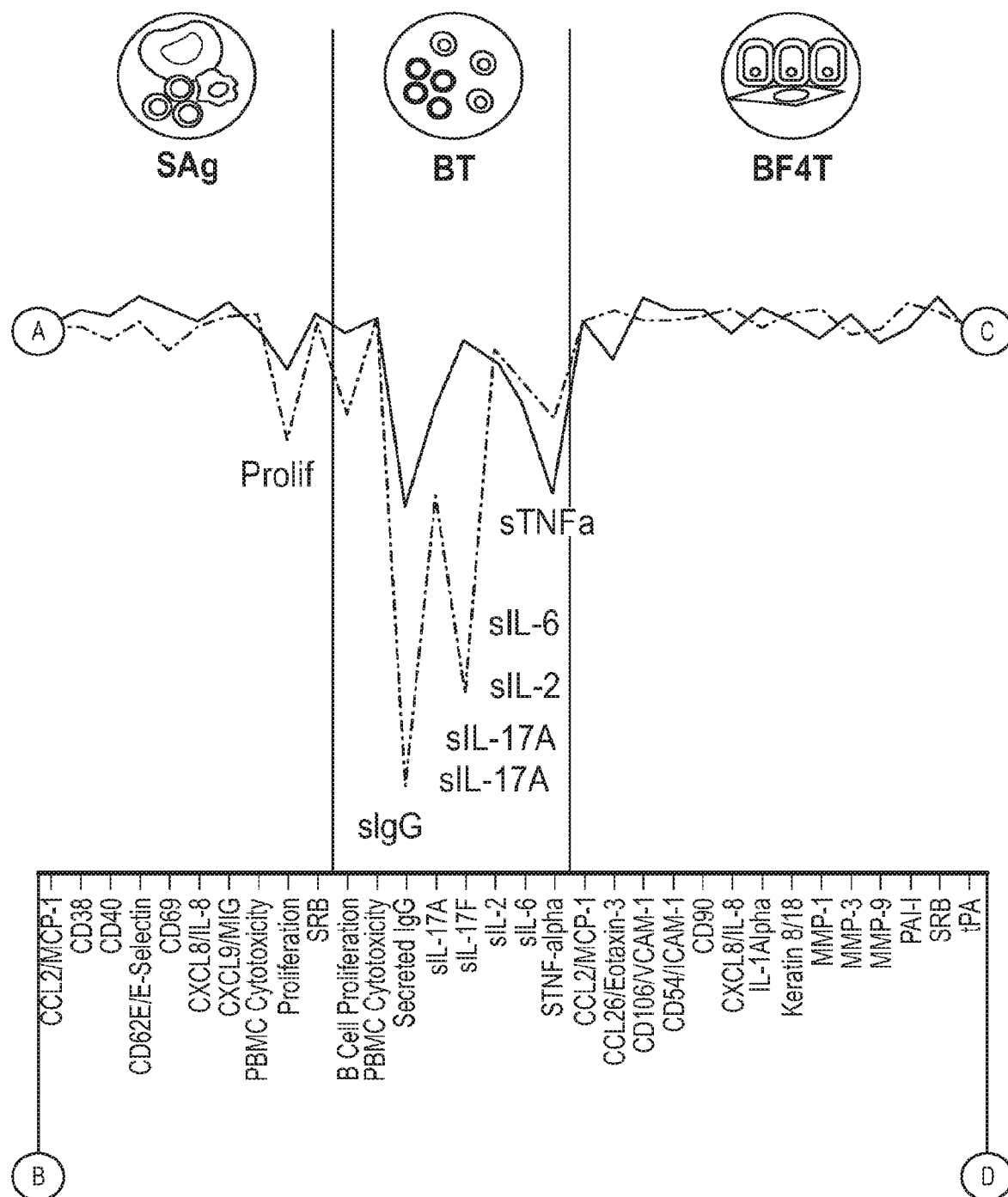


FIG. 53
(Continued)

42/207

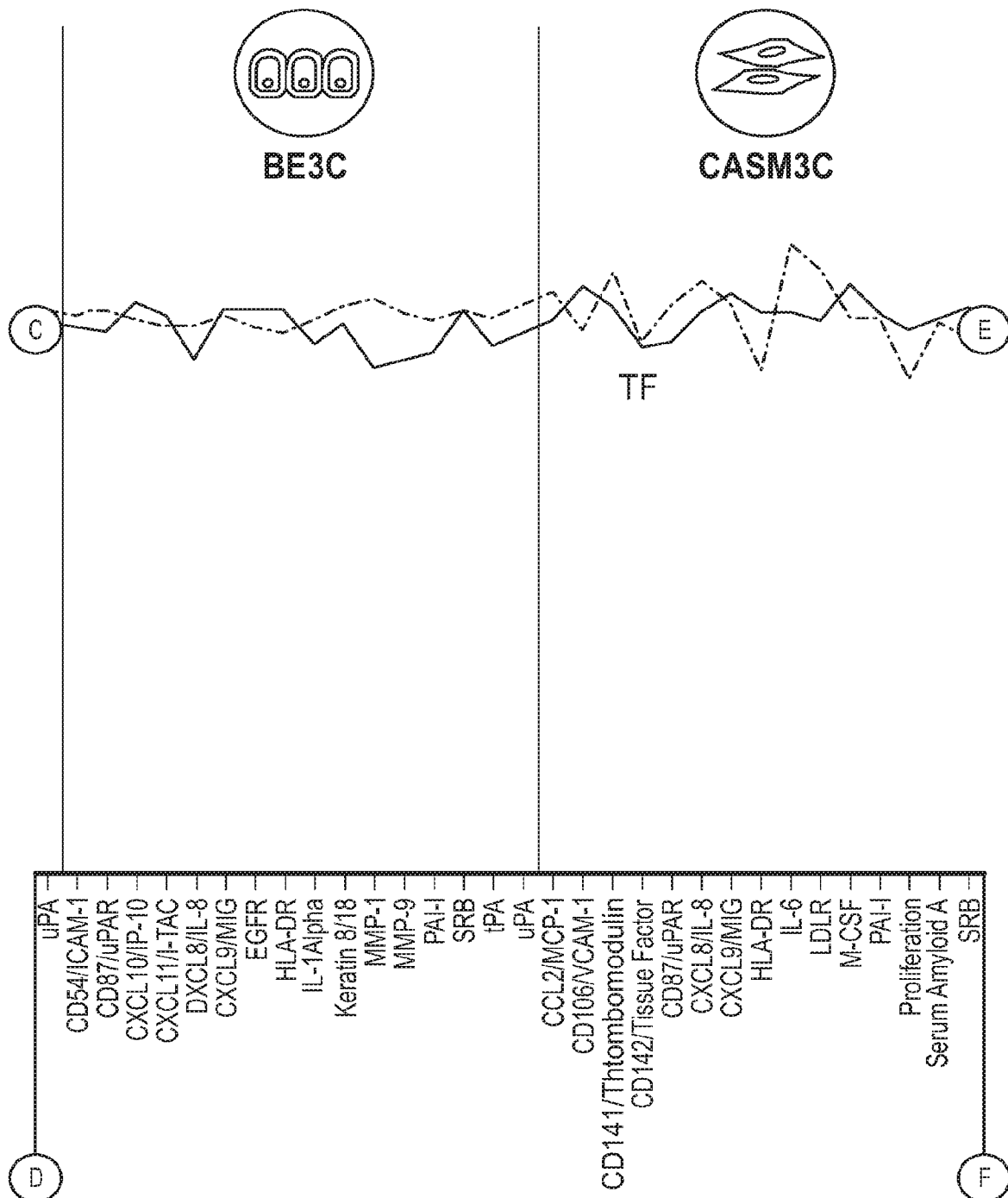


FIG. 53
(Continued)

43/207

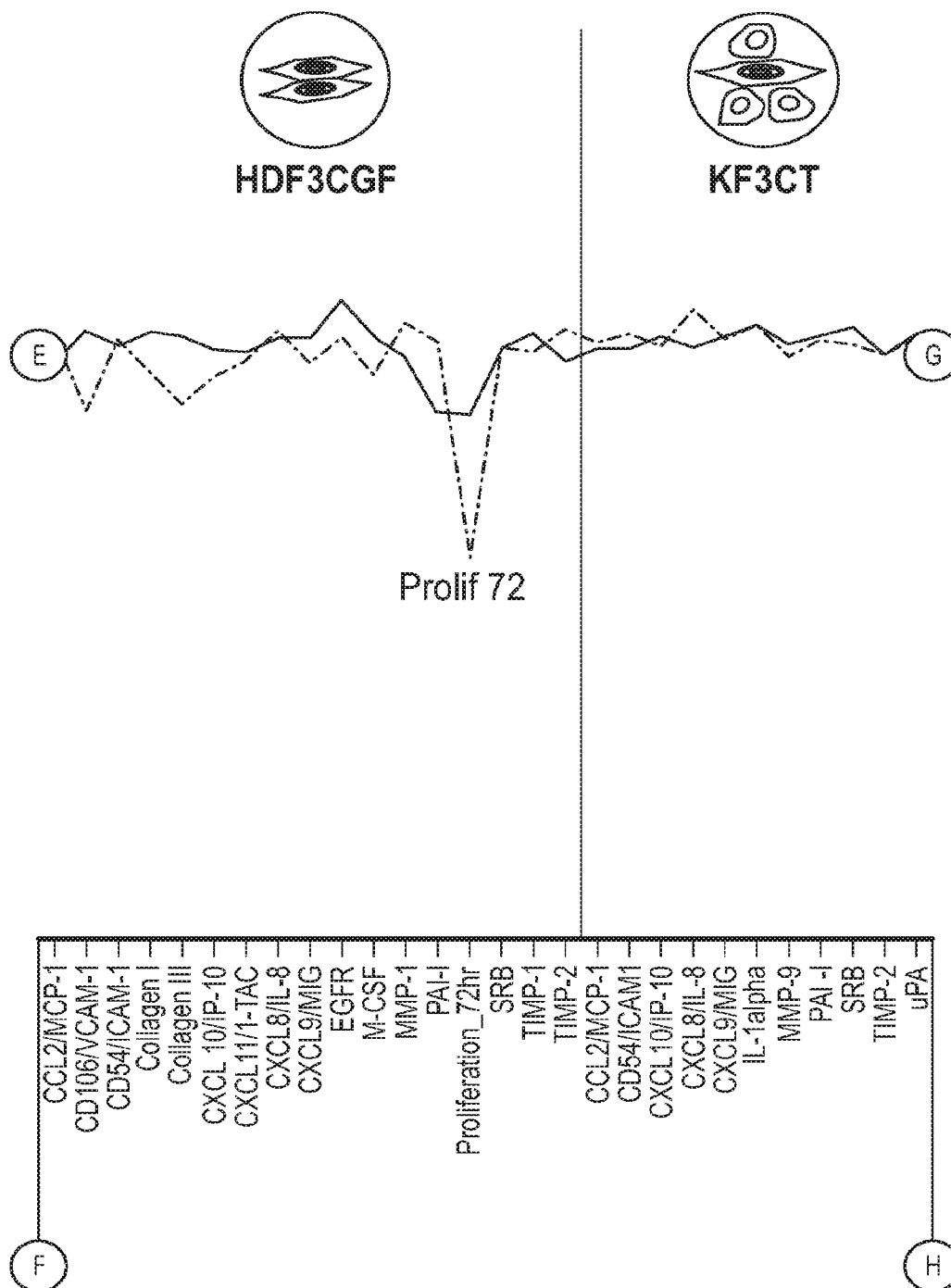


FIG. 53
(Continued)

44/207

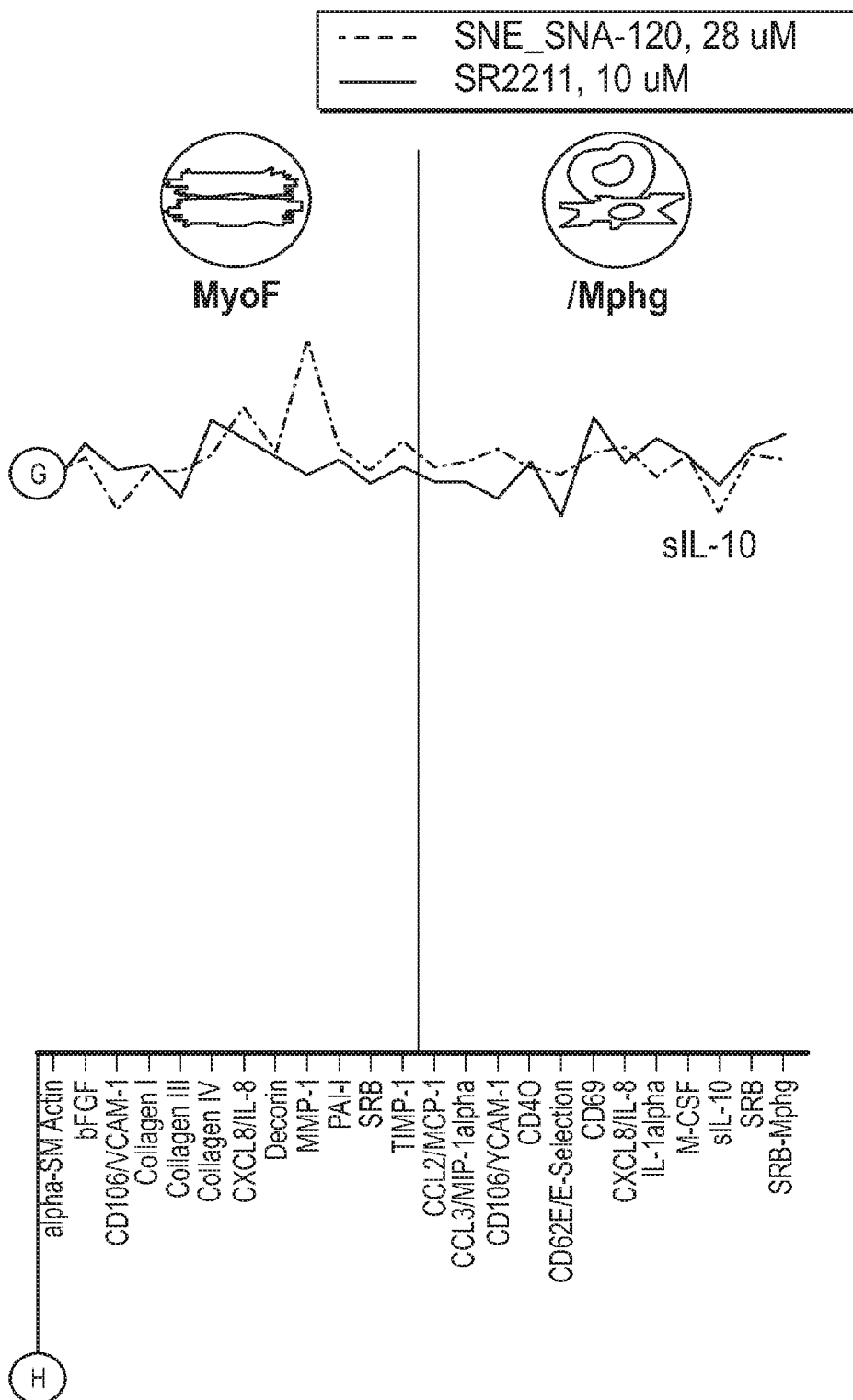


FIG. 53
(Continued)

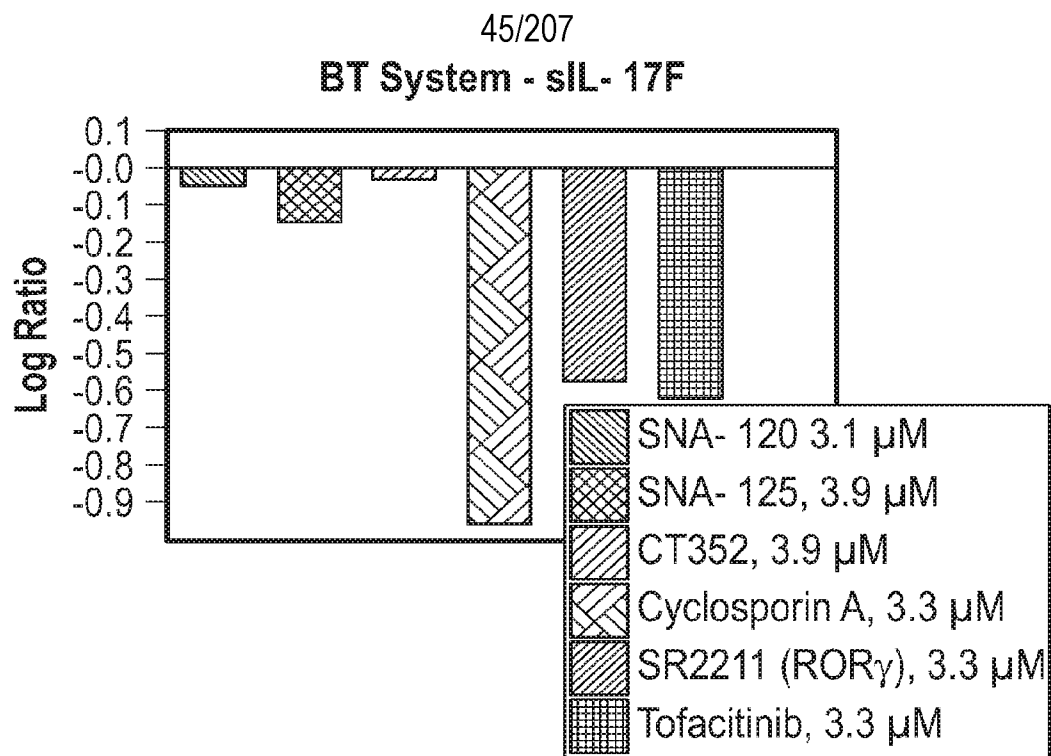


FIG. 54A

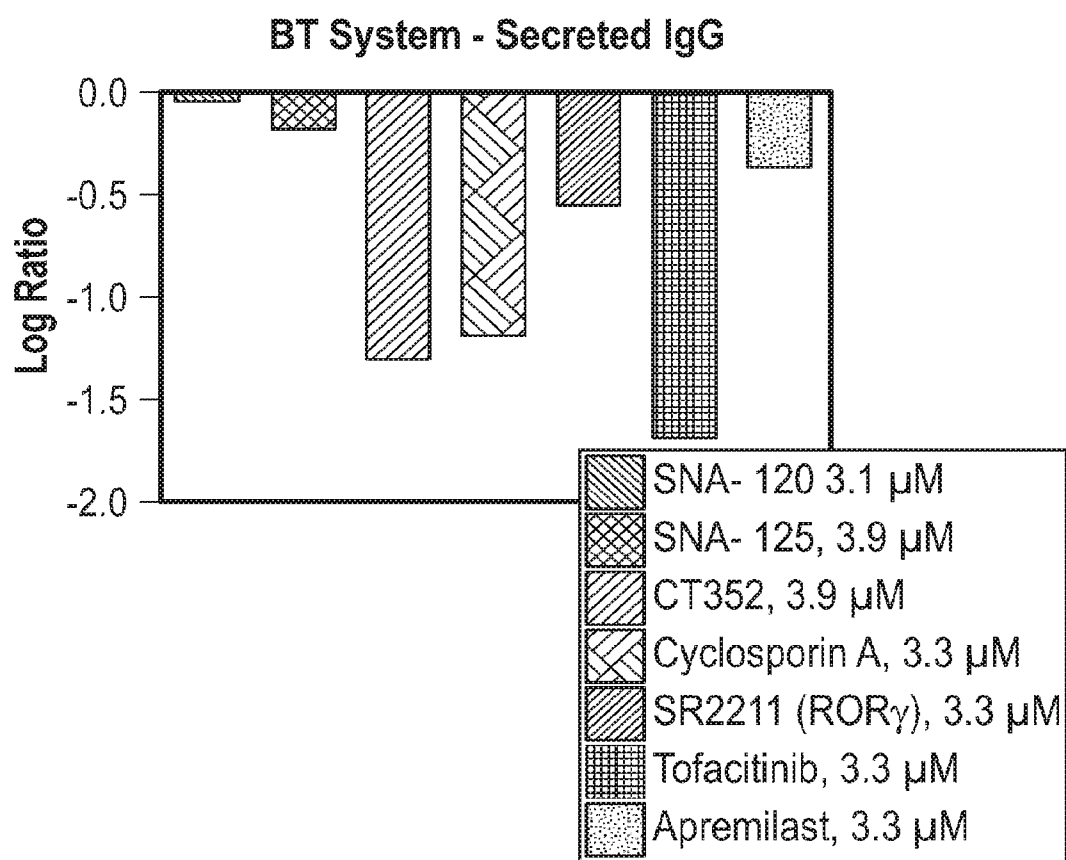
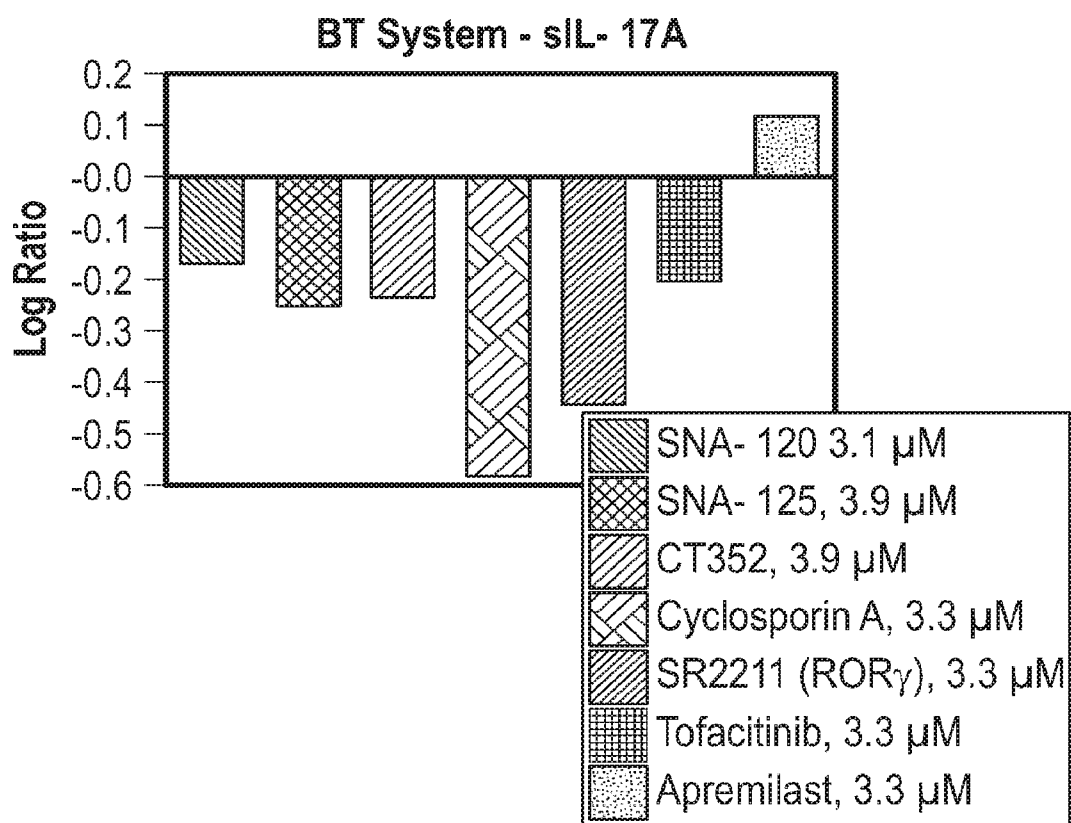
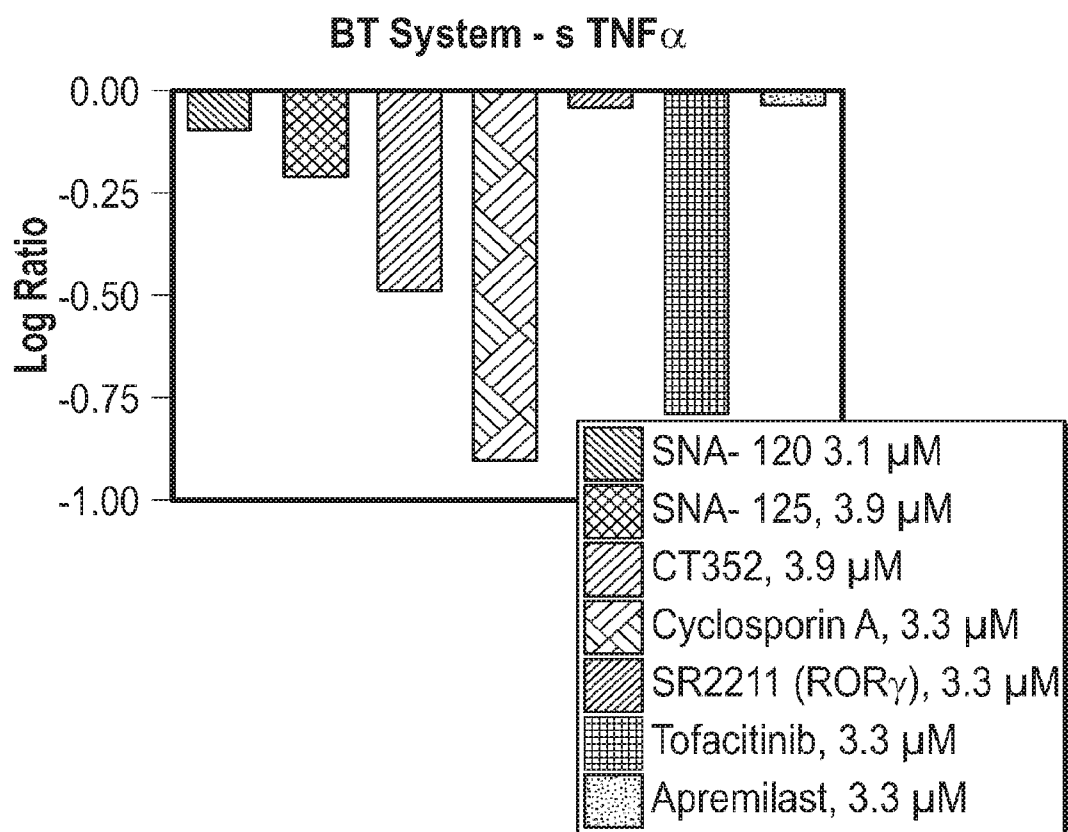


FIG. 54B

46/207



47/207

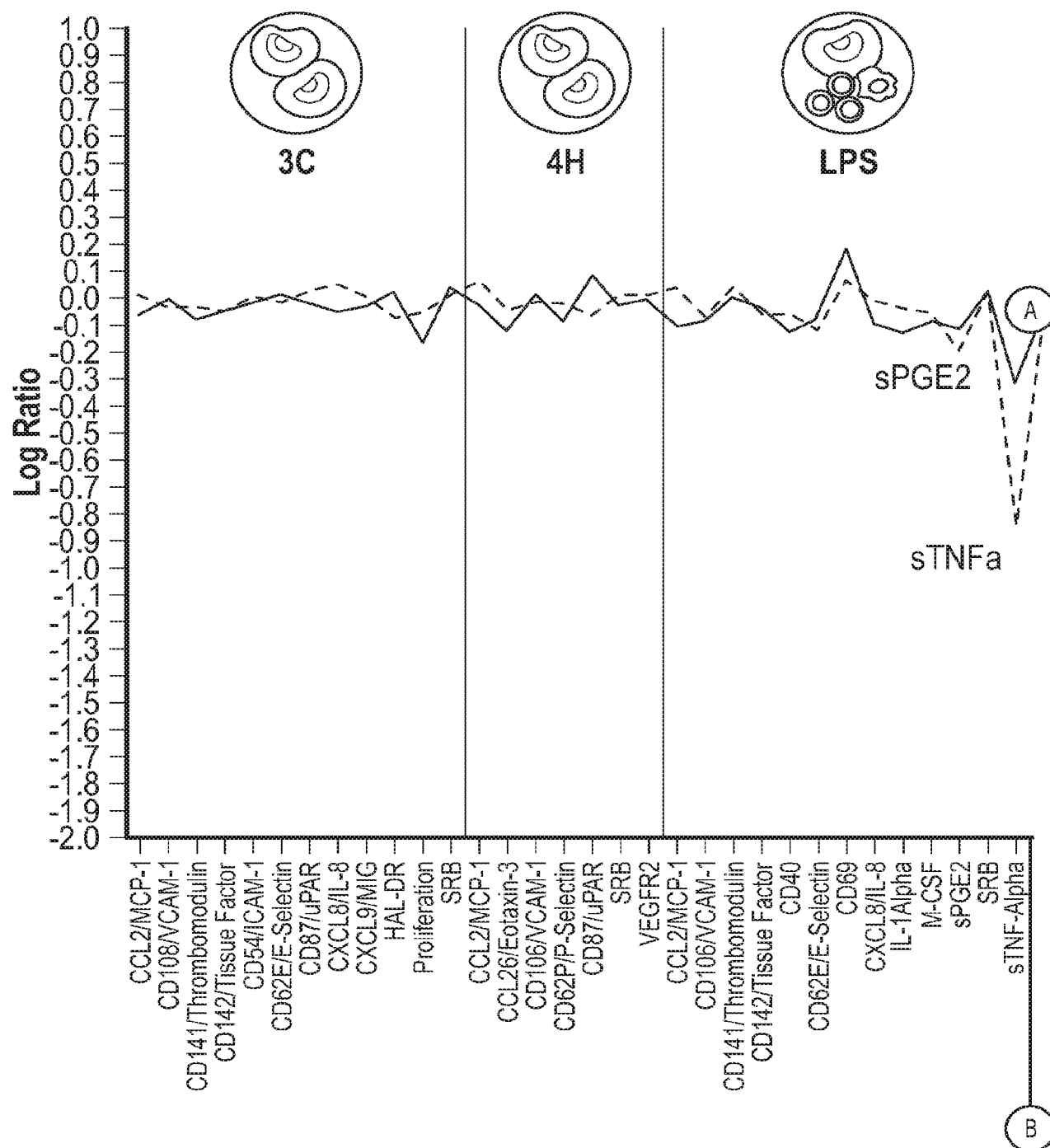


FIG. 55

48/207

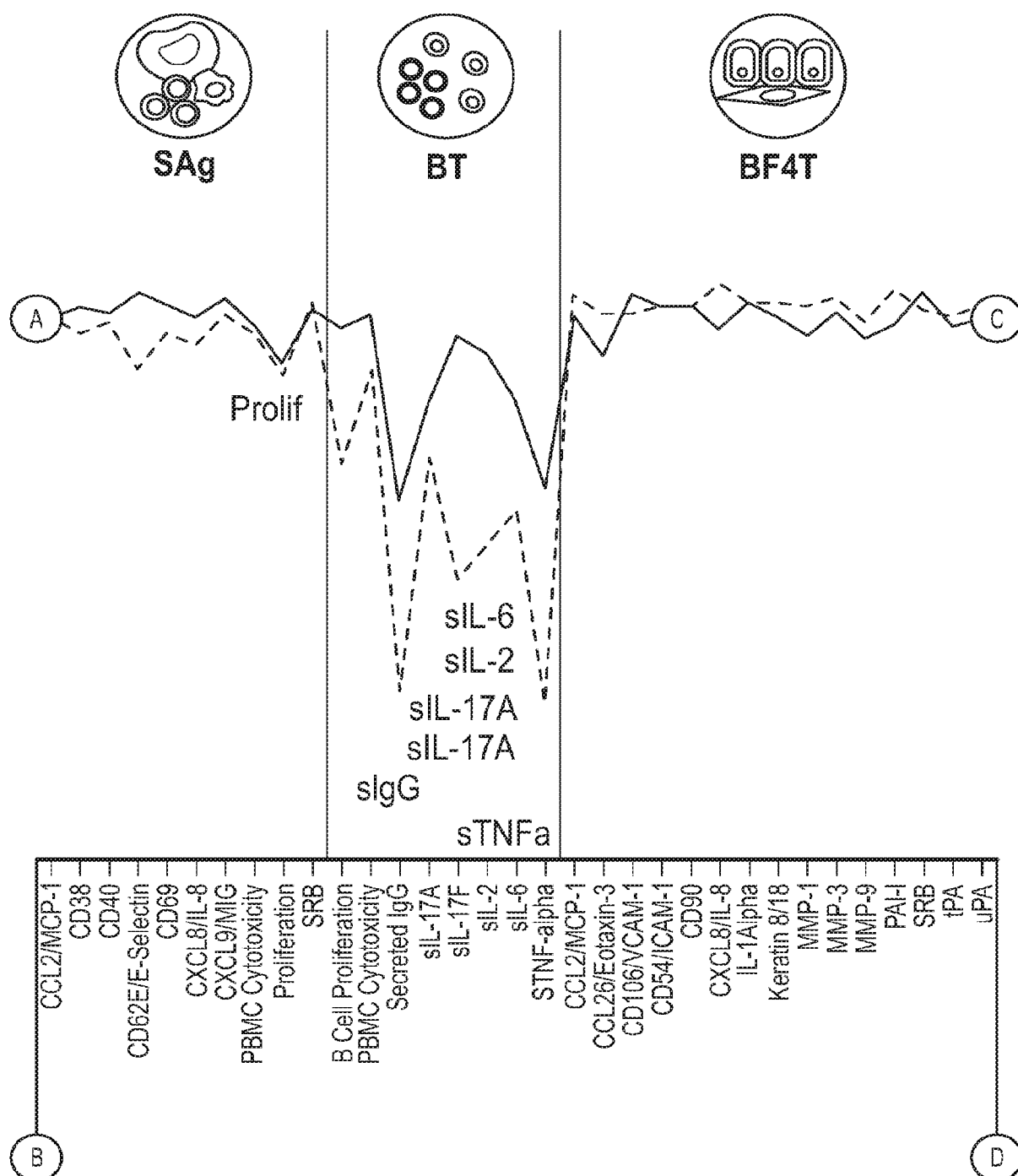


FIG. 55
(Continued)

49/207

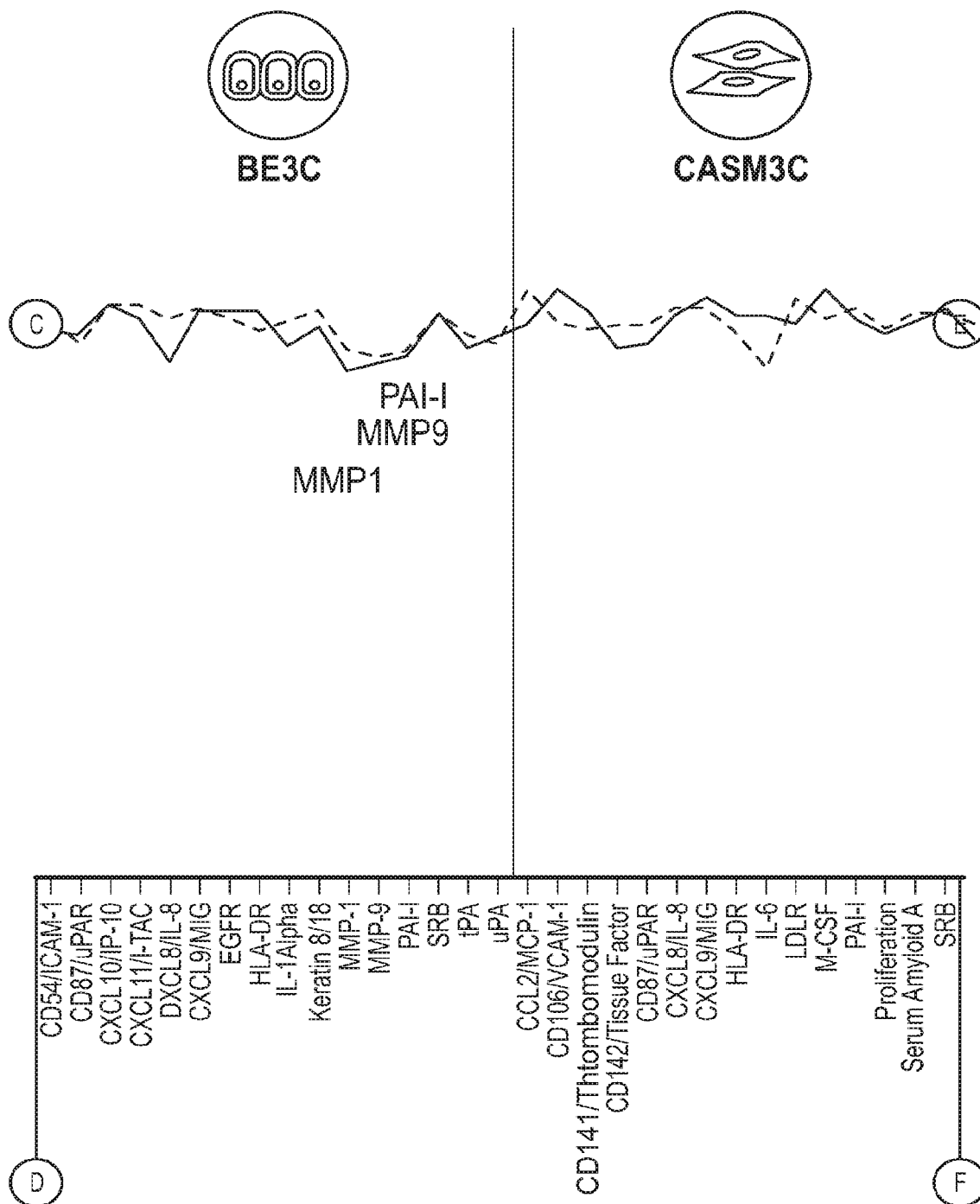


FIG. 55
(Continued)

50/207

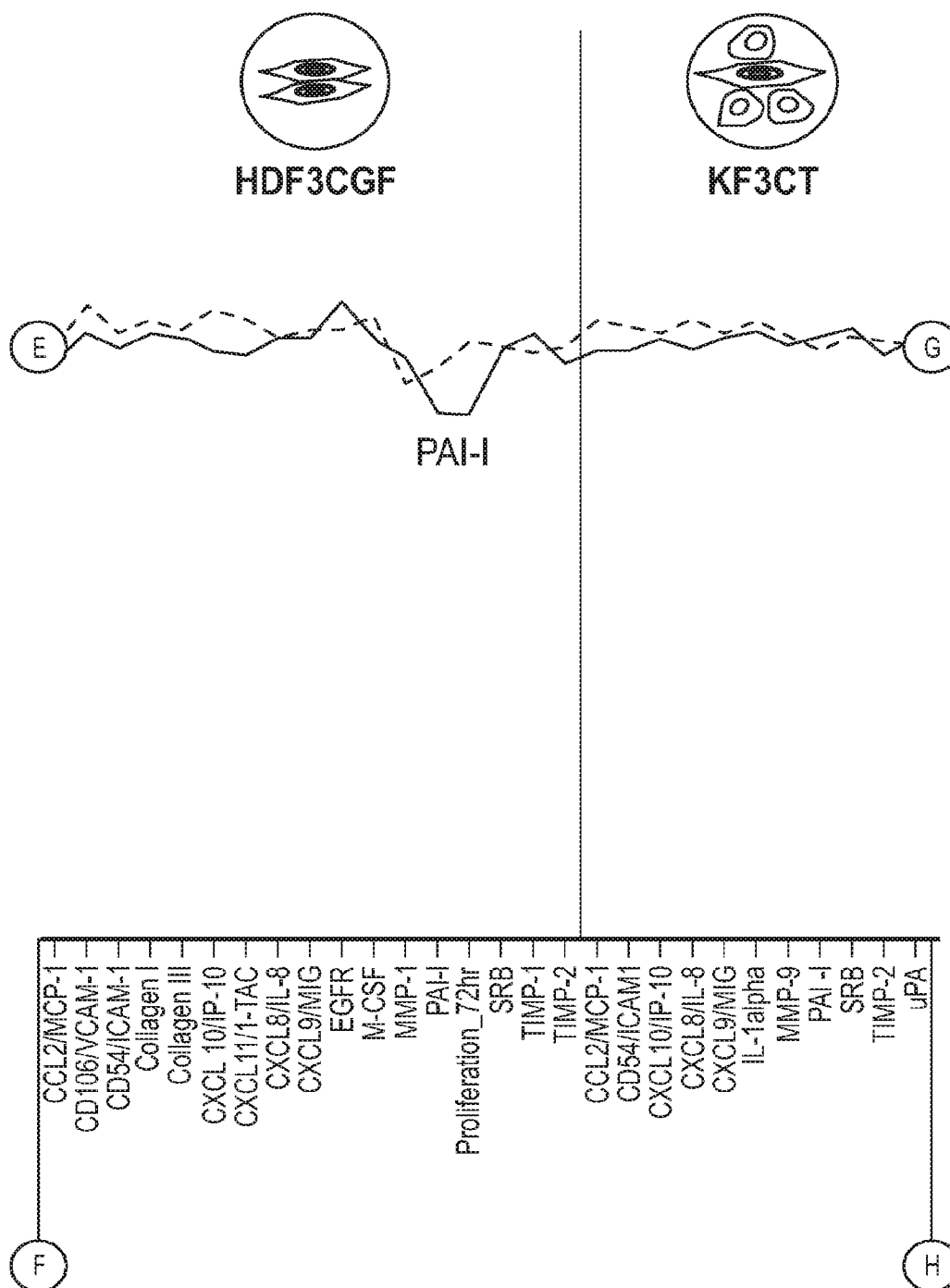


FIG. 55
(Continued)

51/207

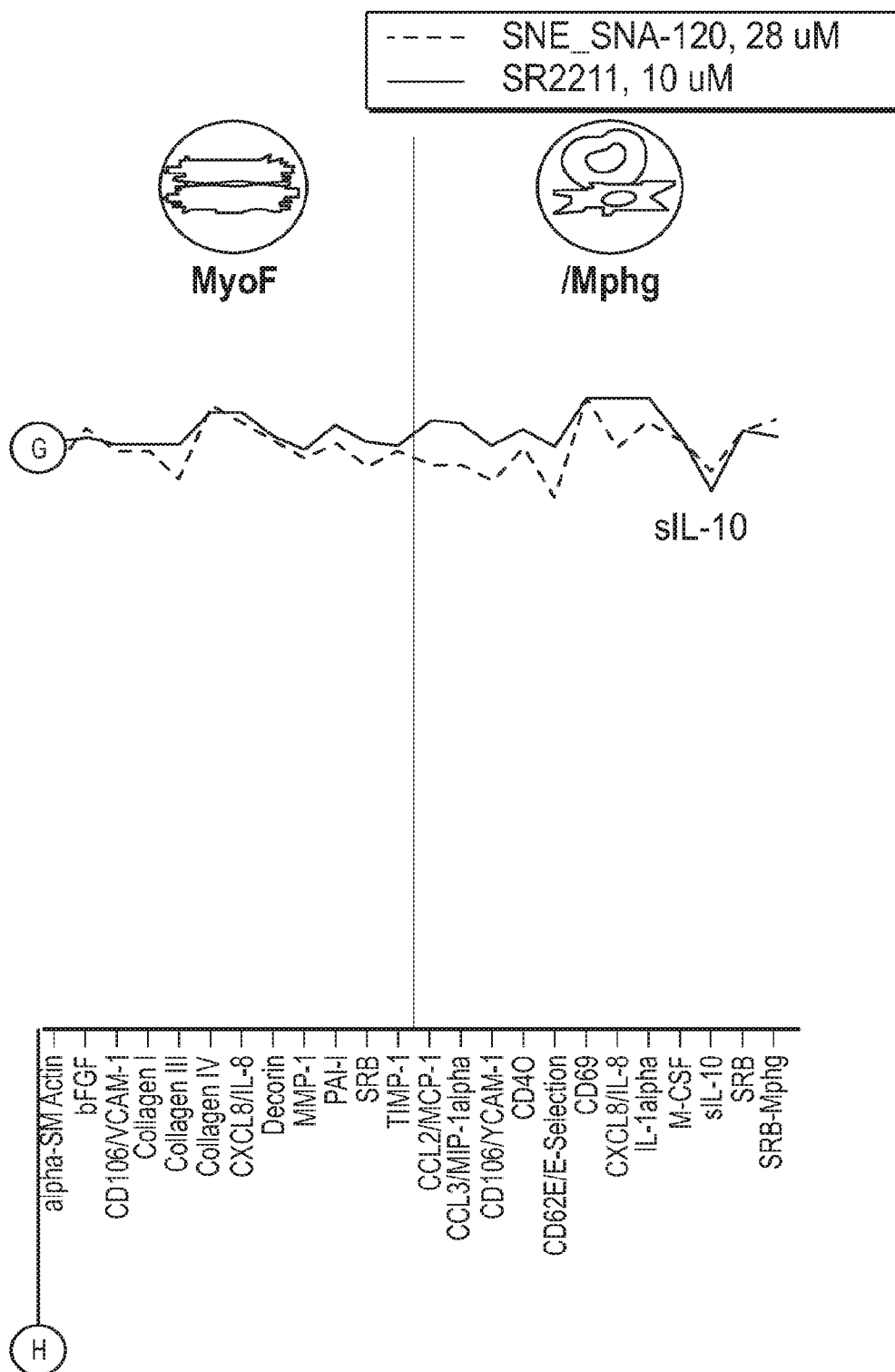


FIG. 55
(Continued)

52/207

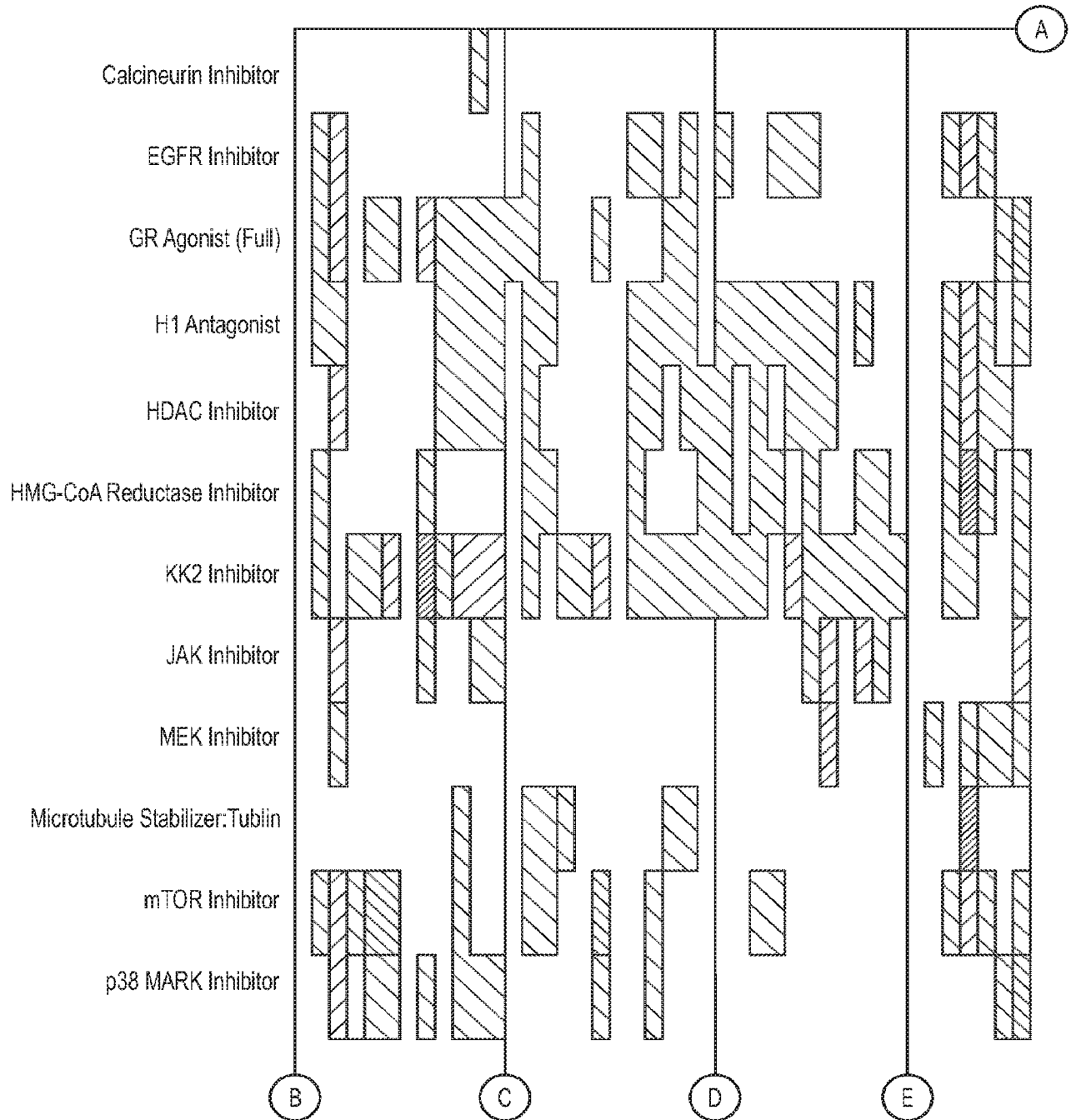


FIG. 56

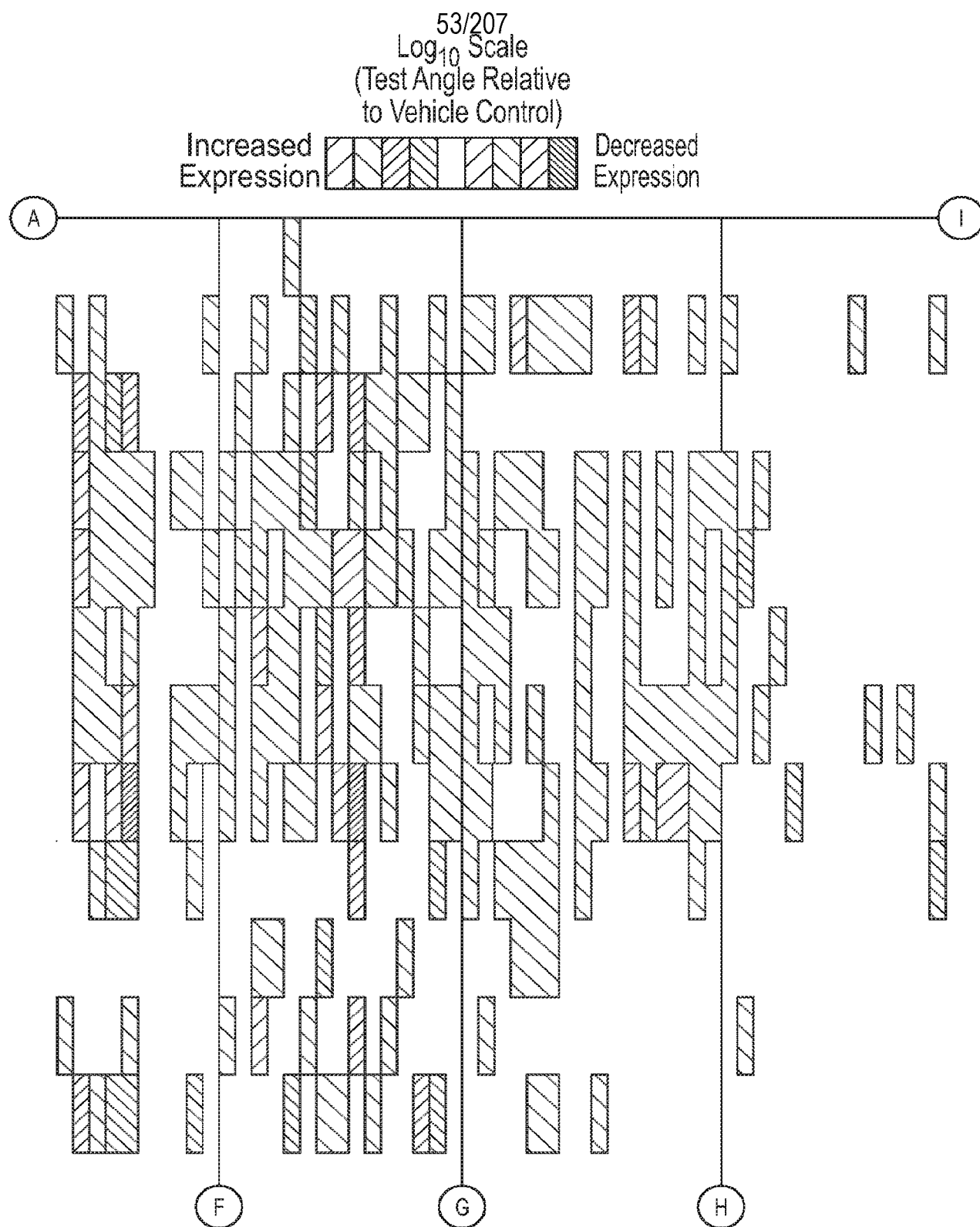


FIG. 56
(continued)

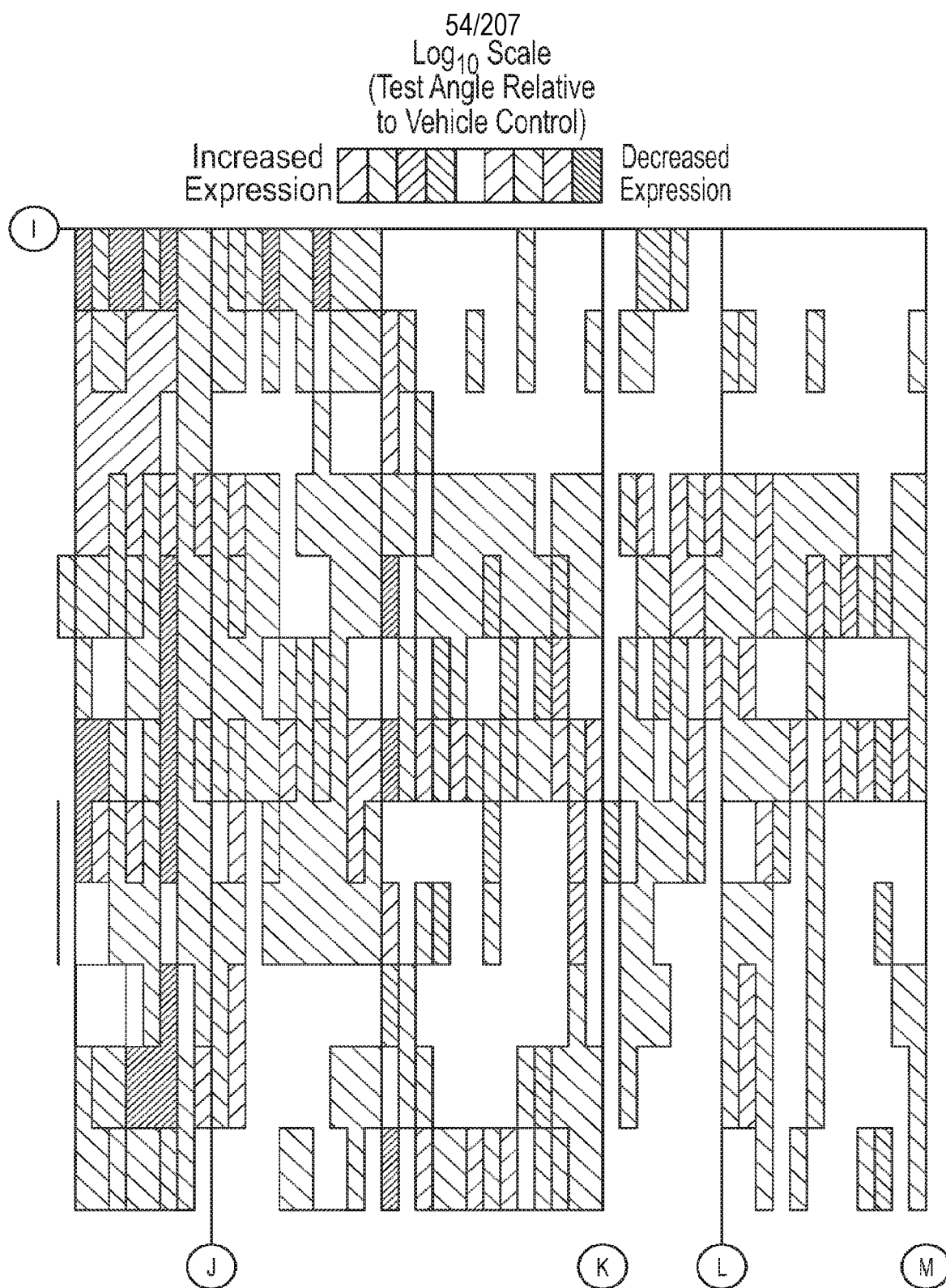


FIG. 56
(continued)

55/207

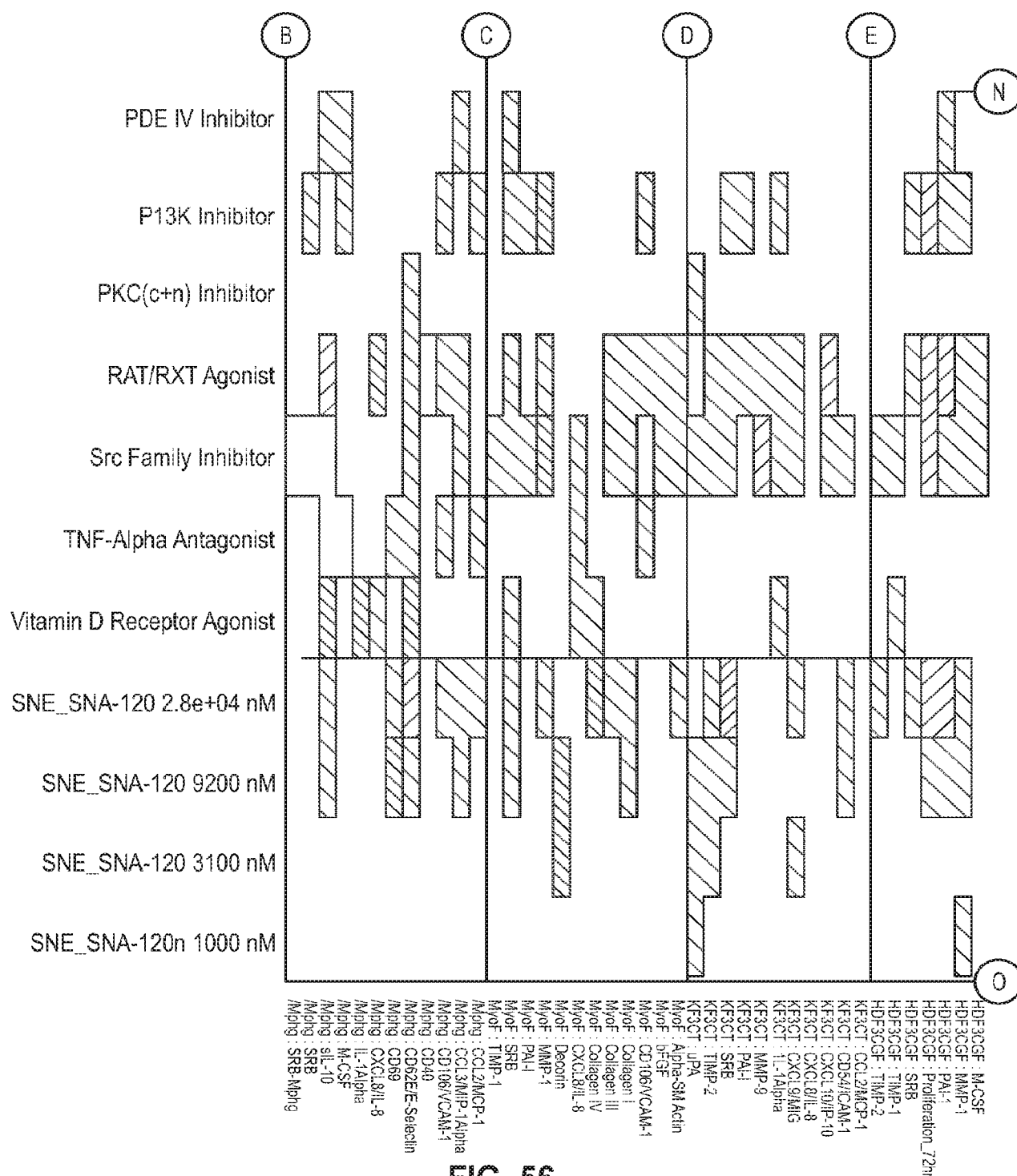


FIG. 56
(continued)

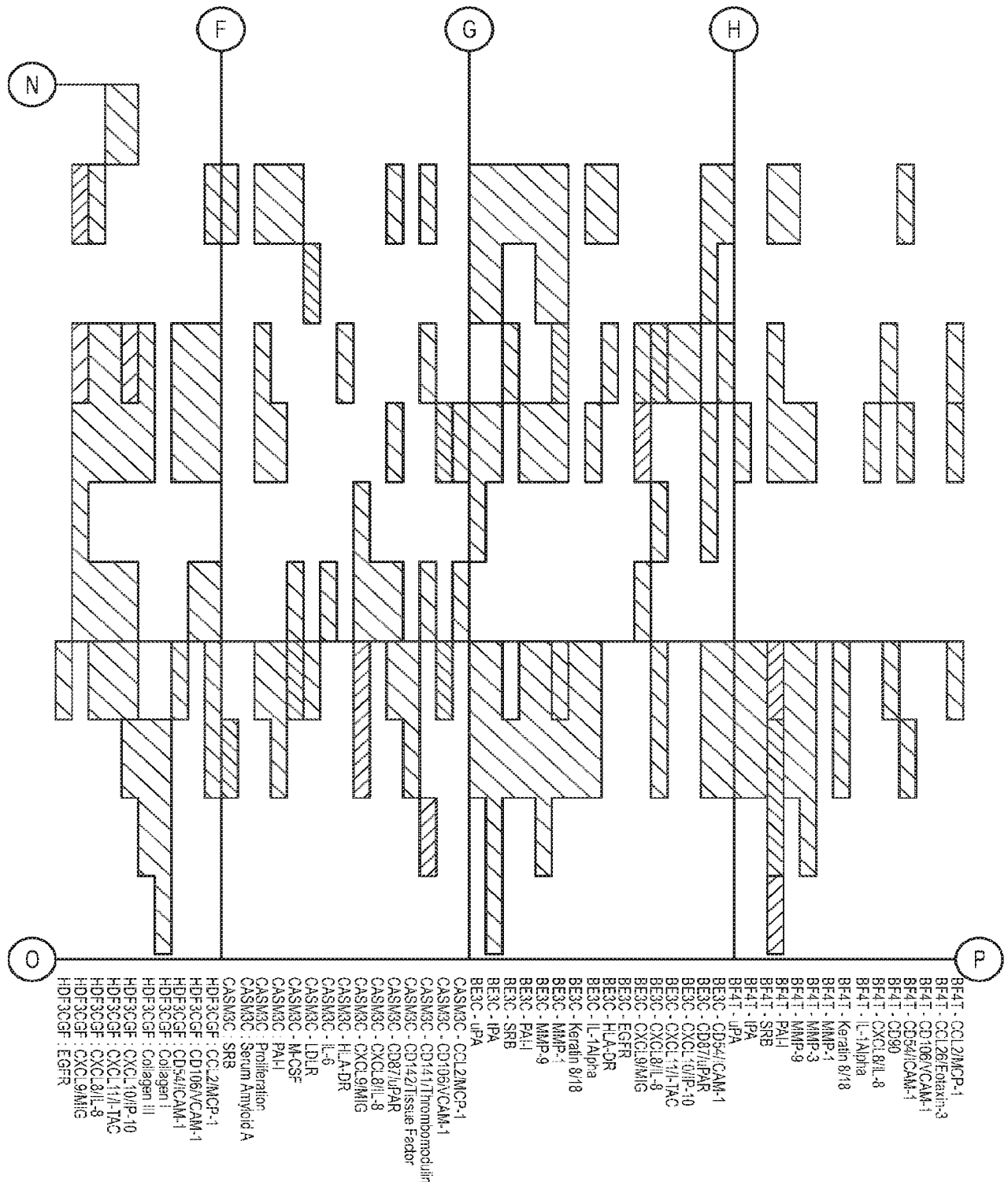


FIG. 56
(continued)

57/207

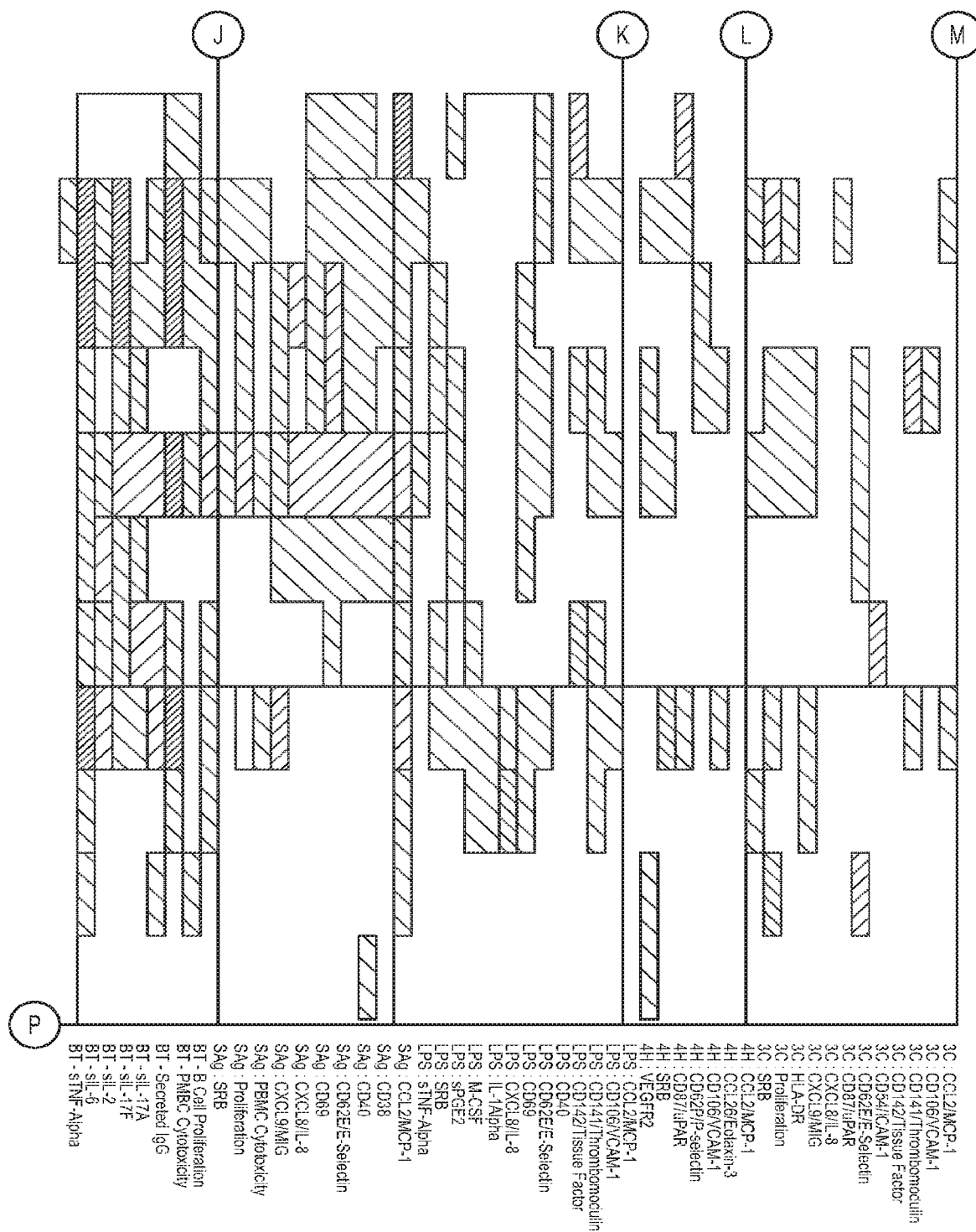


FIG. 56
(continued)

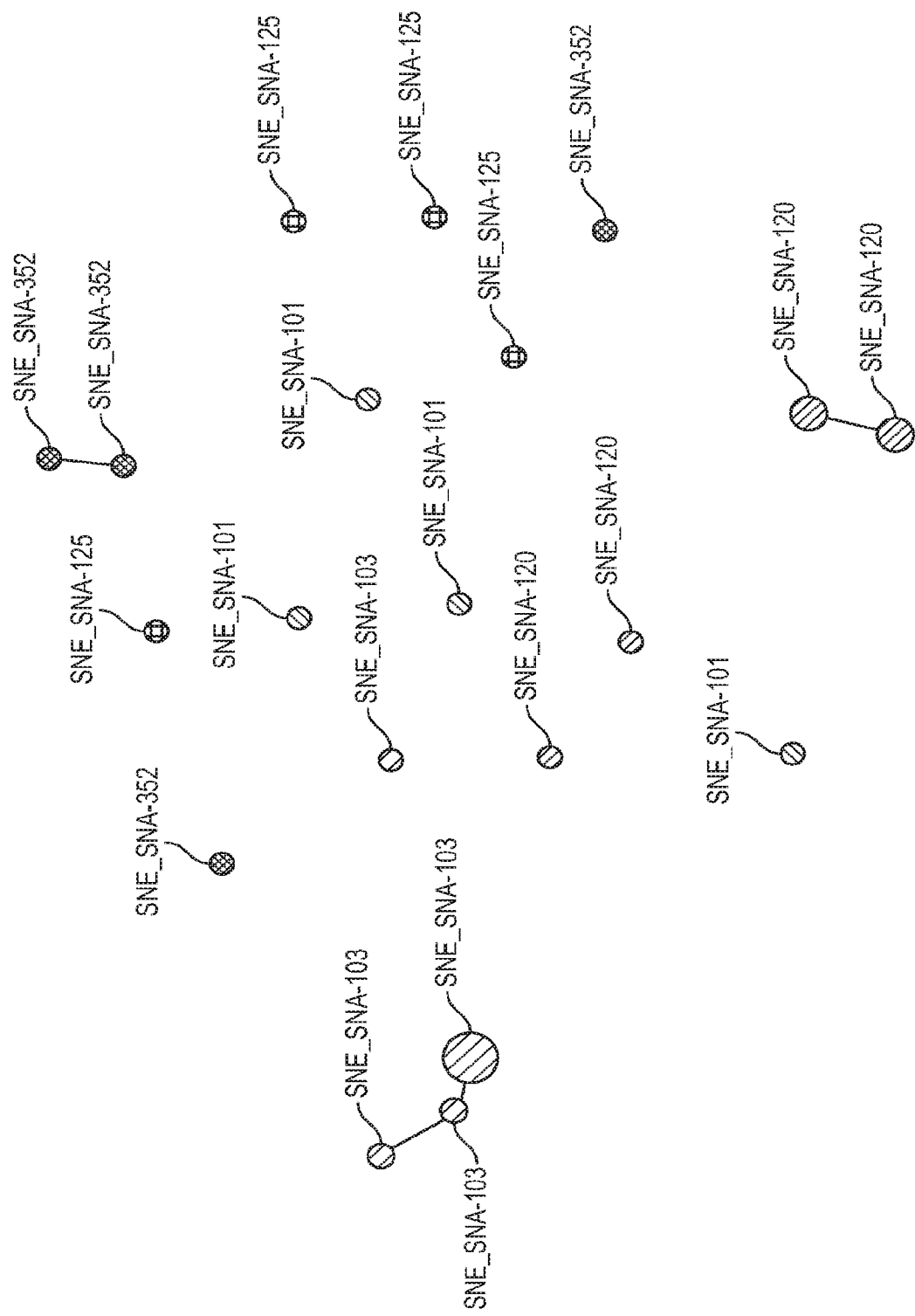


FIG. 57

59/207

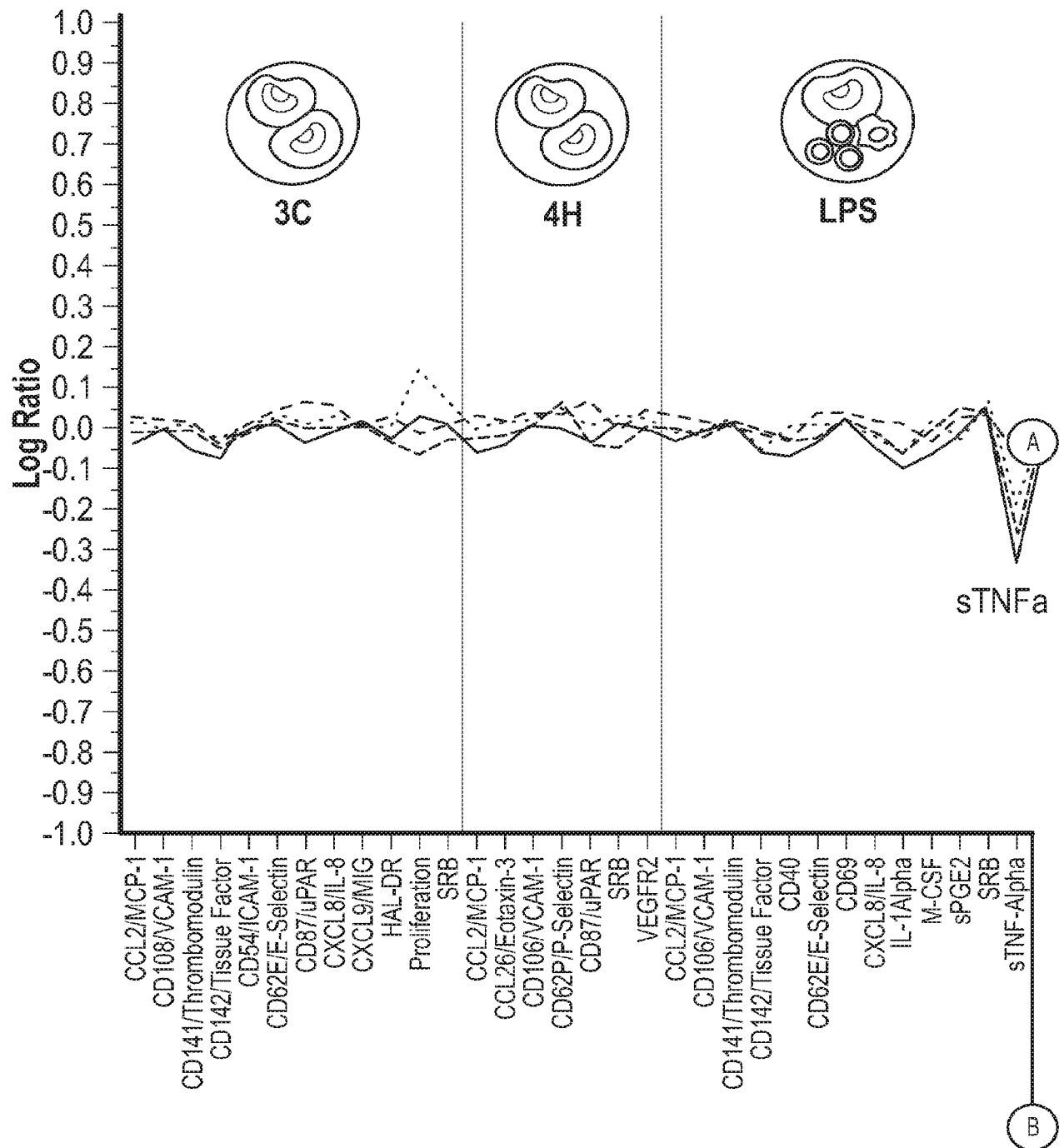


FIG.58

60/207

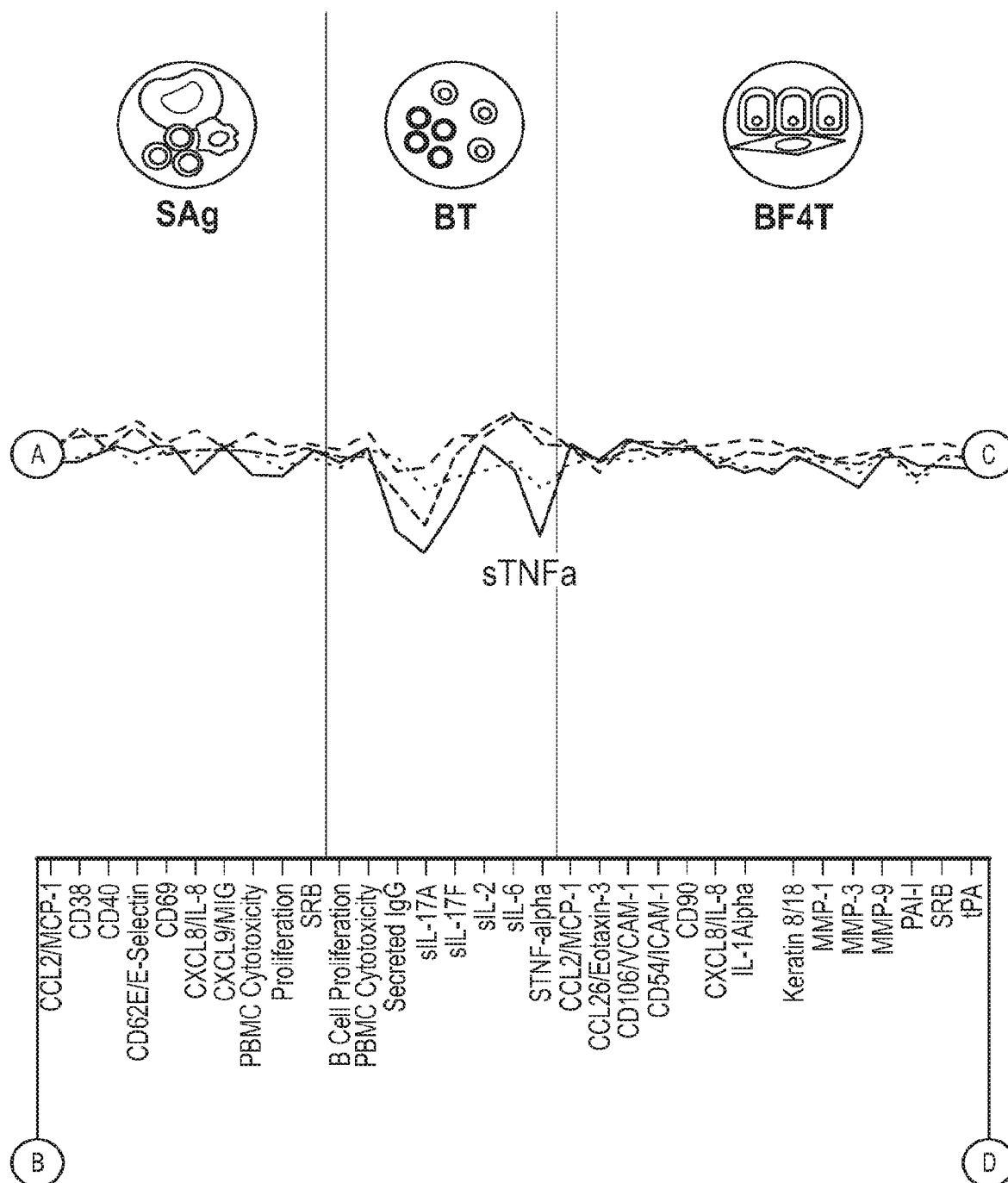


FIG. 58
(Continued)

61/207

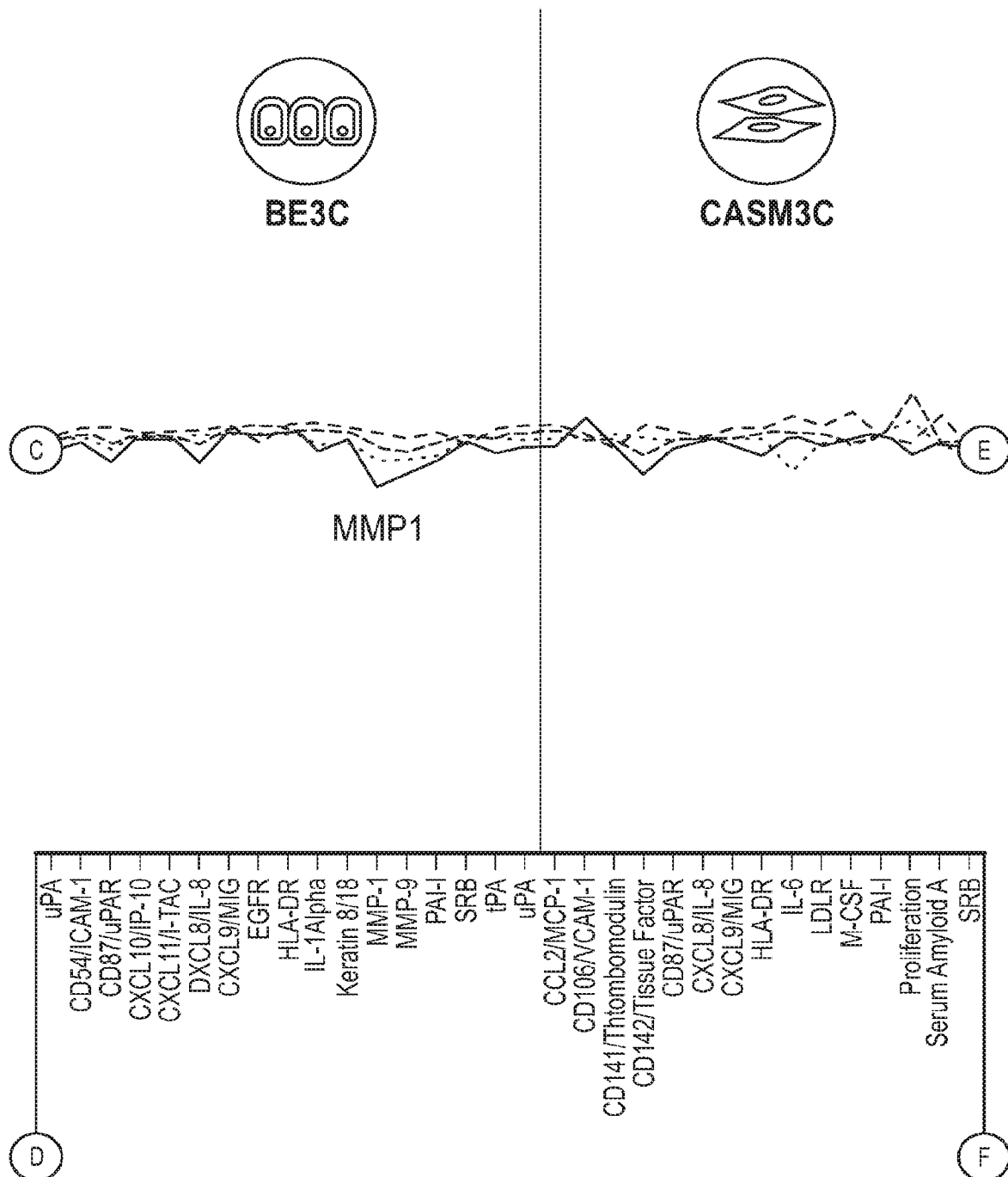


FIG. 58
(Continued)

62/207

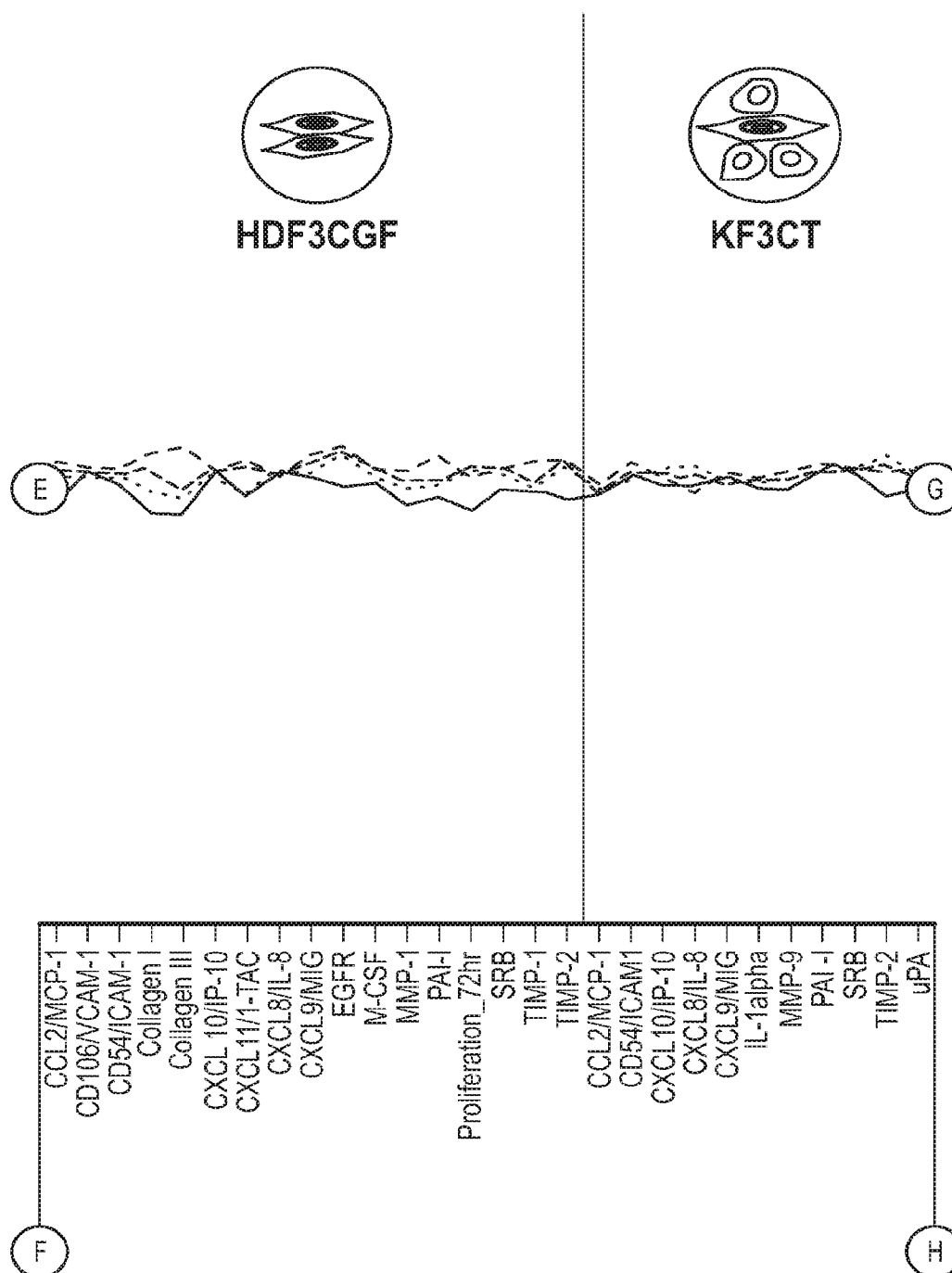


FIG. 58
(Continued)

63/207

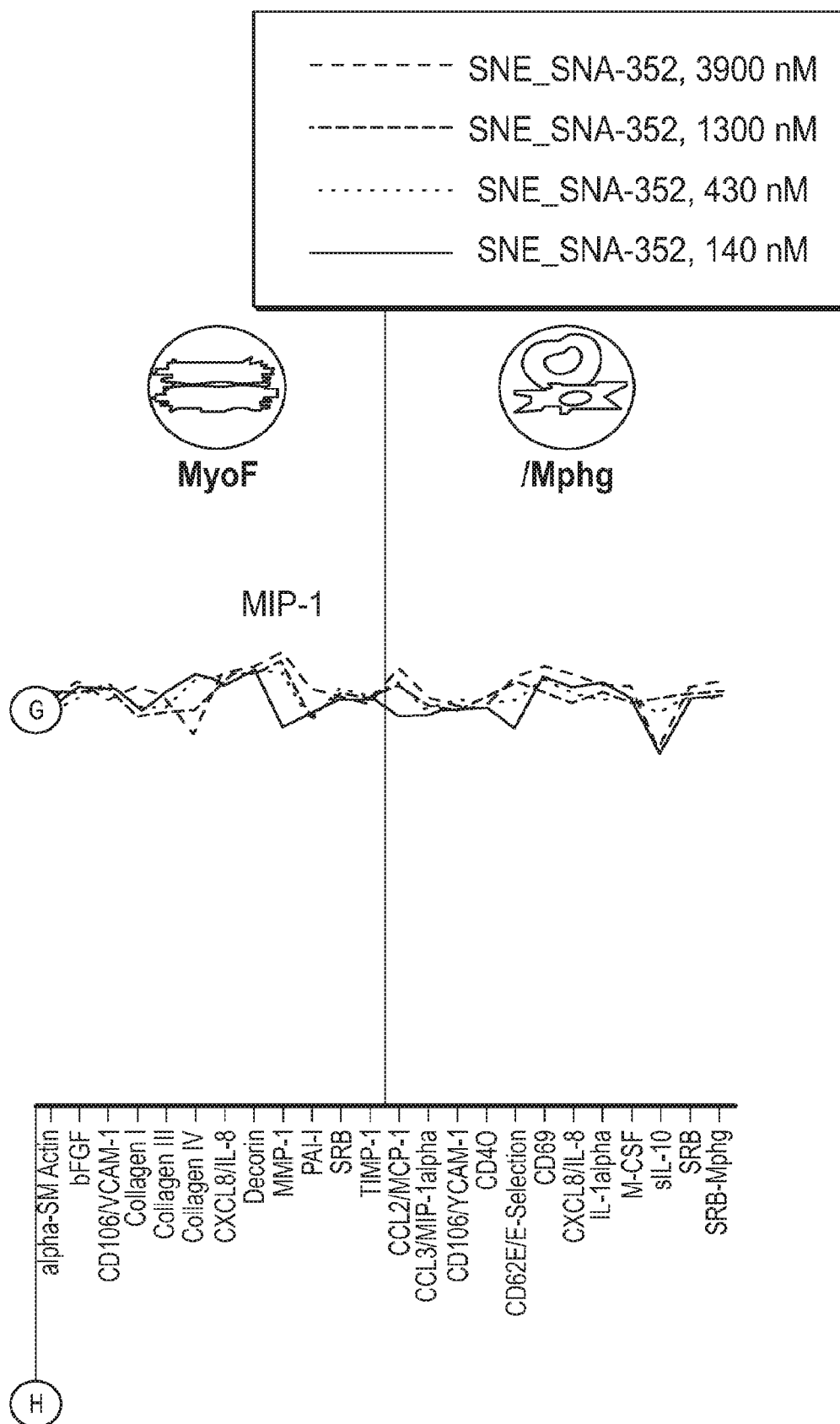


FIG. 58
(Continued)

64/207

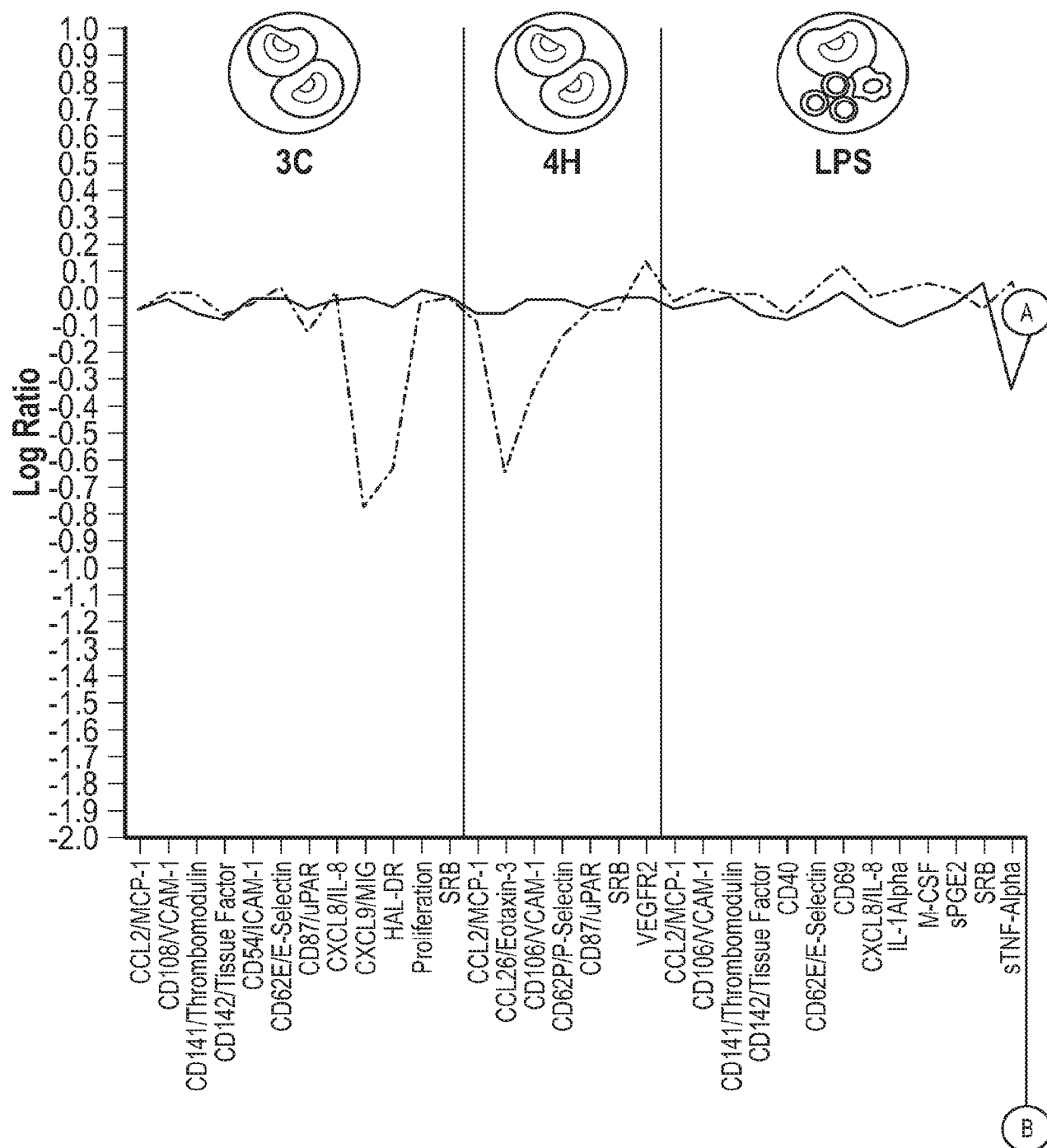


FIG. 59

65/207

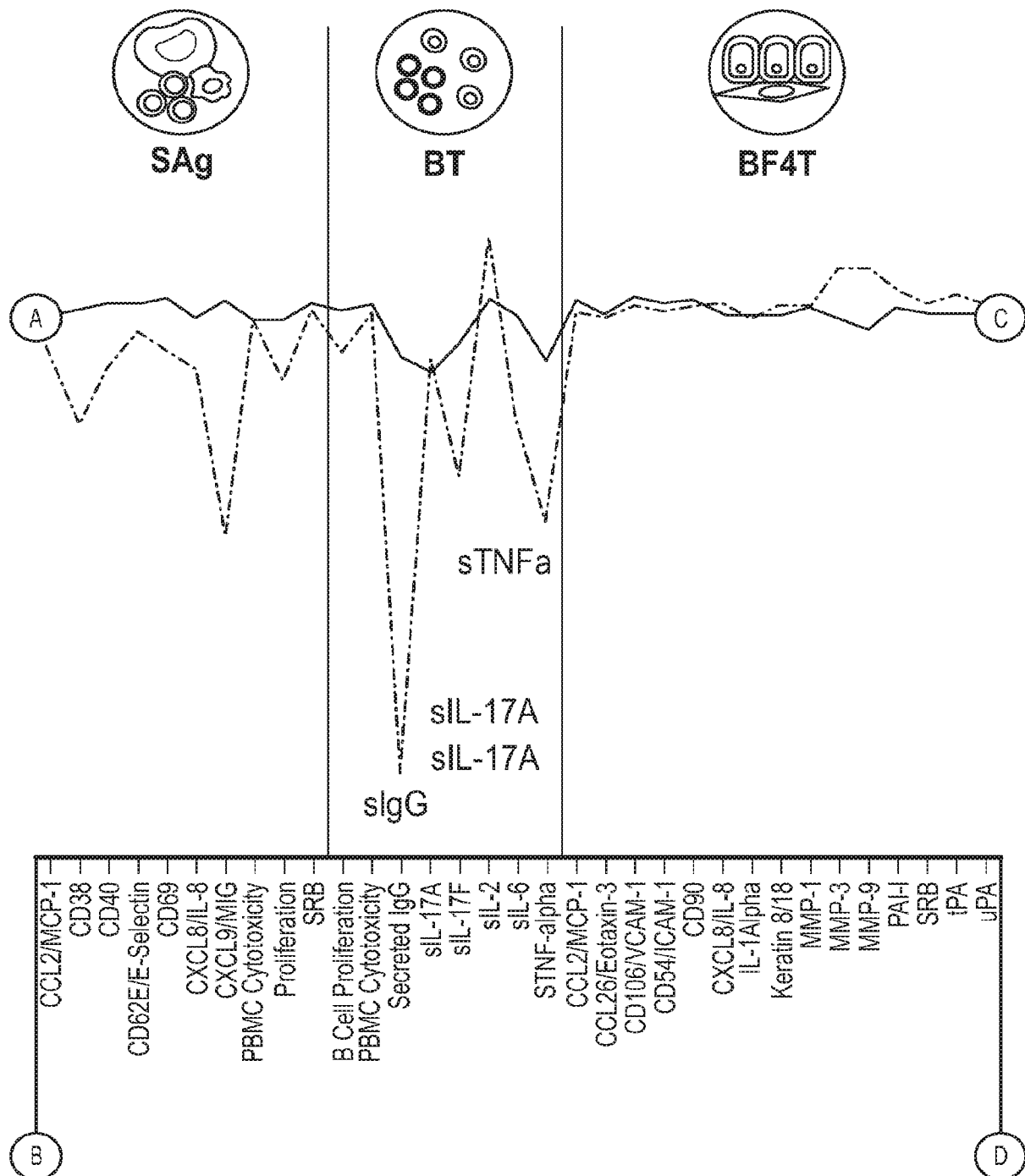


FIG. 59
(Continued)

66/207

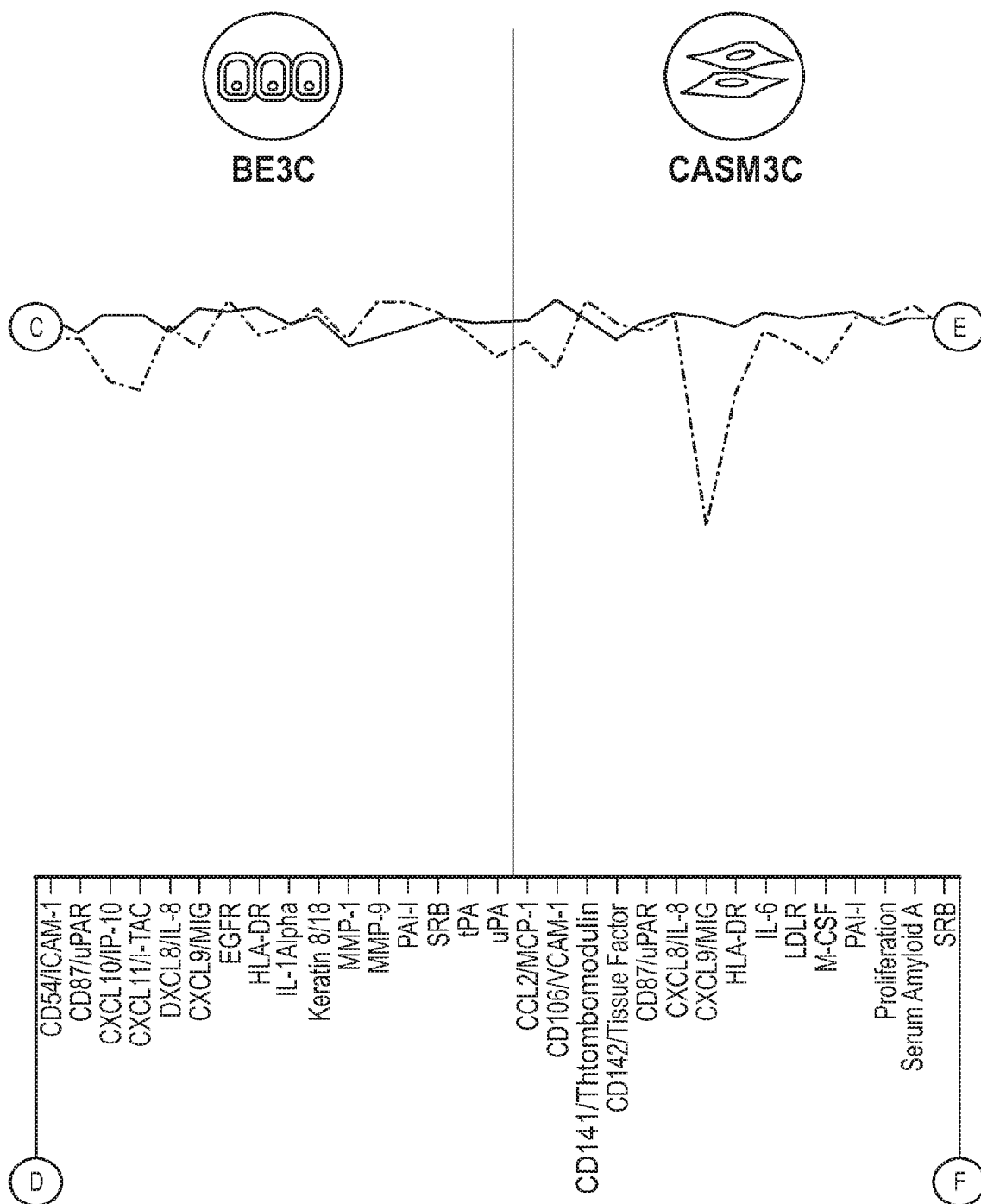


FIG. 59
(Continued)

67/207

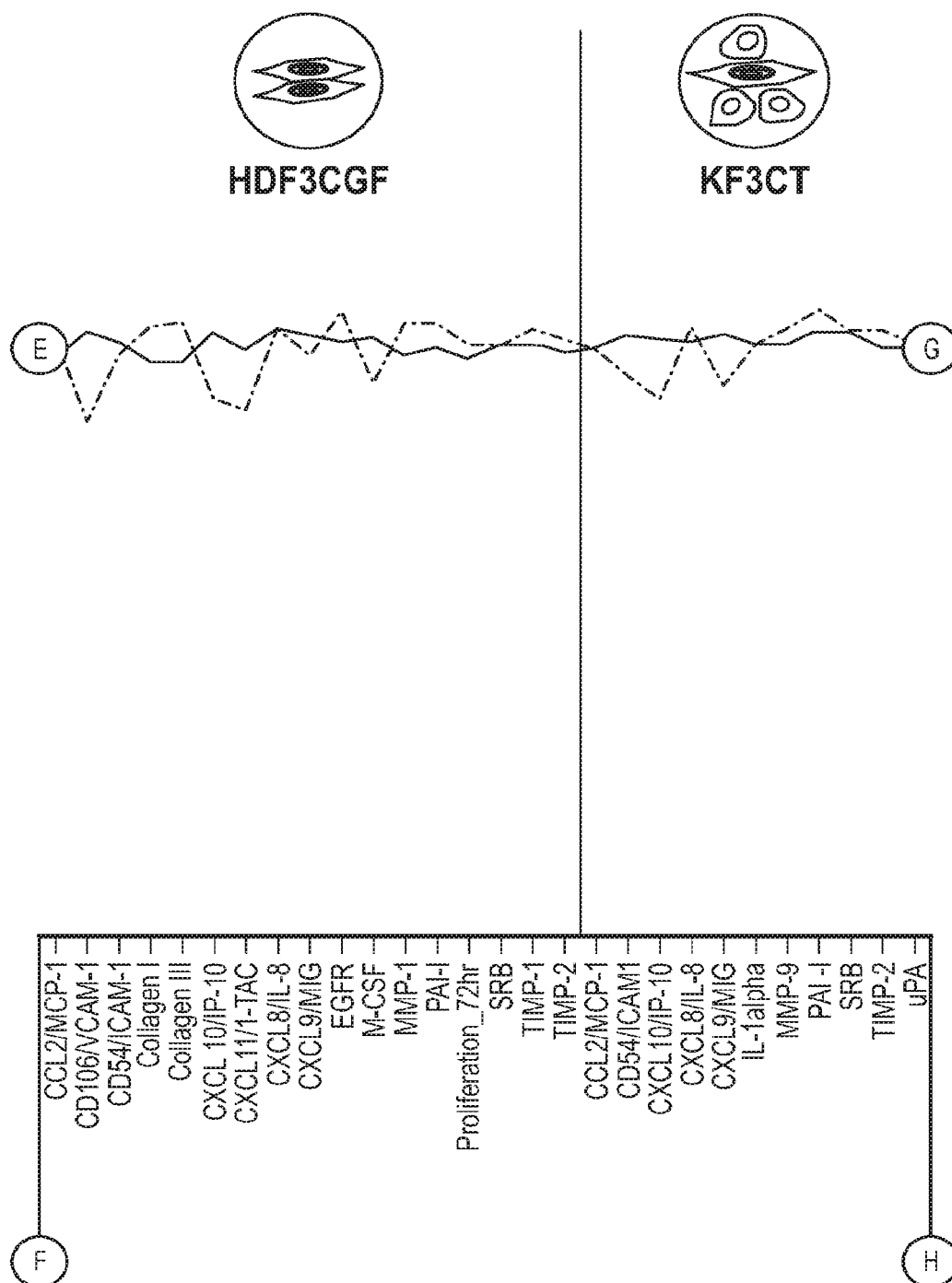


FIG. 59
(Continued)

68/207

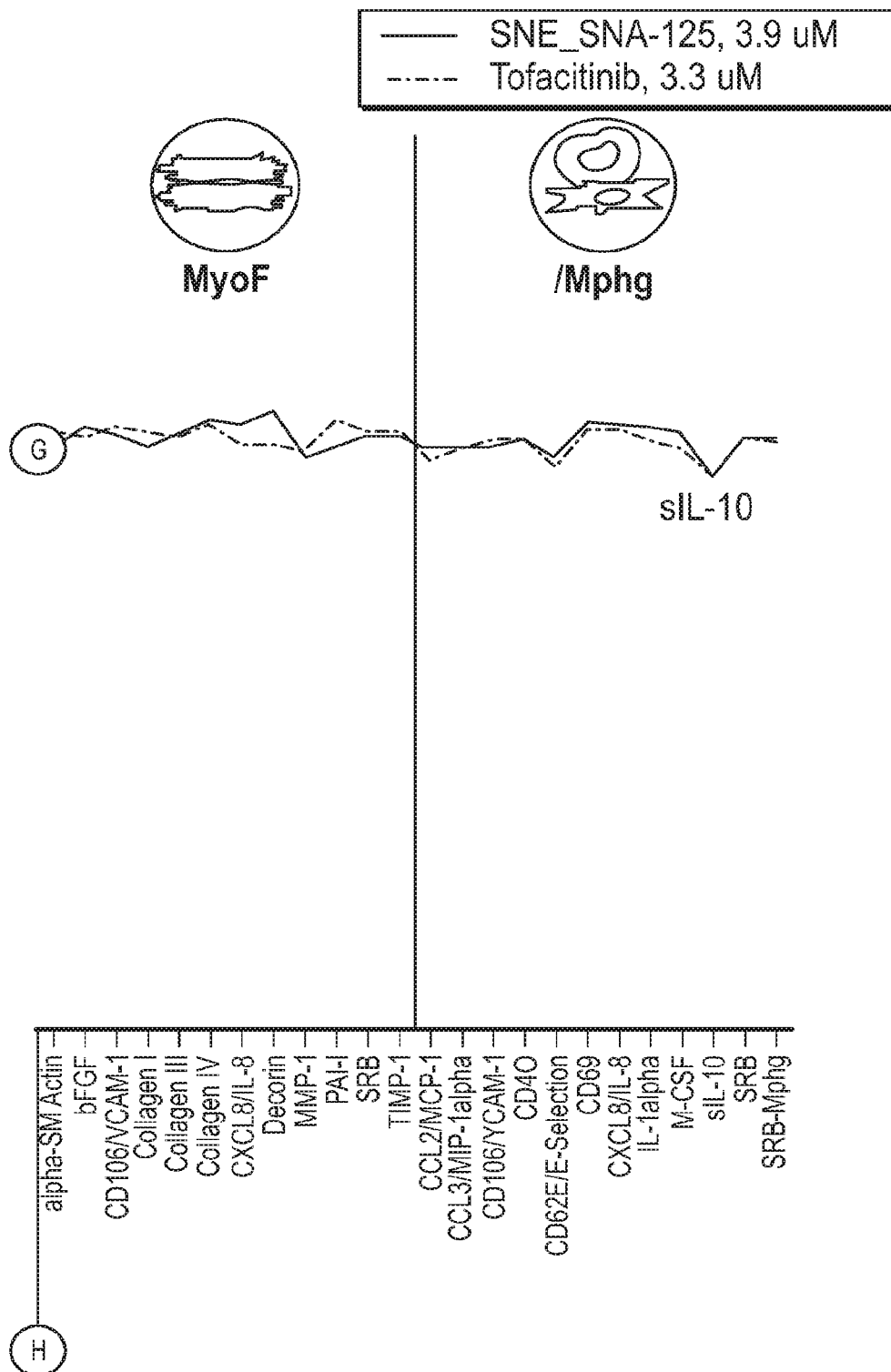


FIG. 59
(Continued)

69/207

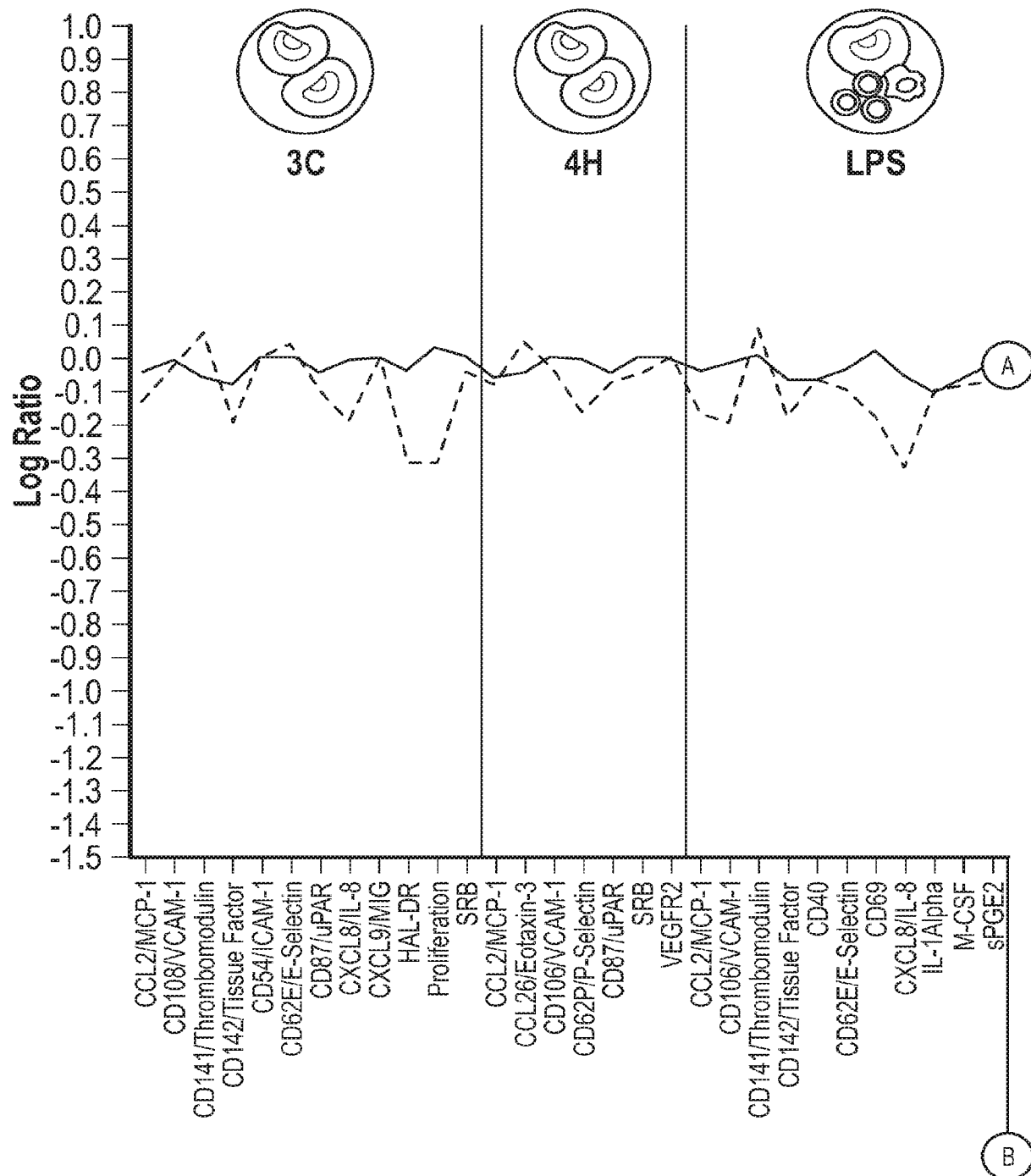


FIG. 60

70/207

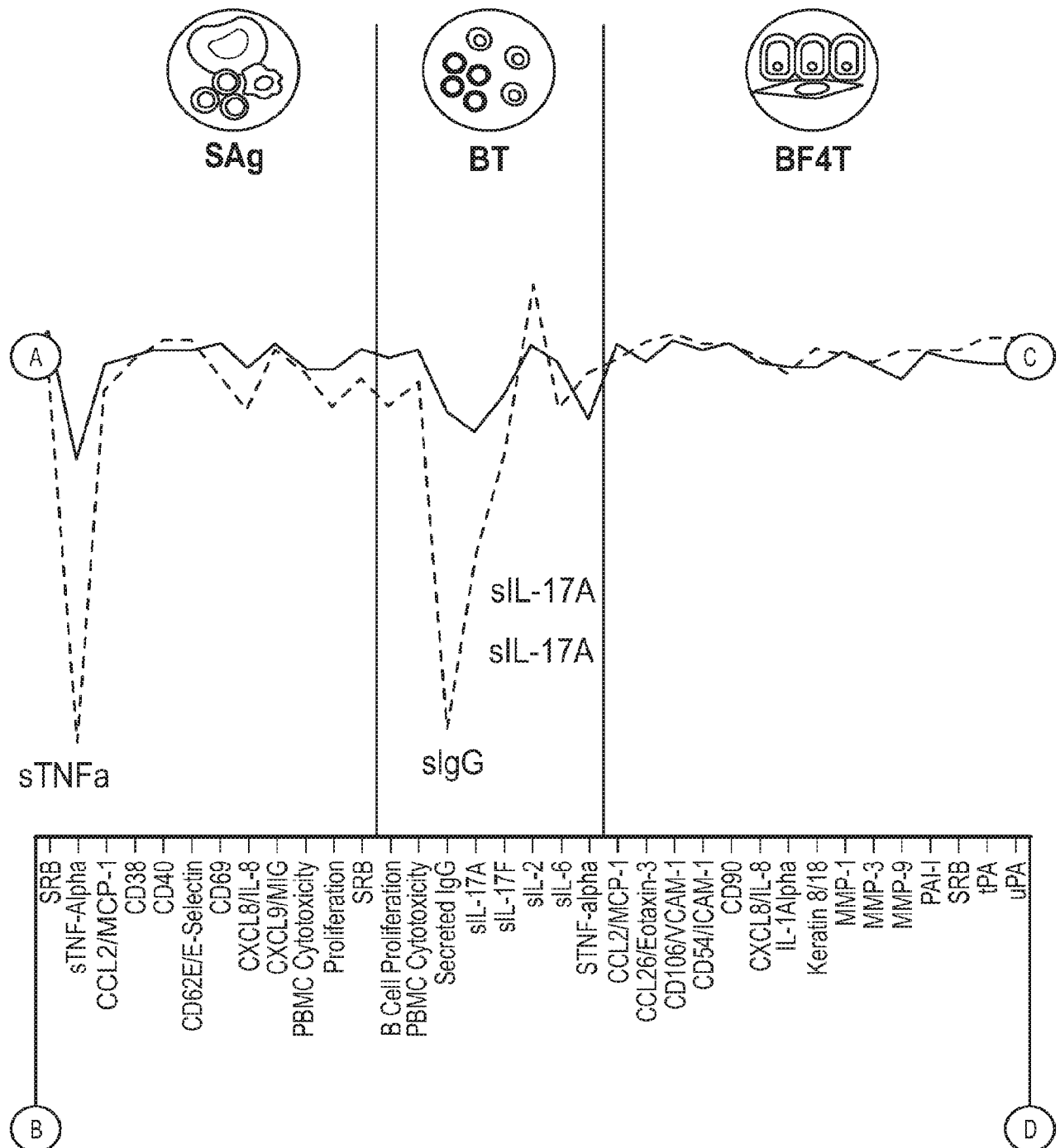


FIG. 60
(Continued)

71/207

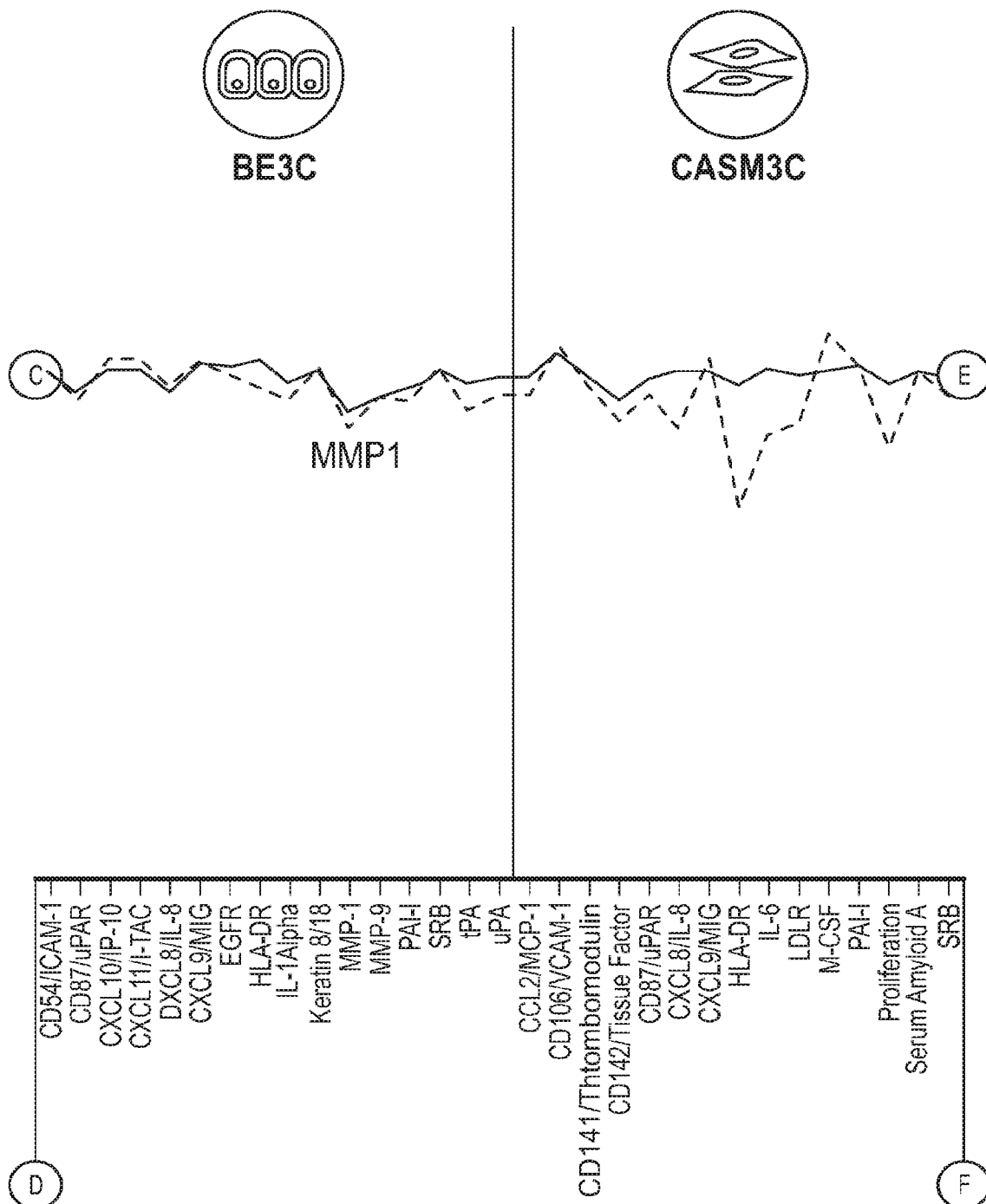


FIG. 60
(Continued)

72/207

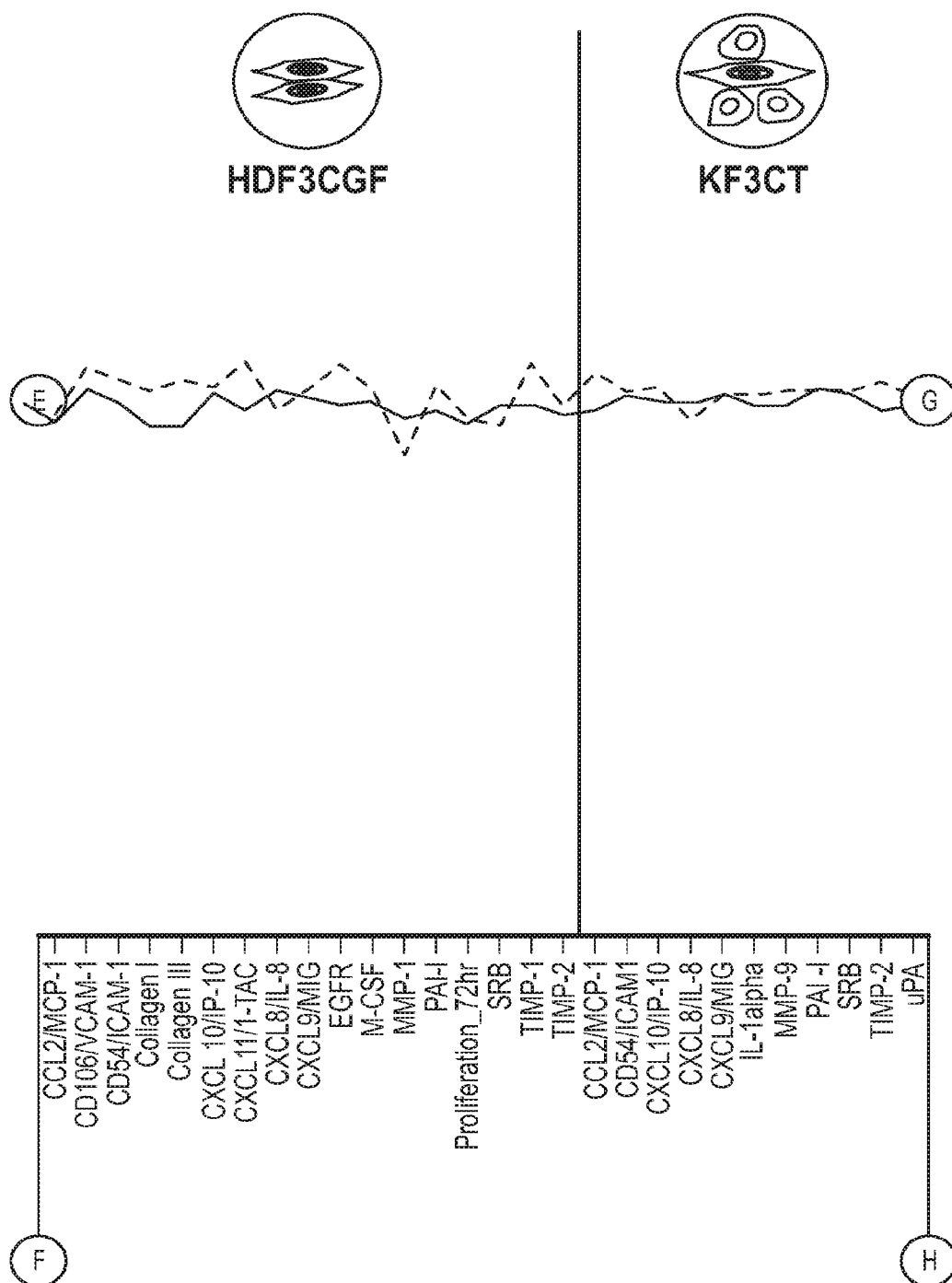


FIG. 60
(Continued)

73/207

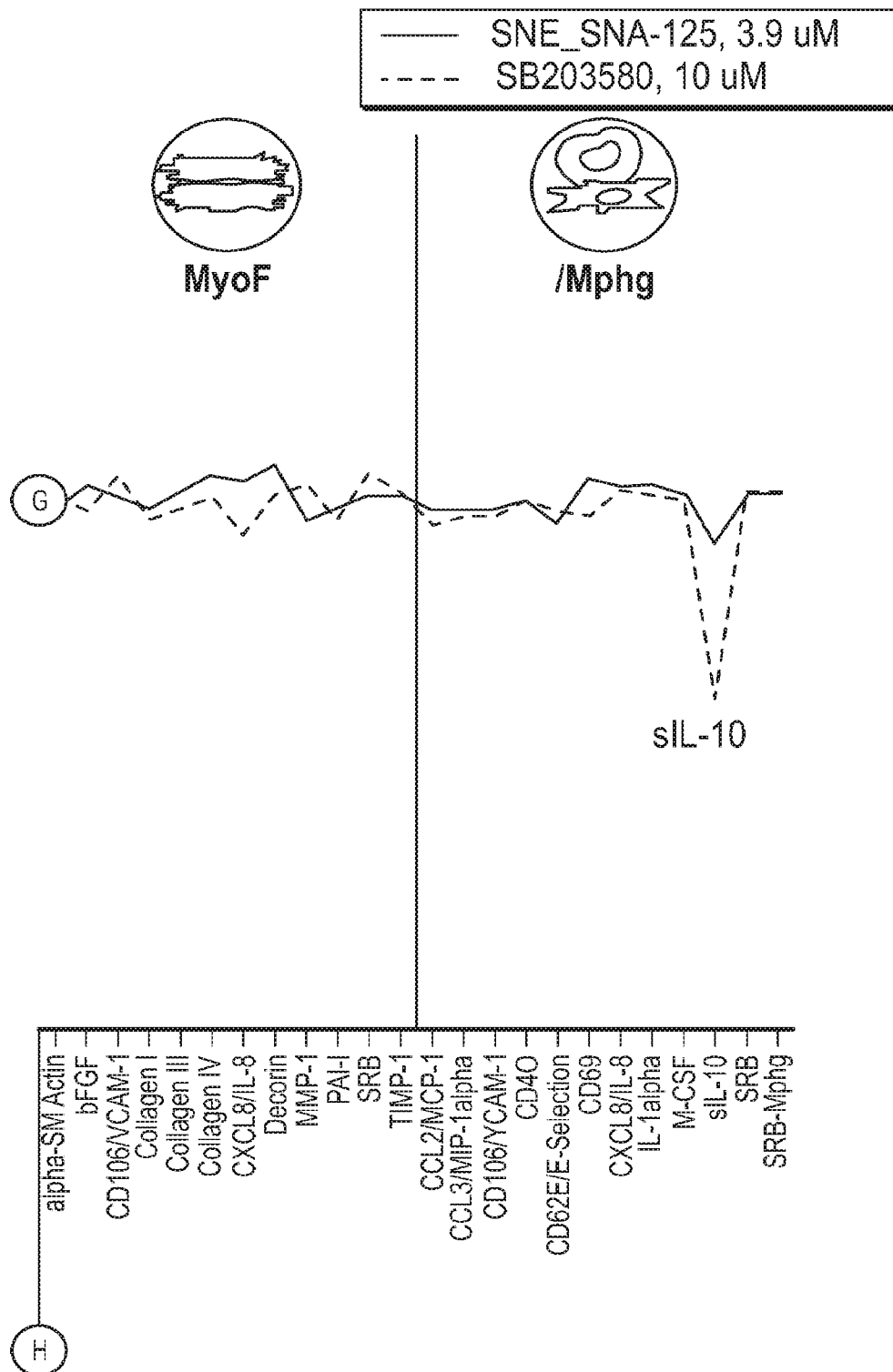


FIG. 60
(Continued)

74/207

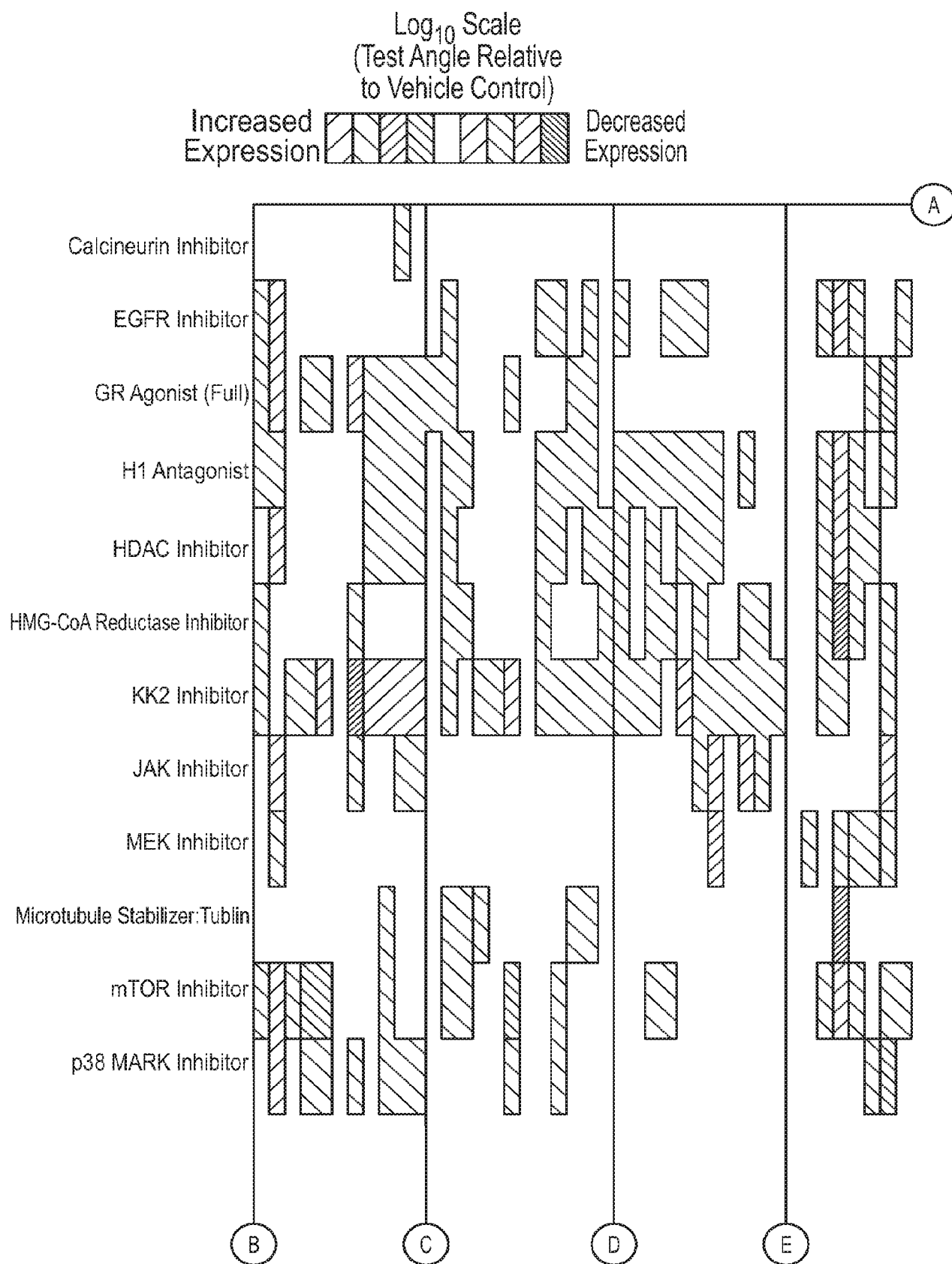


FIG. 61

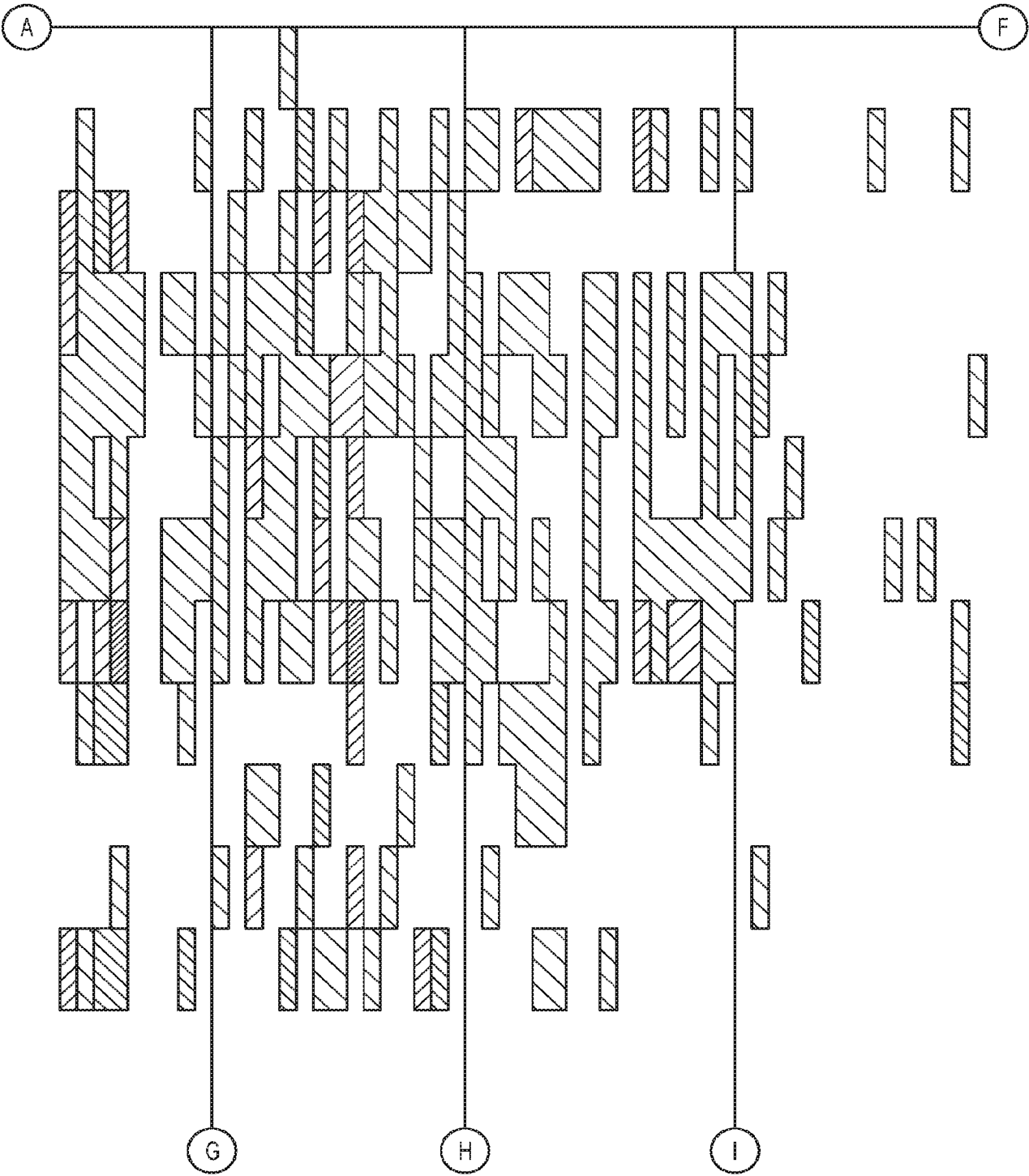


FIG. 61
(Continued)

SUBSTITUTE SHEET (RULE 26)

76/207

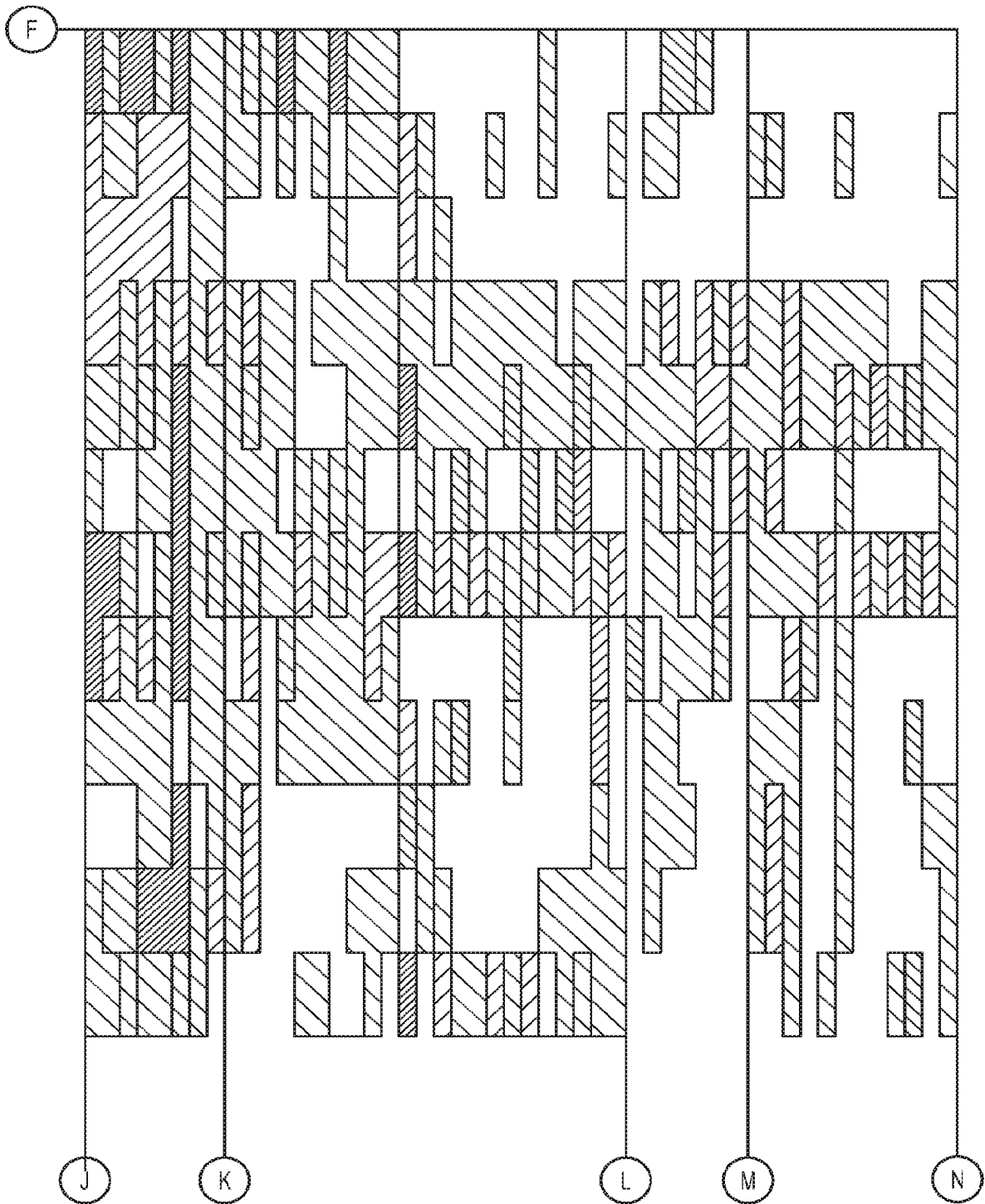


FIG. 61
(Continued)

SUBSTITUTE SHEET (RULE 26)

77/207

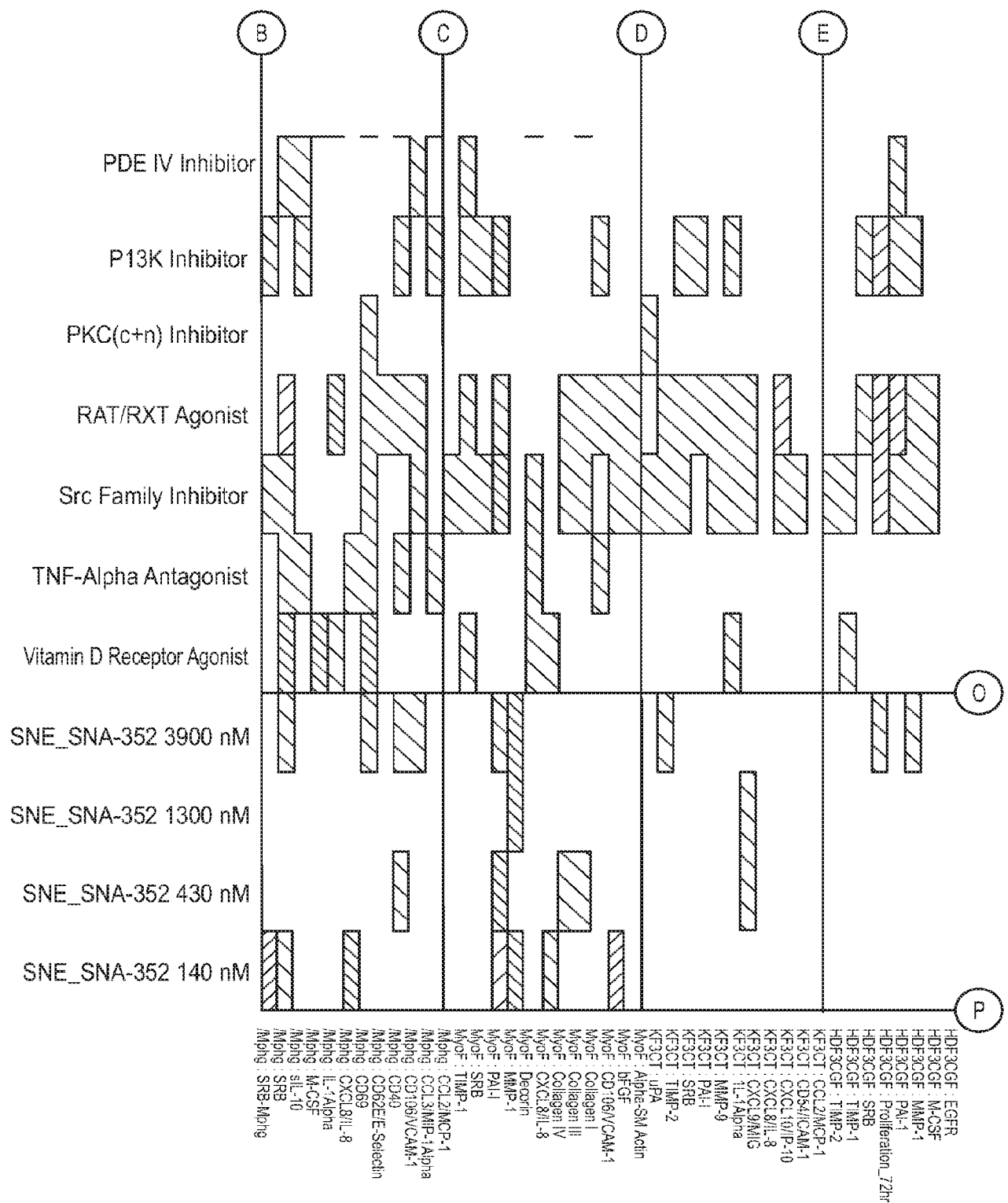


FIG. 61
(Continued)

78/207

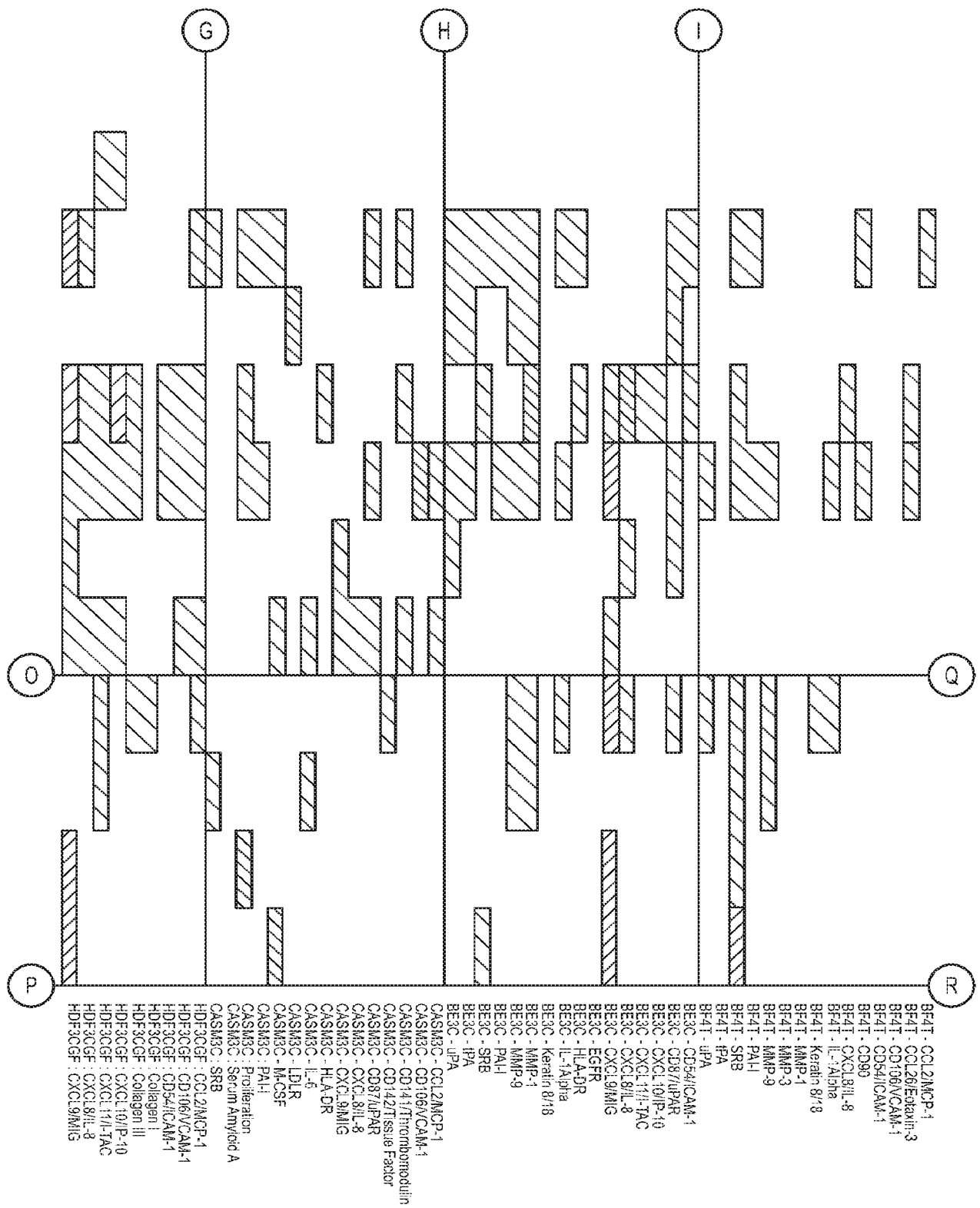


FIG. 61
(Continued)

79/207

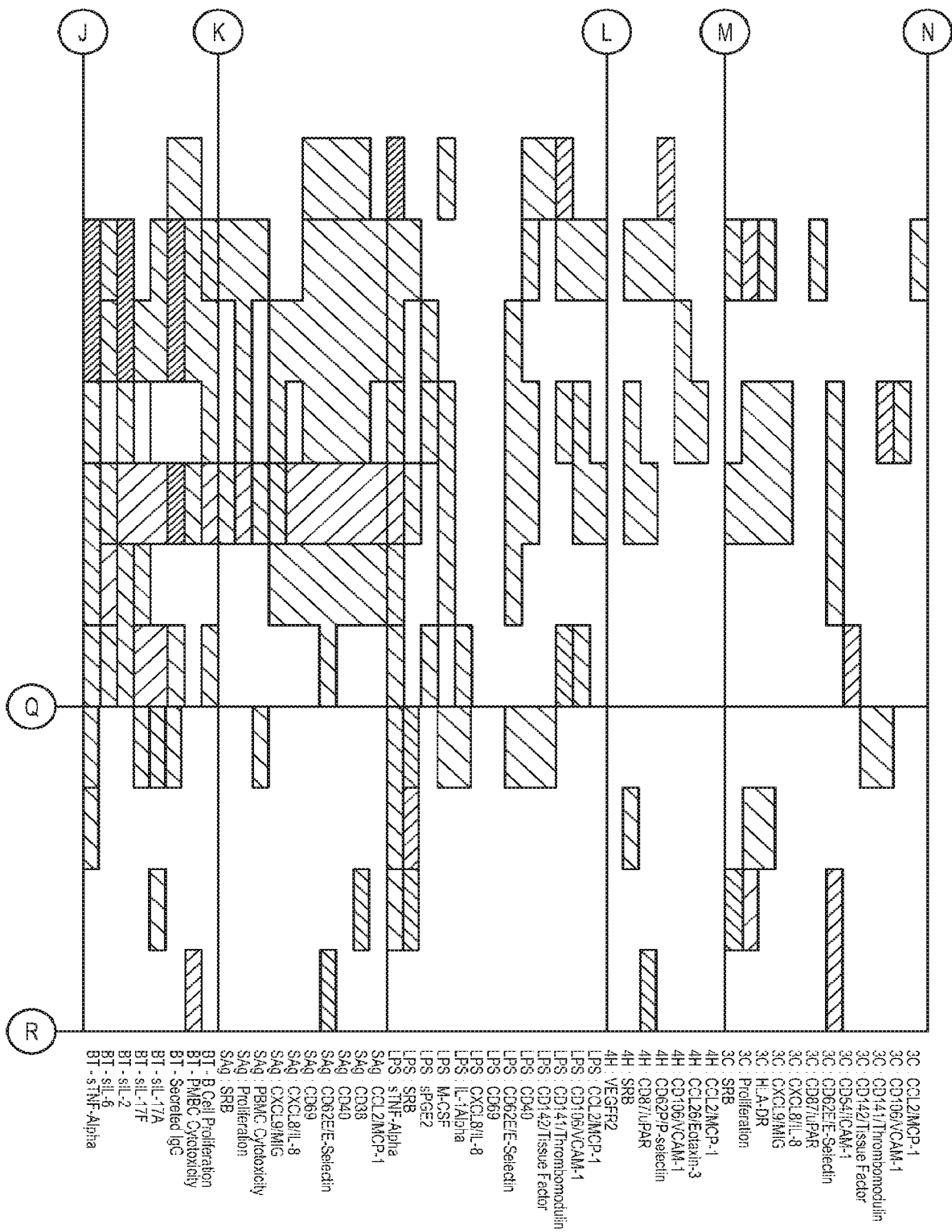


FIG. 61
(Continued)

80/207

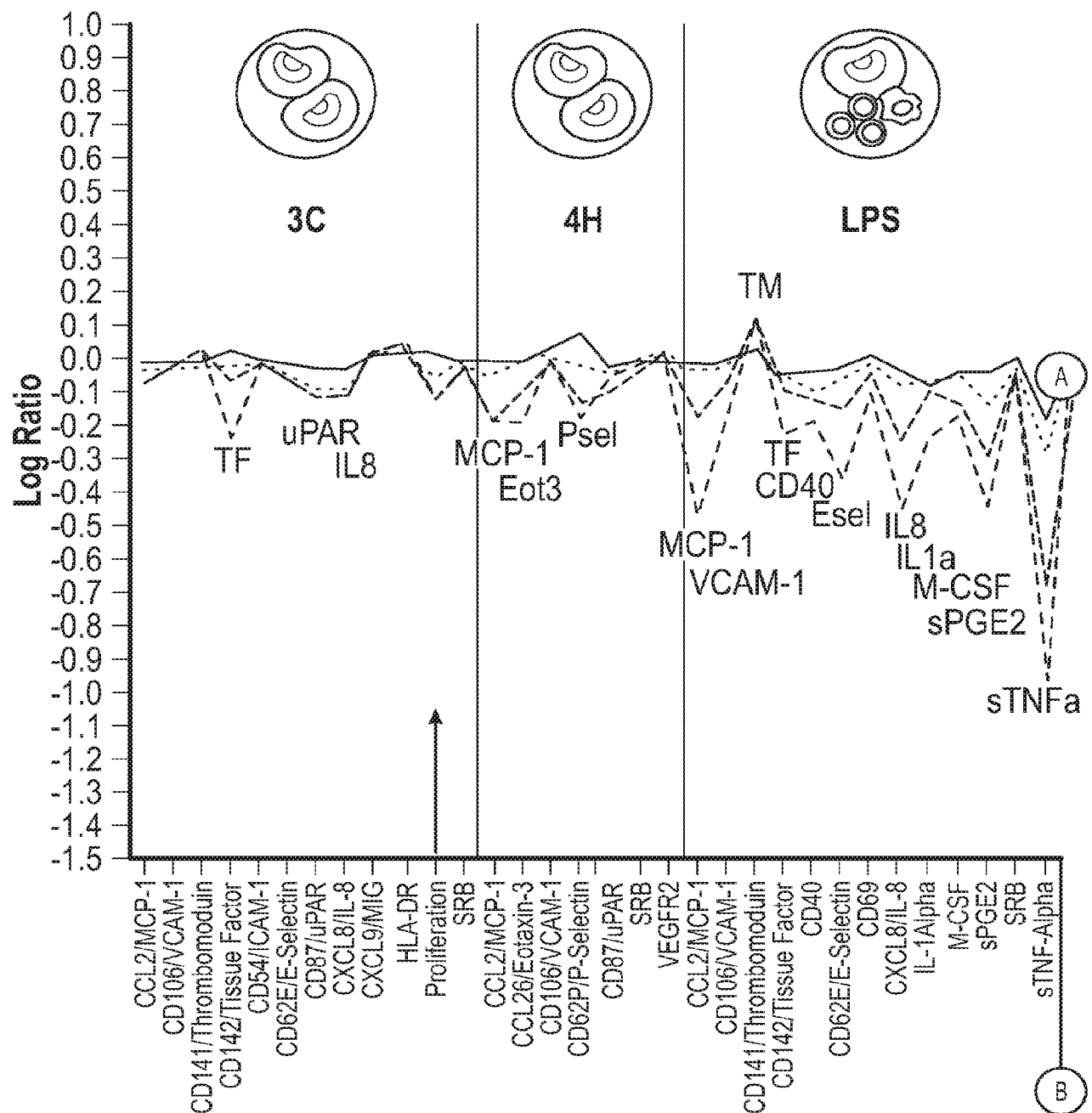


FIG. 62

81/207

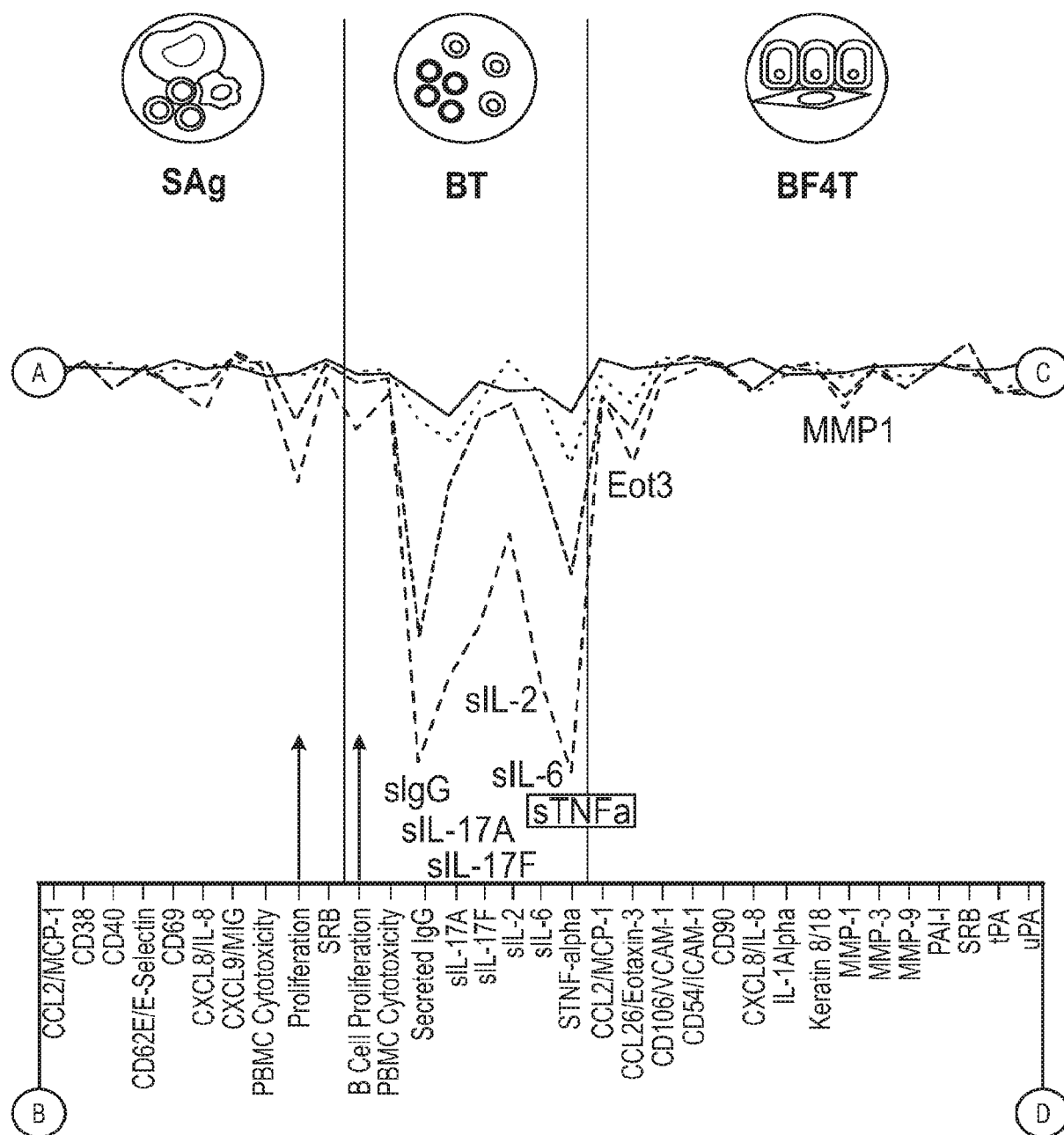


FIG. 62
(Continued)

82/207

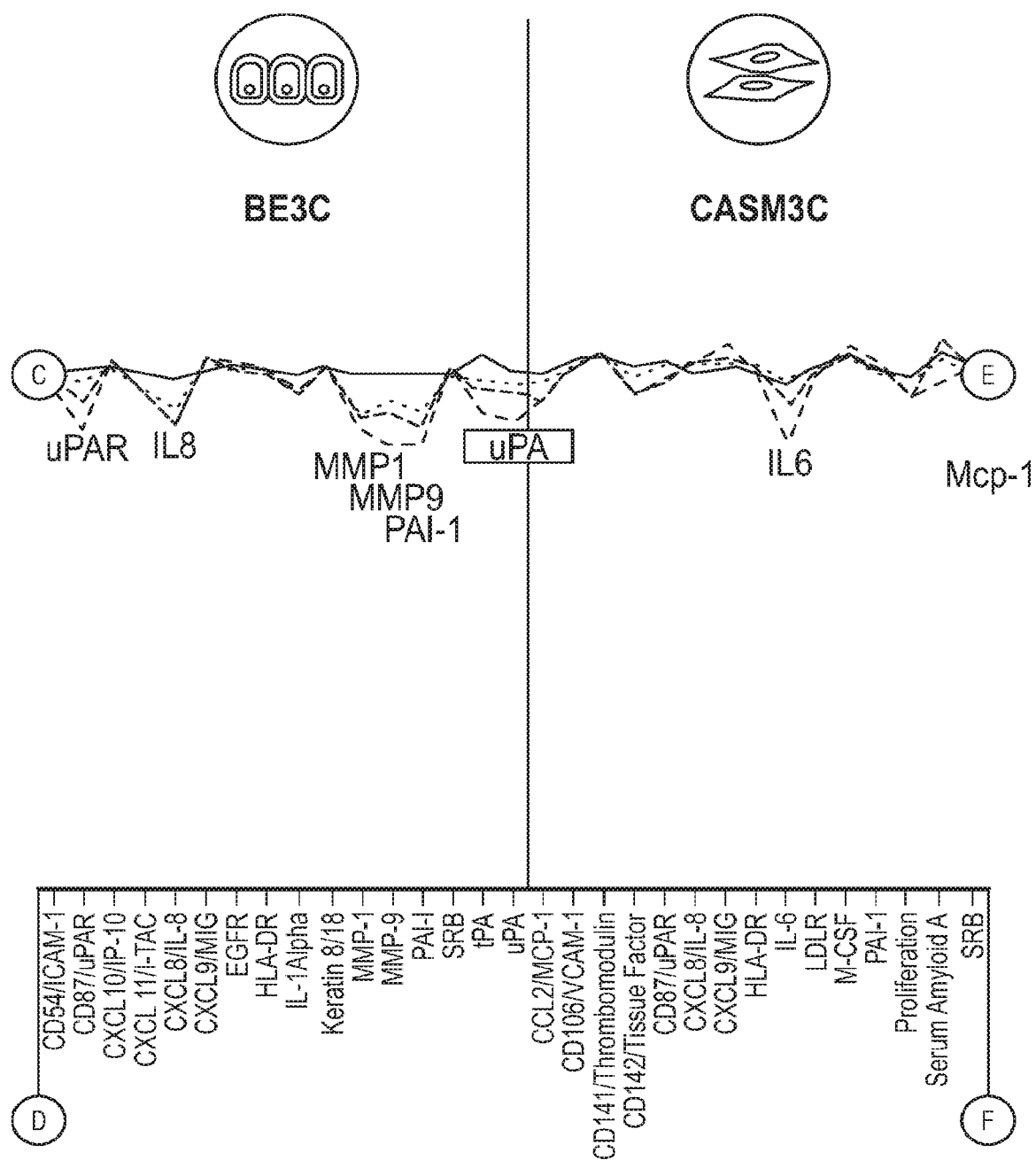


FIG. 62
(Continued)

83/207

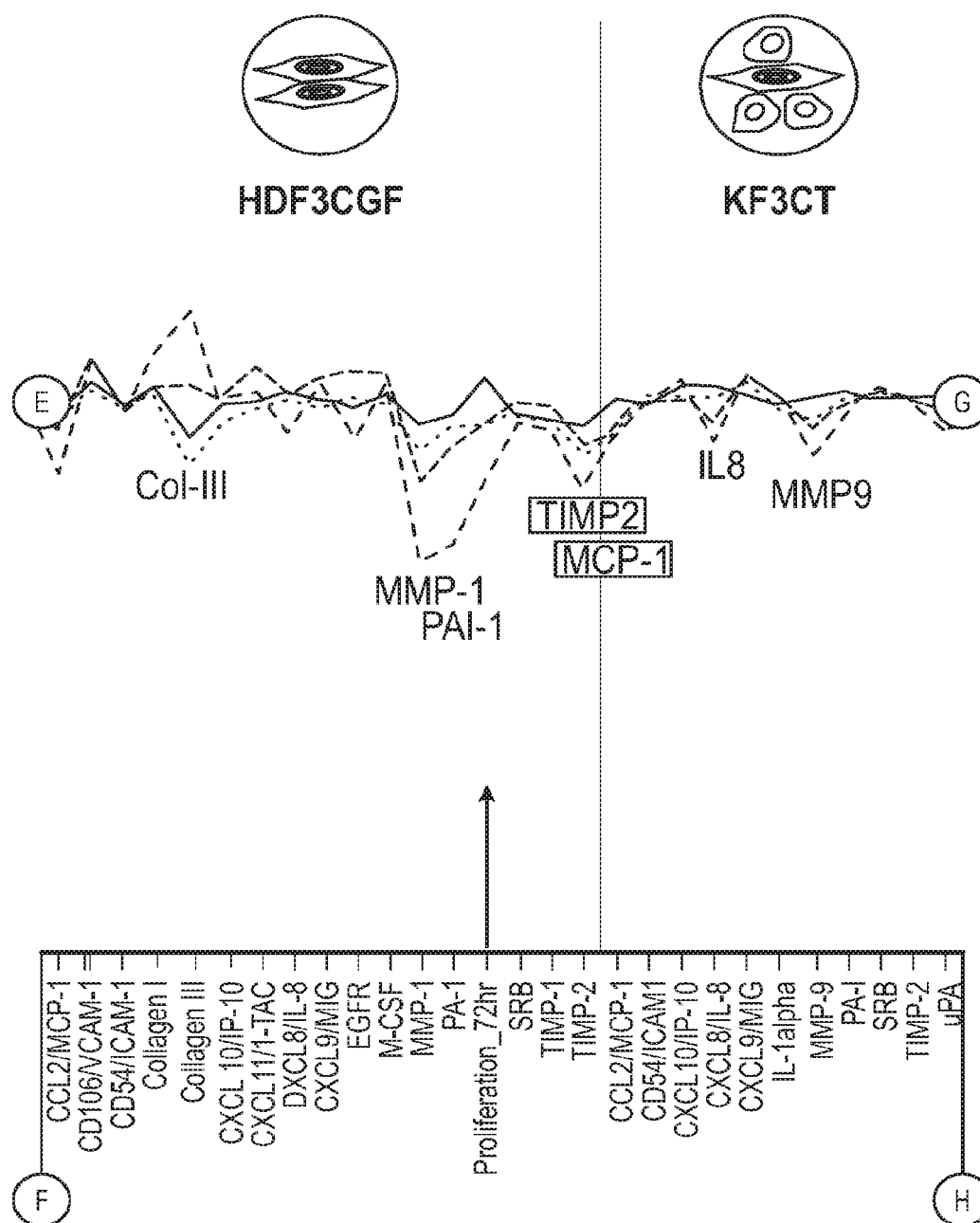


FIG. 62
(Continued)

84/207

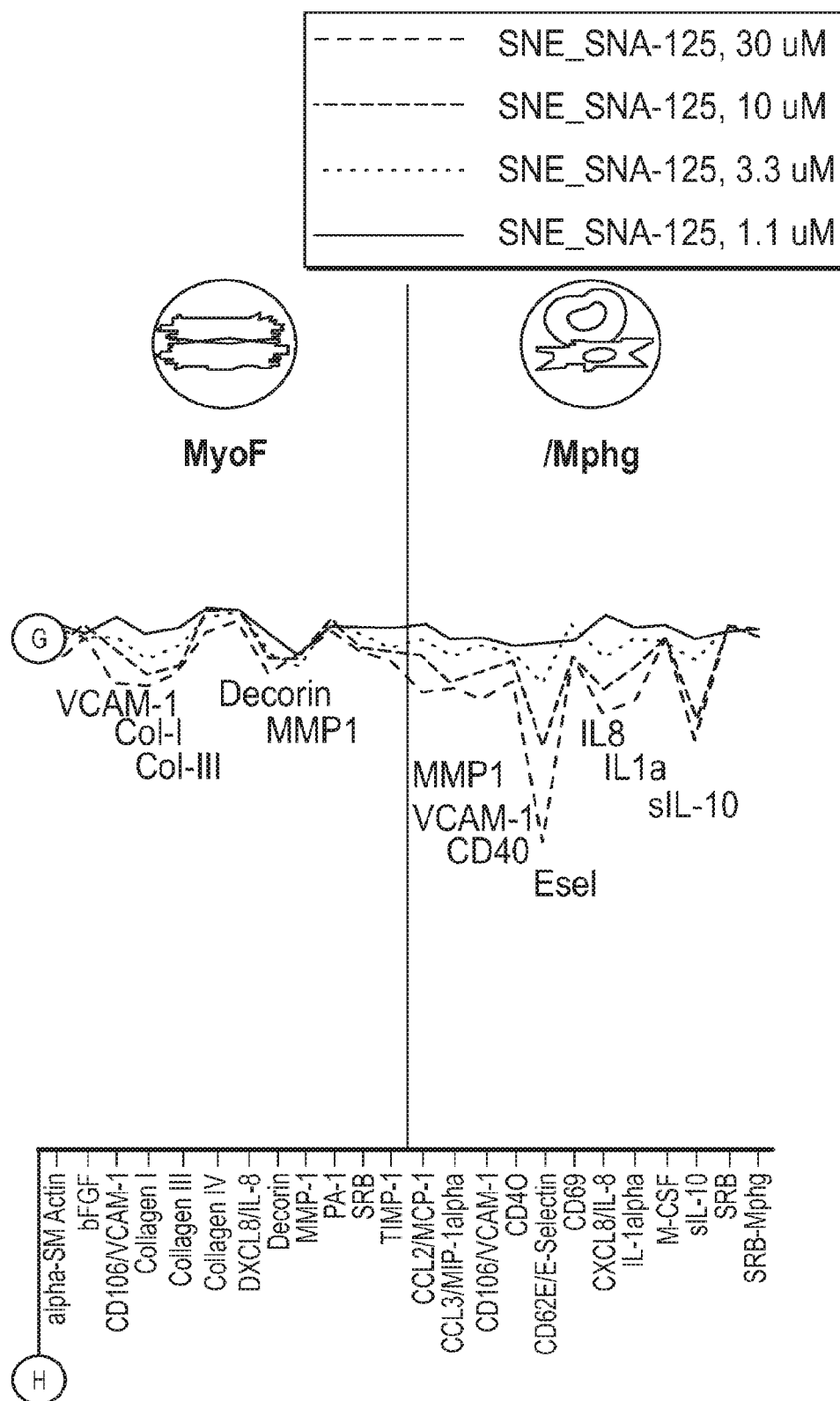
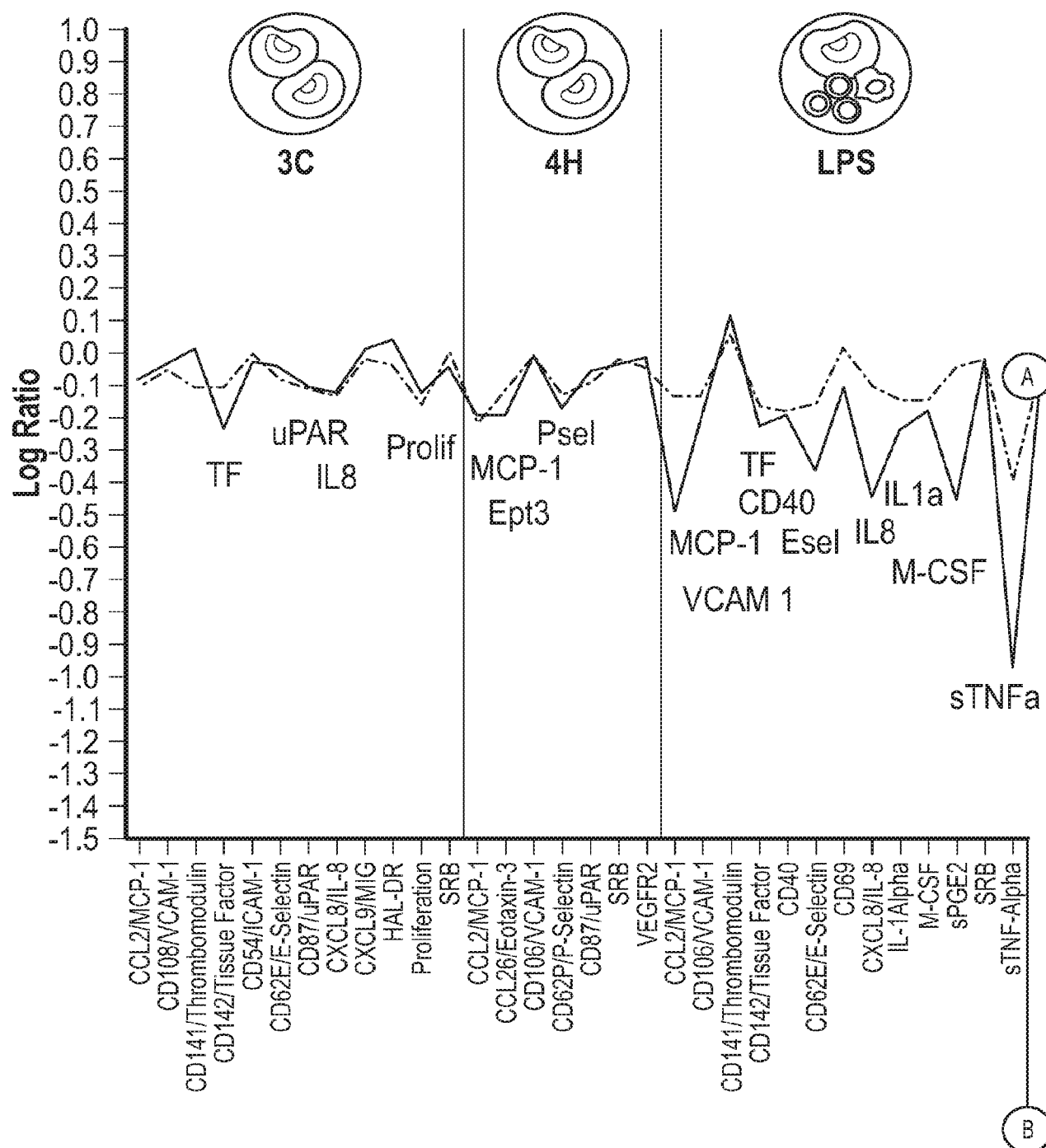


FIG. 62
(Continued)

85/207



86/207

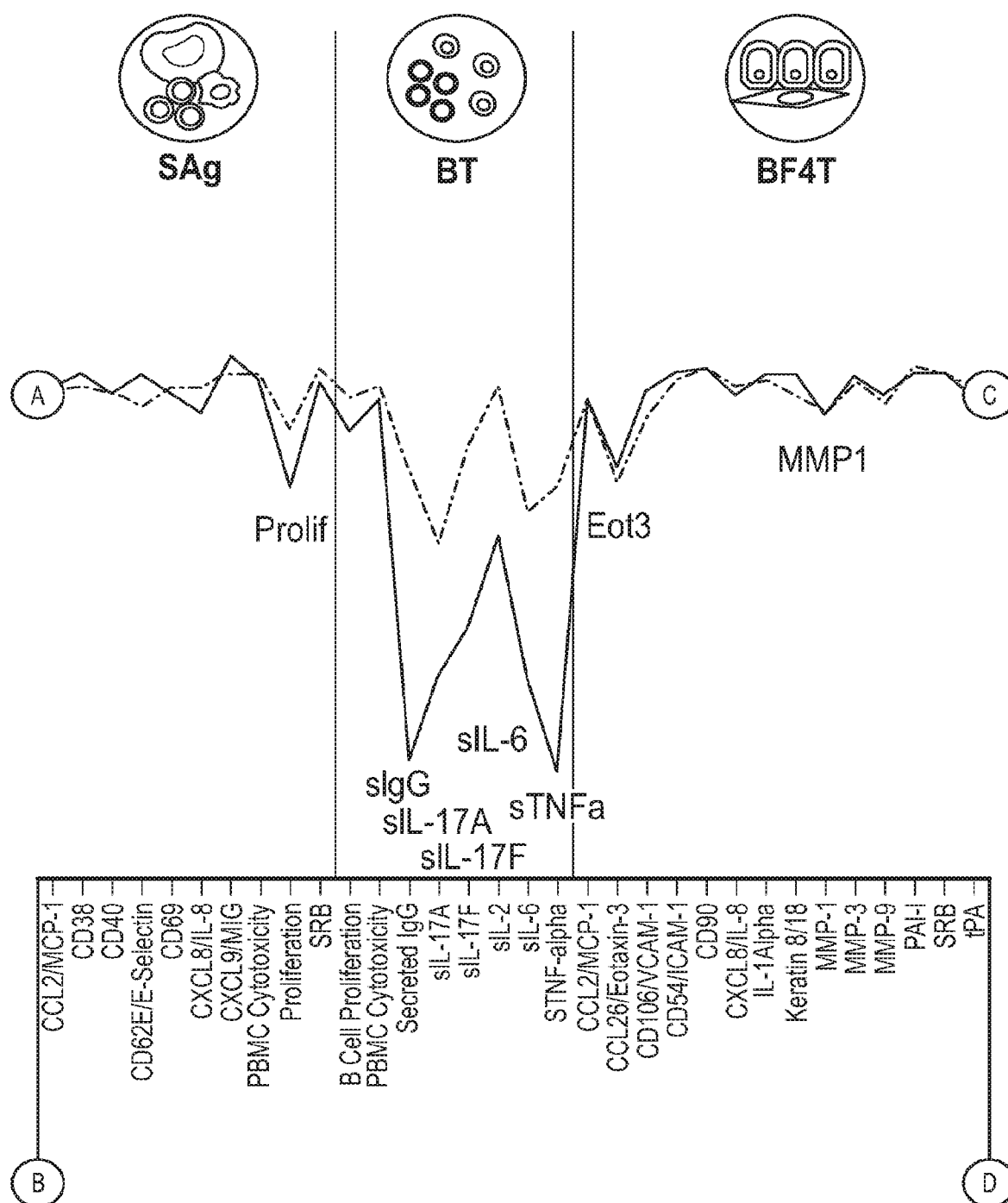


FIG. 63
(Continued)

87/207

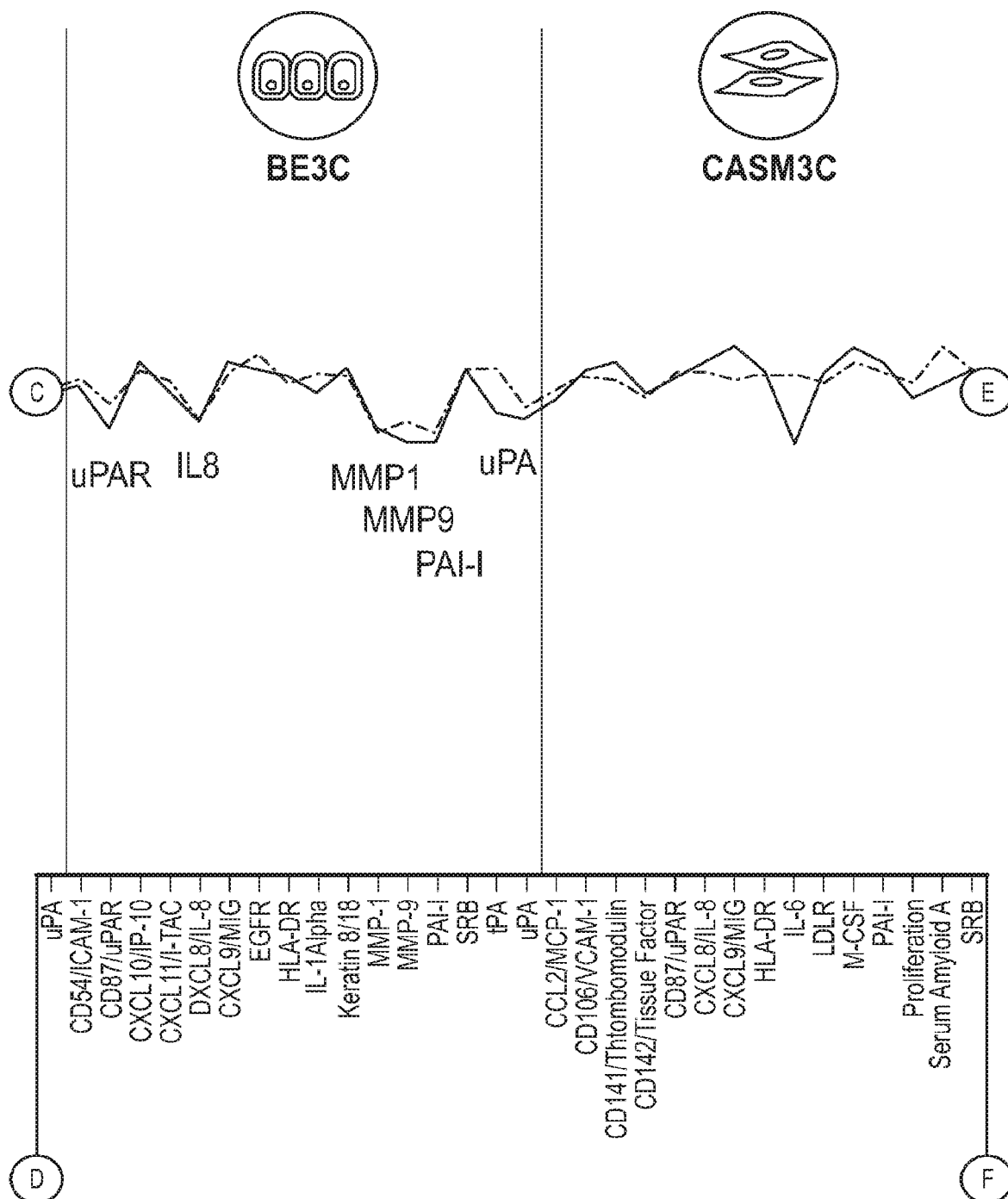


FIG. 63
(Continued)

88/207

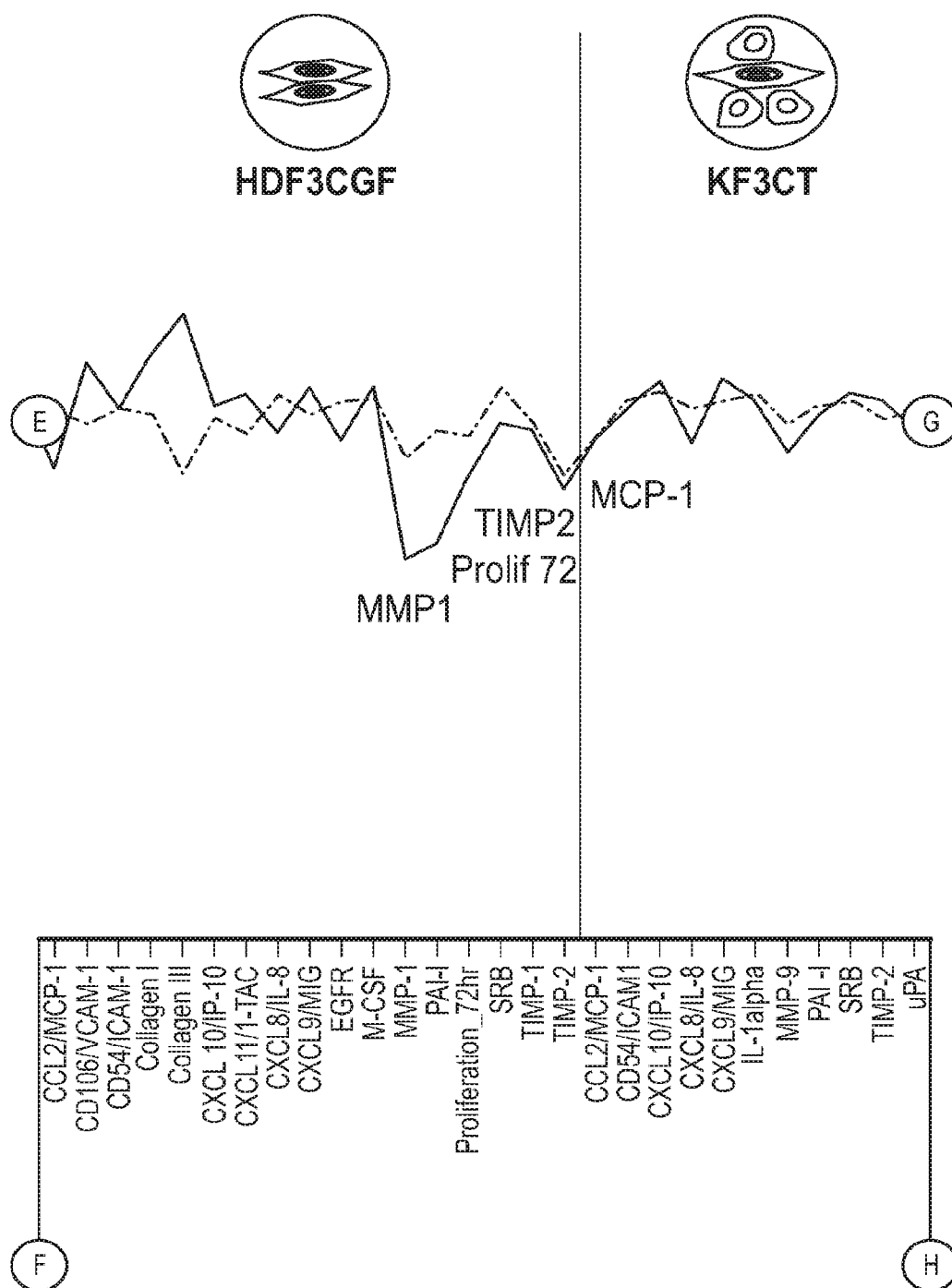


FIG. 63
(Continued)

89/207

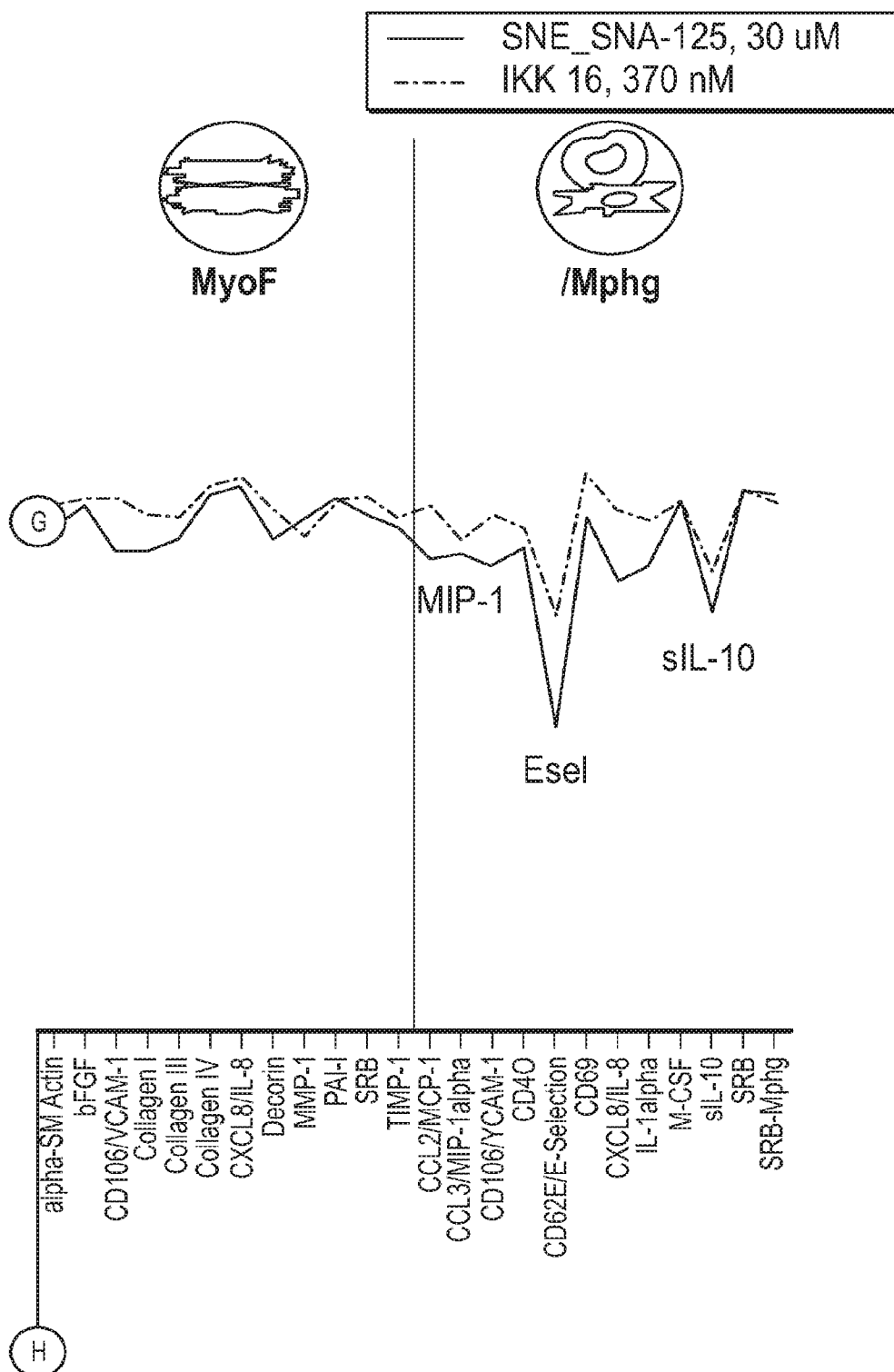


FIG. 63
(Continued)

90/207

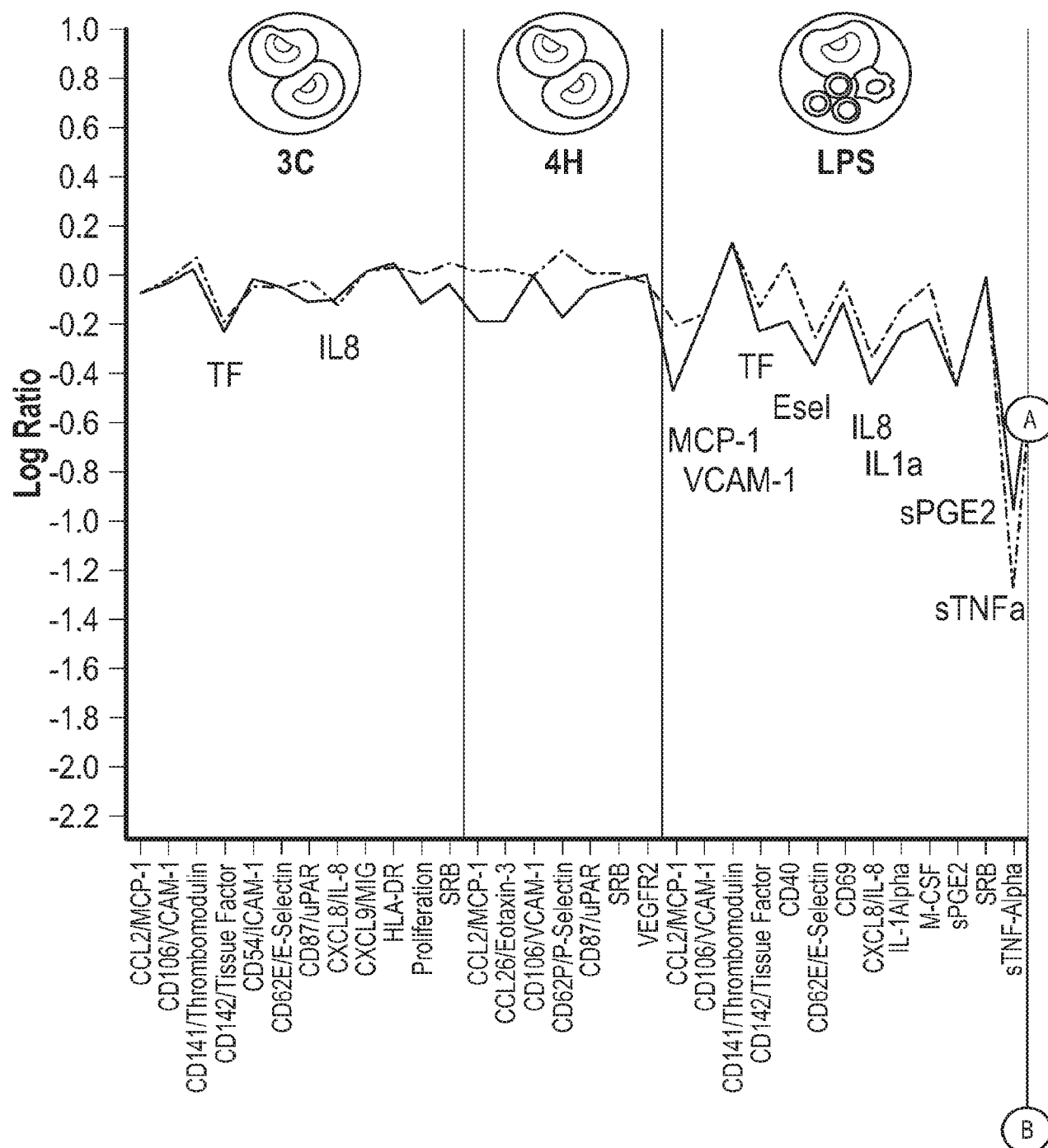


FIG. 64

91/207

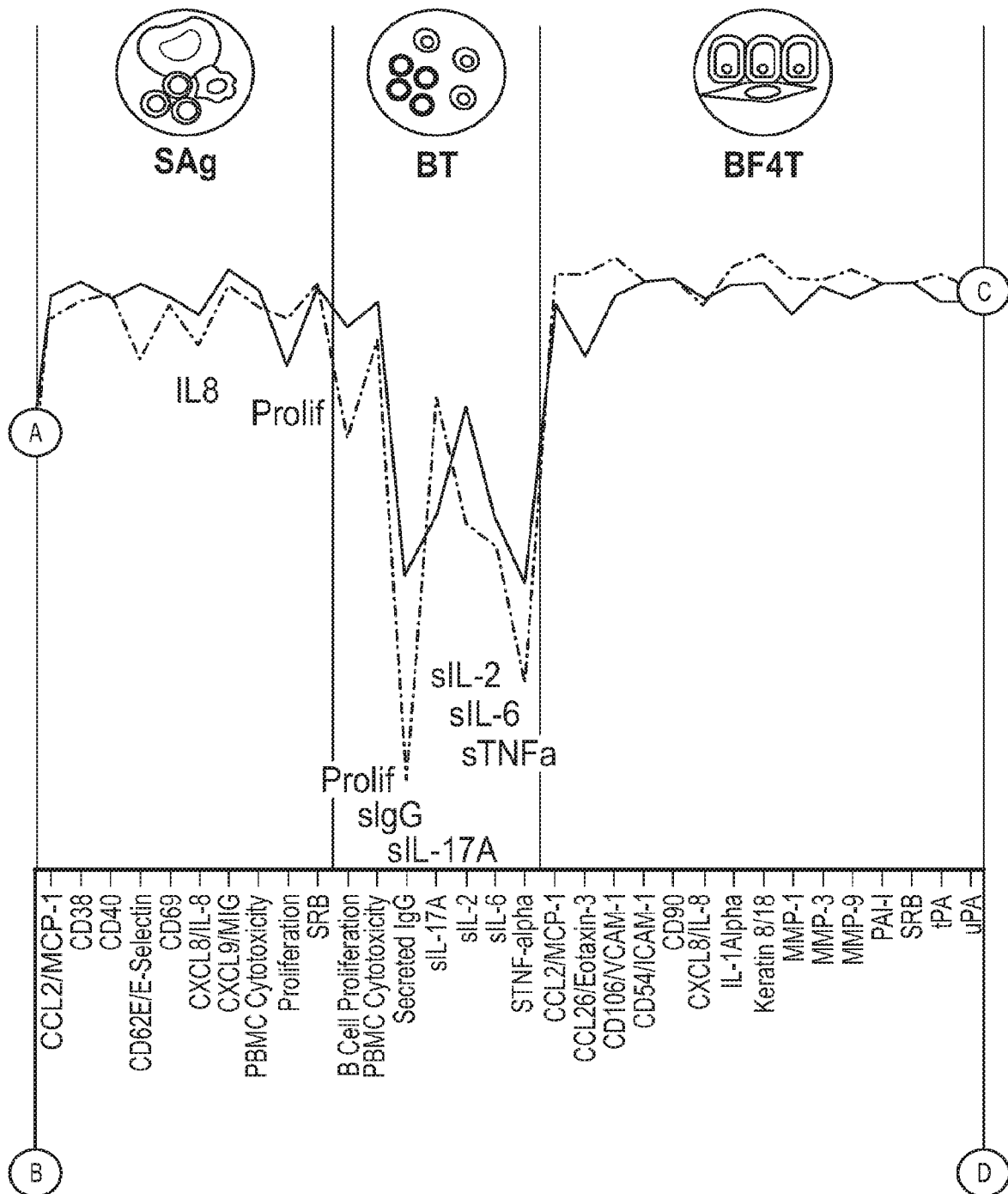


FIG. 64
(Continued)

92/207

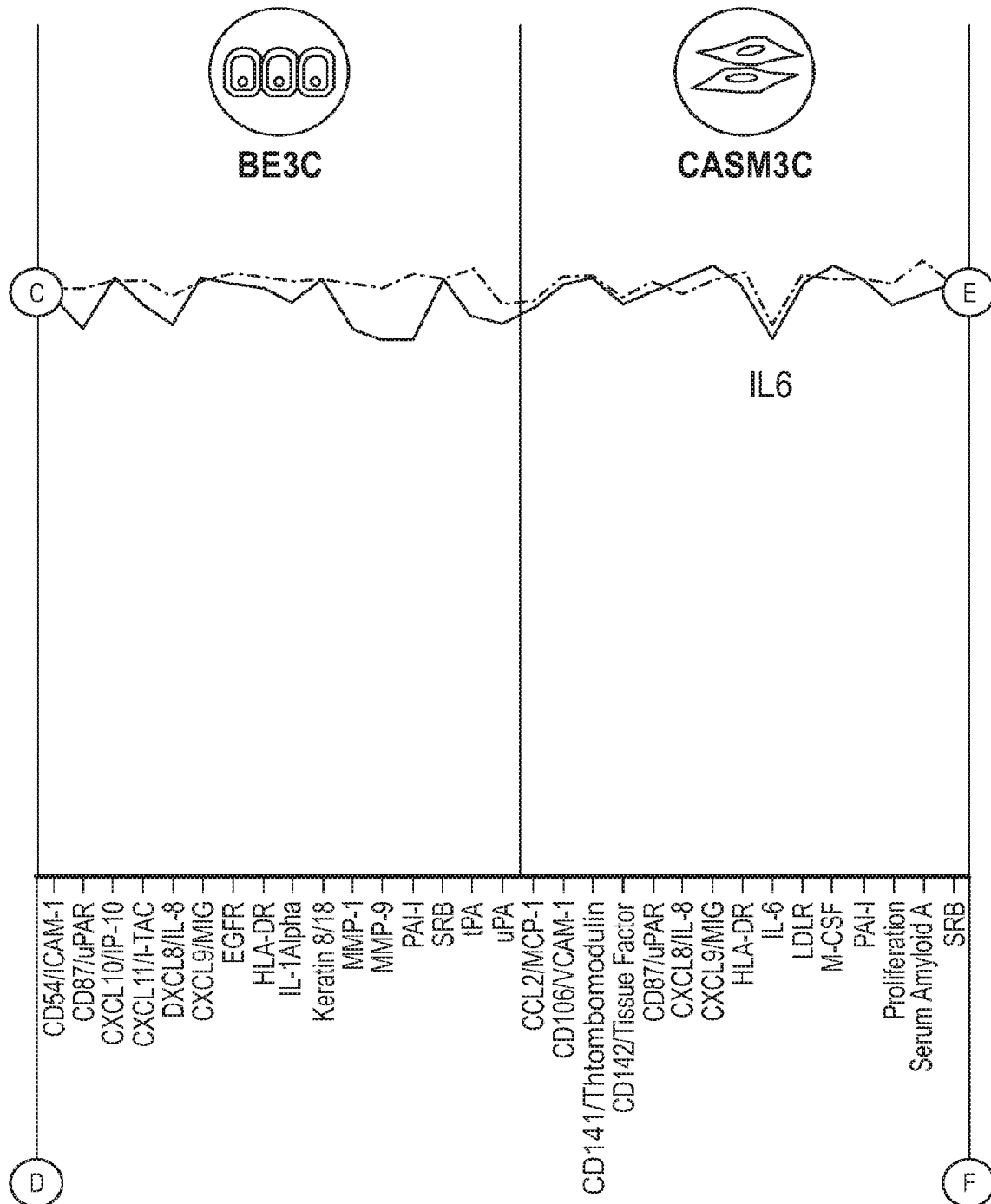


FIG. 64
(Continued)

93/207

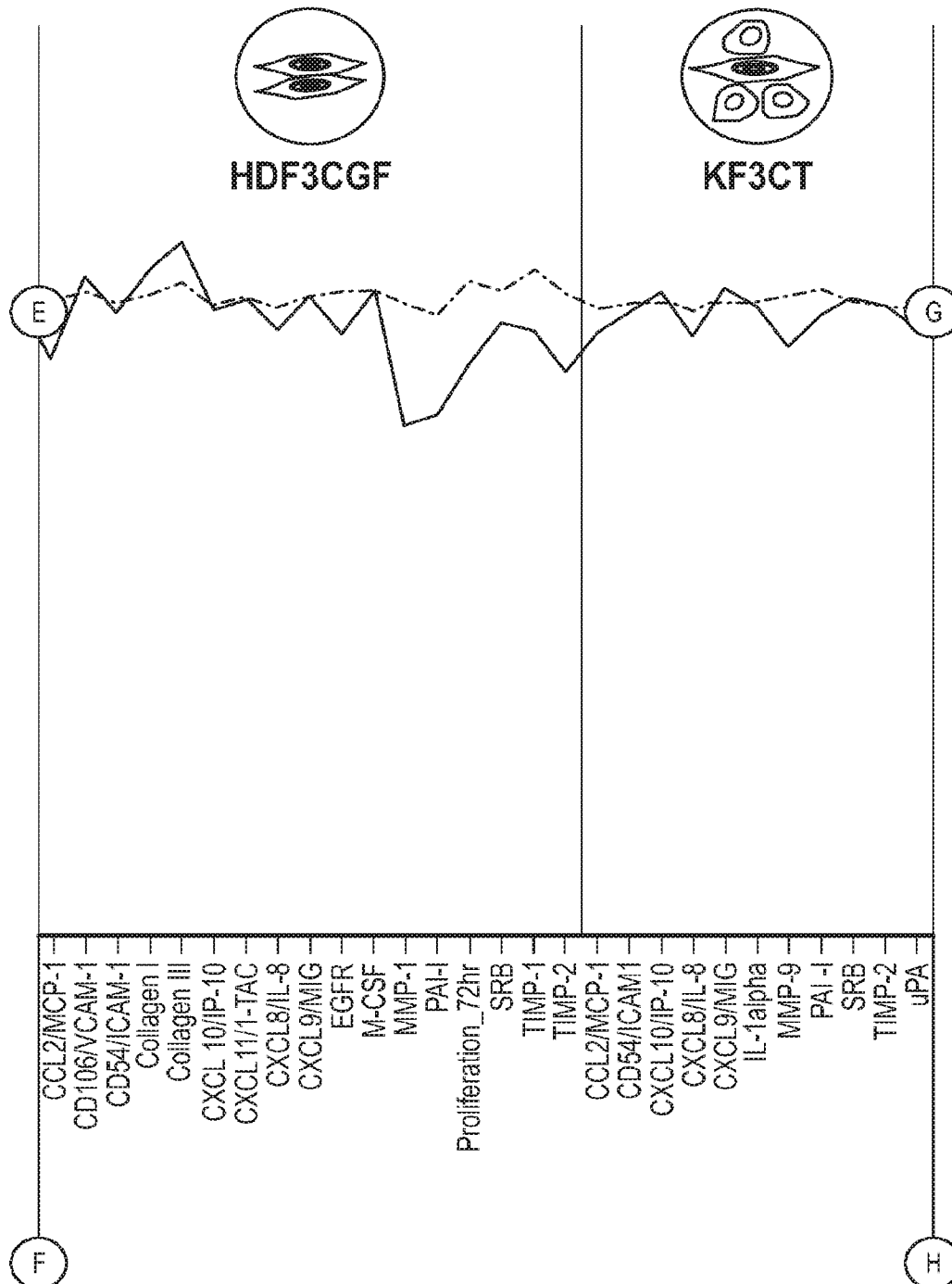


FIG. 64
(Continued)

94/207

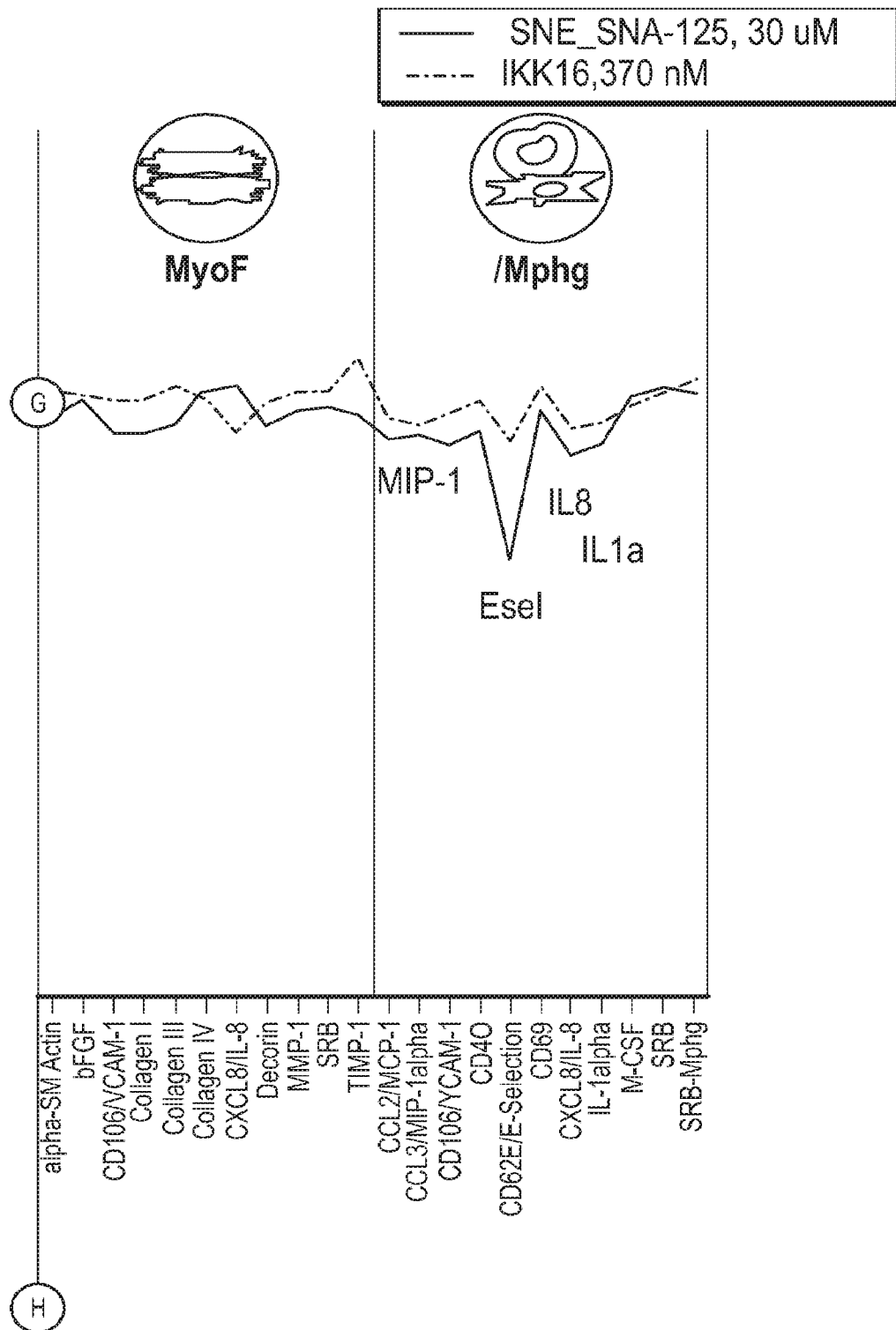


FIG. 64
(Continued)

95/207

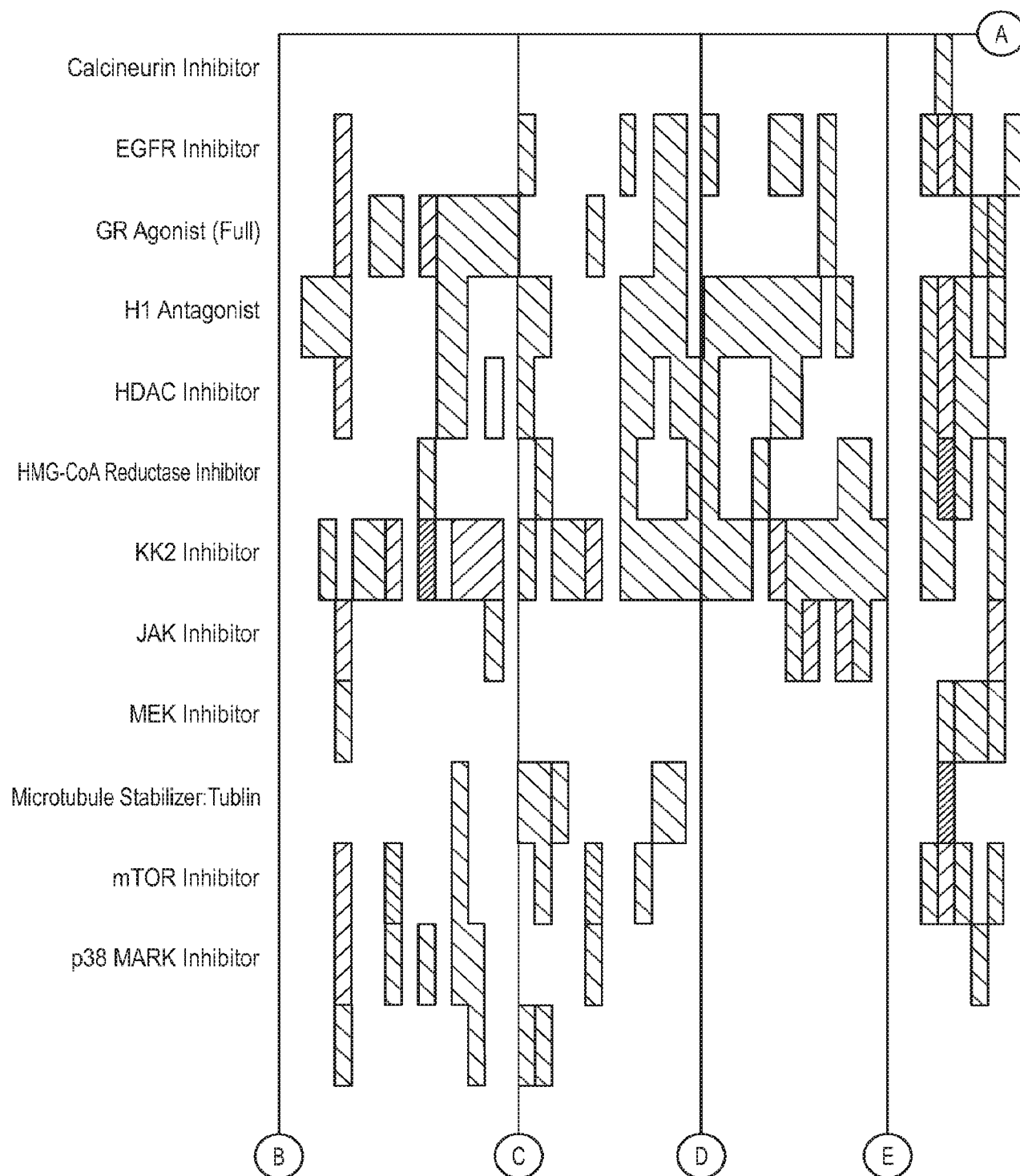


FIG. 65

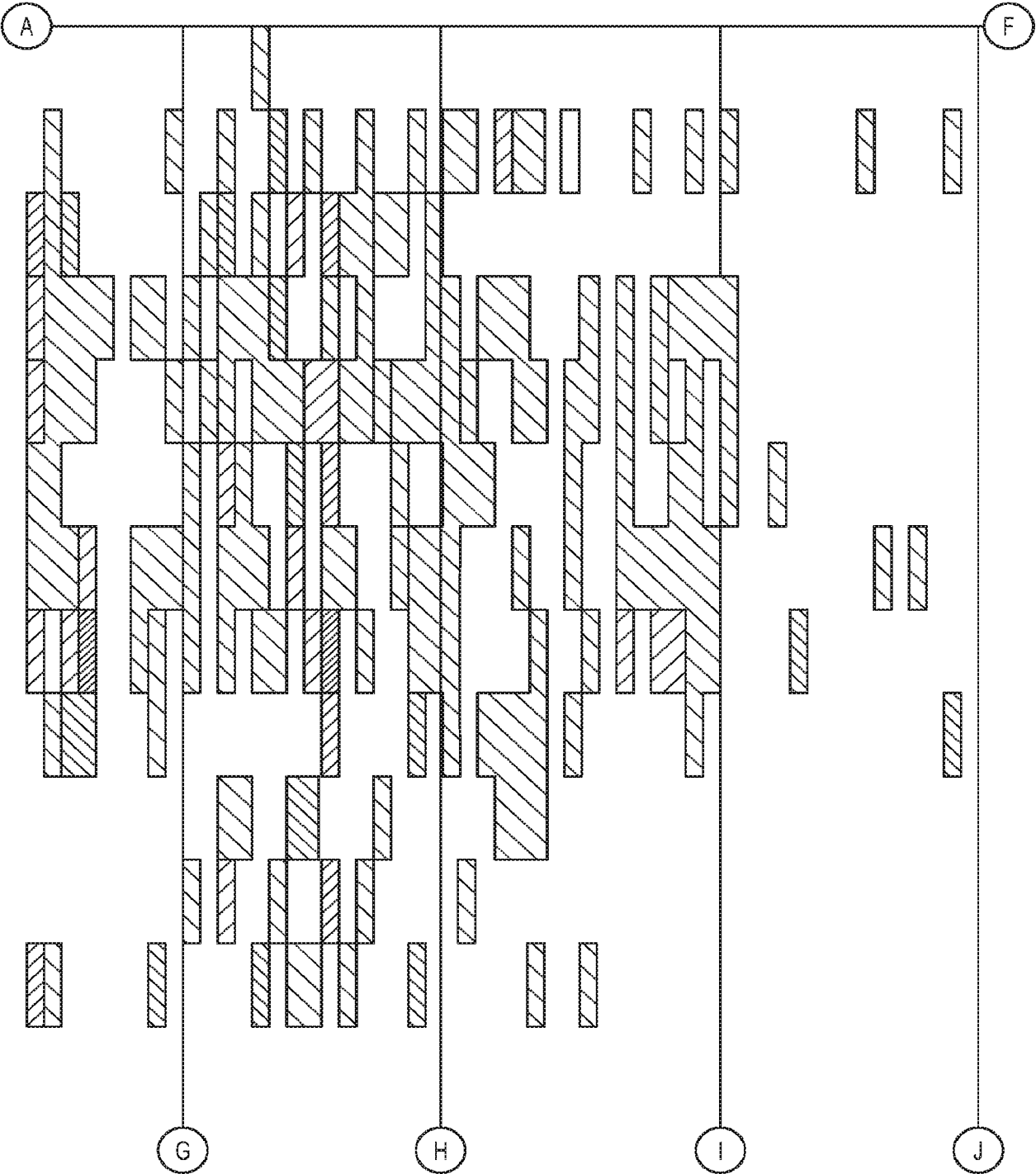


FIG. 65
(Continued)

97/207

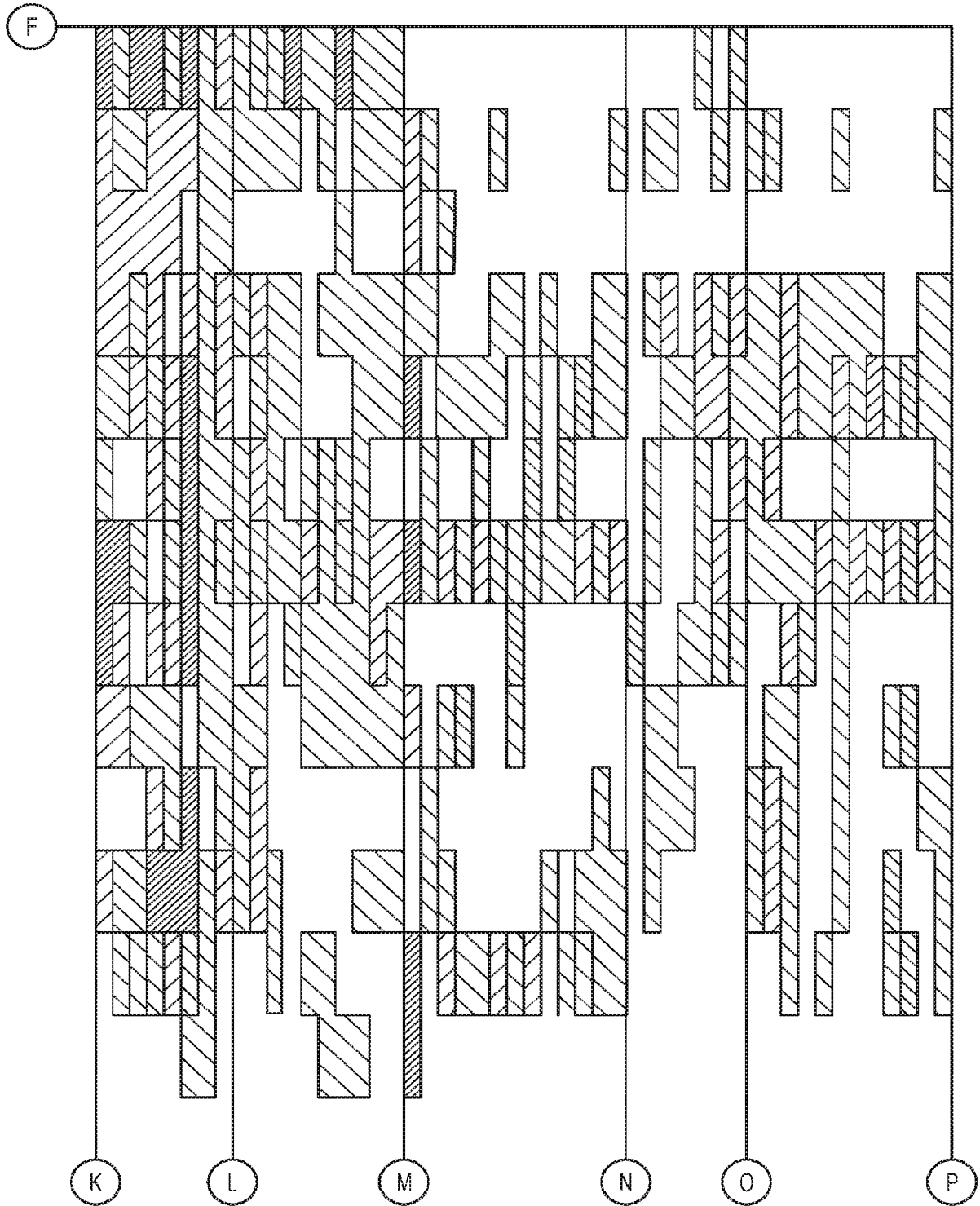


FIG. 65
(Continued)

98/207

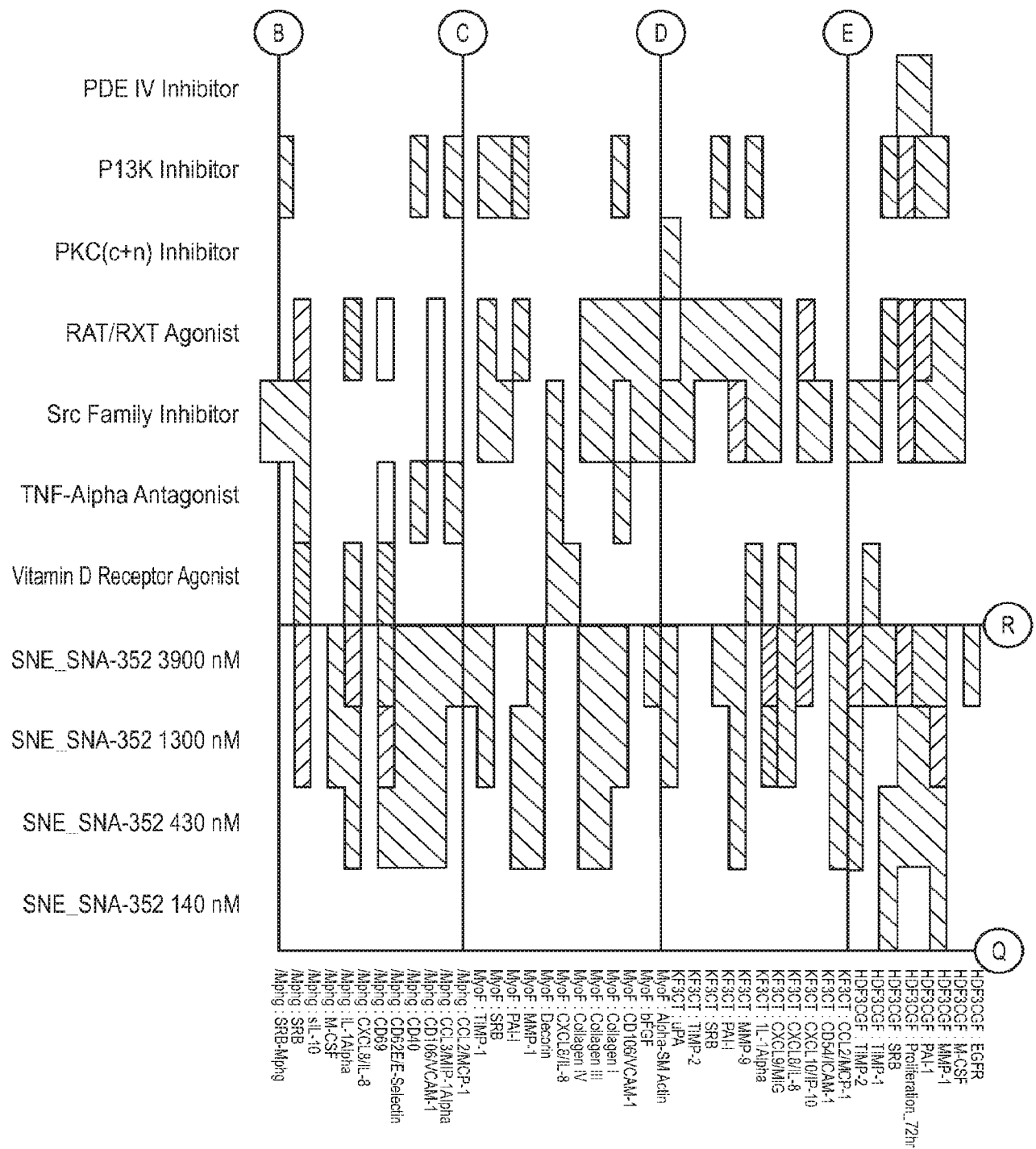
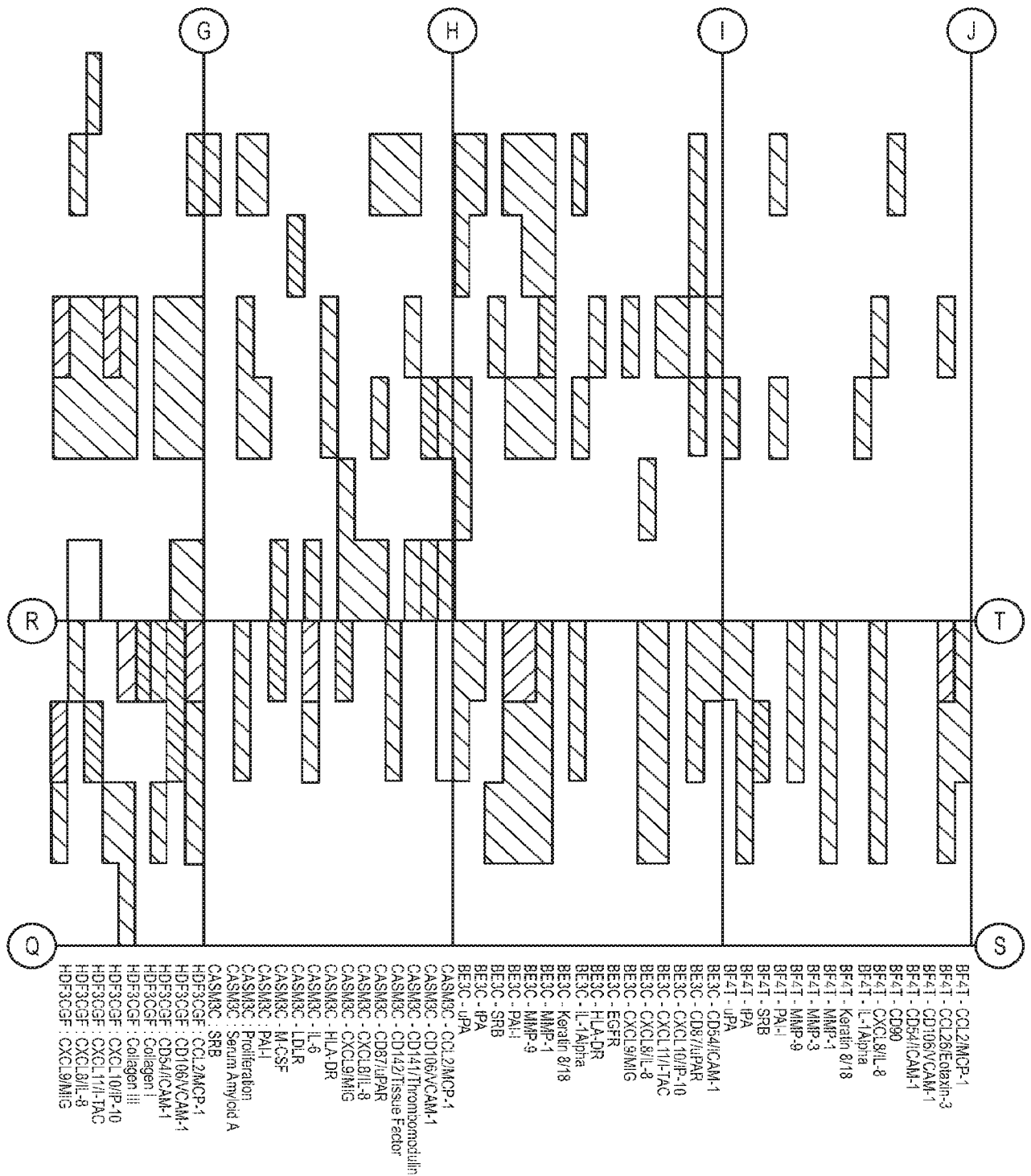


FIG. 65
(Continued)

99/207

FIG. 65
(Continued)

100/207

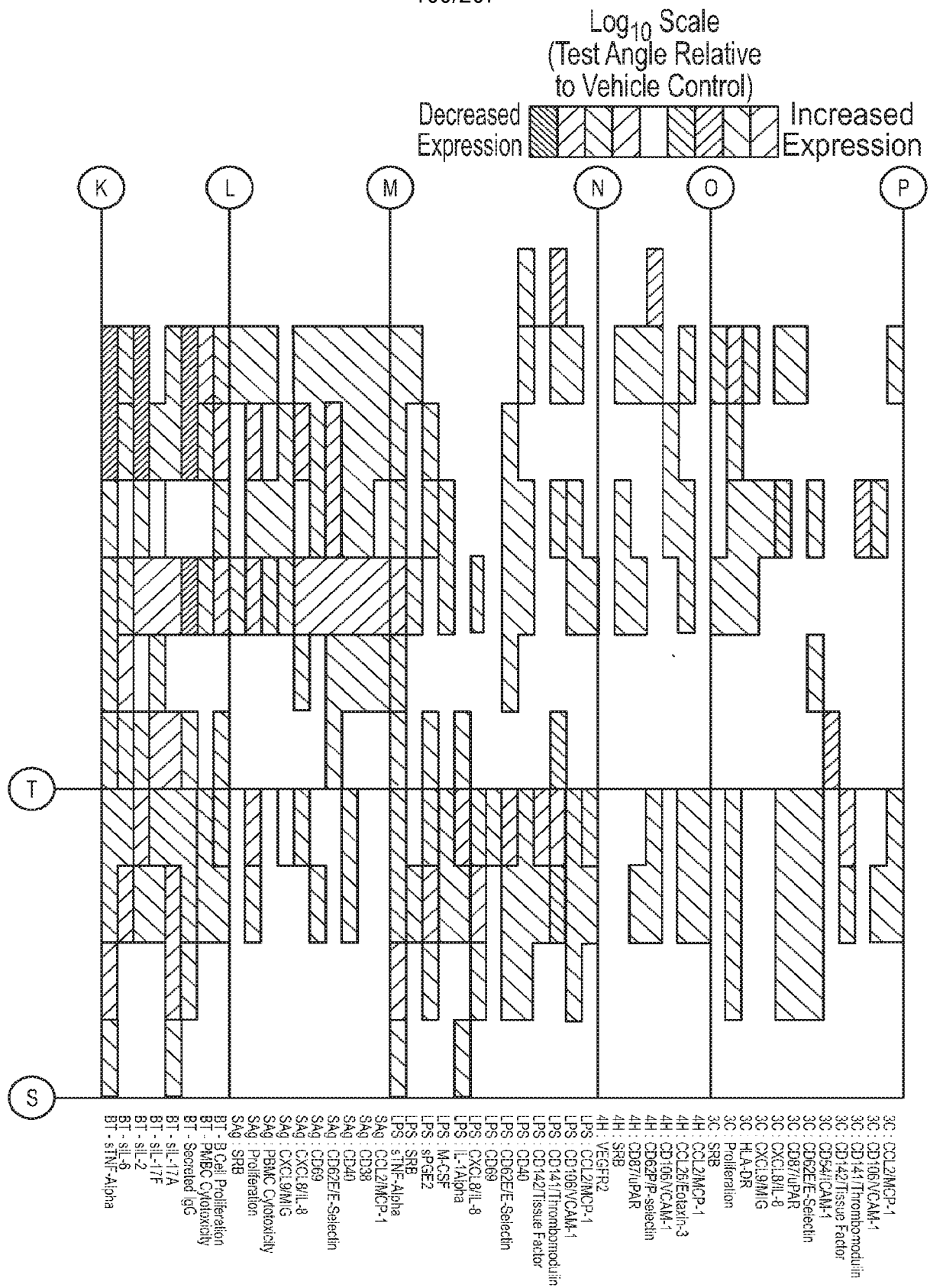


FIG. 65
(Continued)

101/207

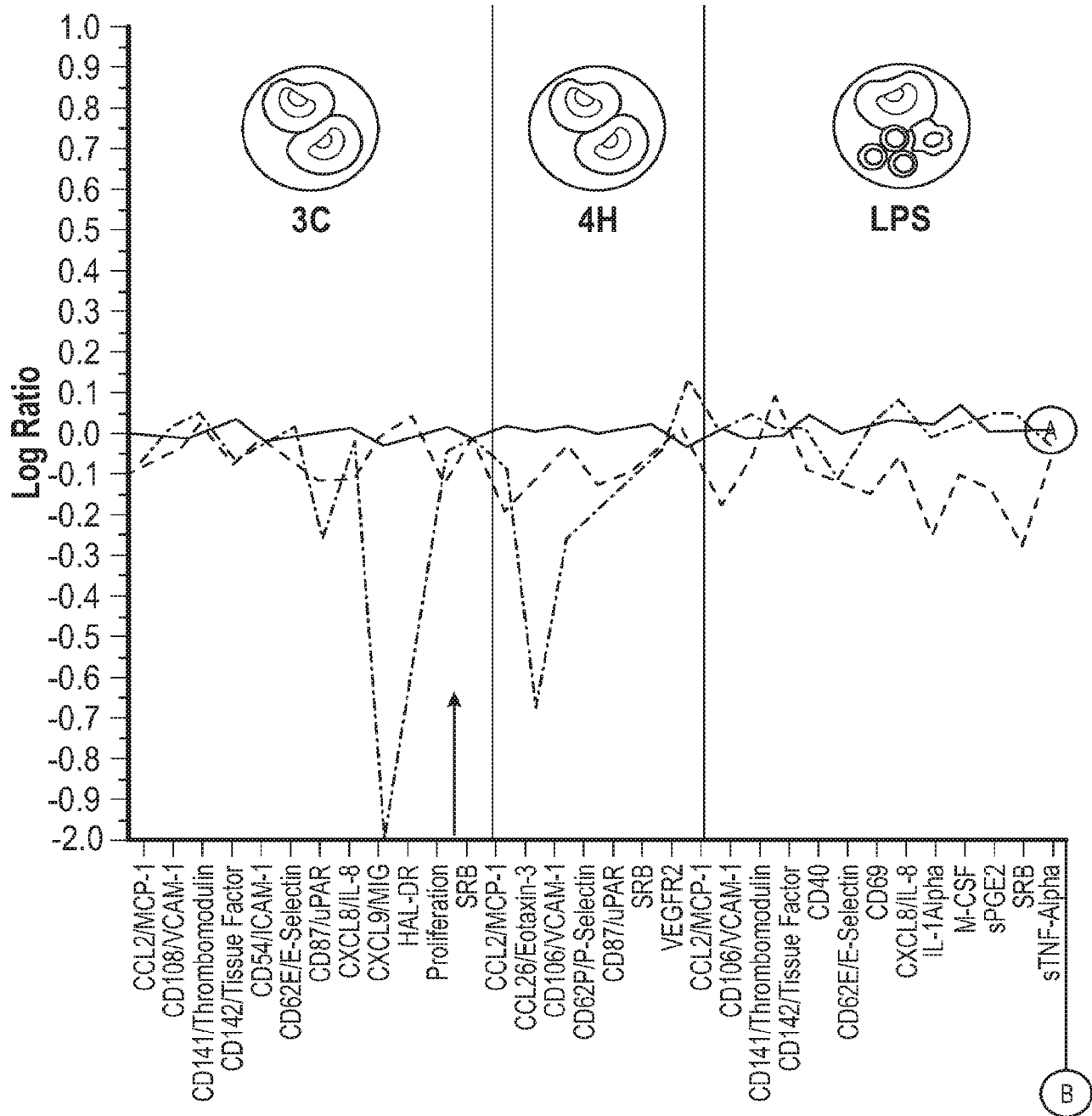
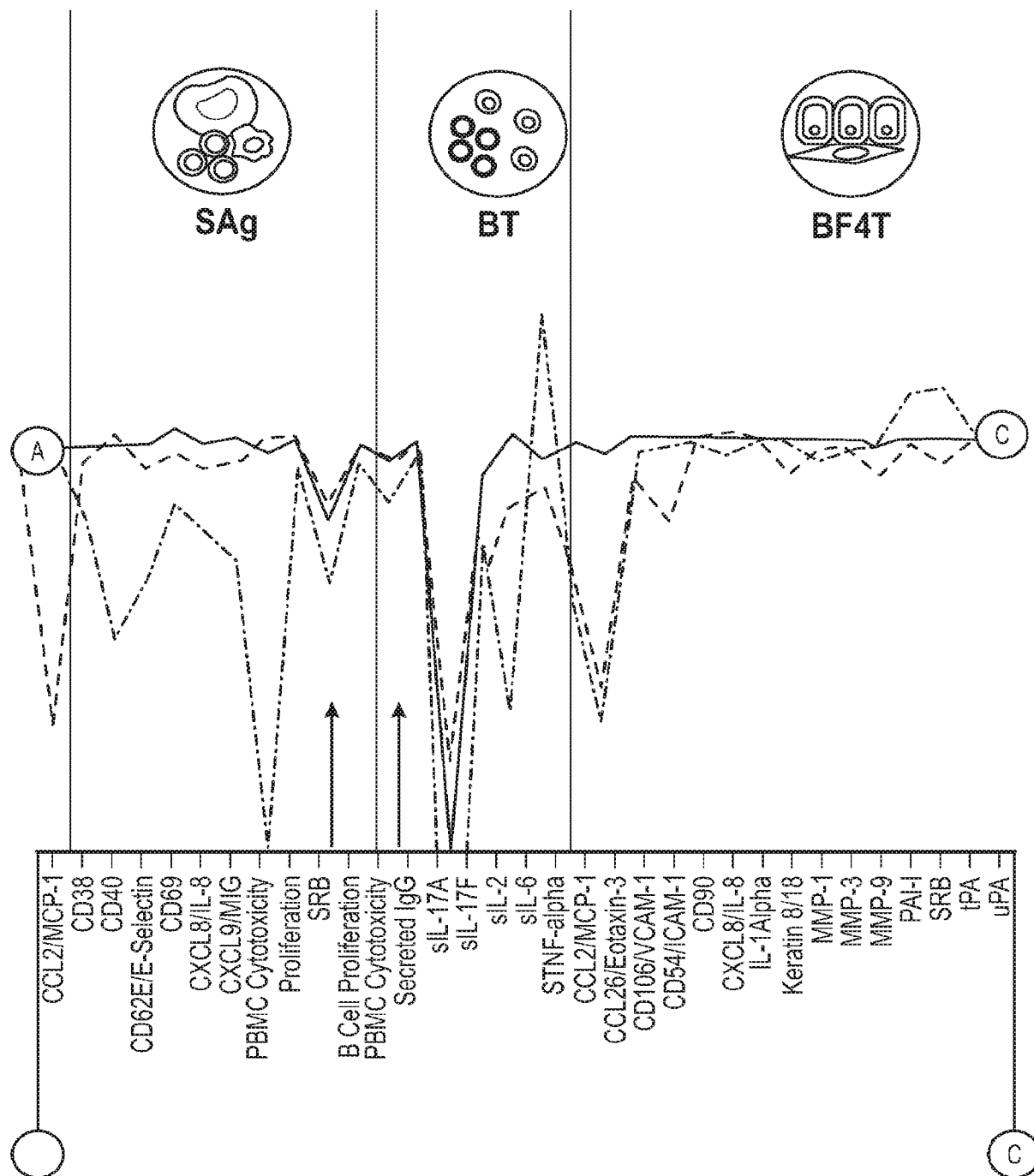


FIG. 66

102/207



103/207

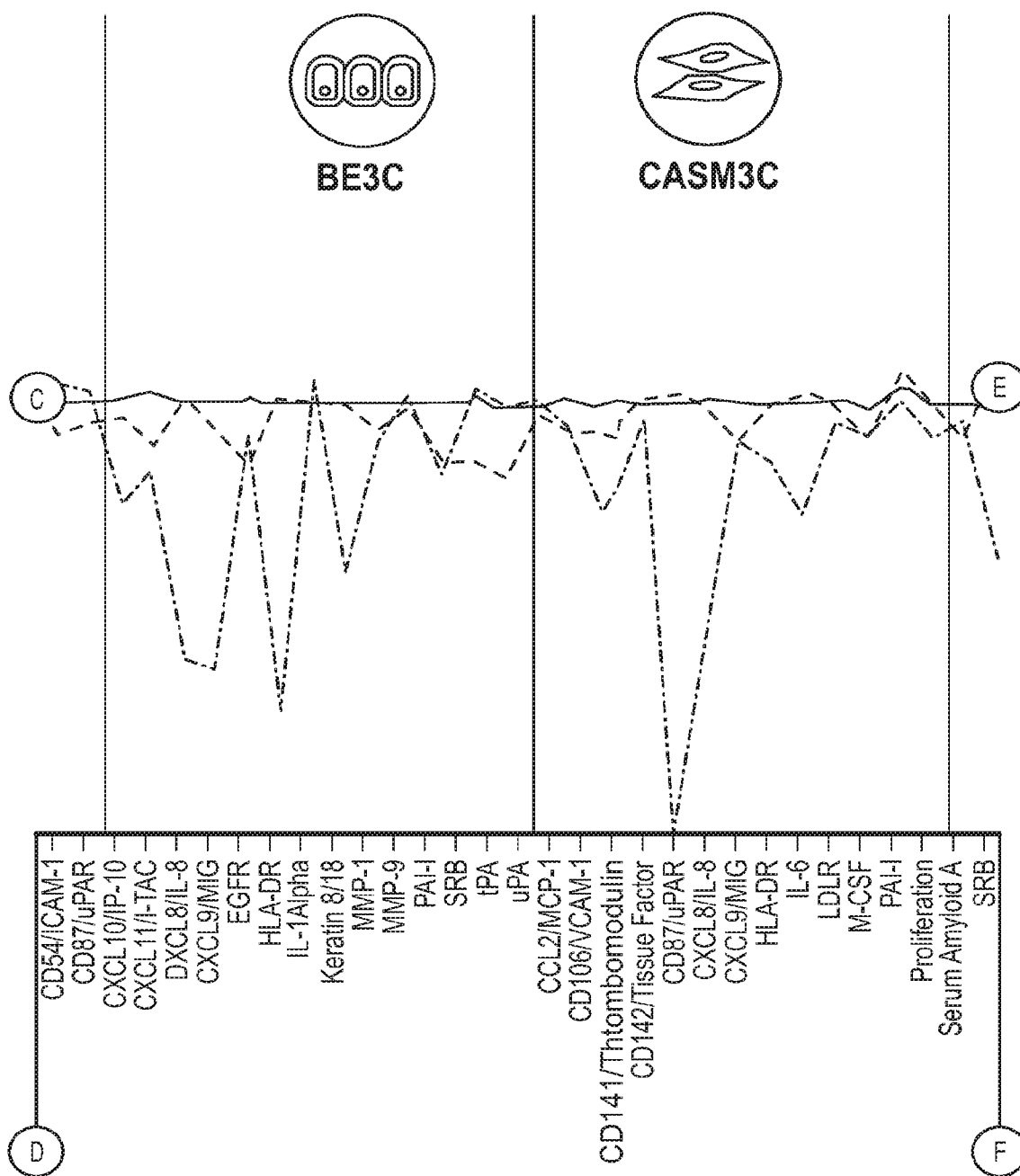


FIG. 66
(Continued)

104/207

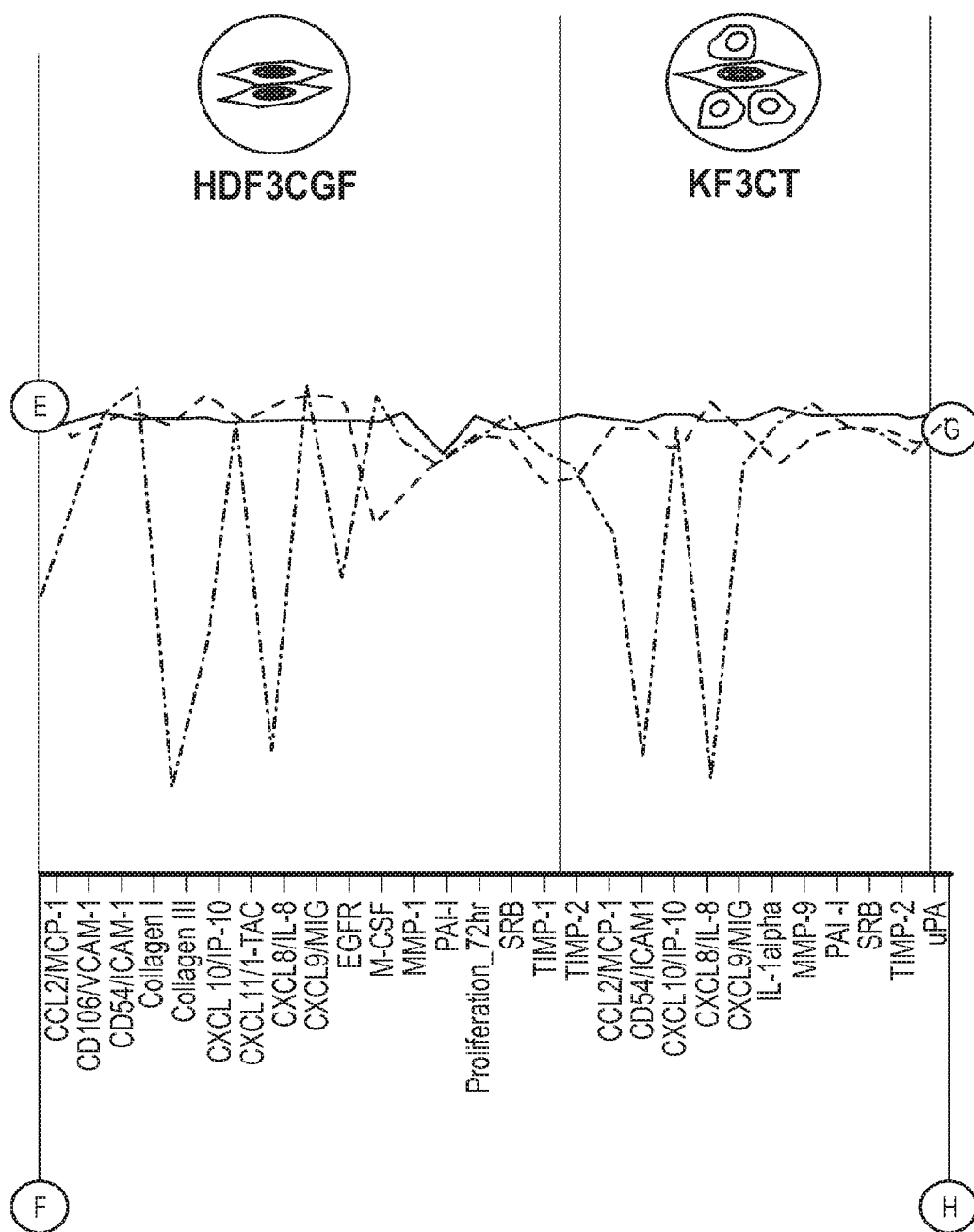


FIG. 66
(Continued)

105/207

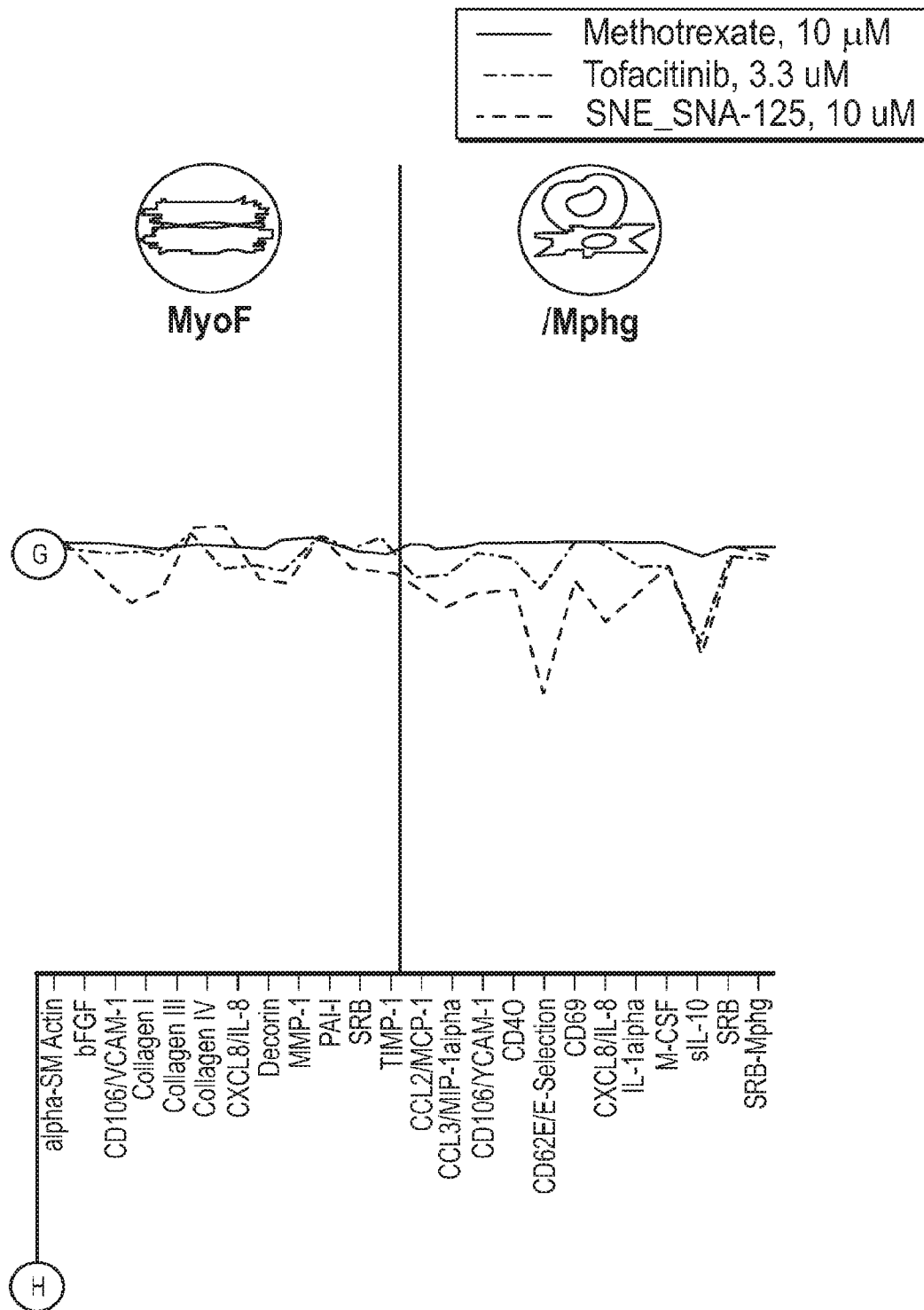
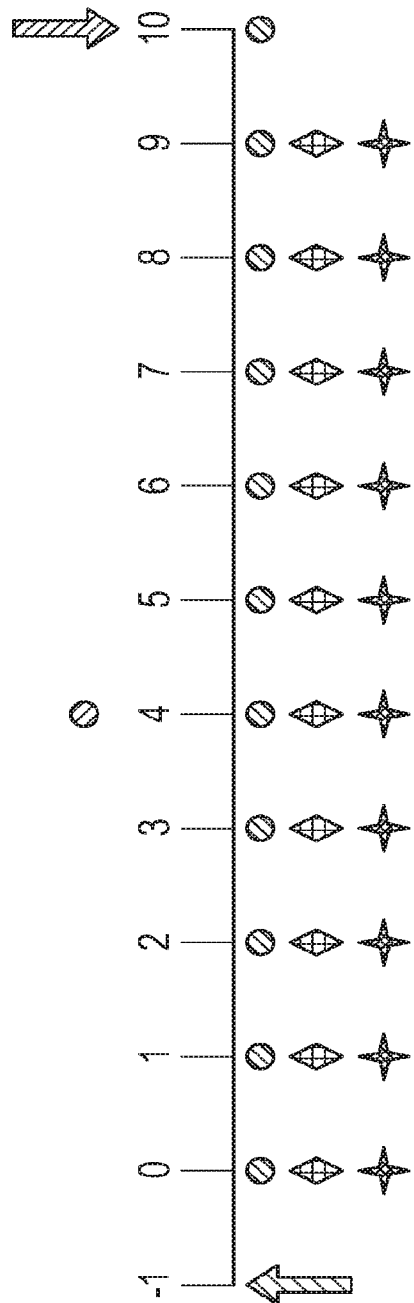
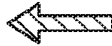


FIG. 66
(Continued)



Animal Shaving

IMQ Cream Application
Daily topical Application of IMQ on Shaved Backs and Onto Ears

Body Weight, Ear Thickness, and Psoriasis Clinical Score
-Ear Thickness Only Days 0,4,6,8, and 10.

Left Ear Biopsy Punch

Treatment:
Test Items Administered

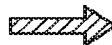
Termination:
Animals will be Terminated on Day 10. Spleen and Back Skin will be Collected

FIG. 67

107/207

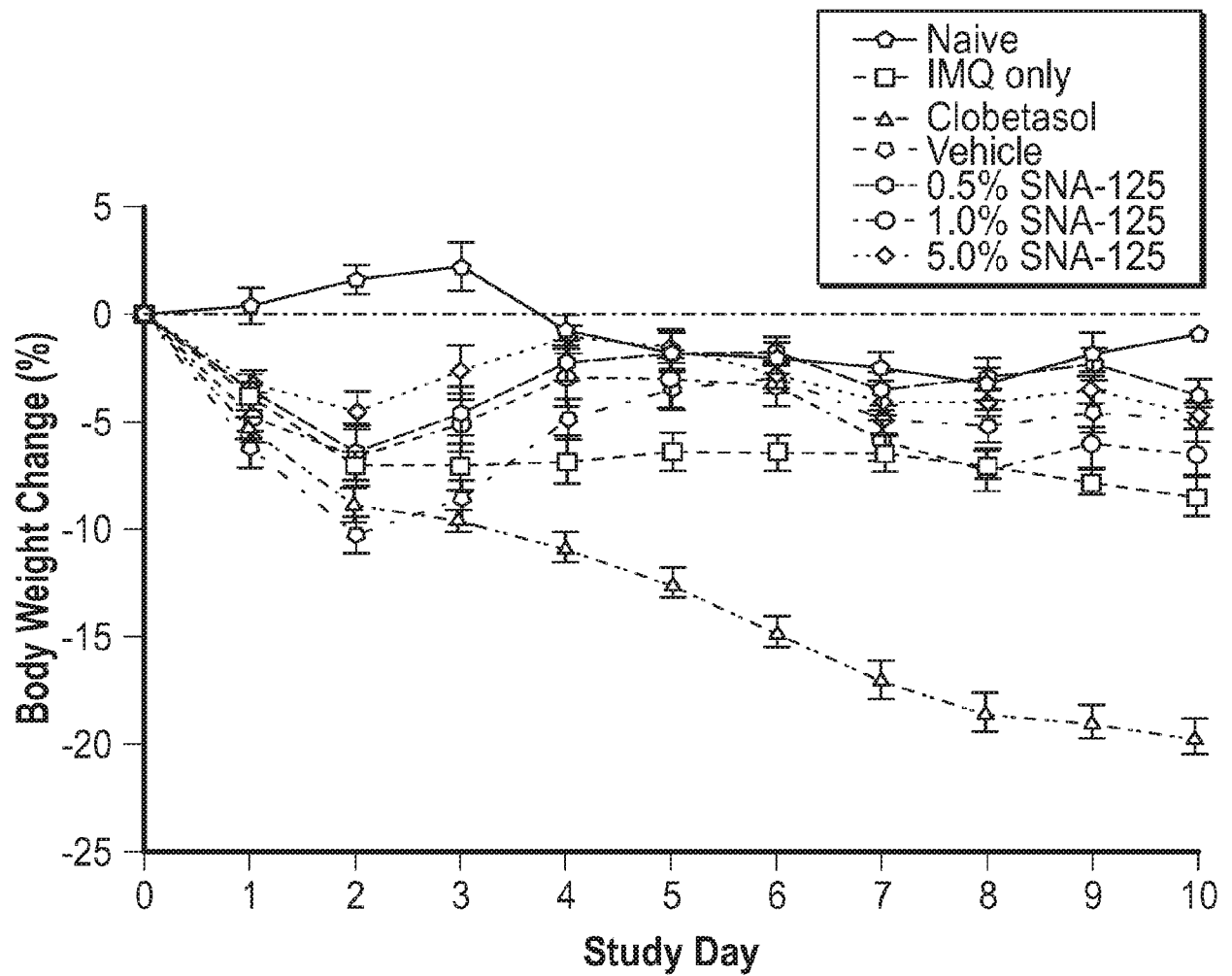


FIG. 68

108/207

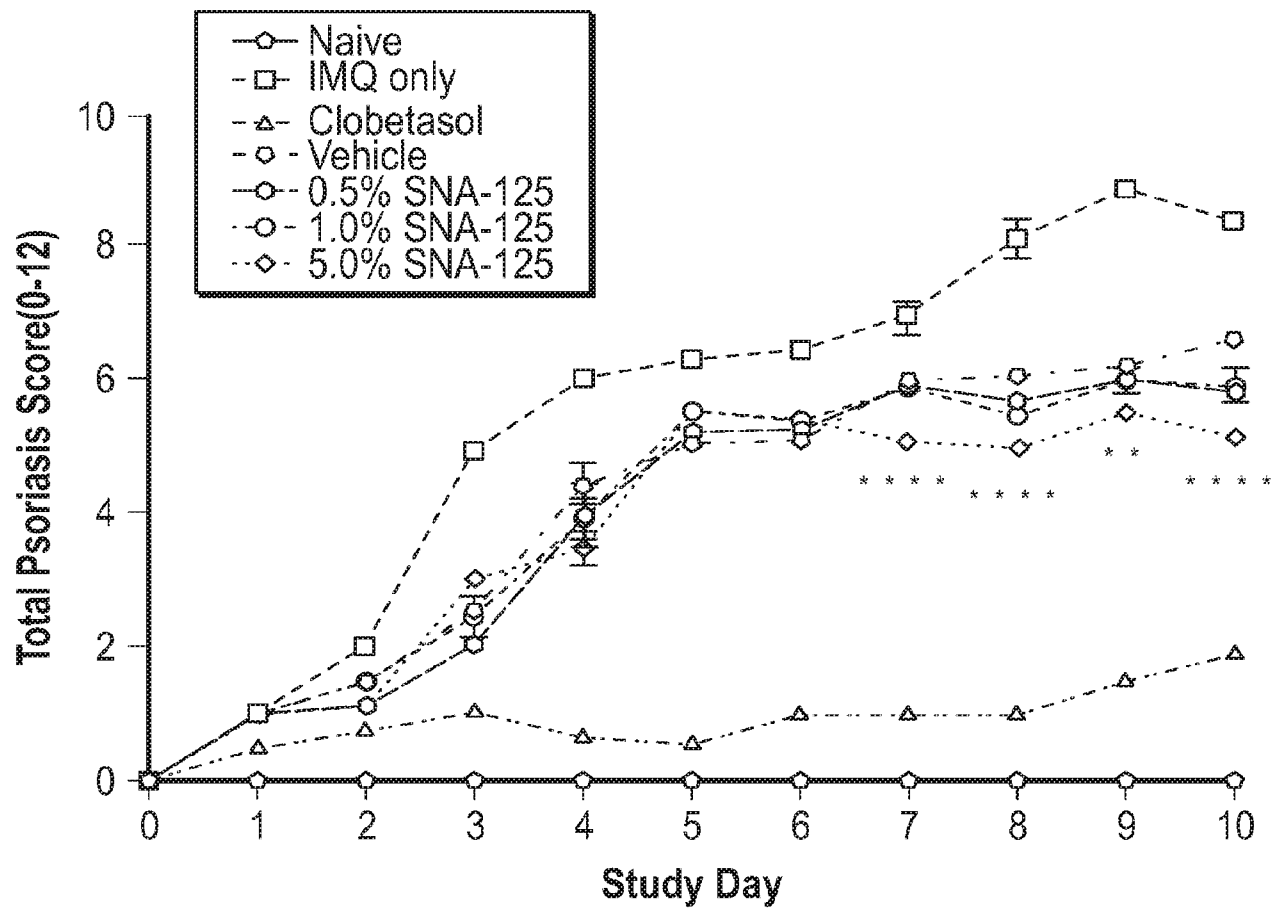


FIG. 69

109/207

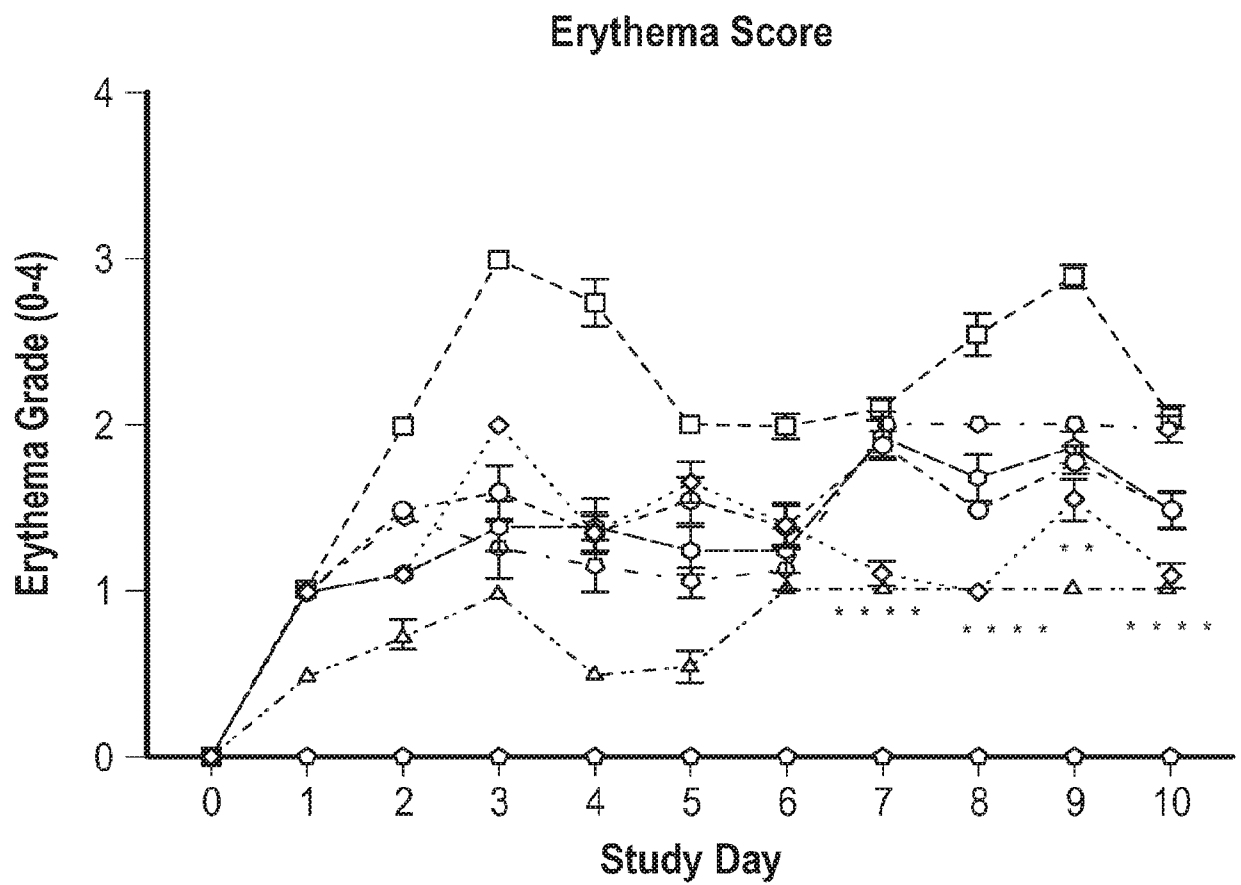


FIG. 70

110/207

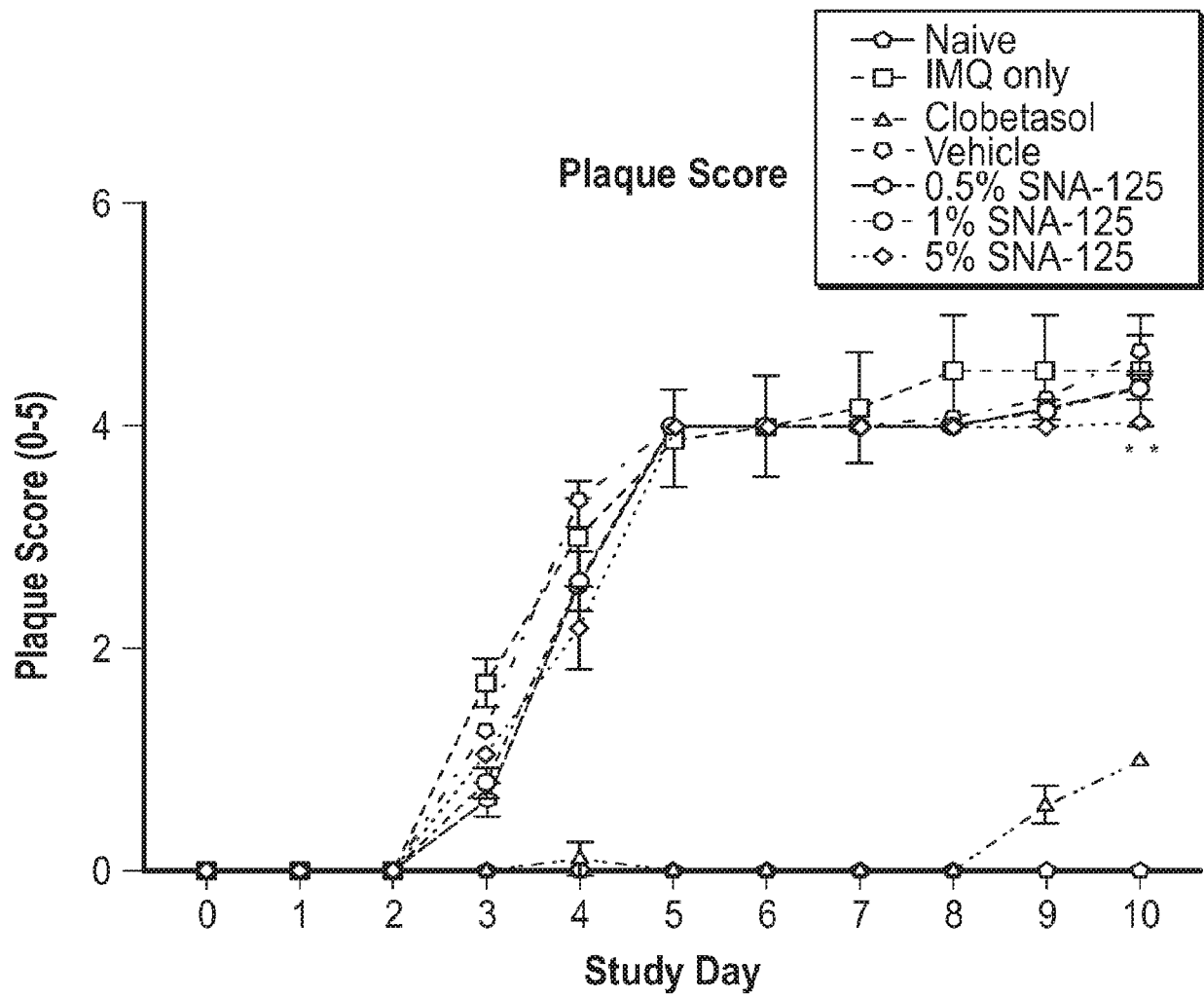


FIG. 71

111/207

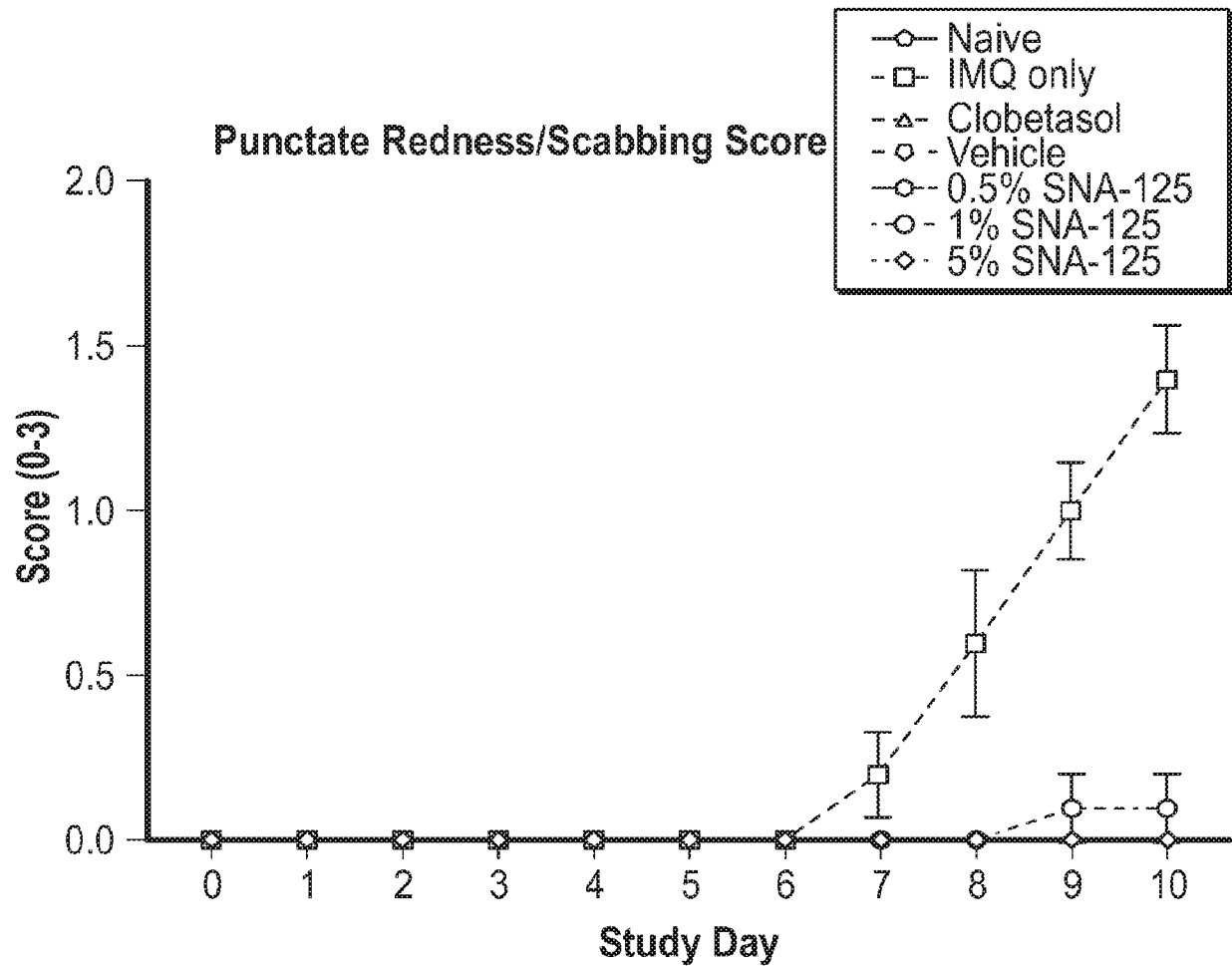


FIG. 72

112/207

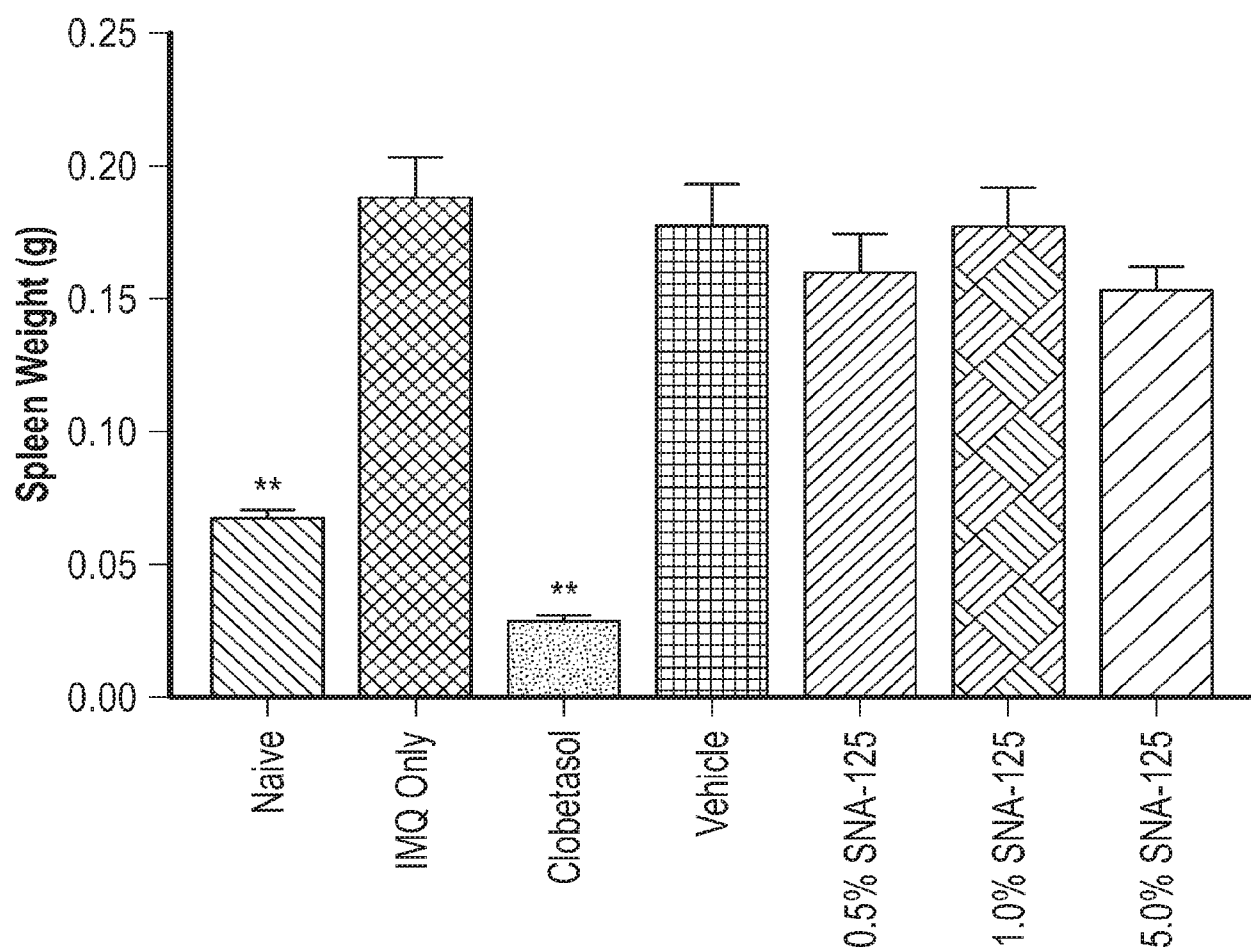


FIG. 73

113/207

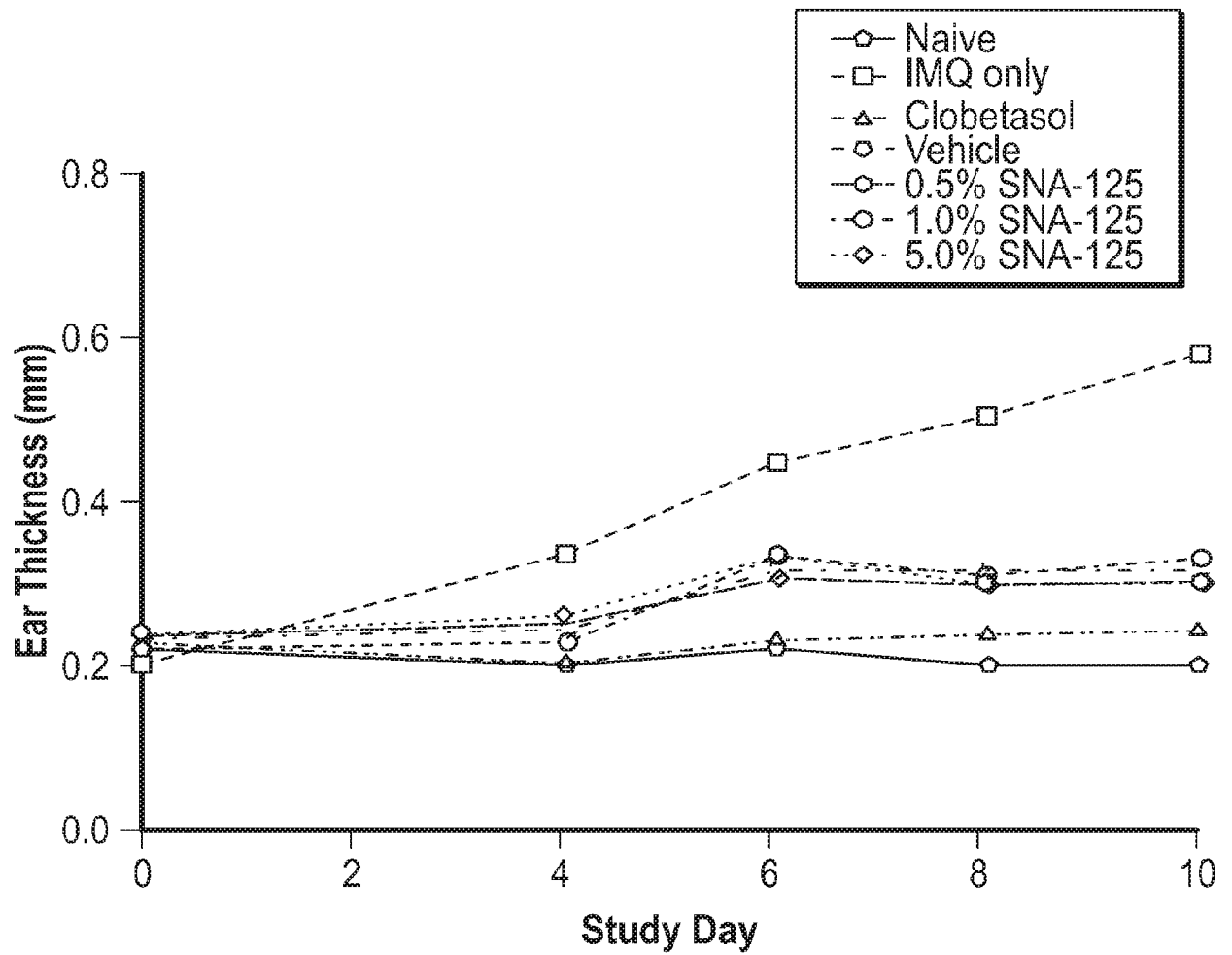


FIG. 74

114/207

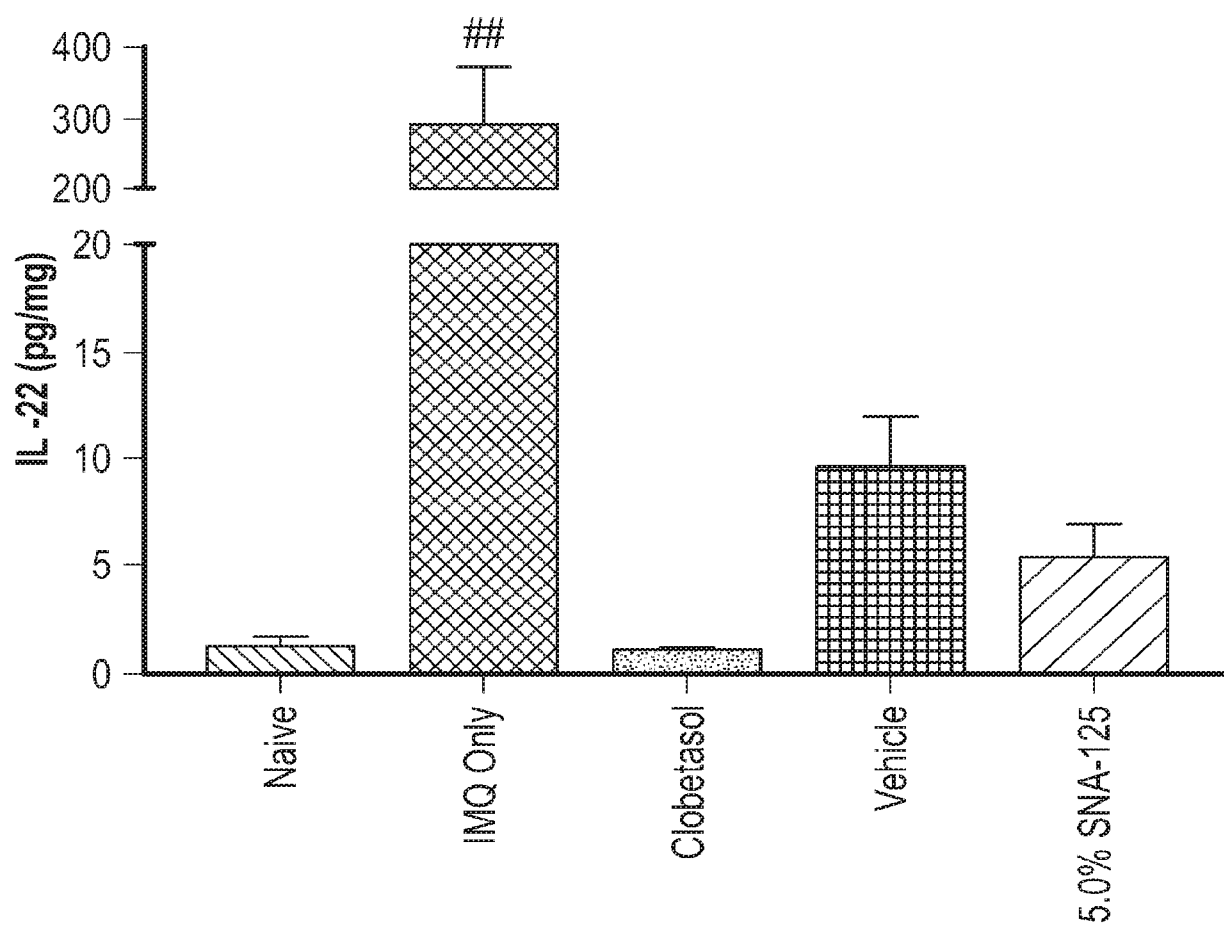


FIG. 75A

115/207

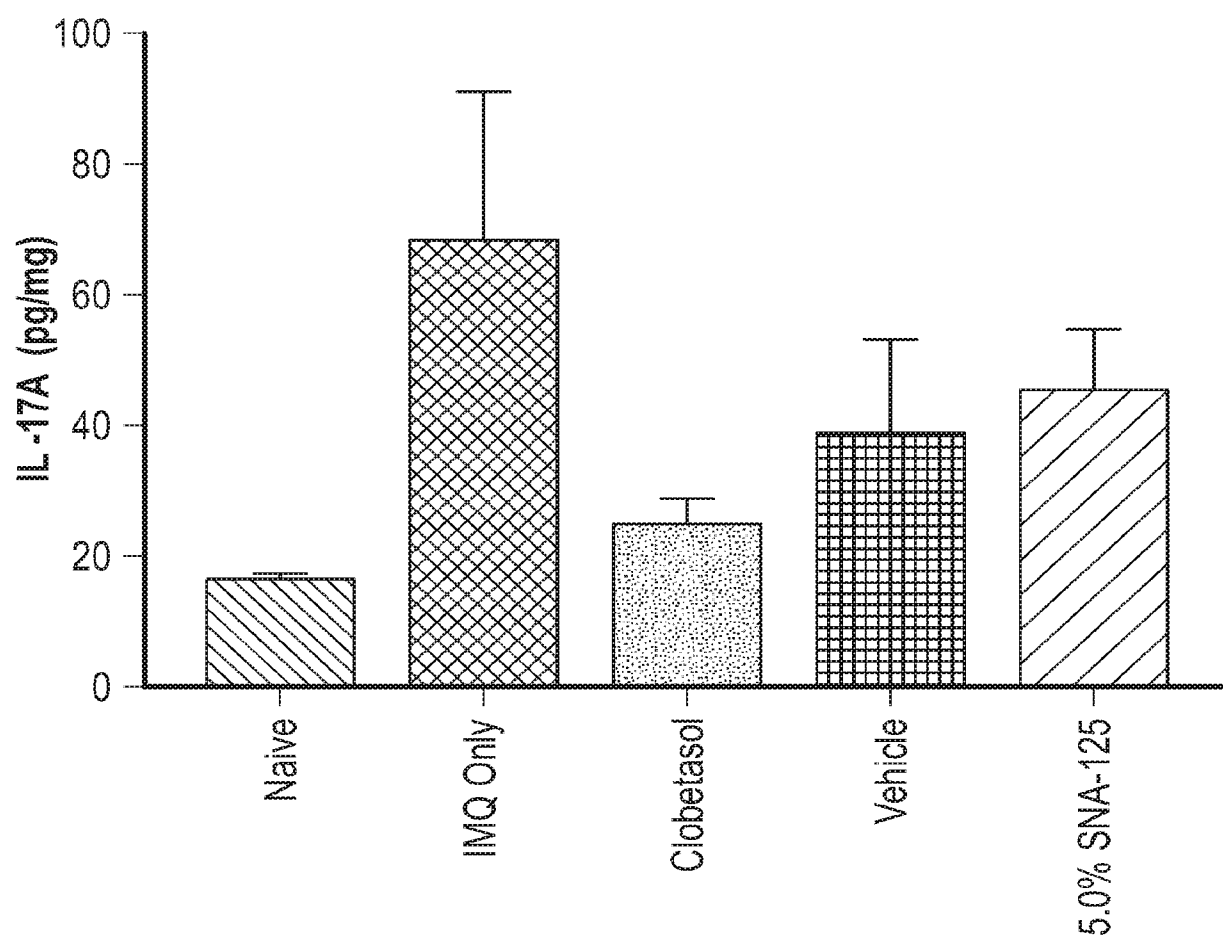


FIG. 75B

IL-17F (pg/mg)

Naive IMQ Only Clobetasol Vehicle 5.0% SNA-125

##

Group	IL-17F (pg/mg)
Naive	~2.0
IMQ Only	~250
Clobetasol	~2.0
Vehicle	~4.5
5.0% SNA-125	~4.0

FIG. 75C

117/207

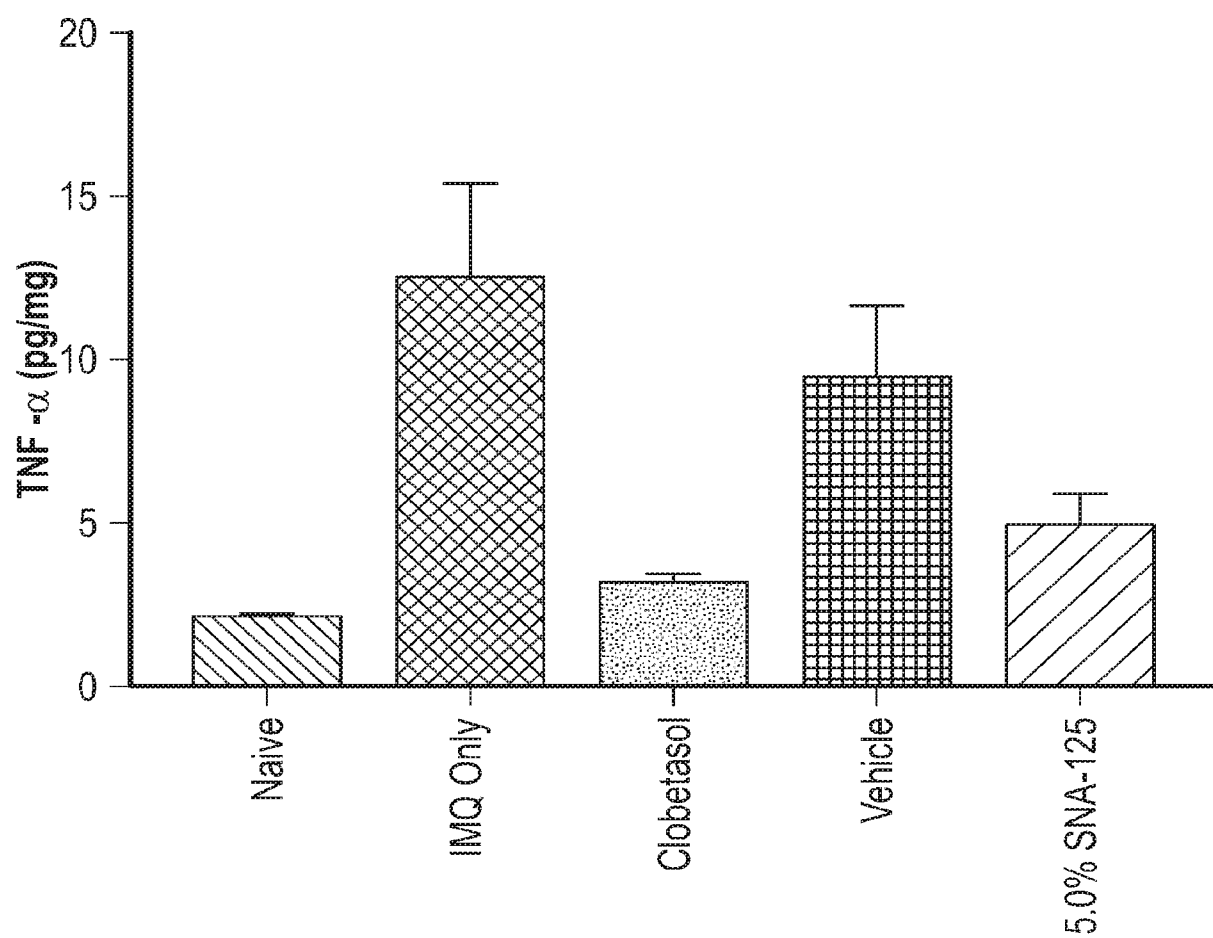


FIG. 75D

118/207

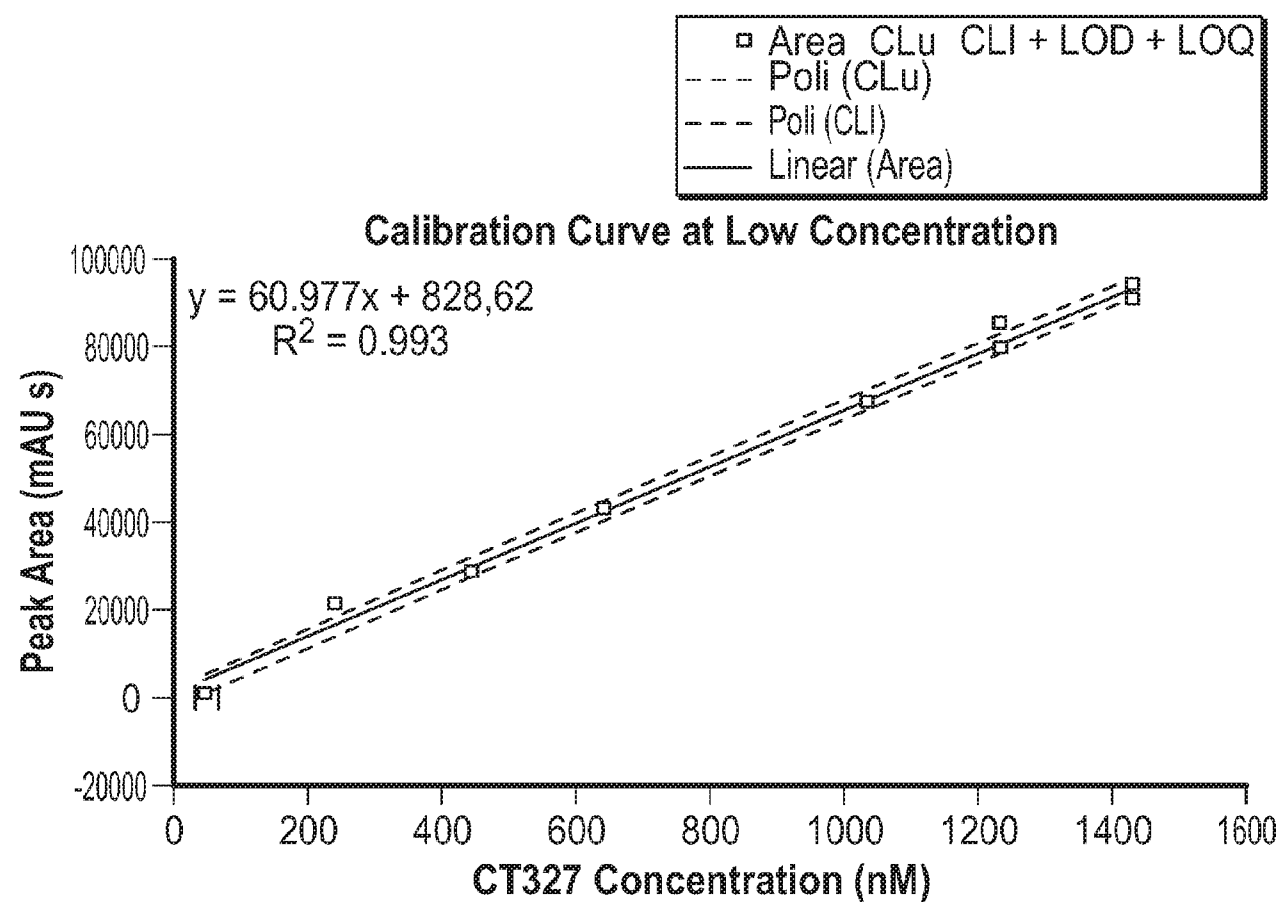
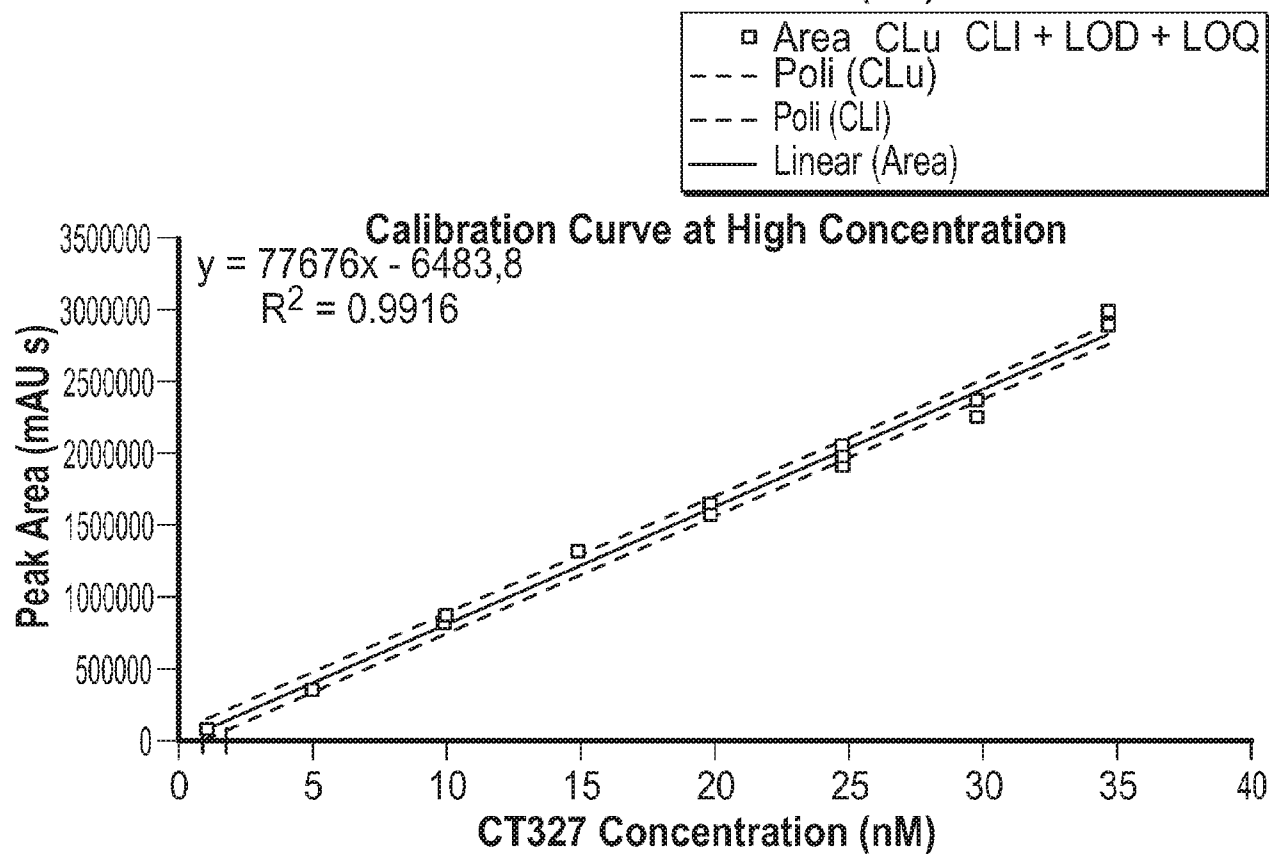
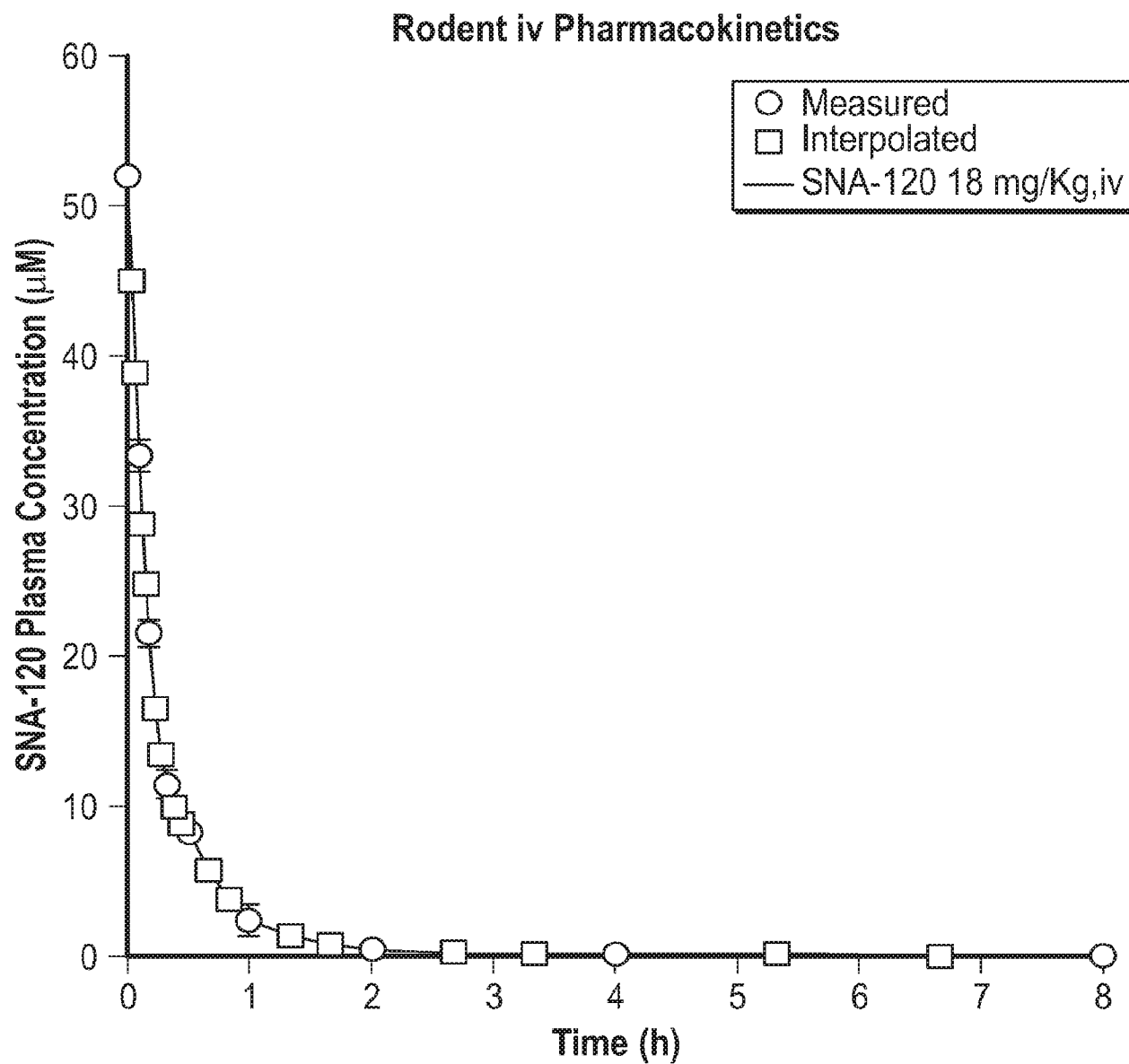


FIG. 76

119/207

**FIG. 77**

120/207

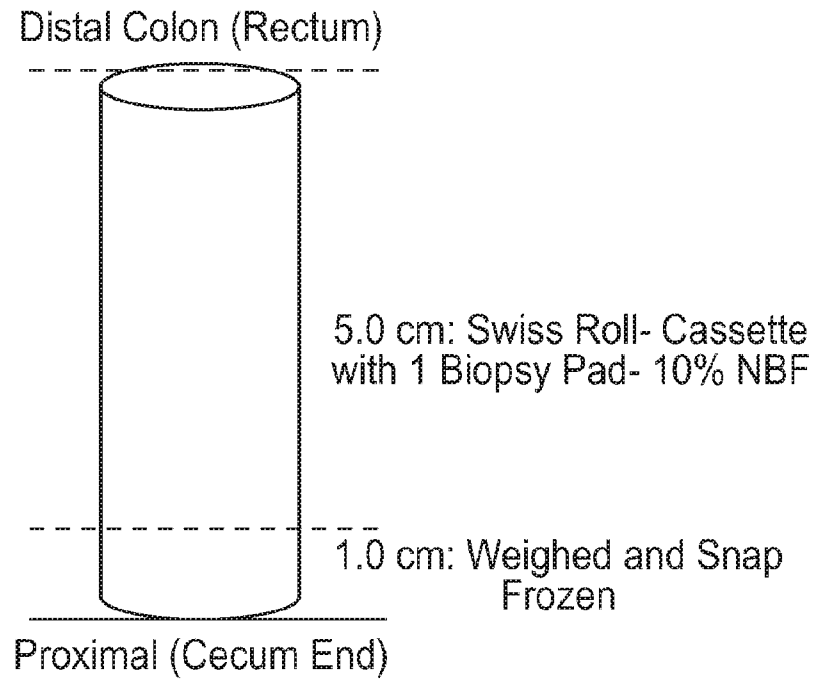


FIG. 78A

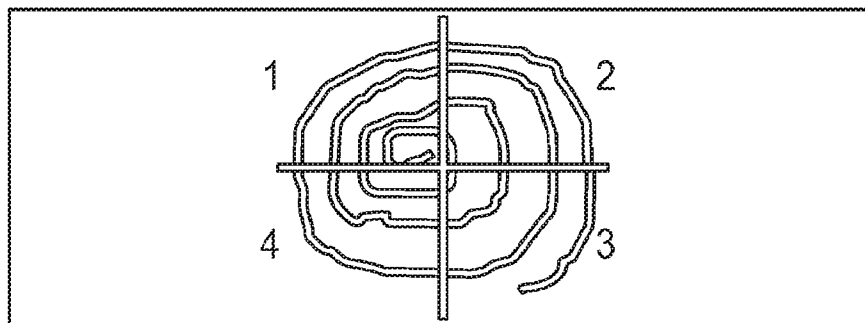


FIG. 78B

121/207

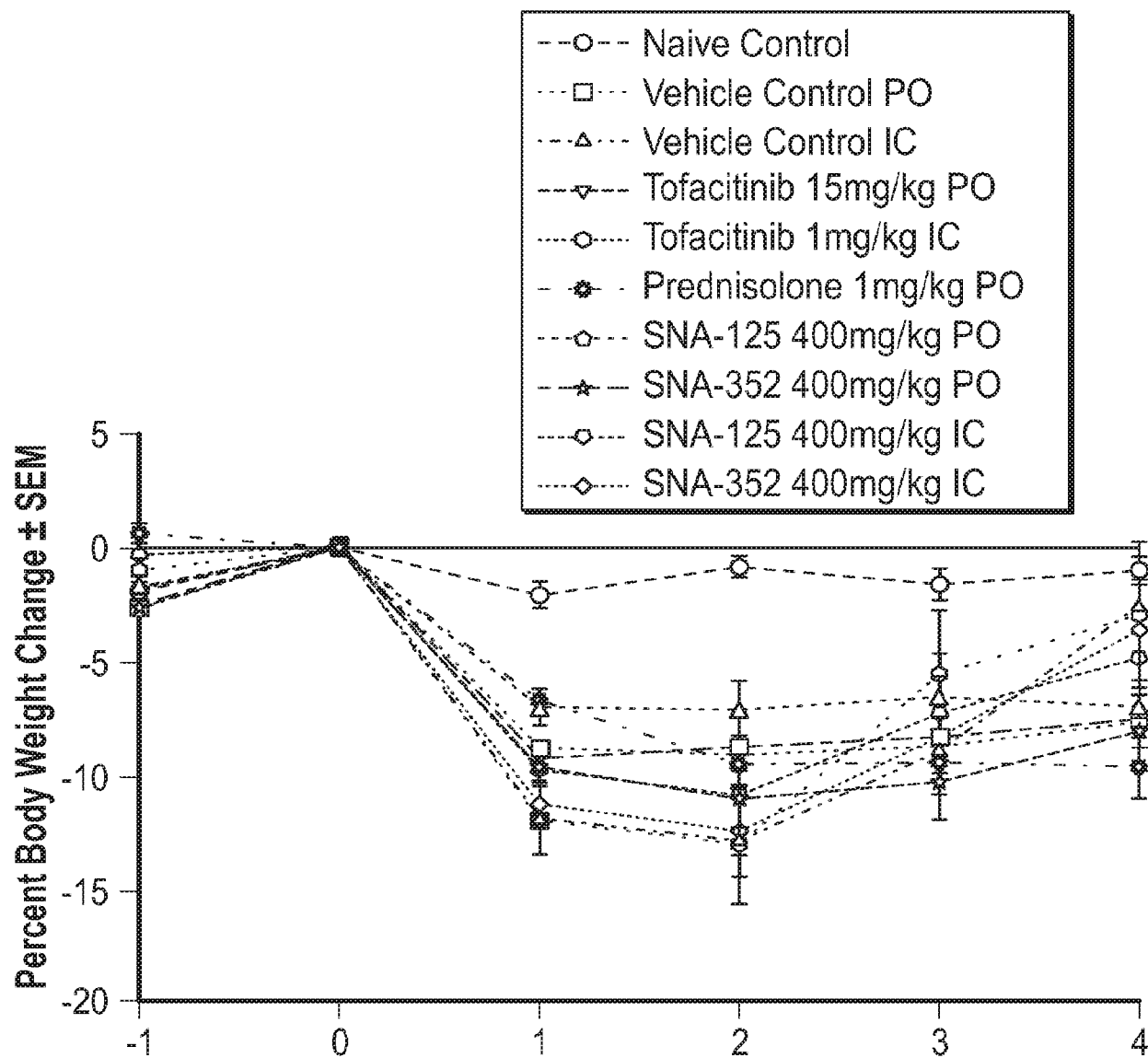


FIG. 79A

122/207

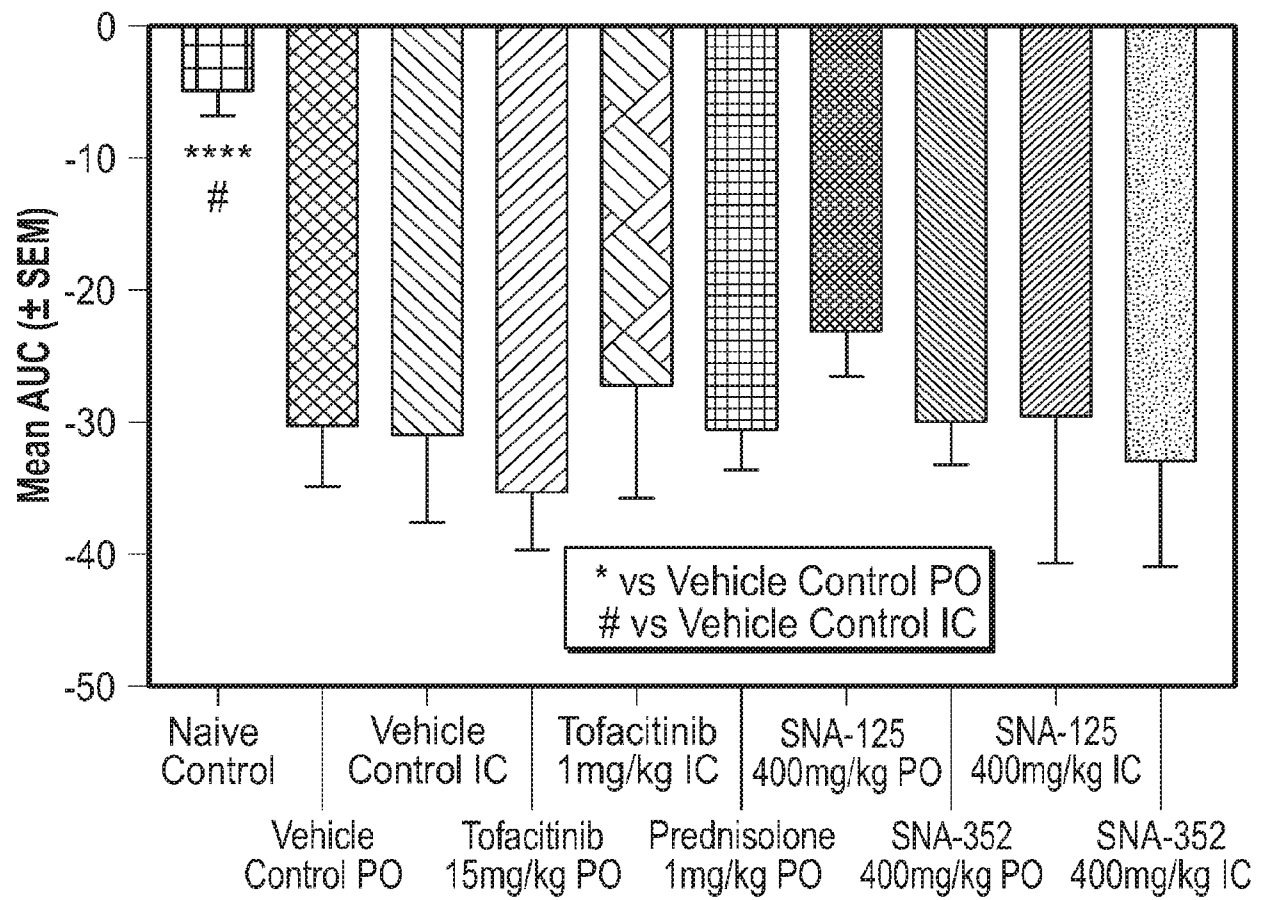


FIG. 79B

123/207

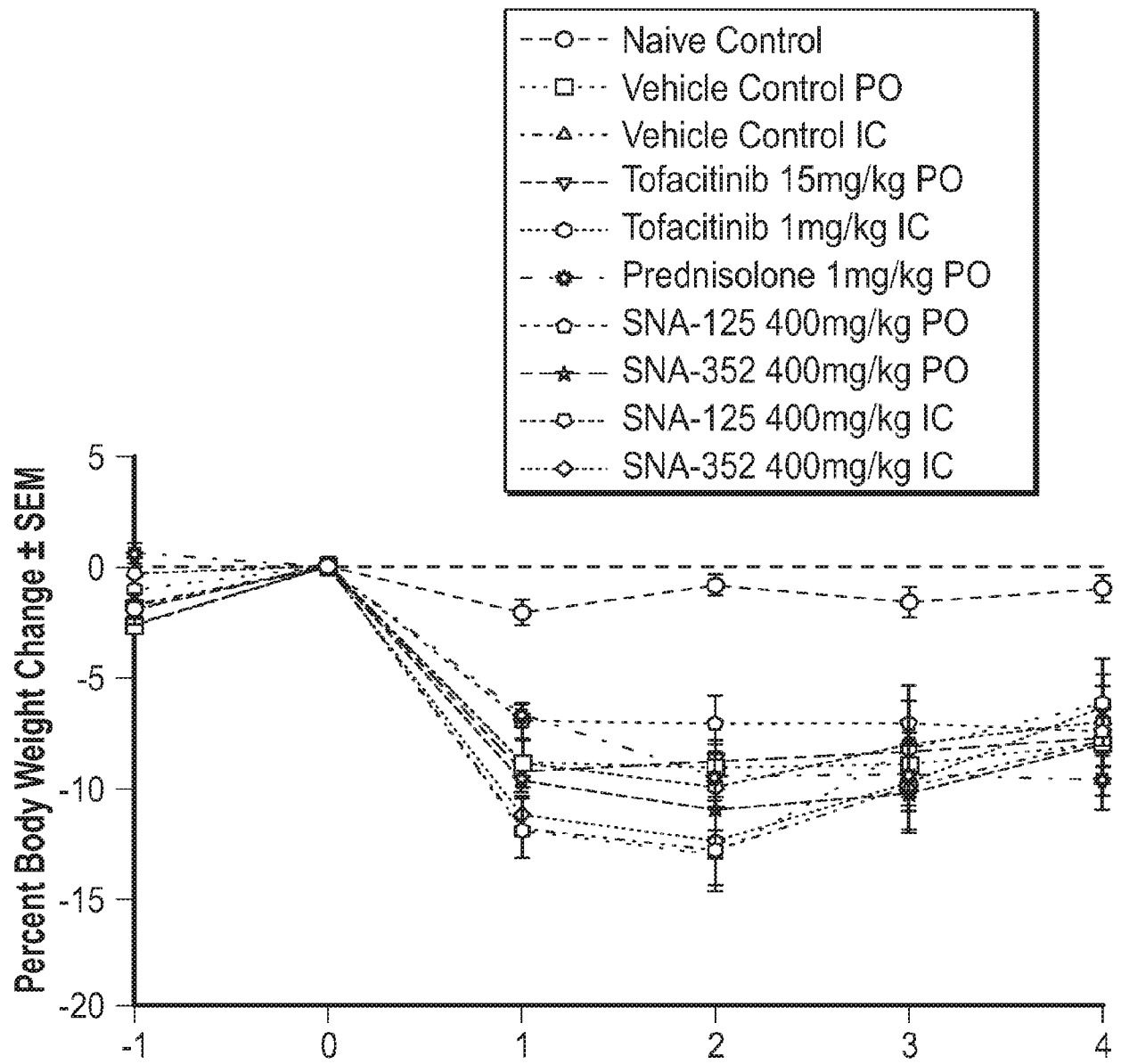


FIG. 80A

124/207

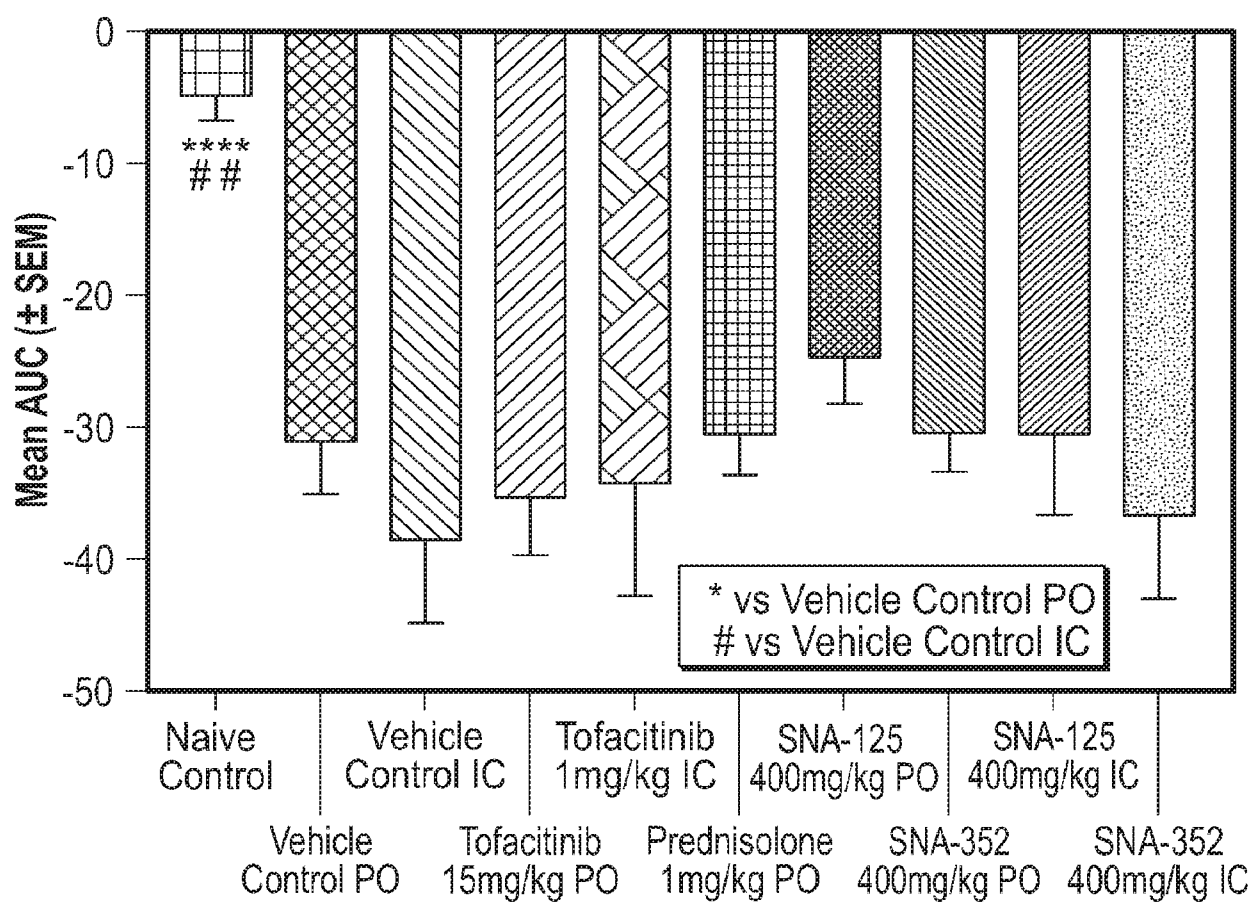


FIG. 80B

125/207

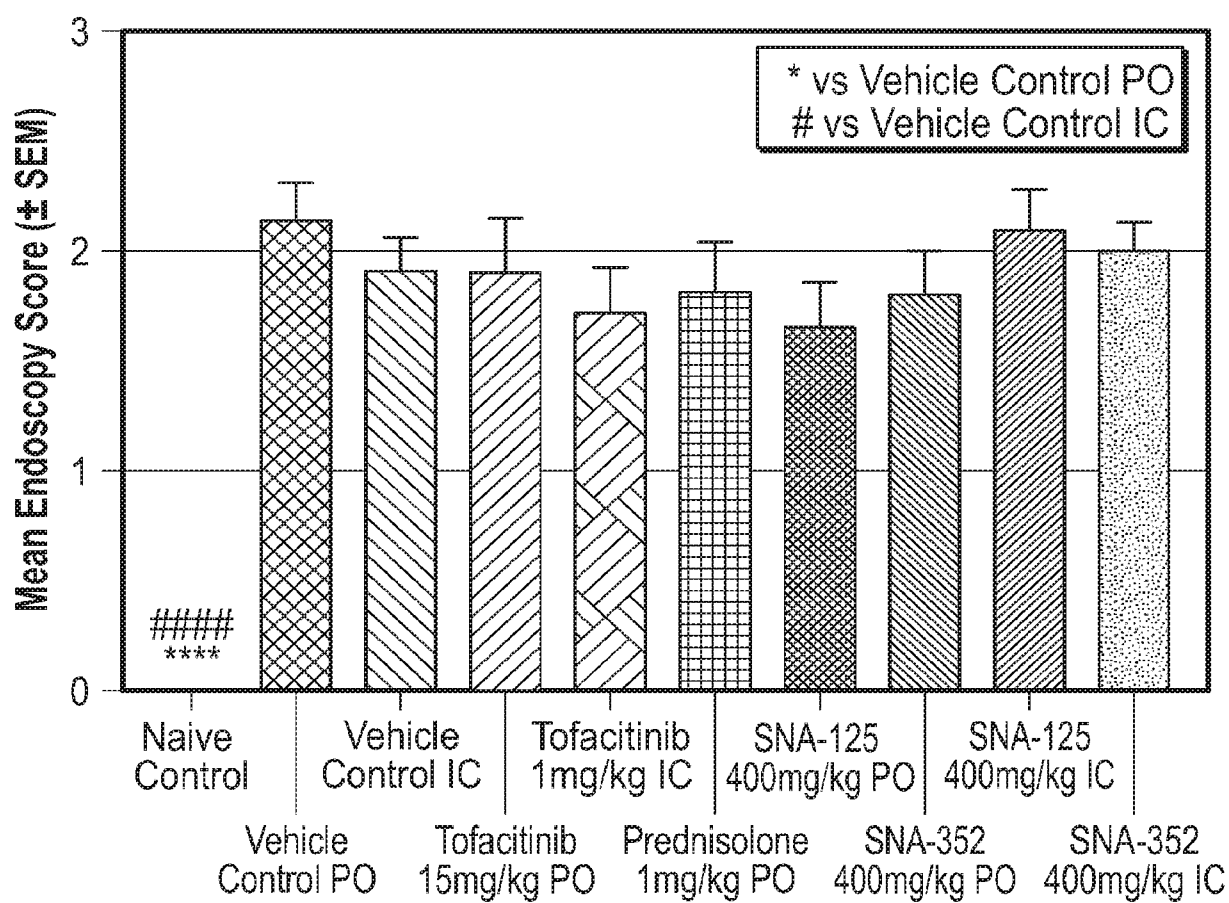


FIG. 81A

126/207

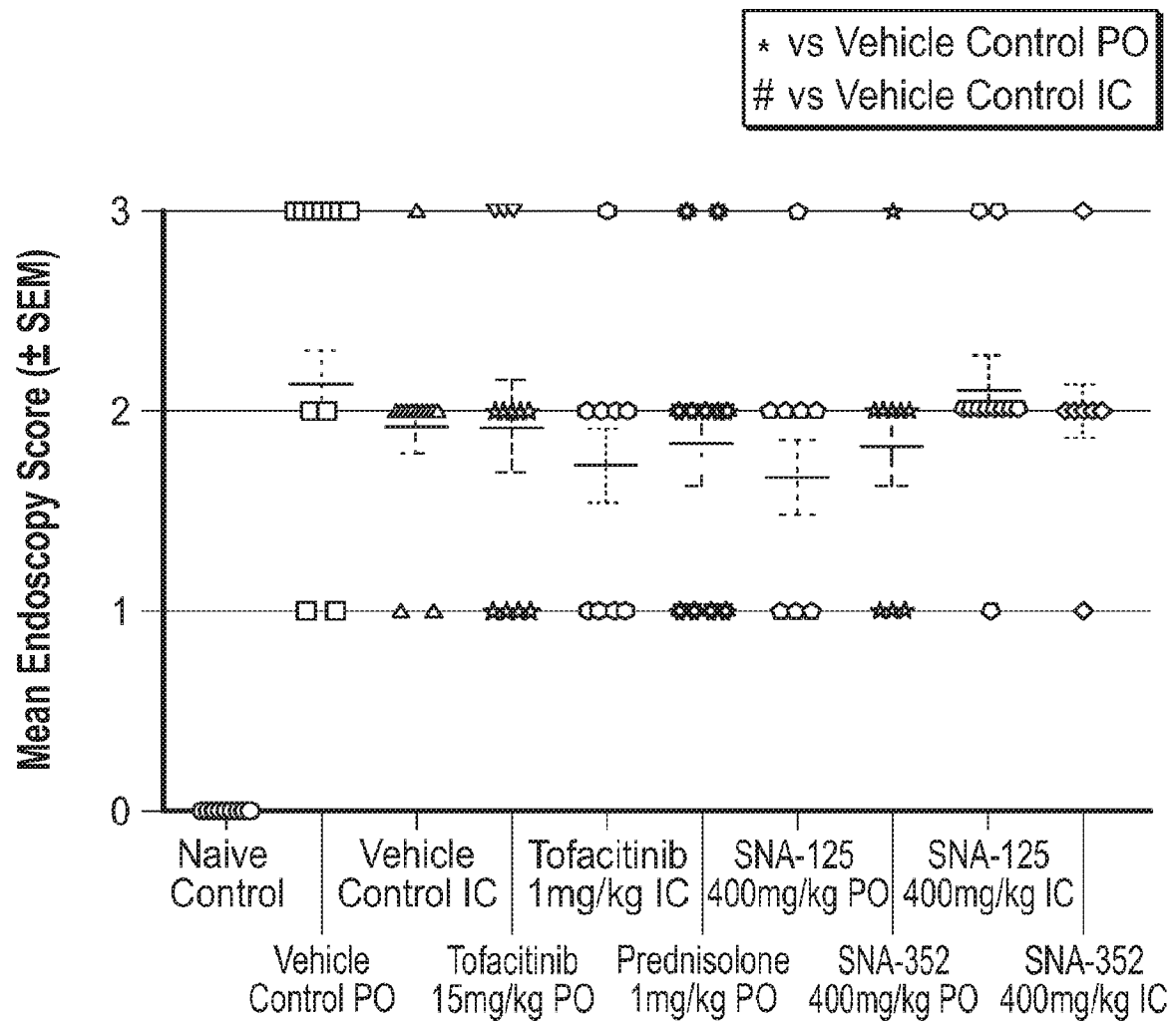
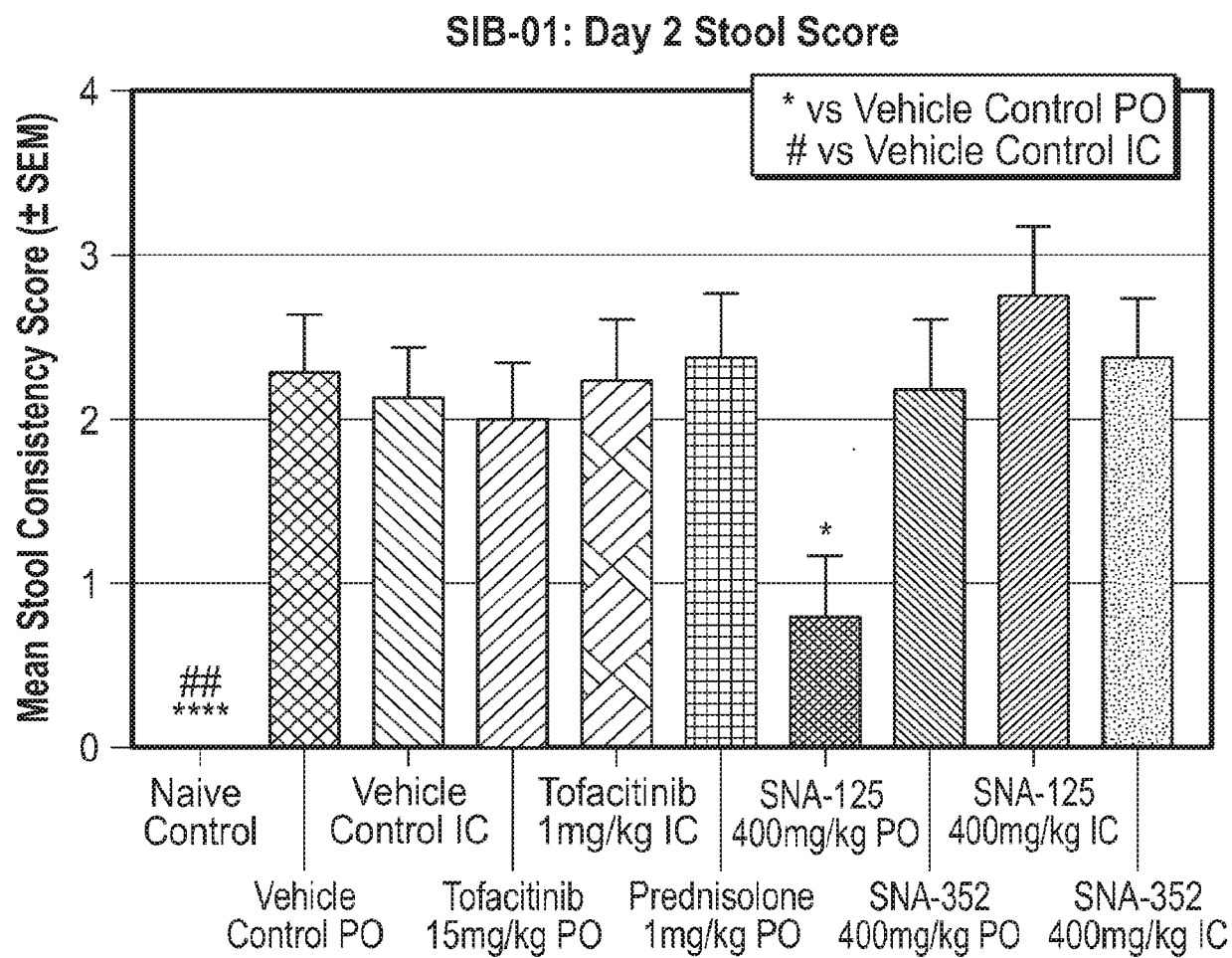
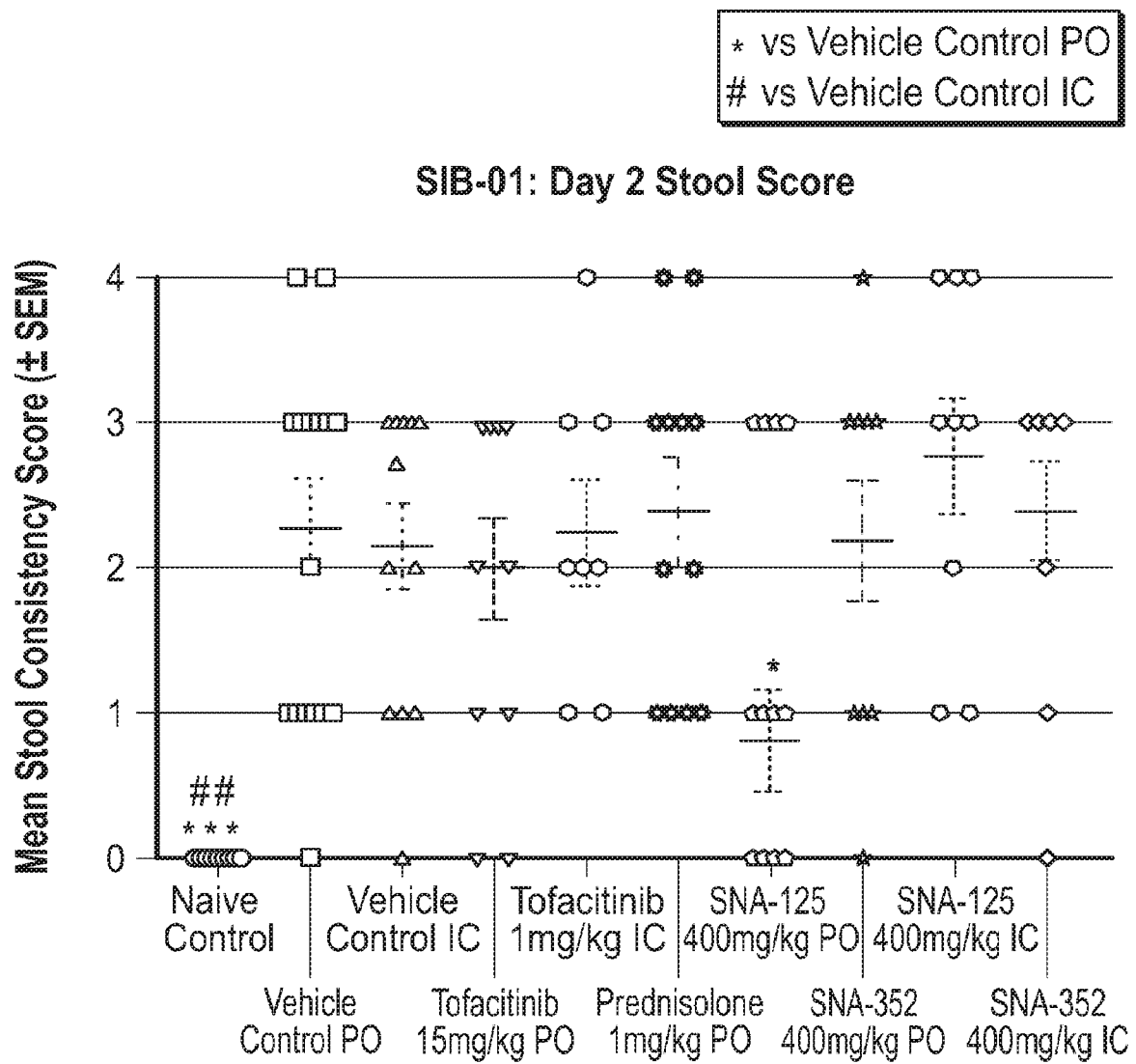


FIG. 81B

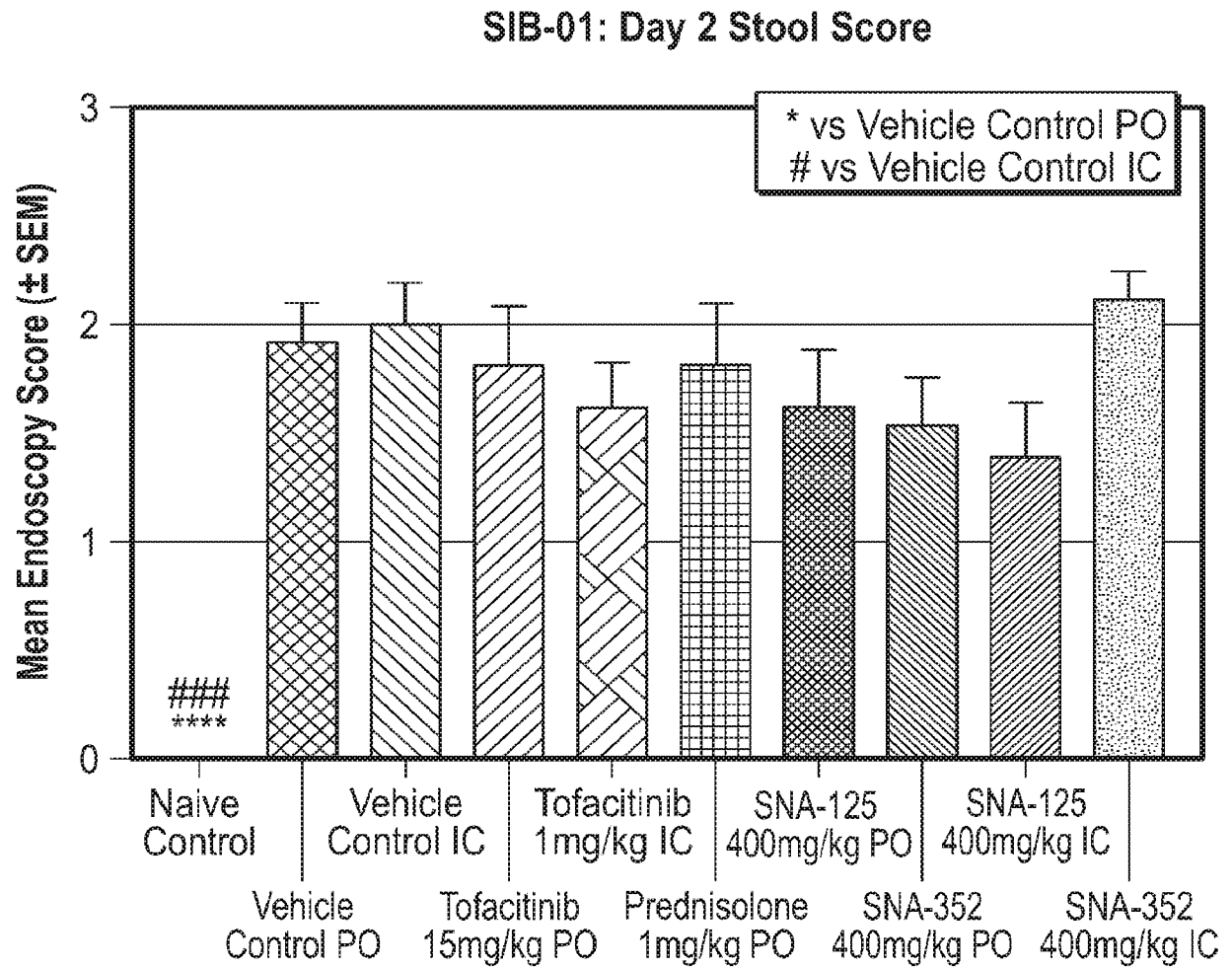
127/207

**FIG. 82A**

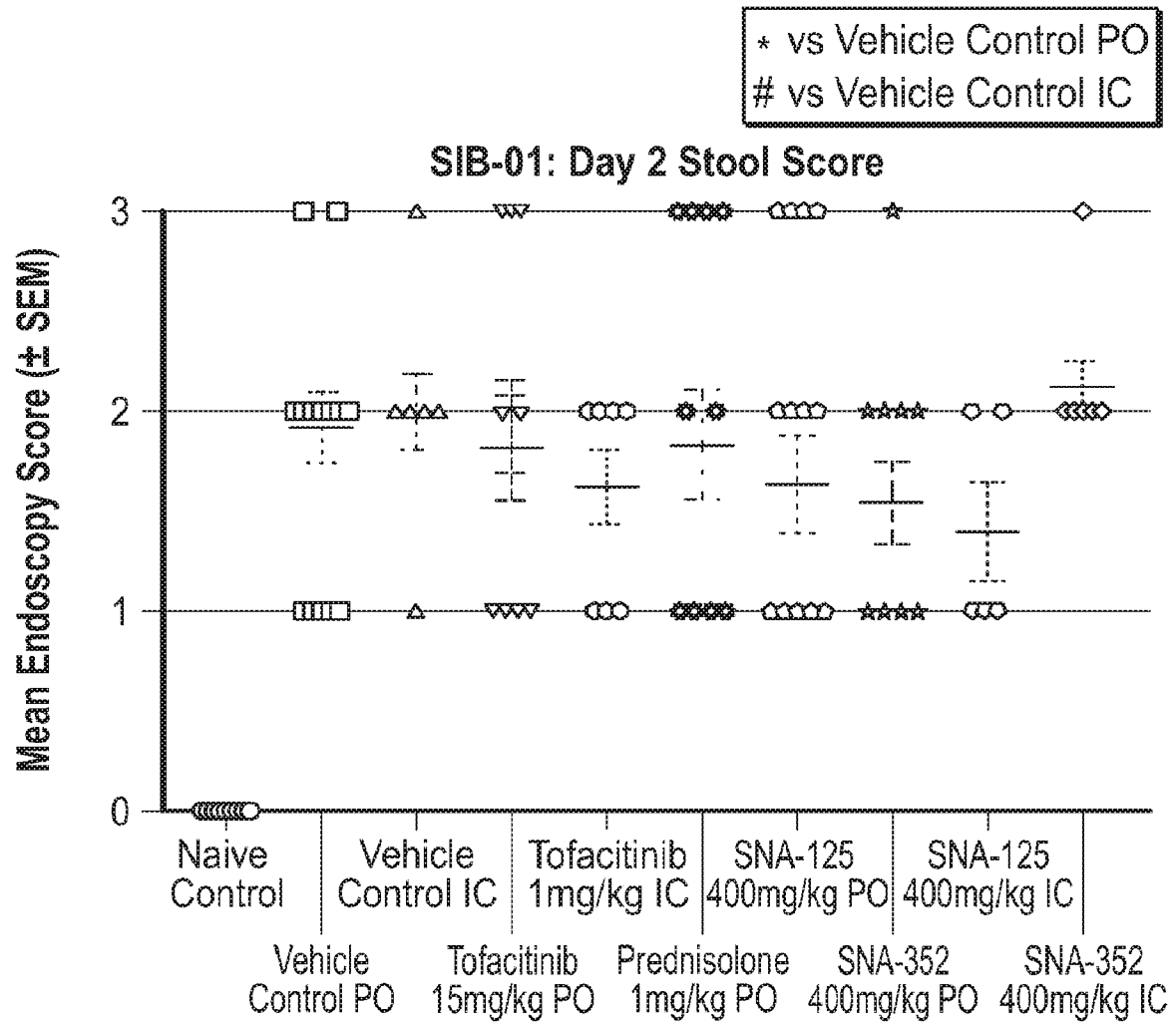
128/207

**FIG. 82B**

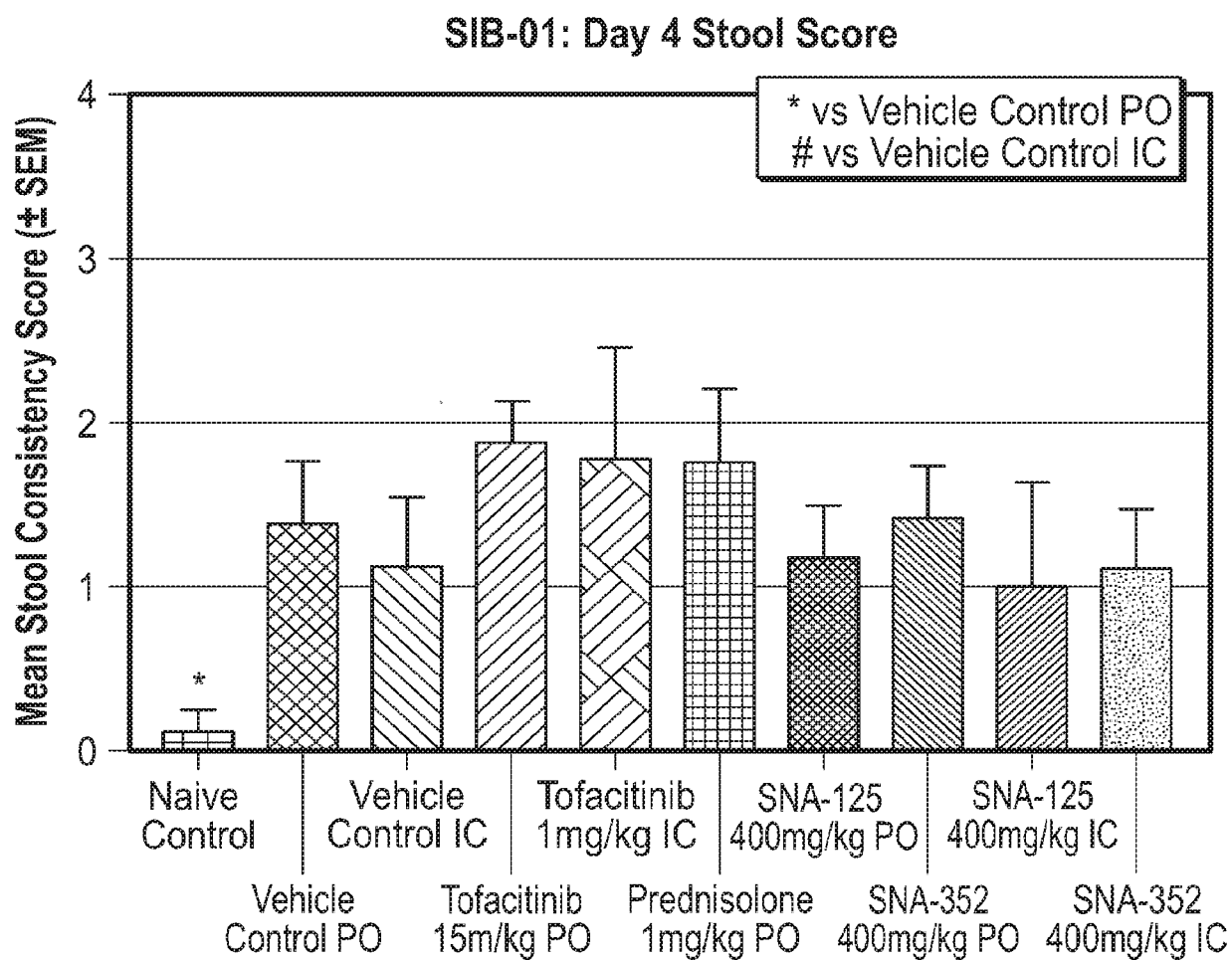
129/207

**FIG. 83A**

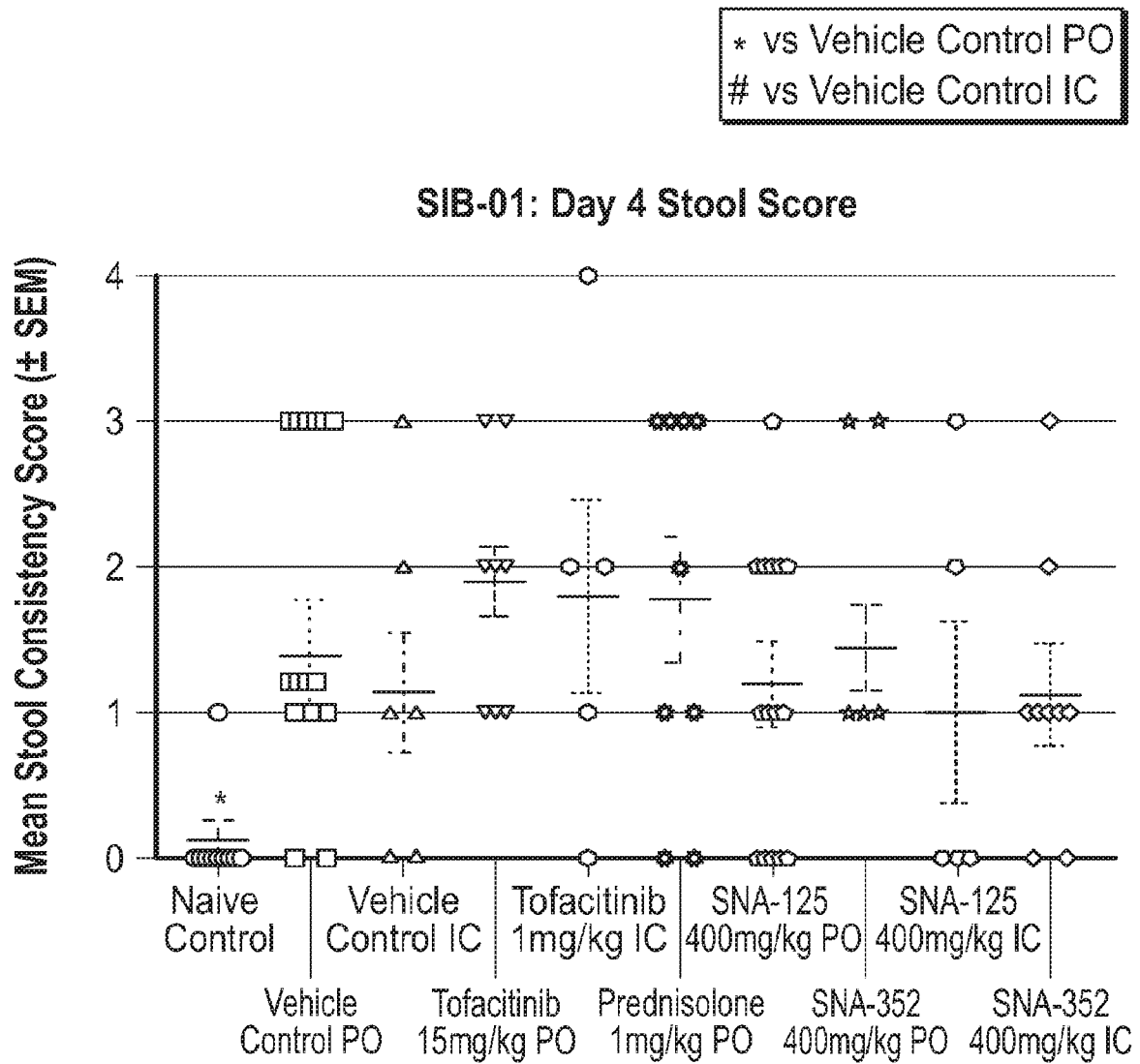
130/207

**FIG. 83B**

131/207

**FIG. 84A**

132/207

**FIG. 84B**

133/207

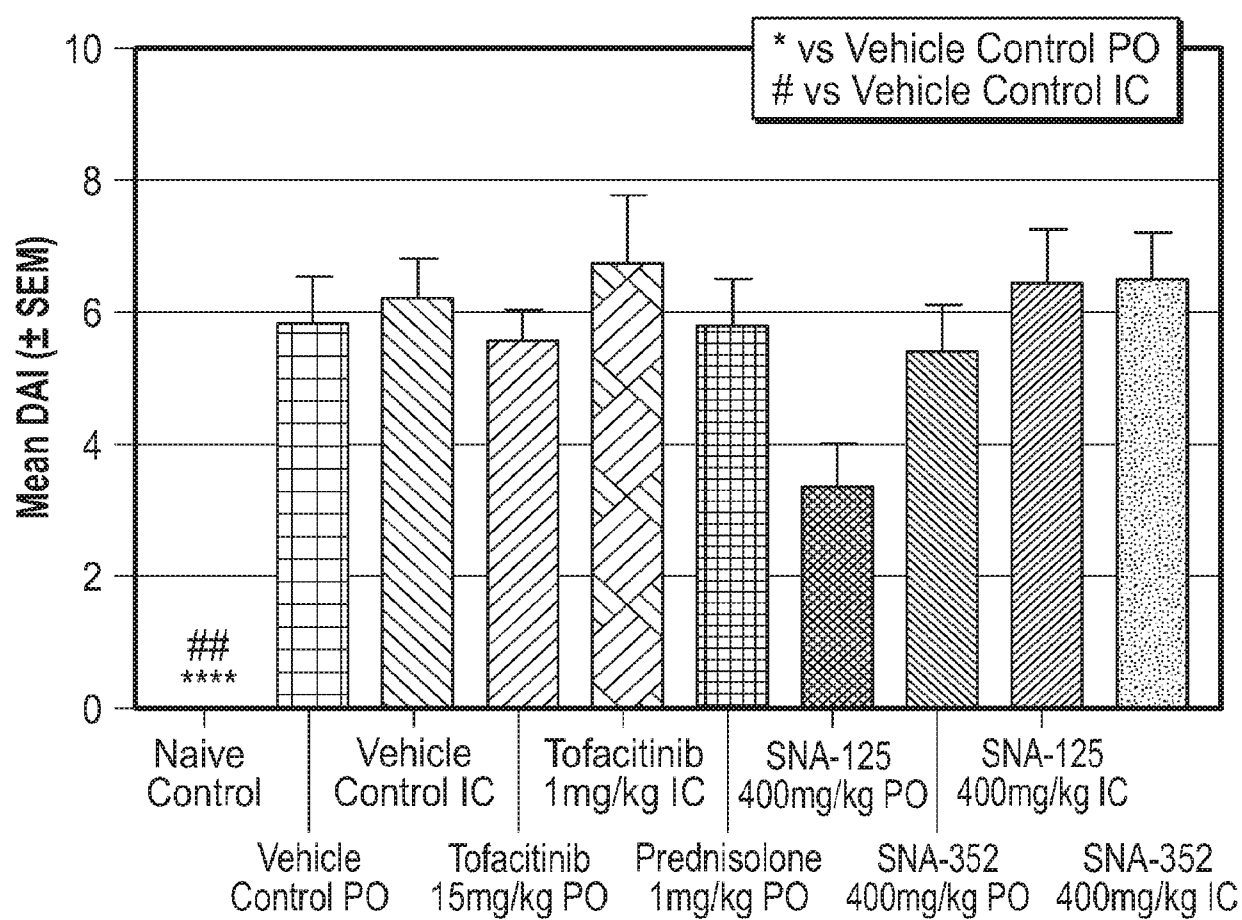


FIG. 85A

134/207

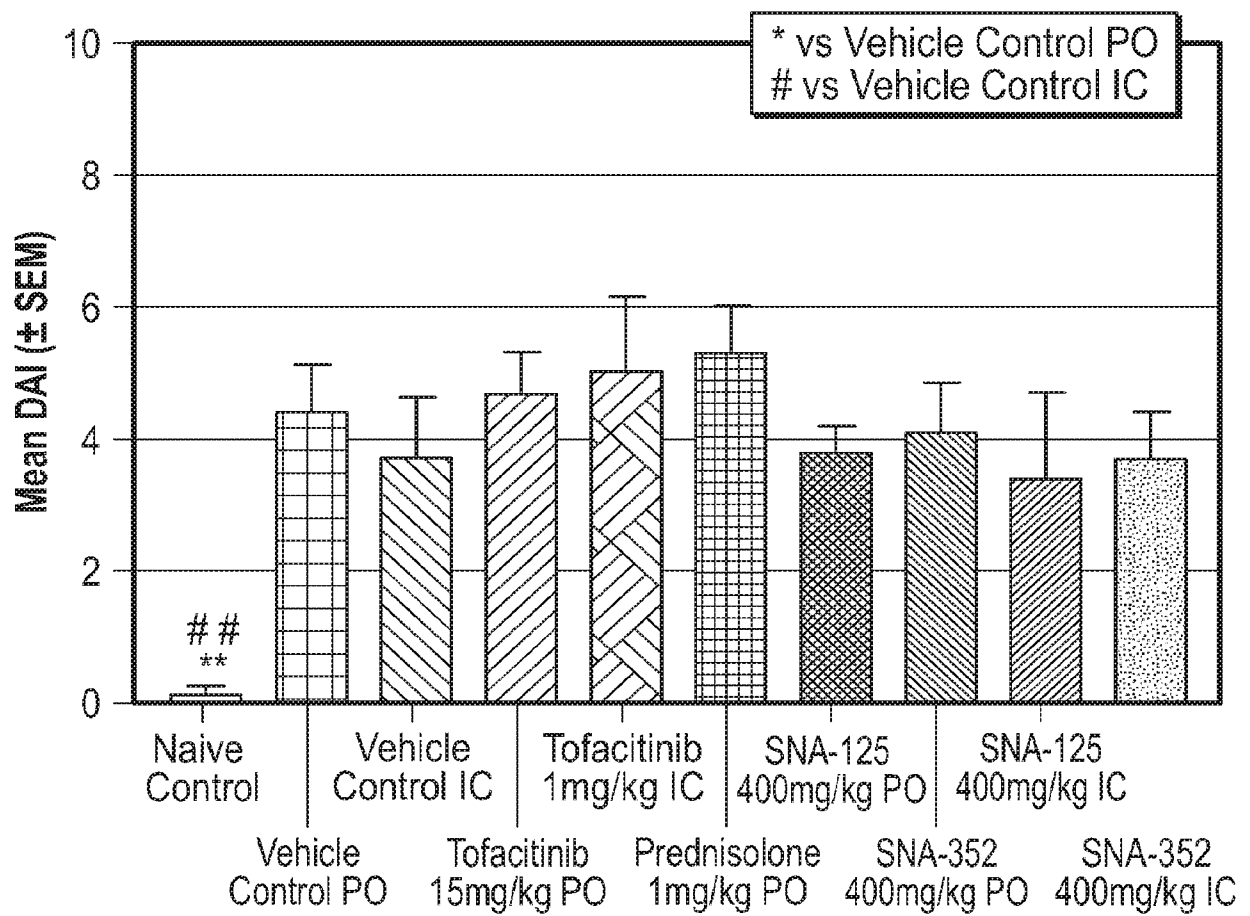
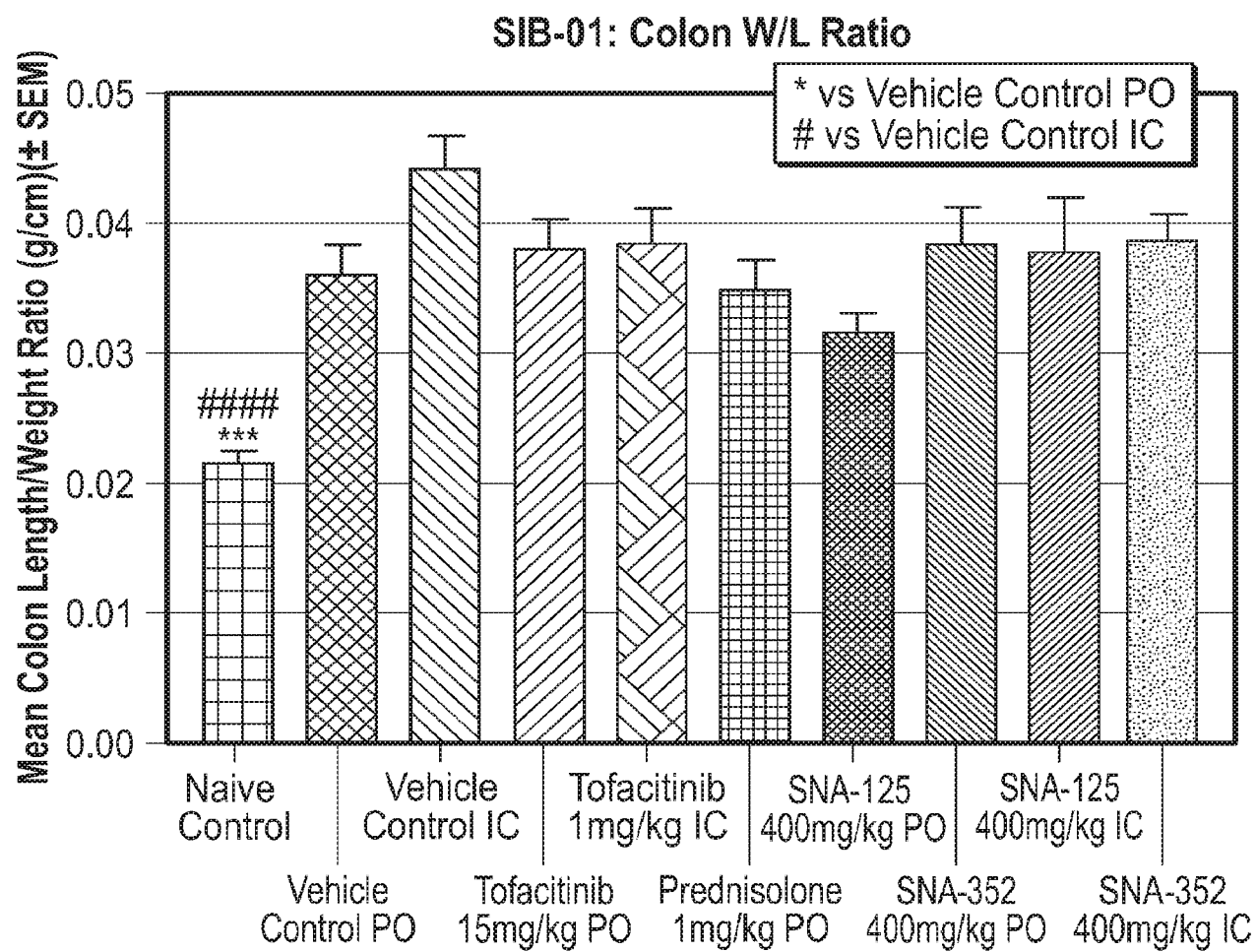
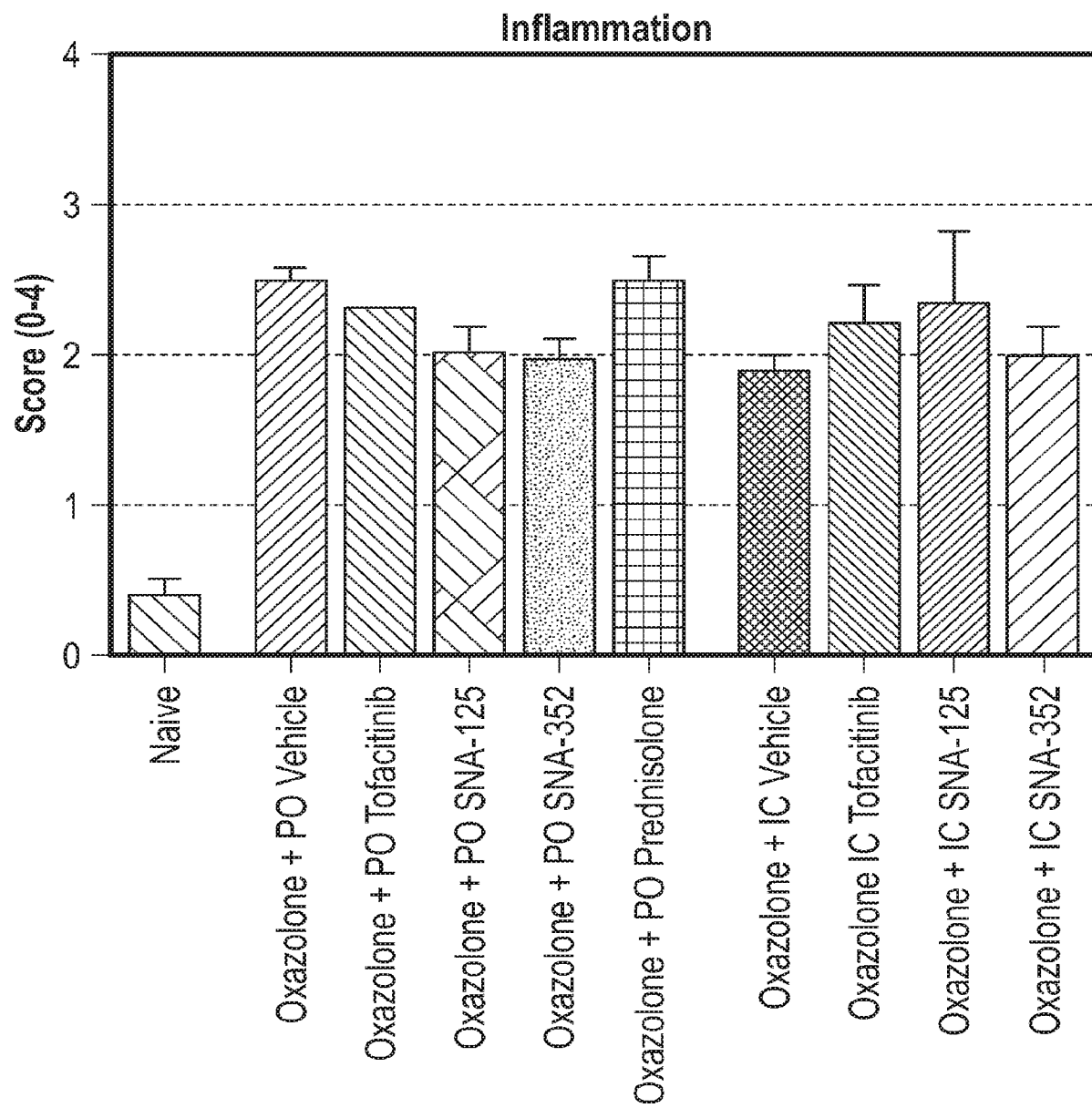


FIG. 85B

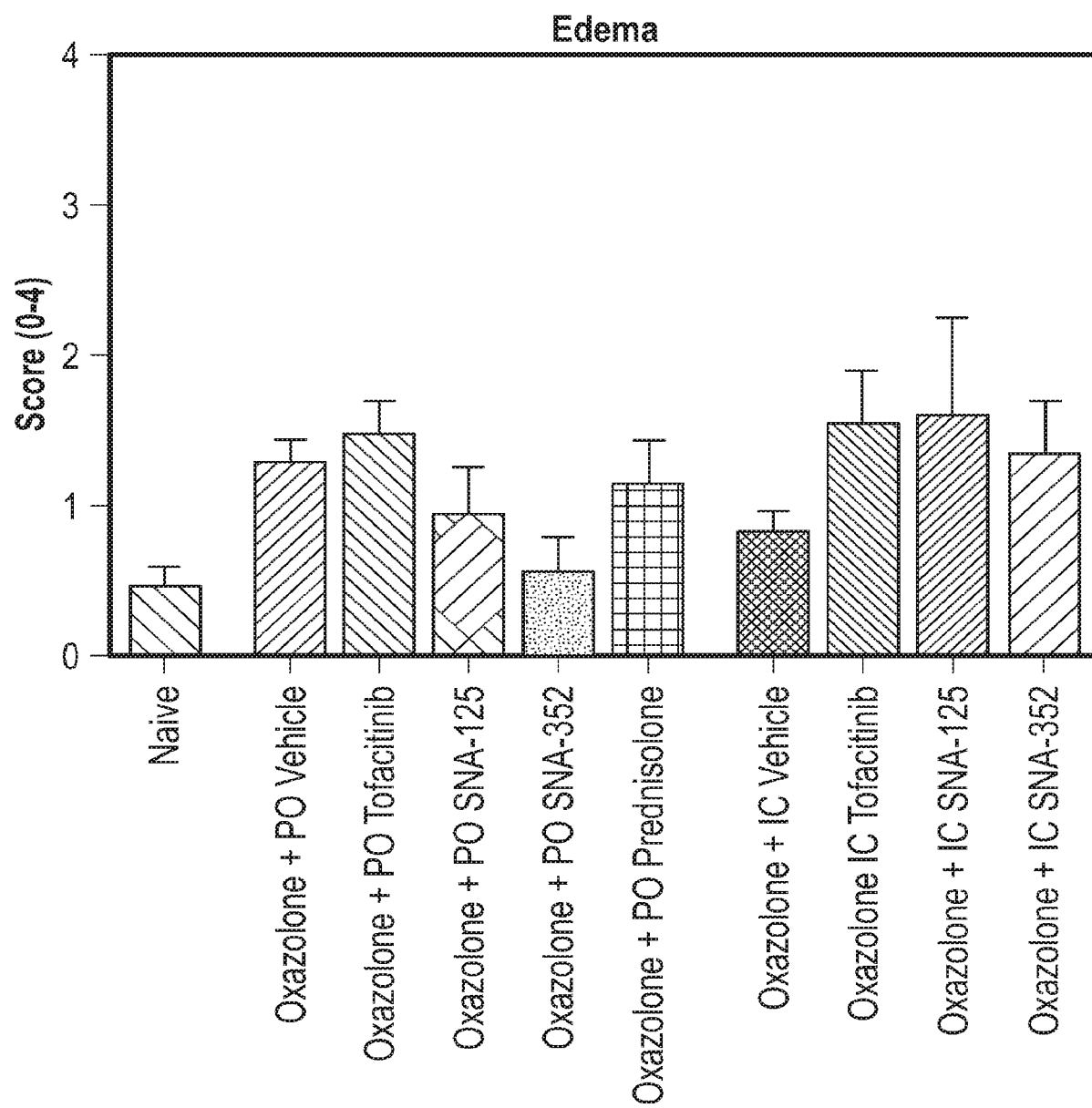
135/207

**FIG. 86**

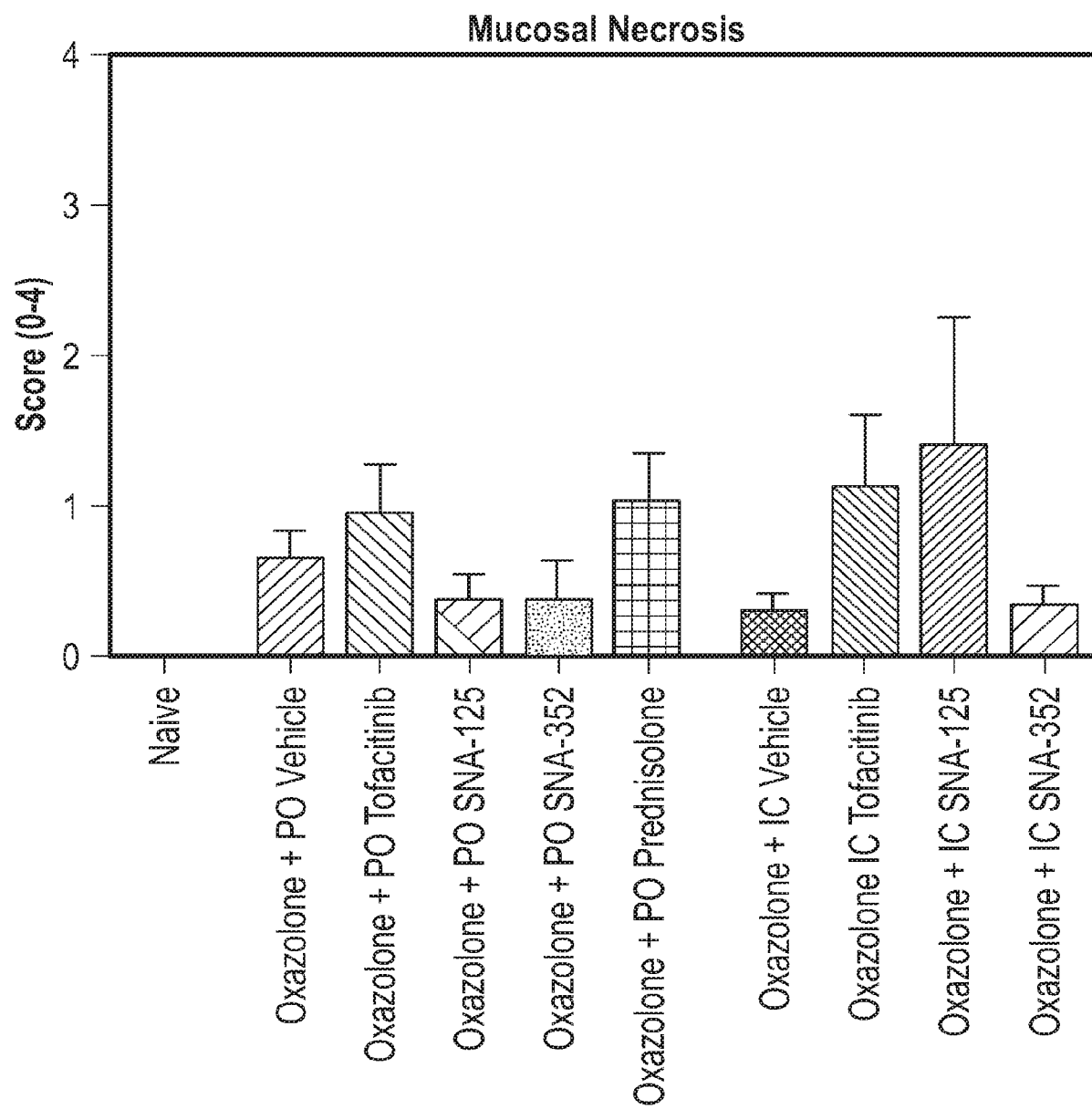
136/207

**FIG. 87**

137/207

**FIG. 88**

138/207

**FIG. 89**

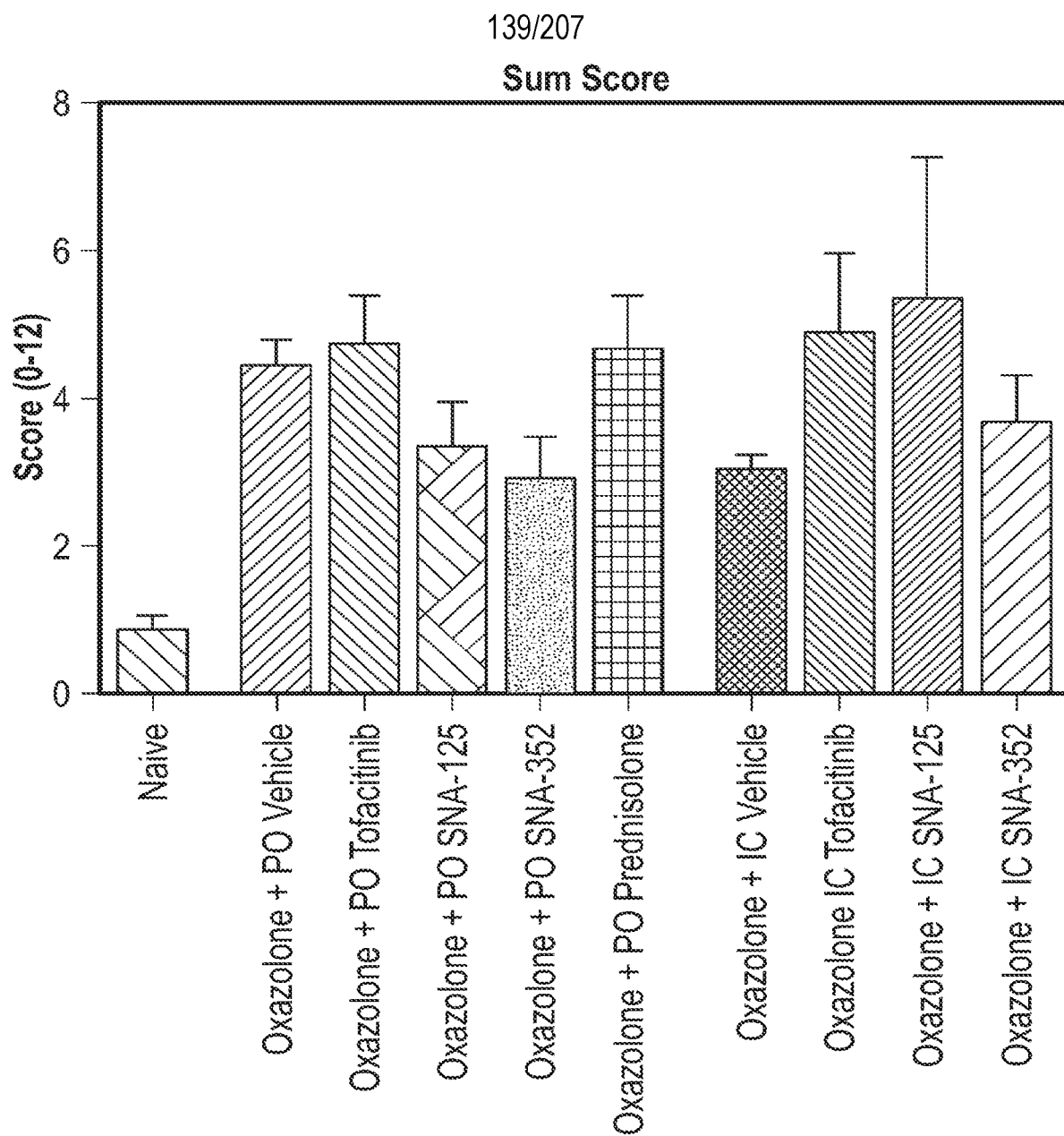


FIG. 90

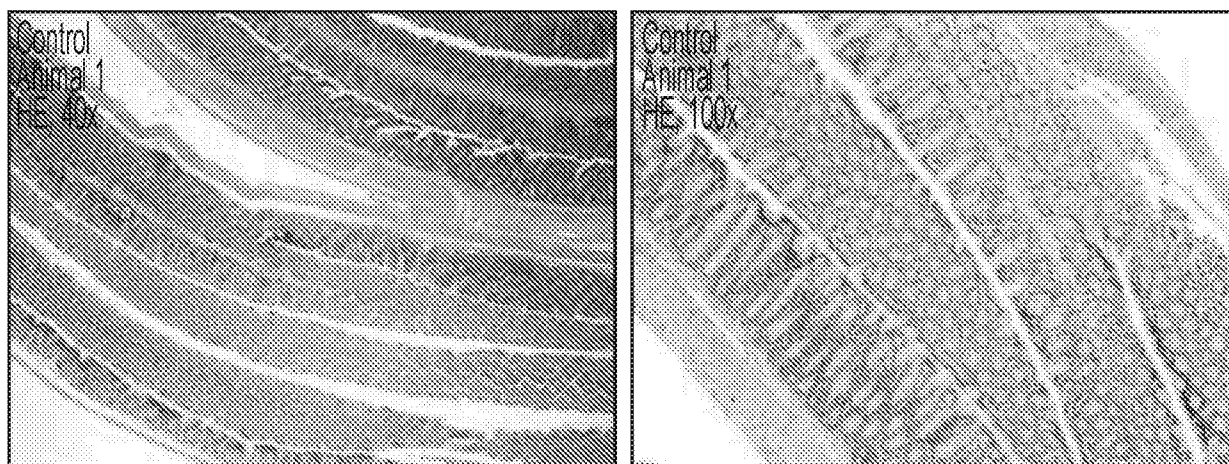


FIG. 91

140/207

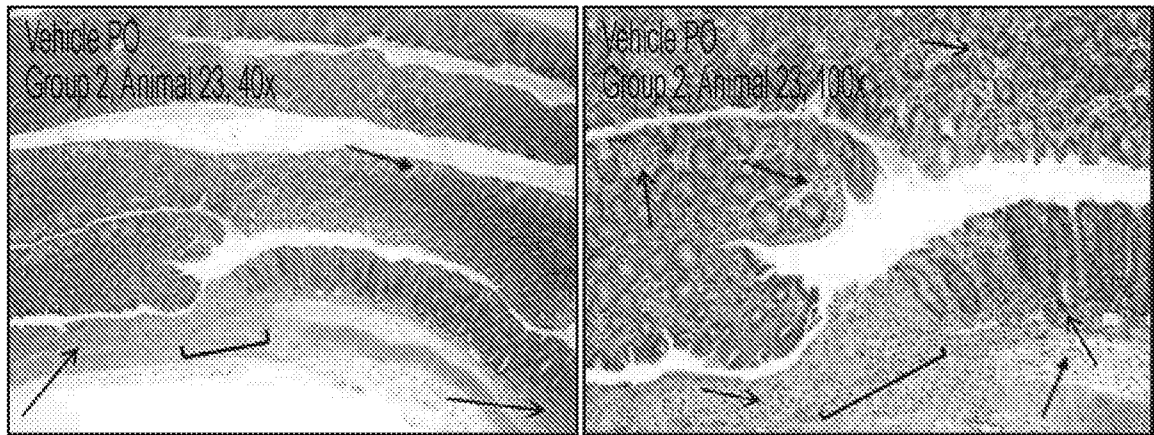


FIG. 92A

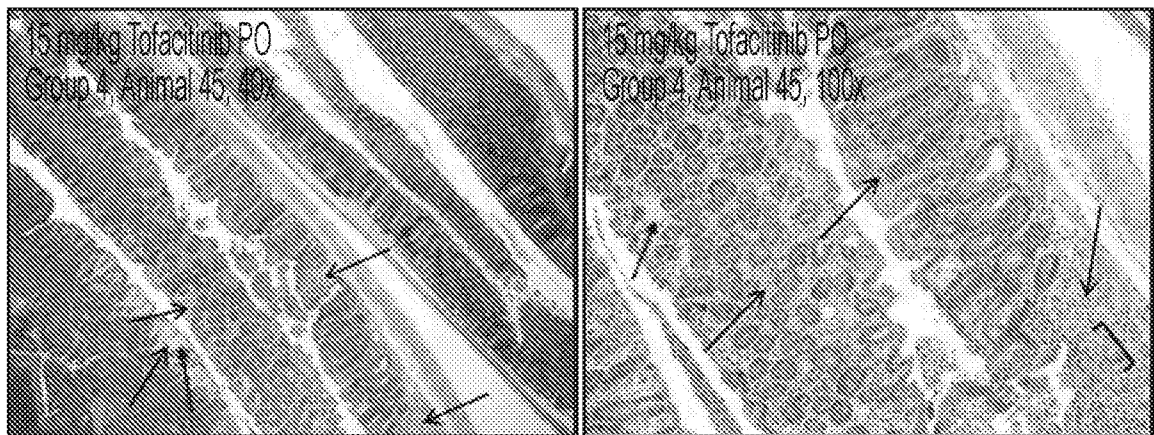


FIG. 92B

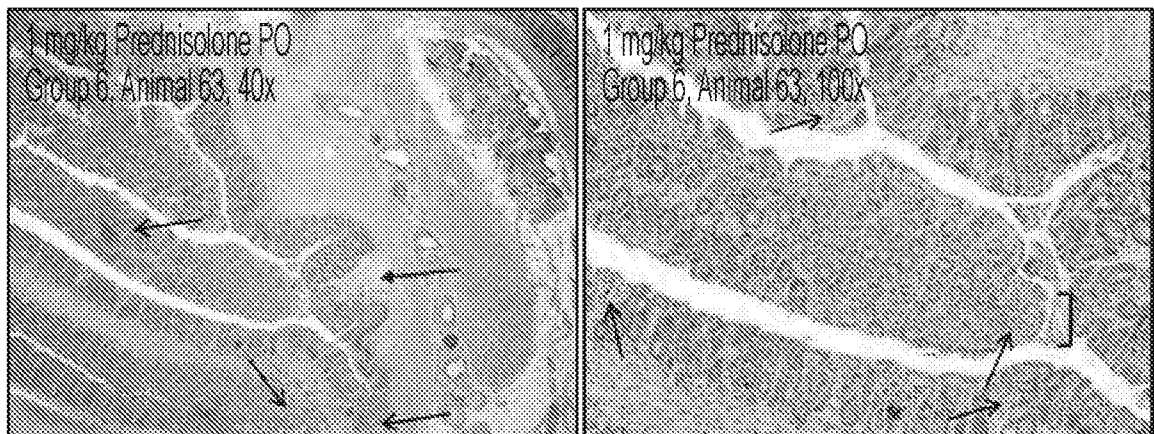


FIG. 92C

141/207

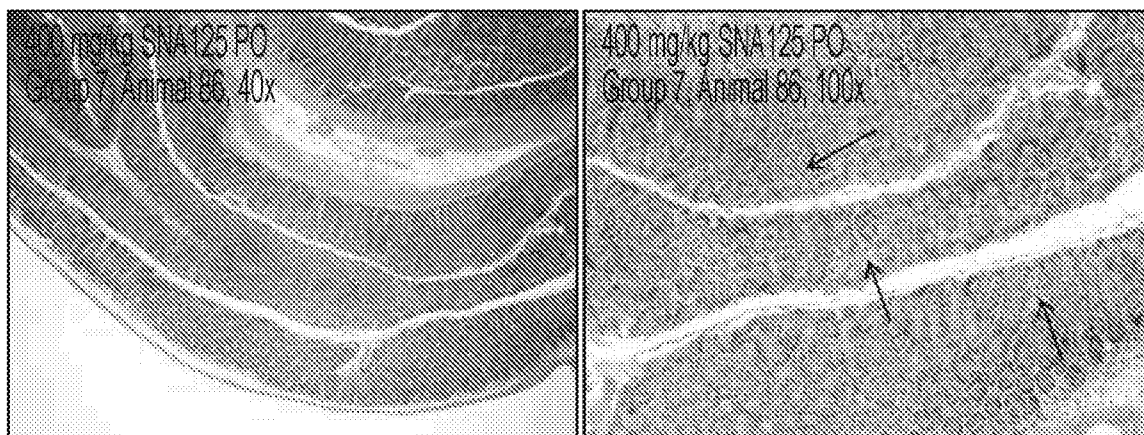


FIG. 92D

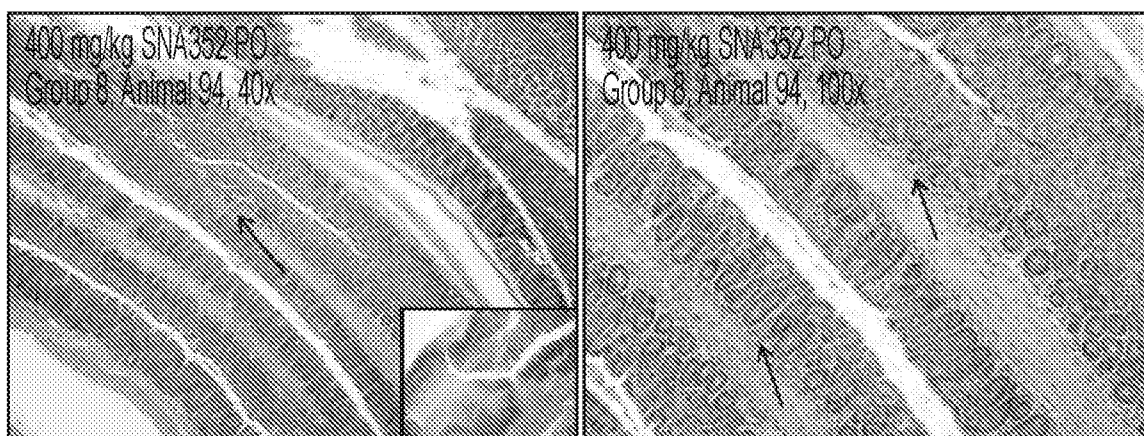


FIG. 92E

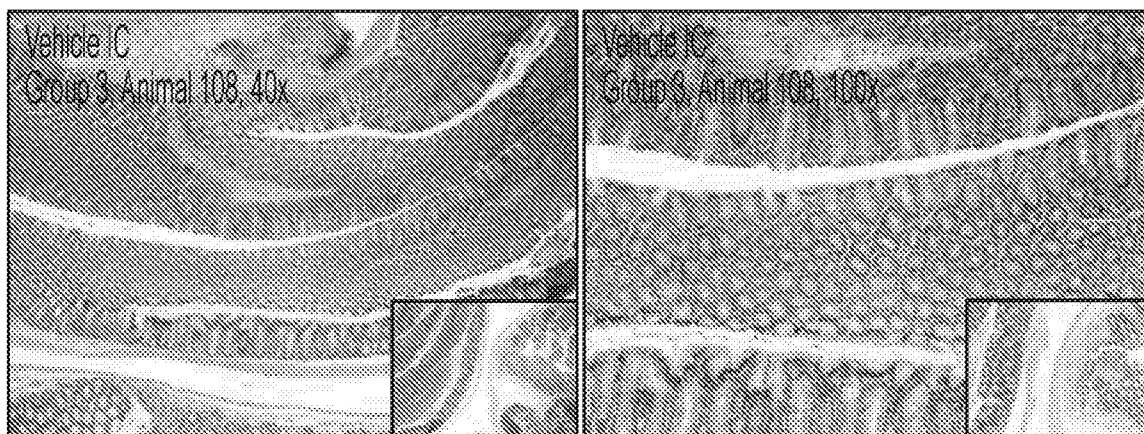


FIG. 93A

142/207

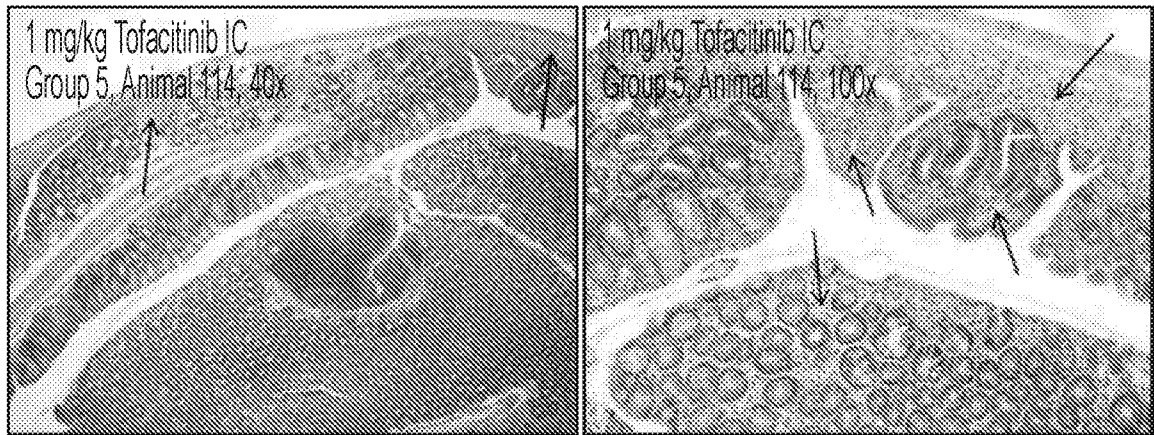


FIG. 93B

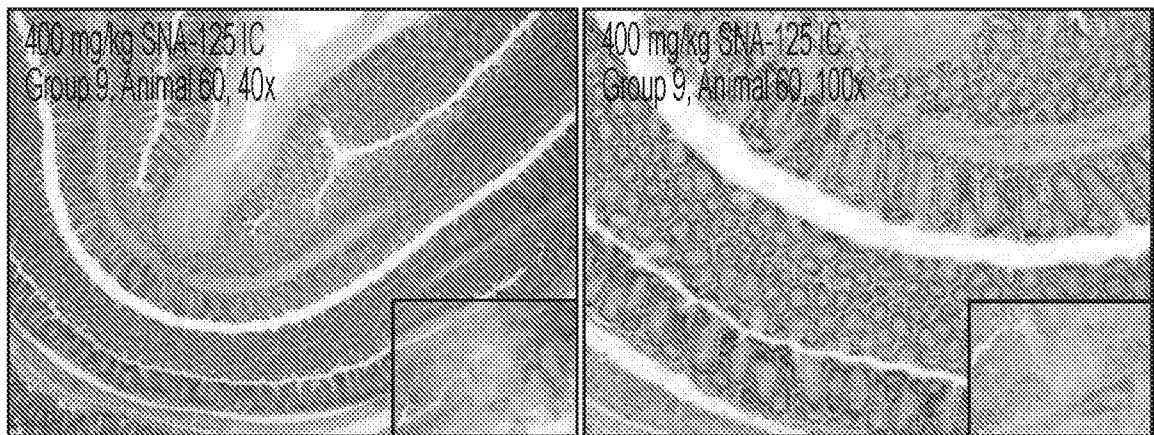


FIG. 93C

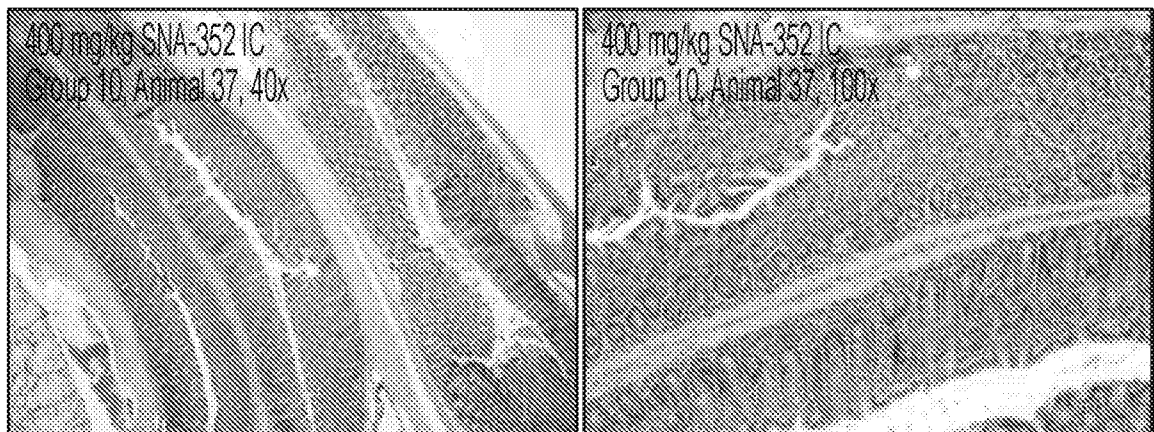


FIG. 93D

143/207

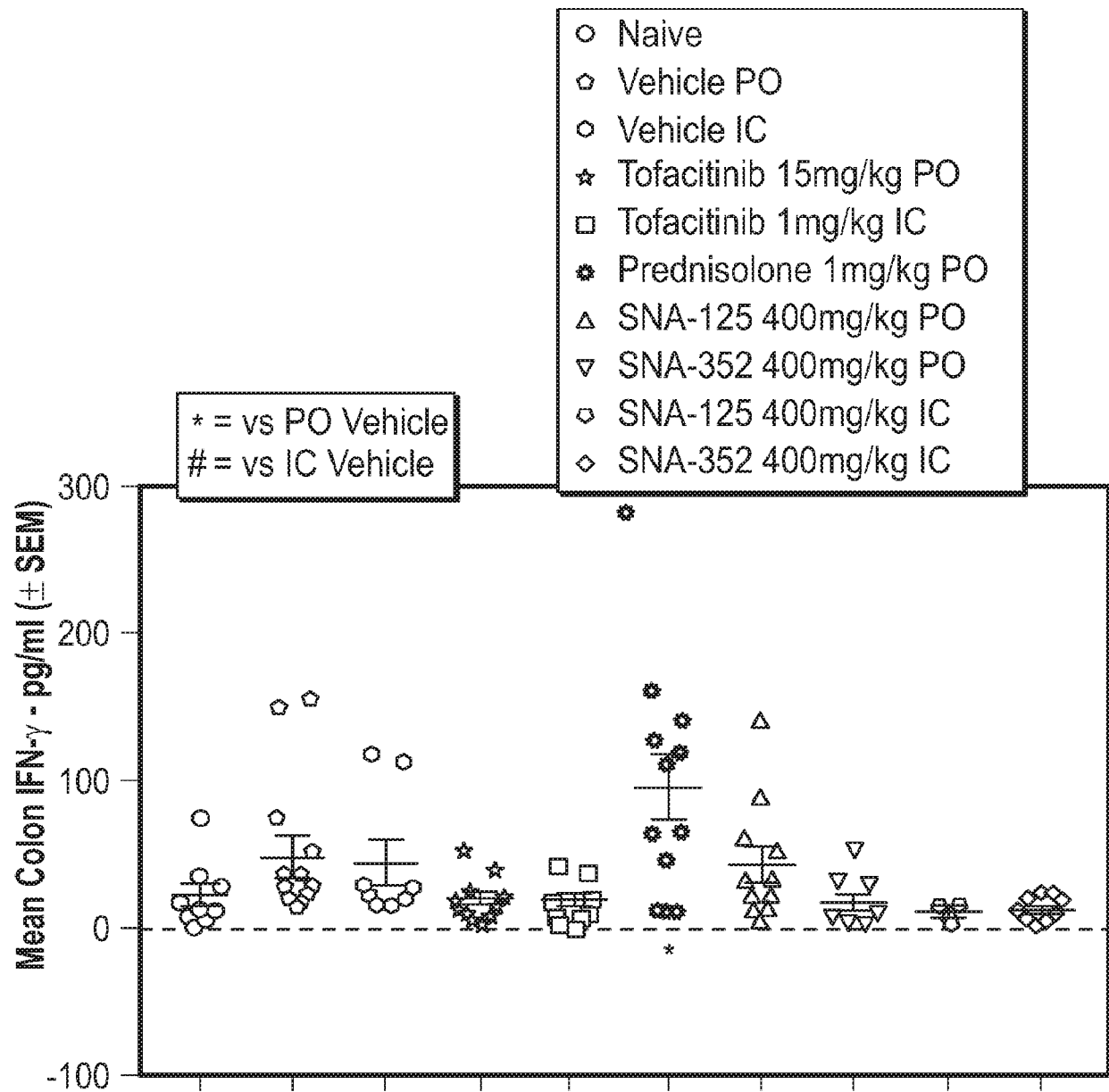


FIG. 94

144/207

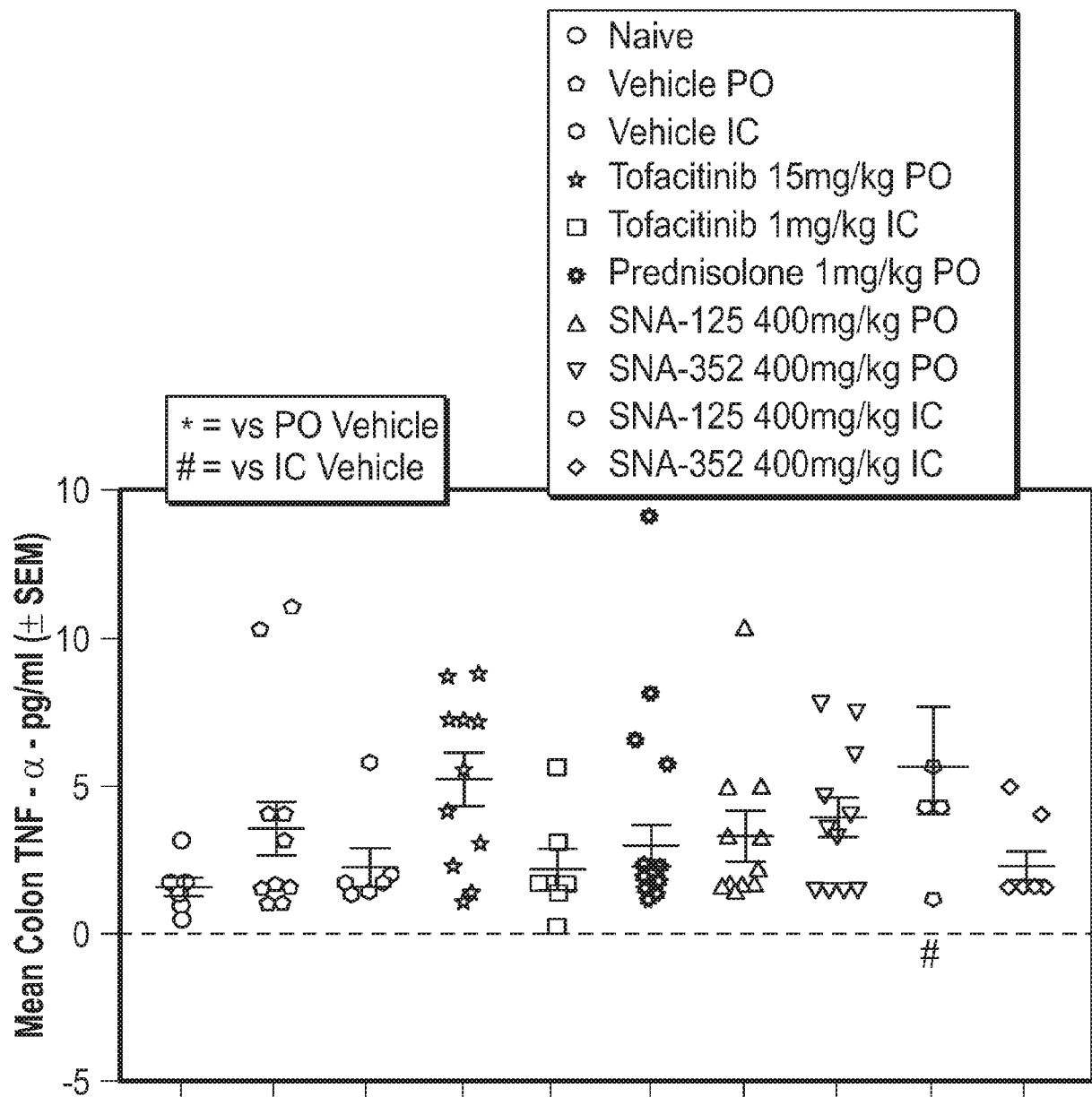


FIG. 95A

145/207

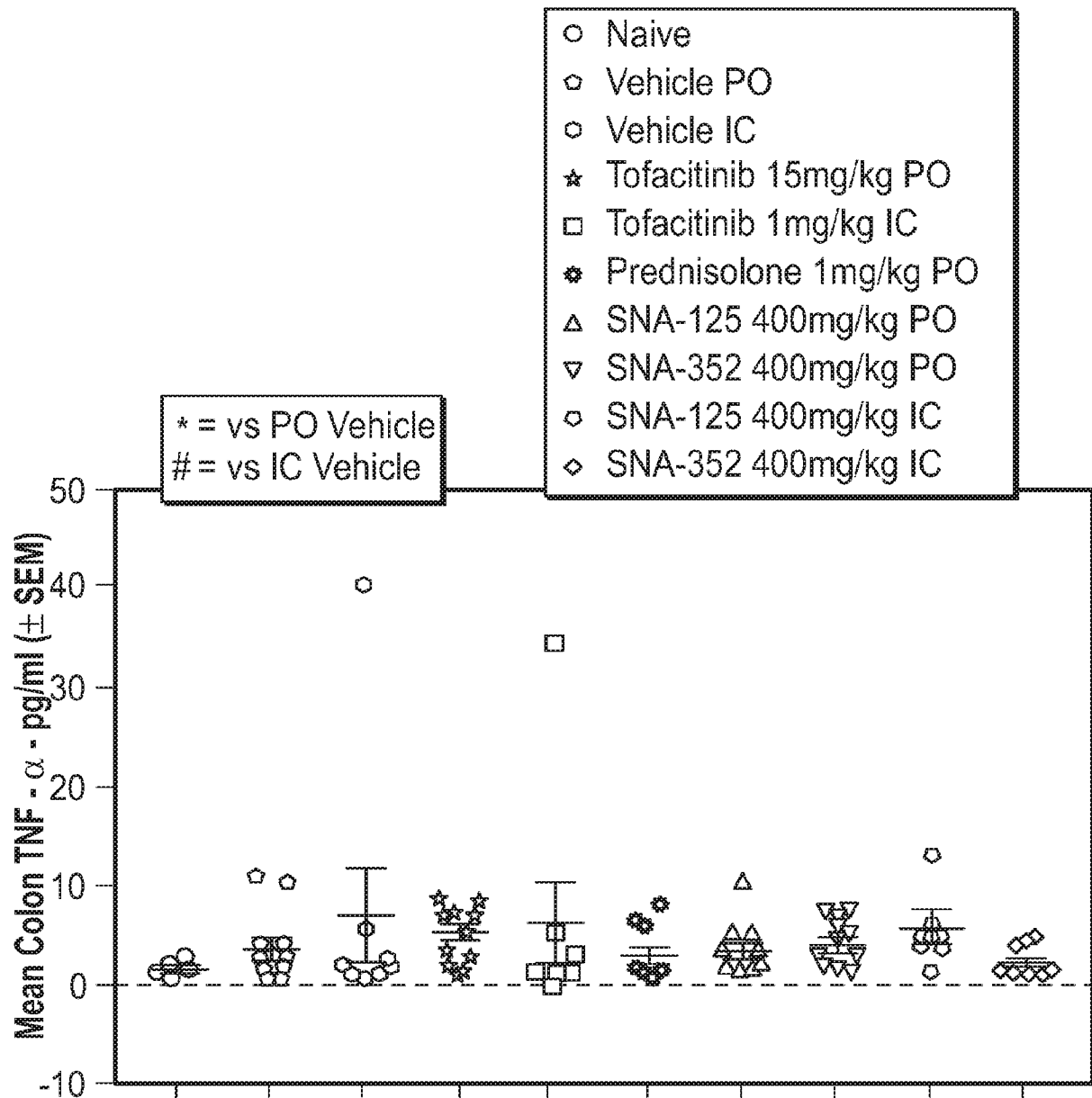


FIG. 95B

146/207

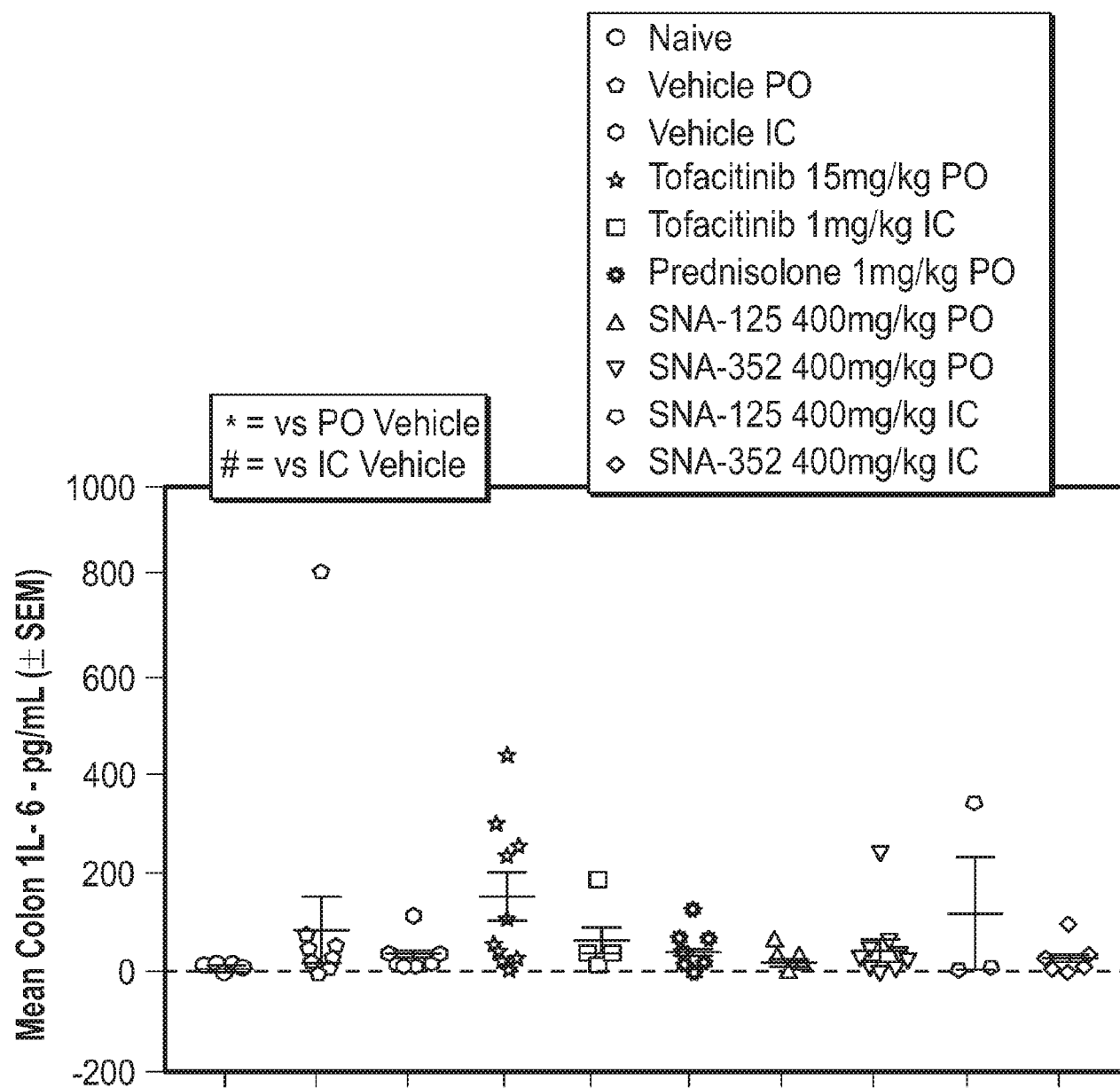


FIG. 96A

147/207

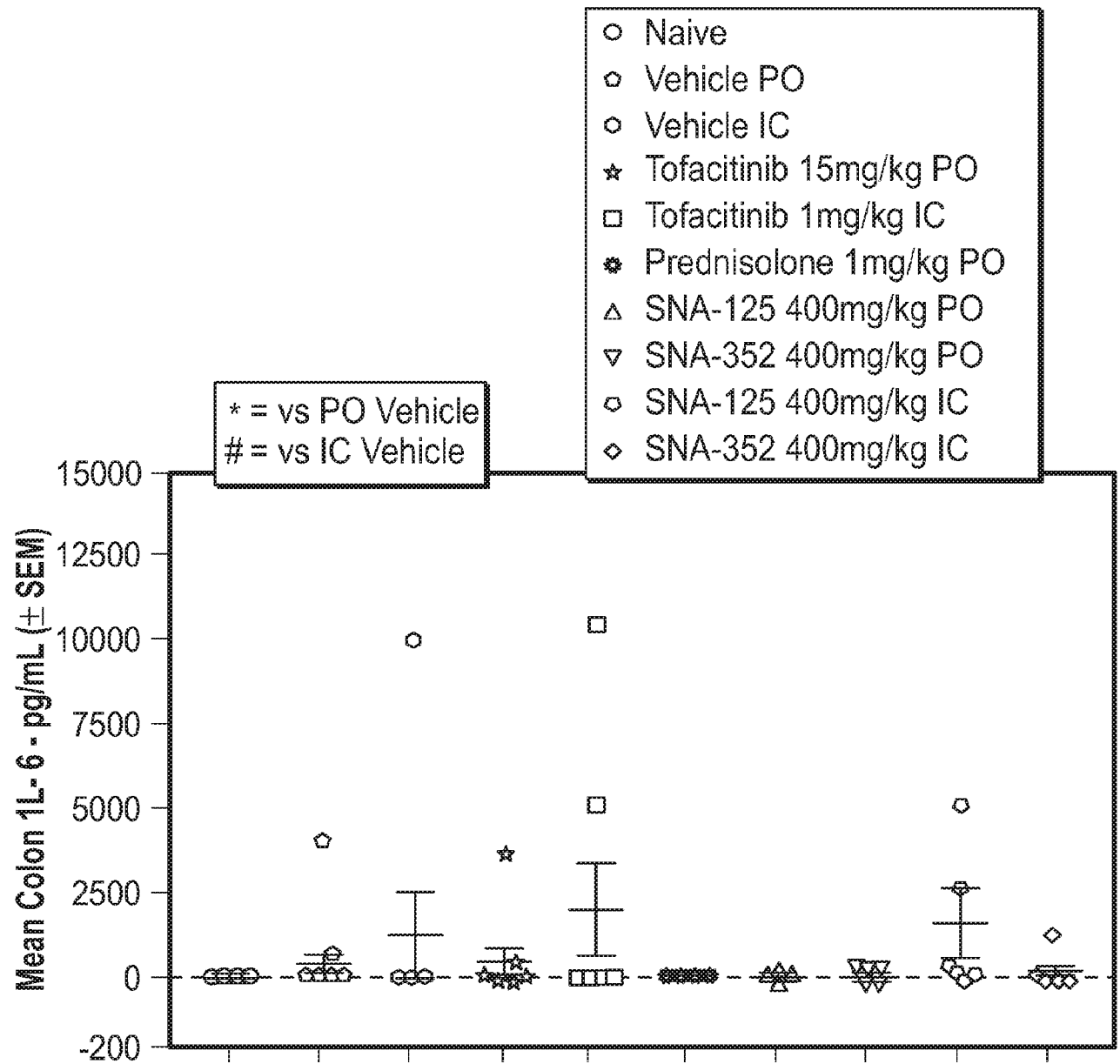


FIG. 96B

148/207

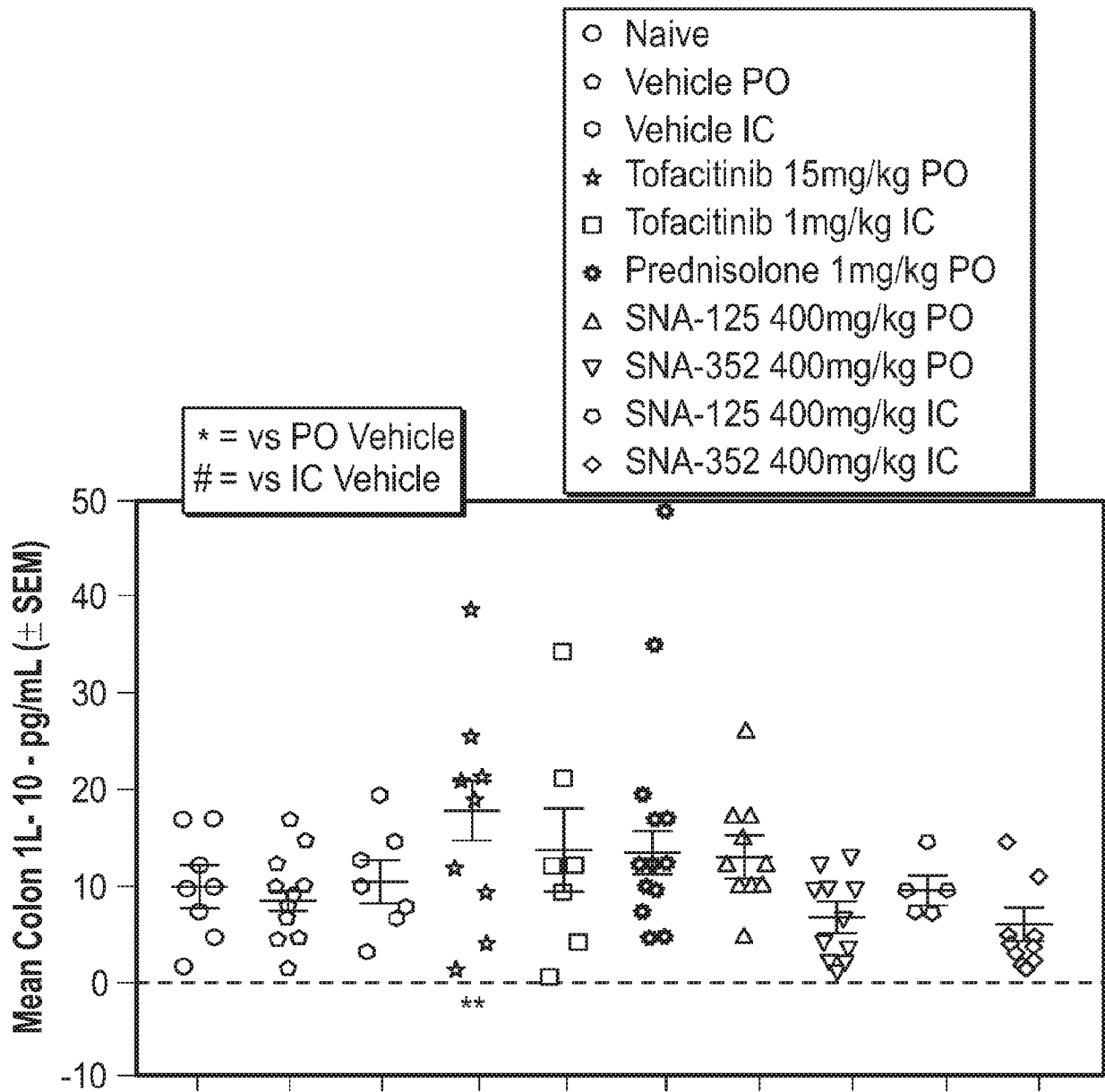


FIG. 97A

149/207

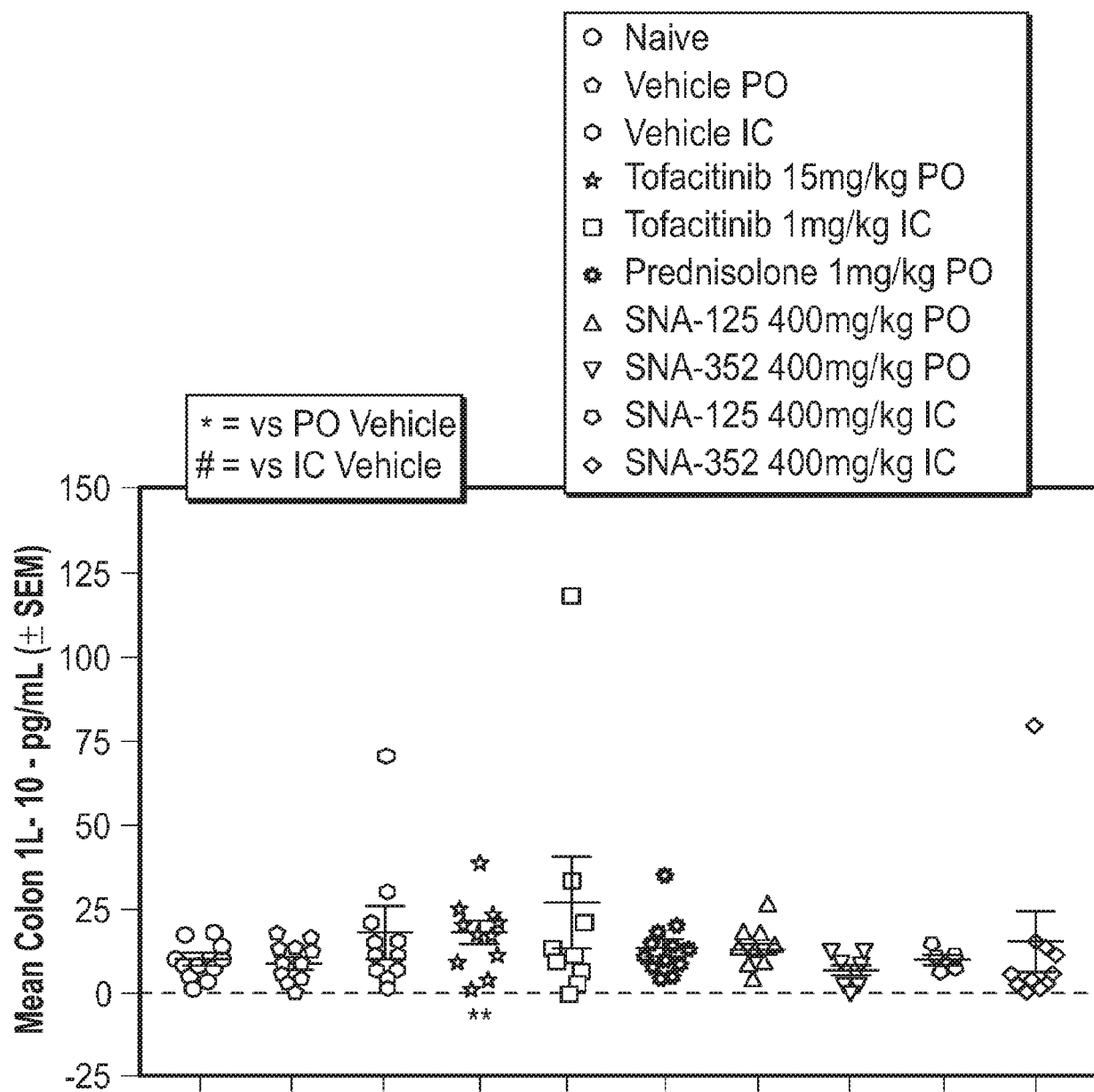


FIG. 97B

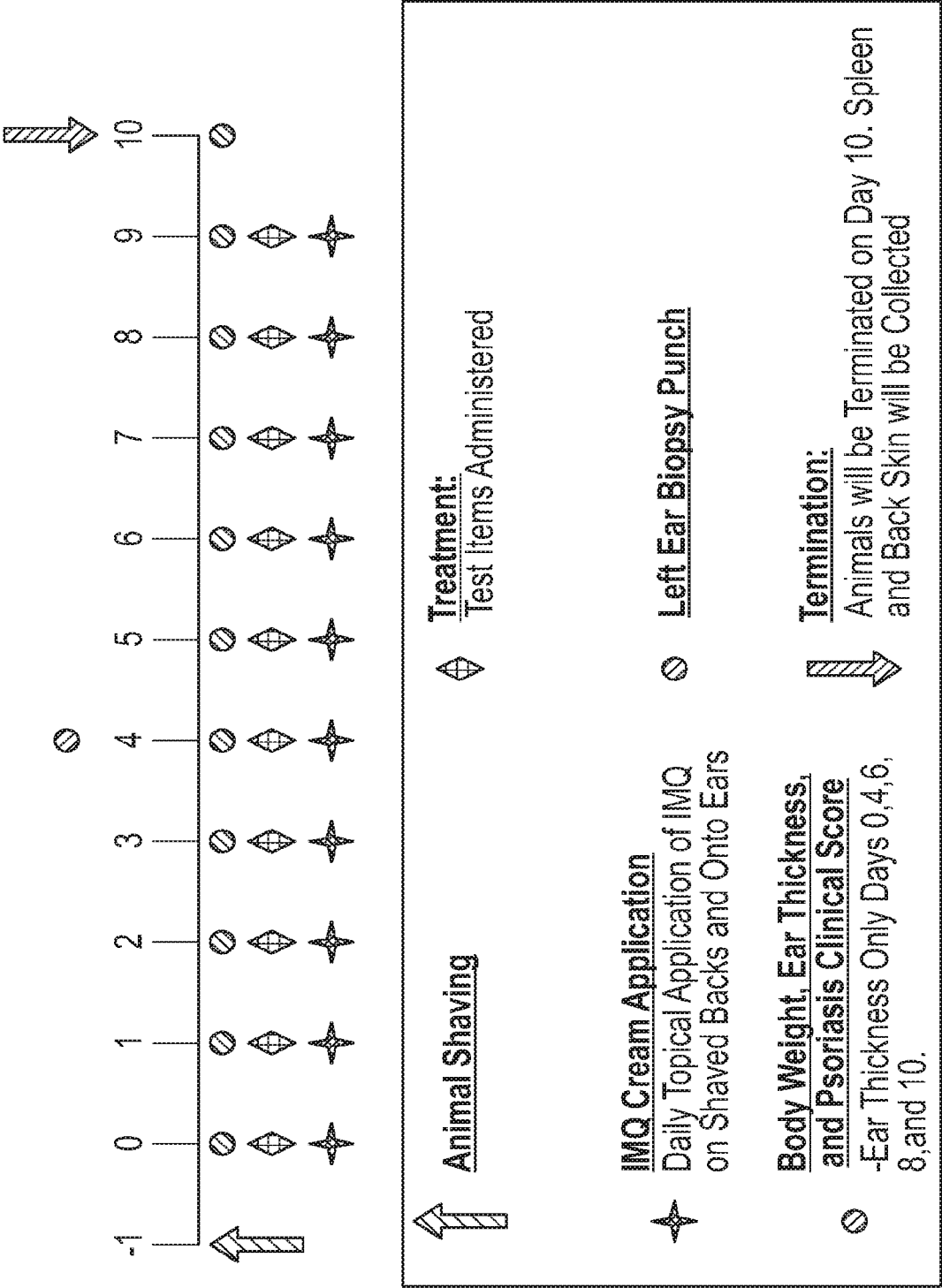


FIG. 98

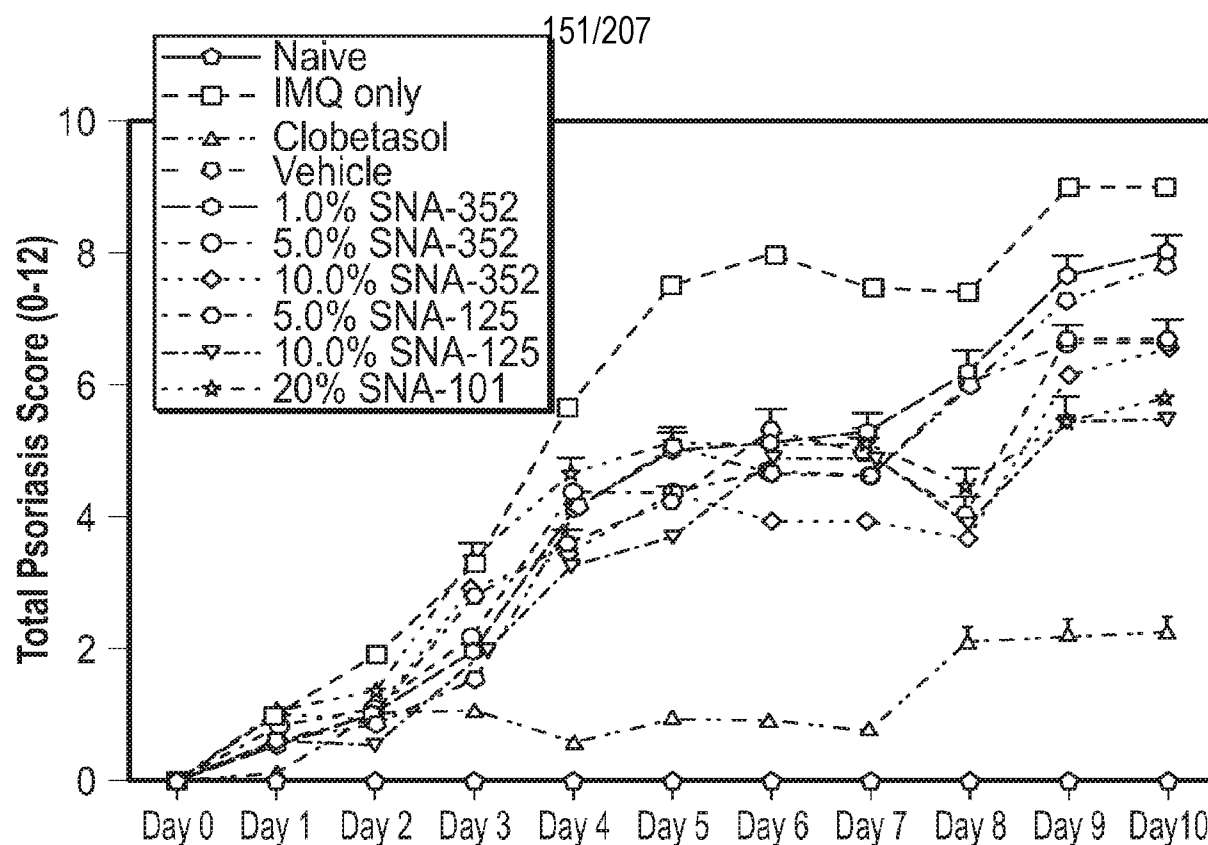


FIG. 99A

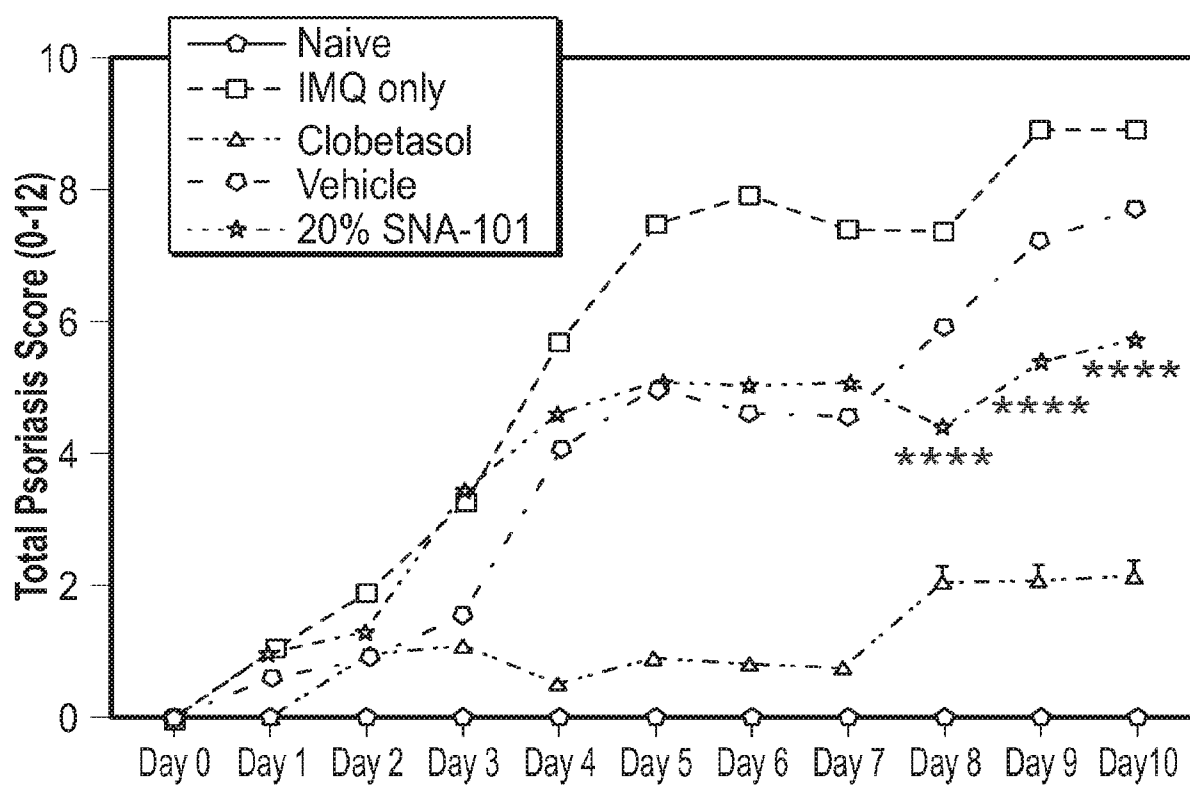


FIG. 99B

152/207

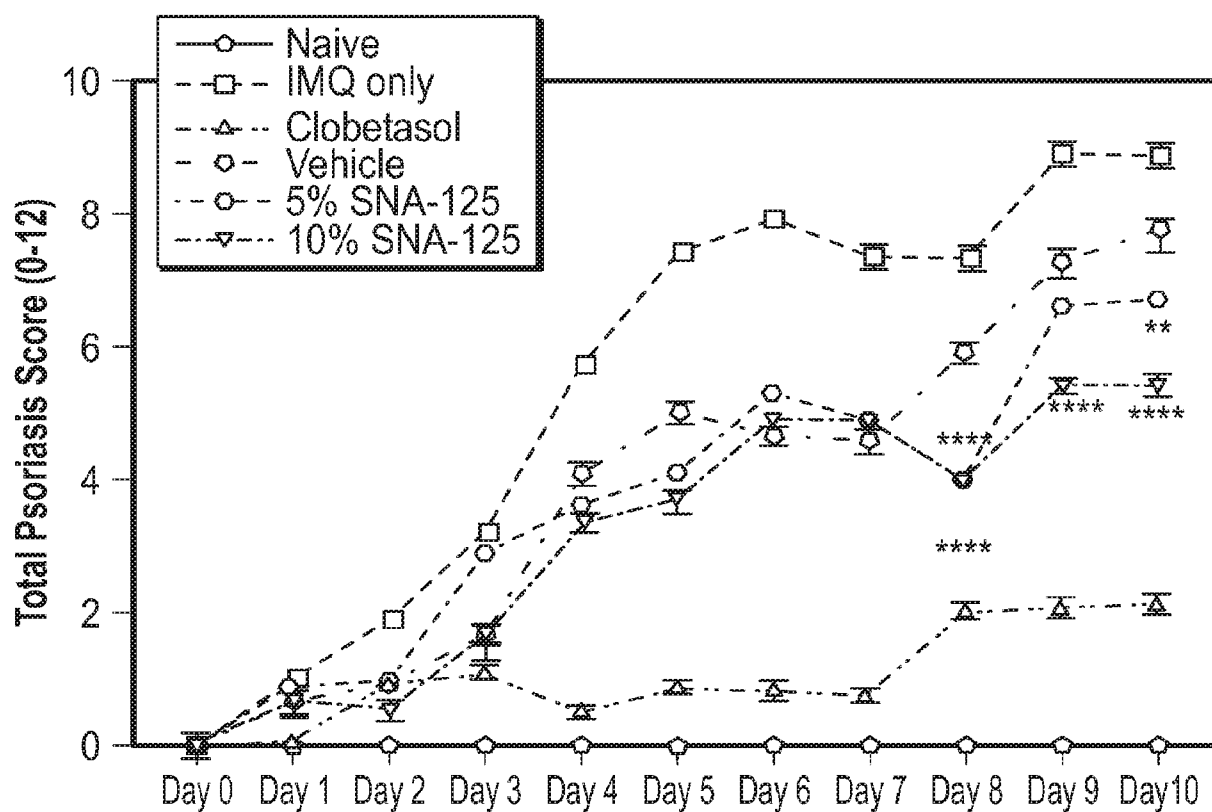


FIG. 99C

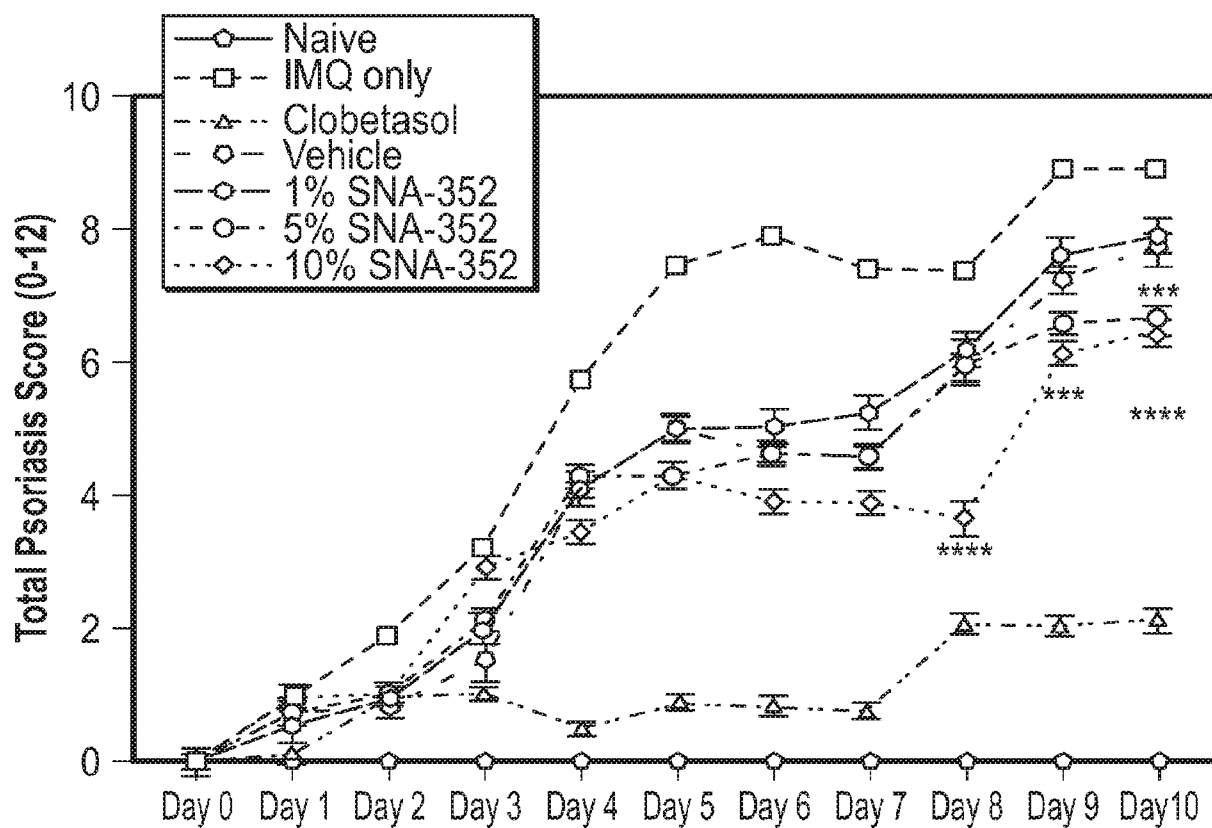


FIG. 99D

153/207

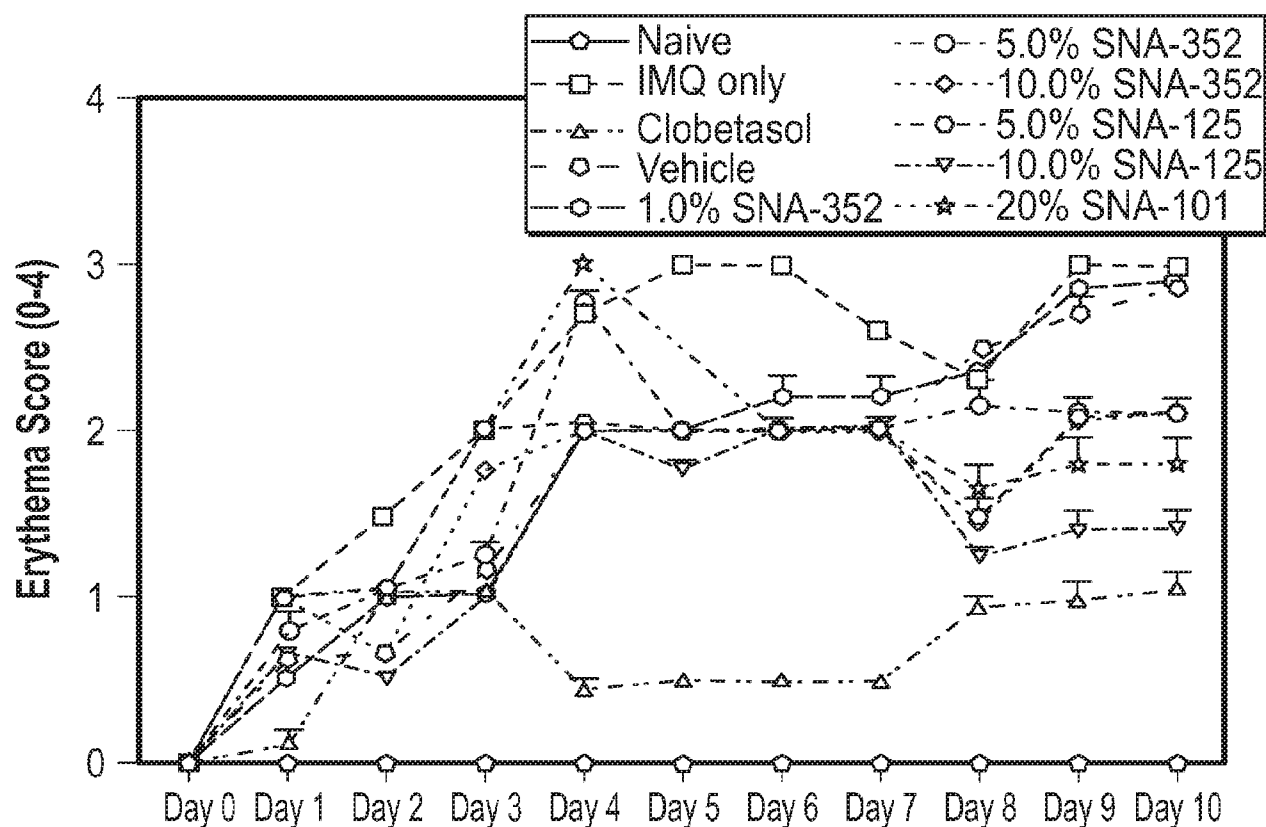


FIG. 100A

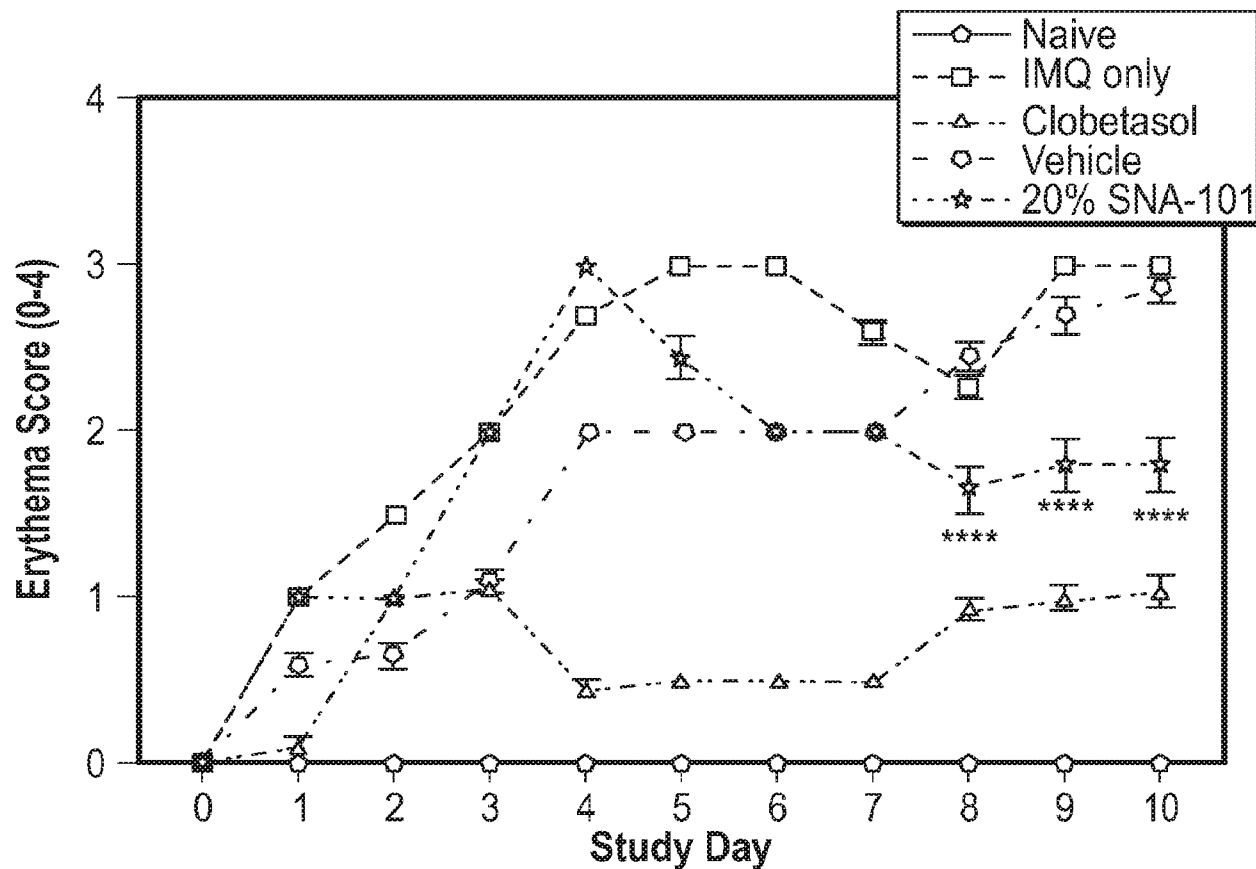
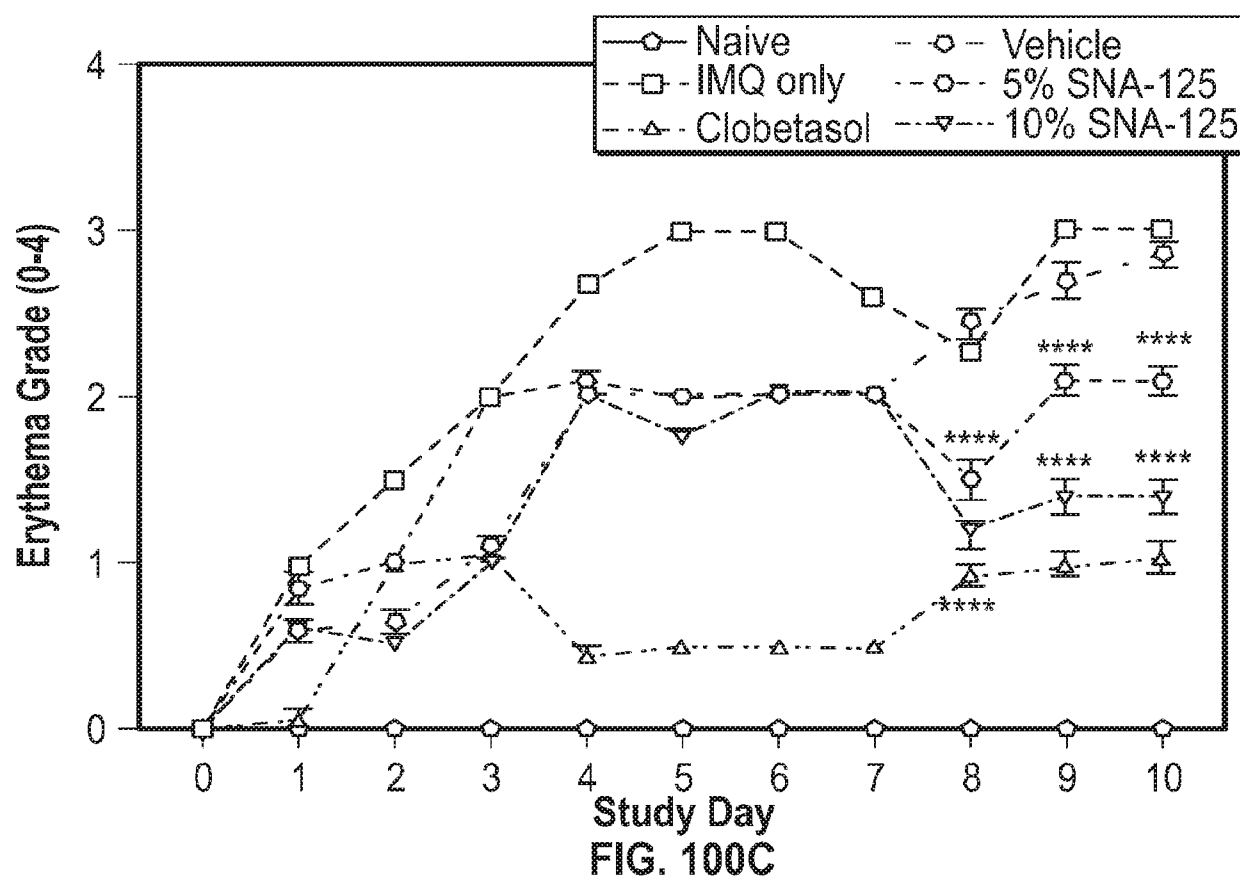
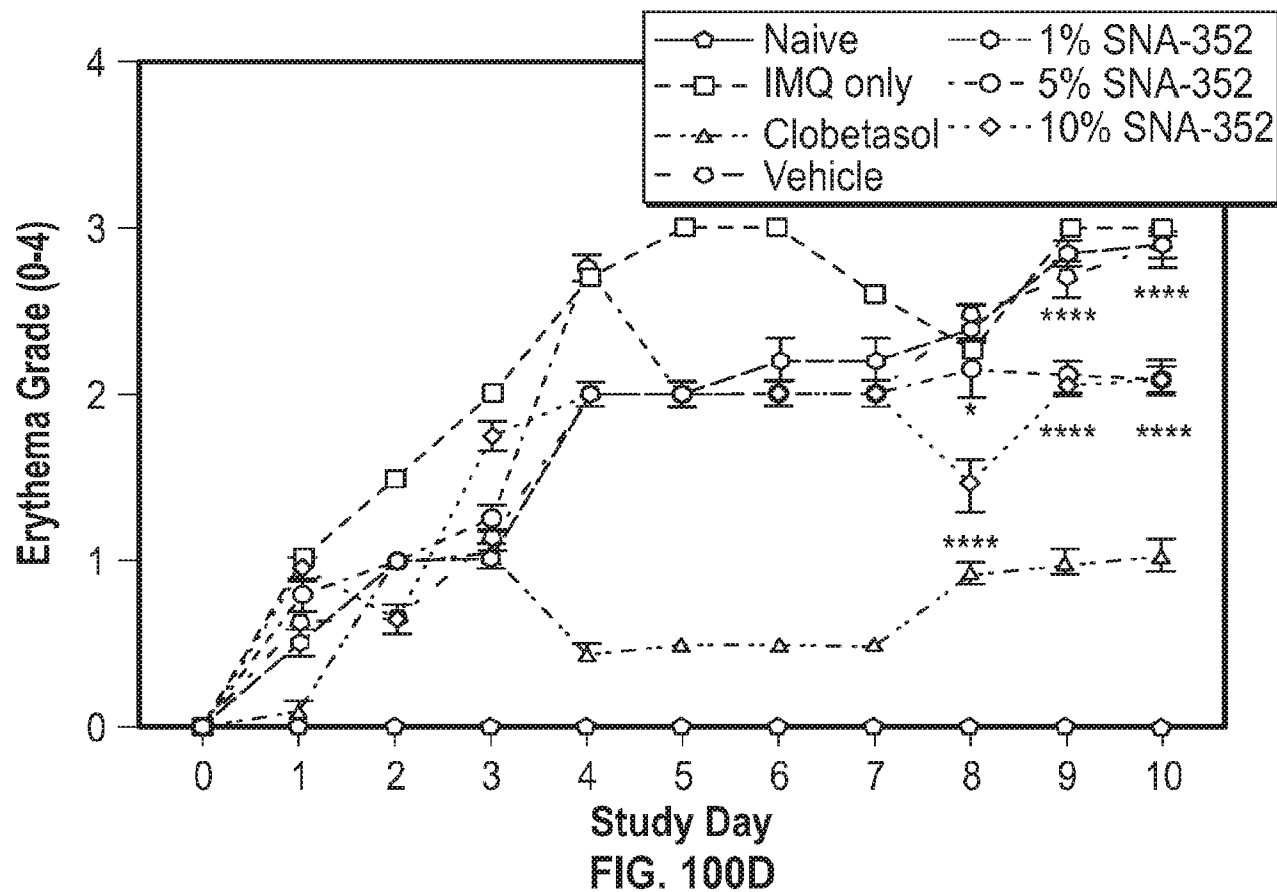


FIG. 100B

154/207



155/207

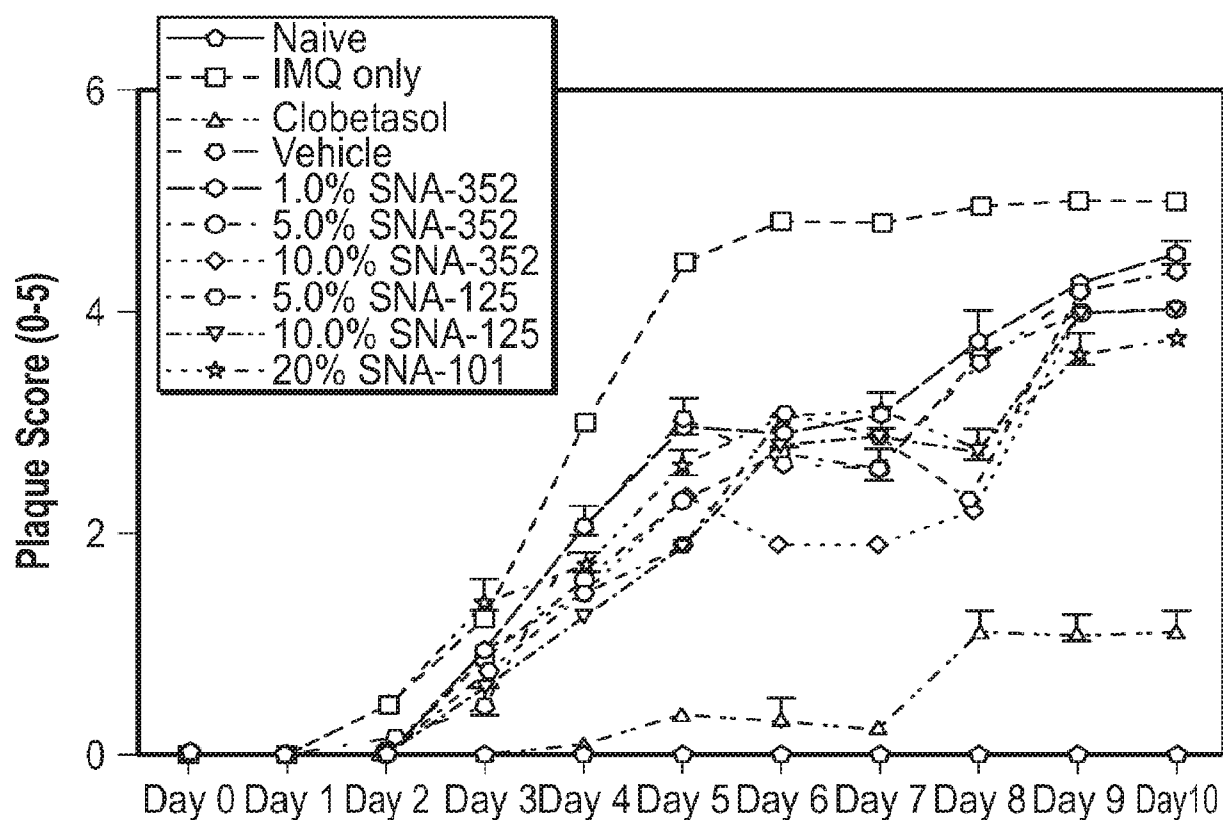


FIG. 101A

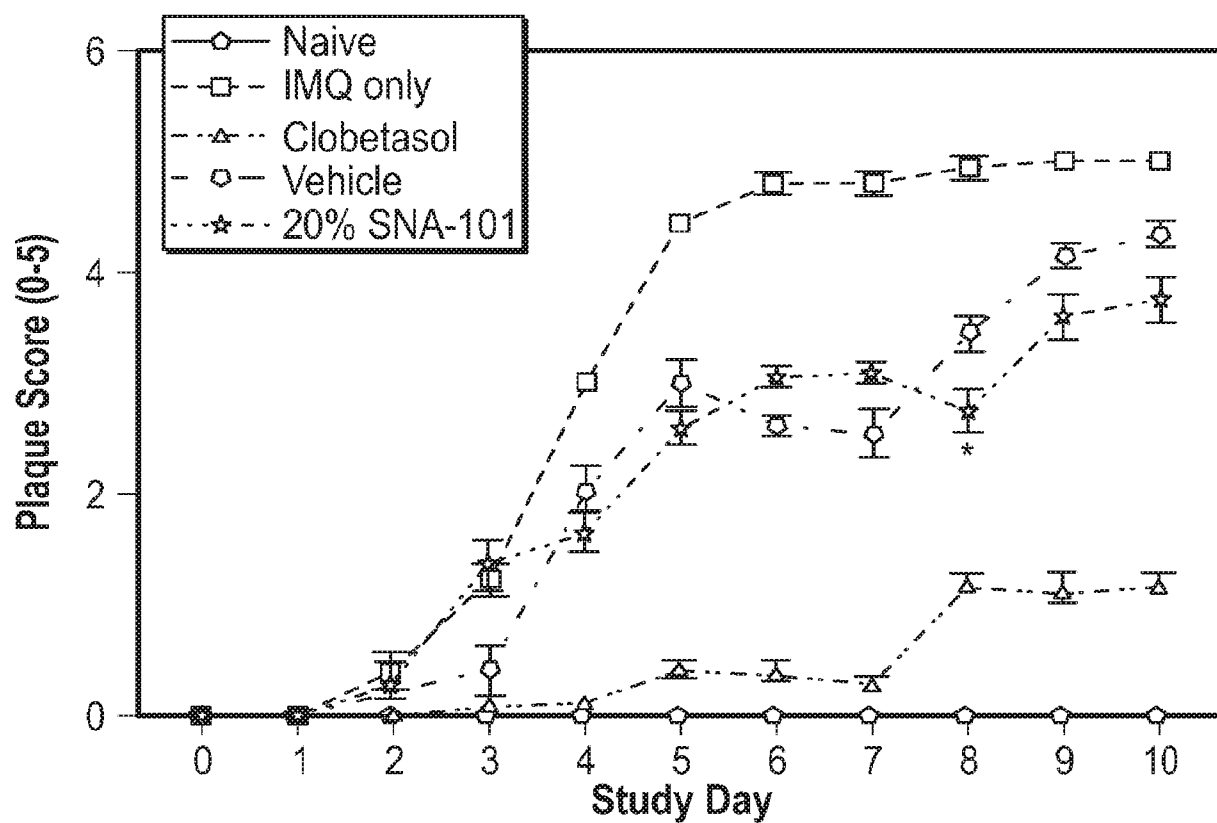
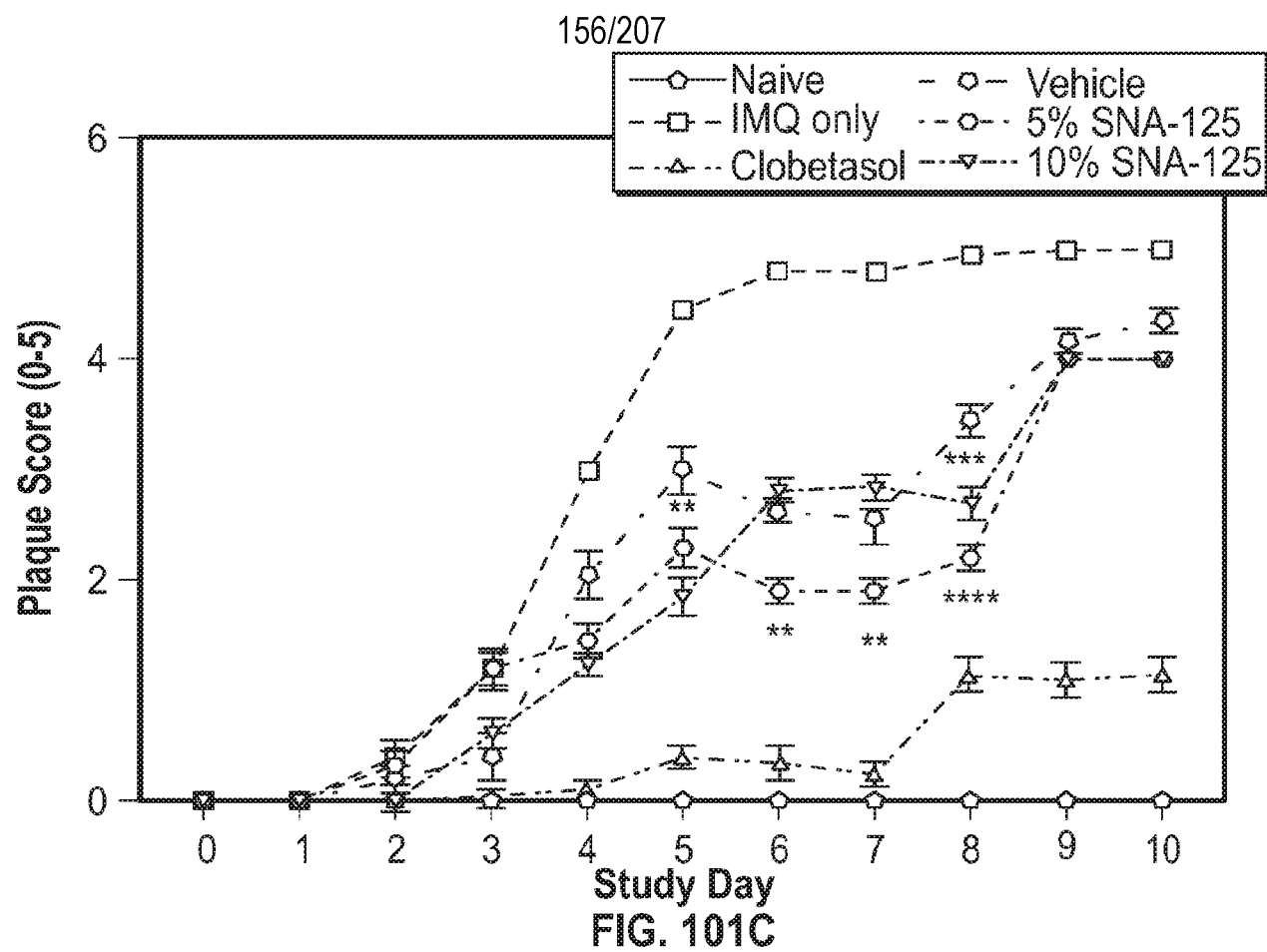
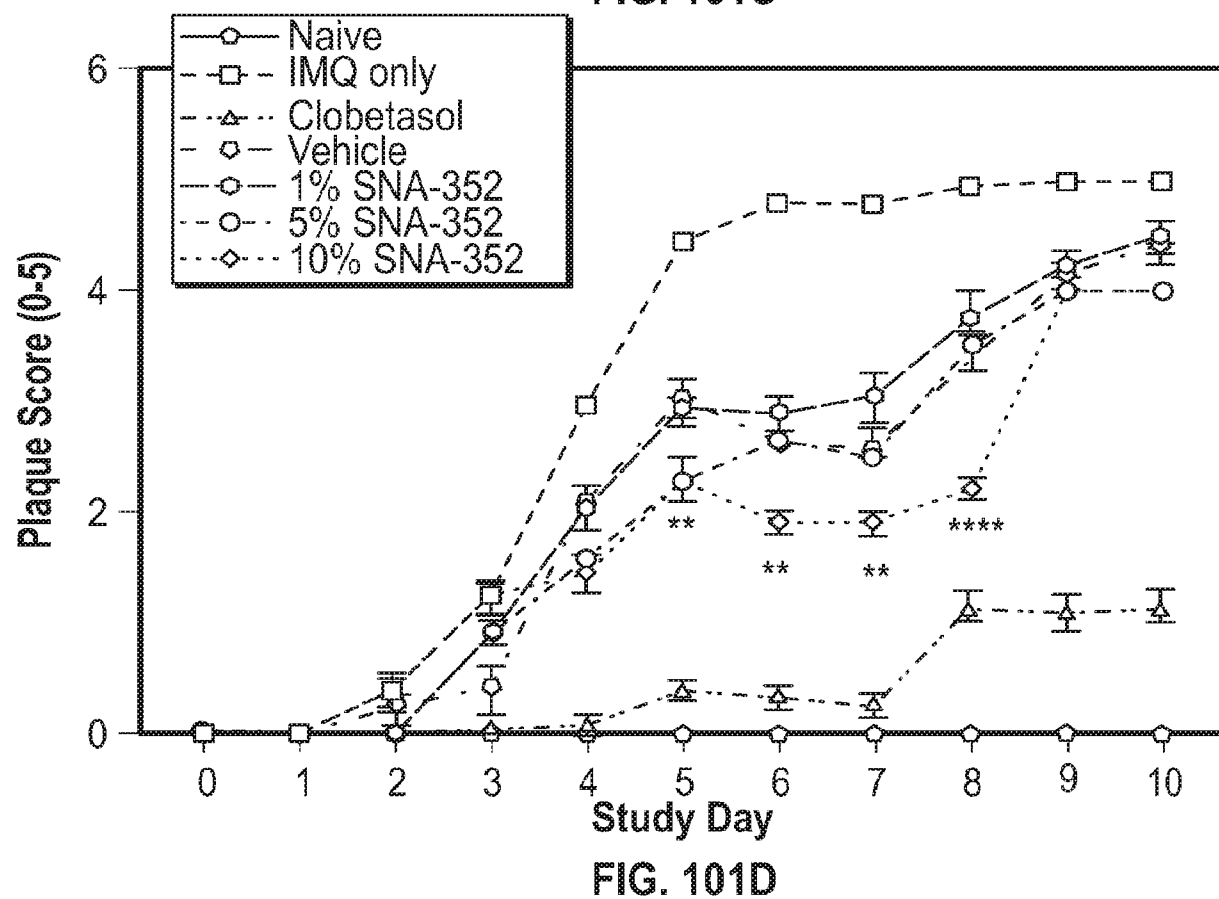


FIG. 101B



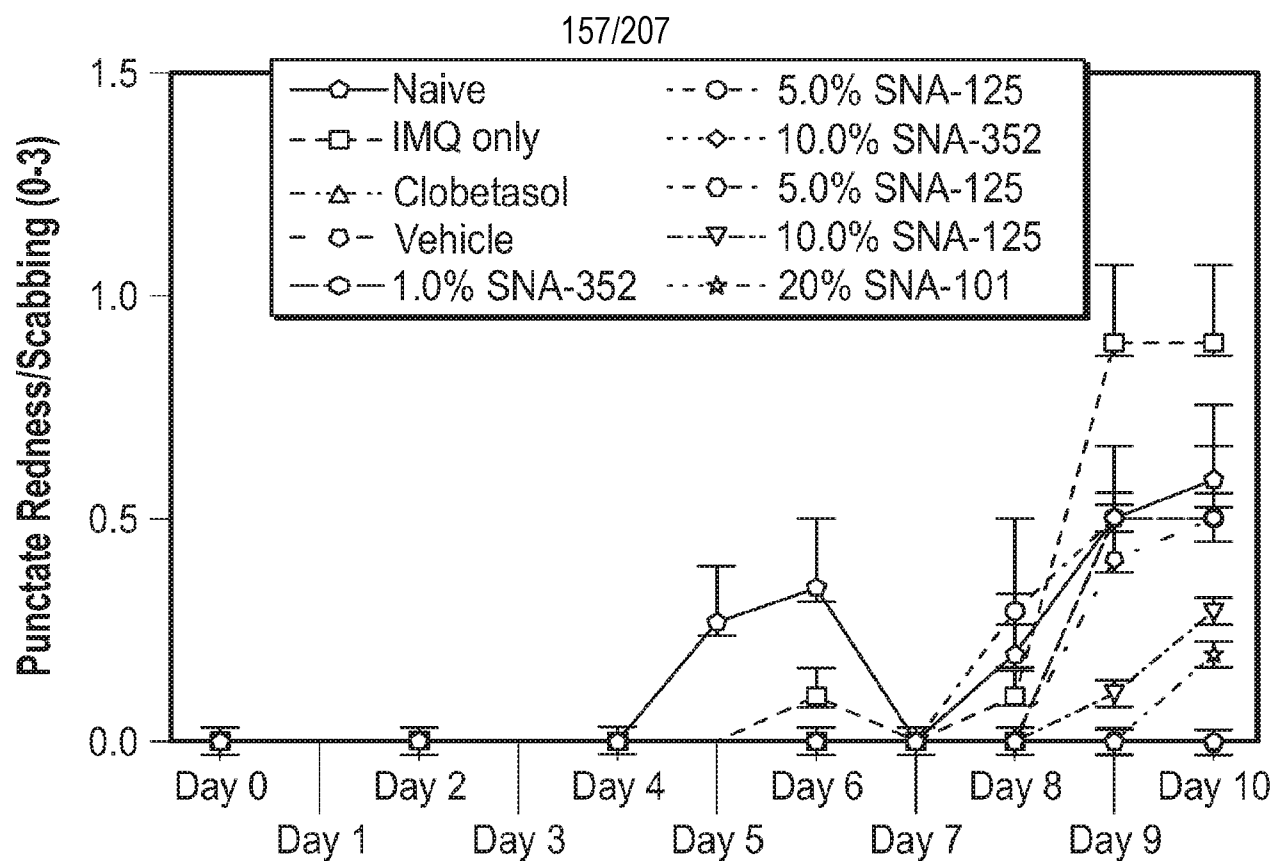


FIG. 102A

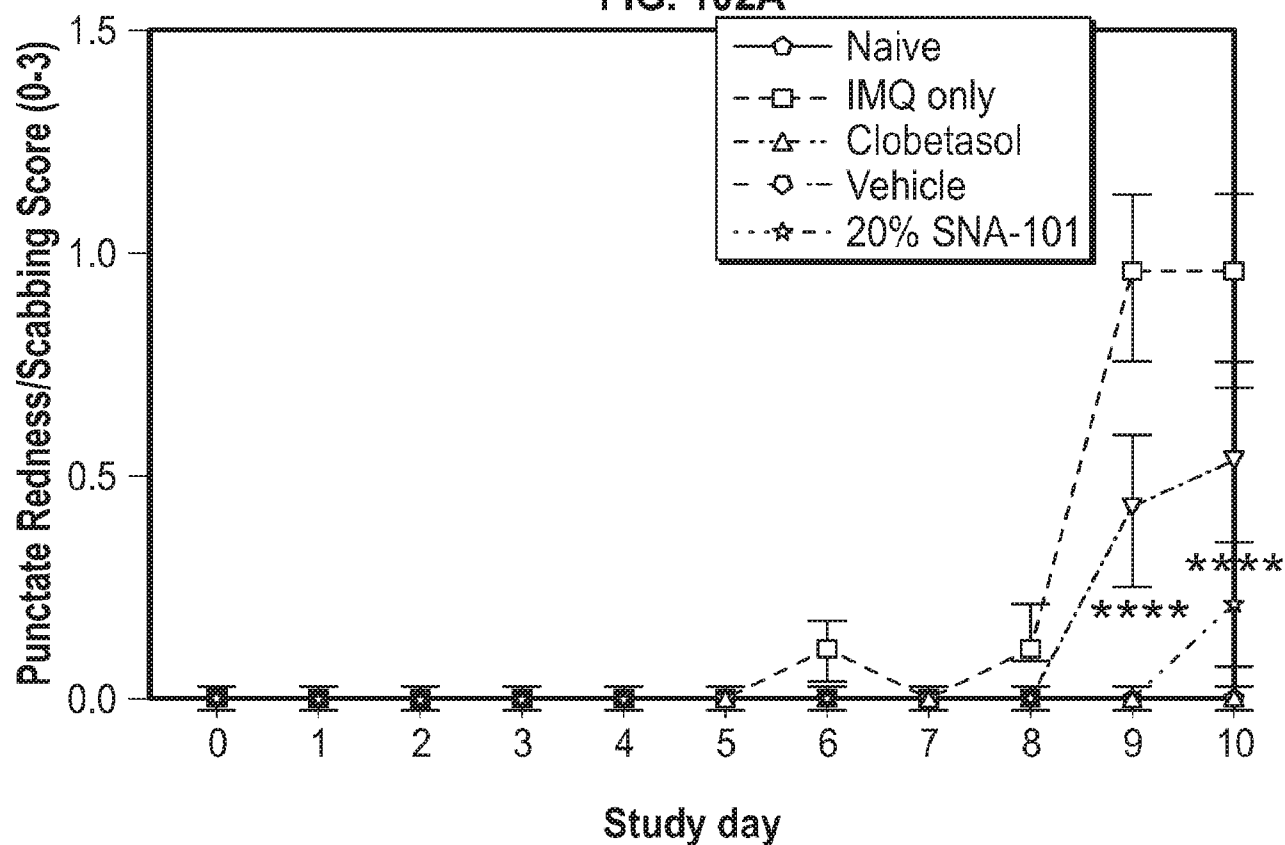


FIG. 102B

158/207

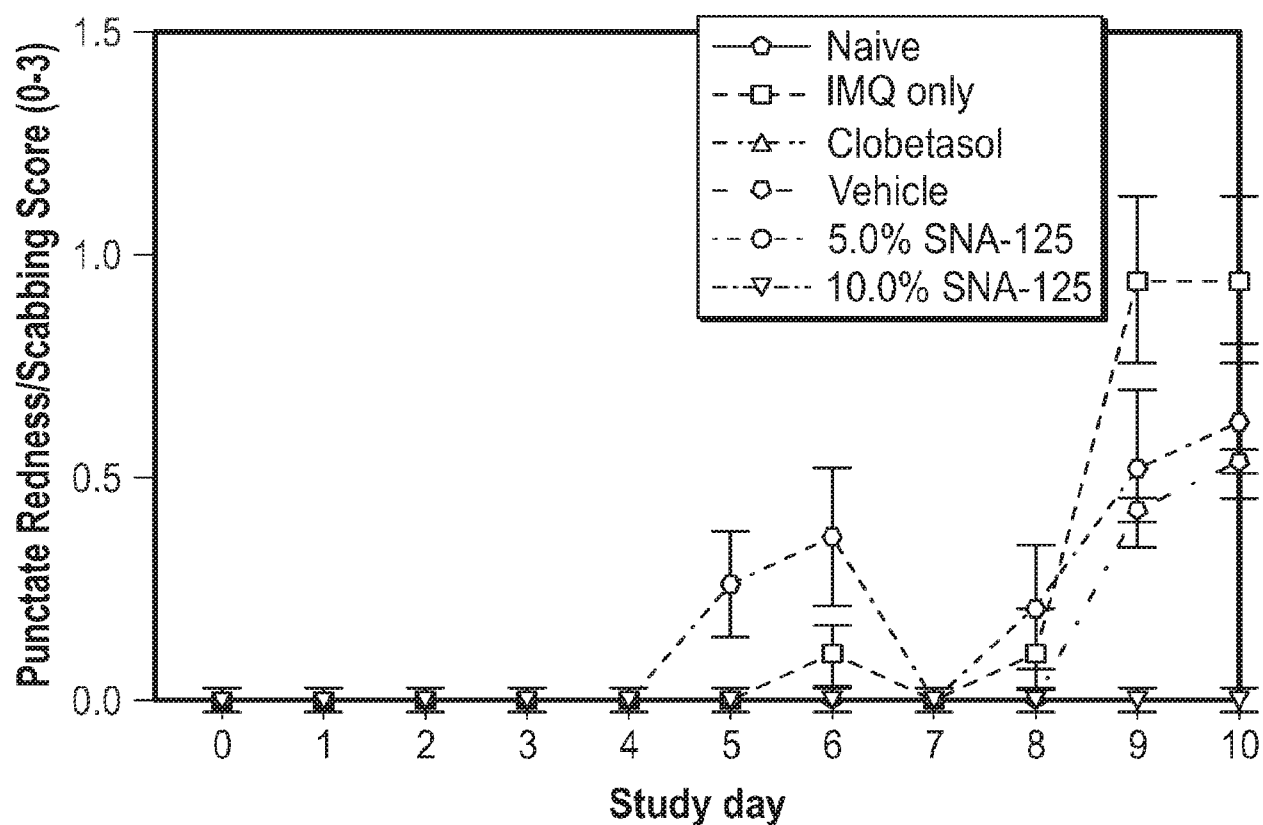


FIG. 102C

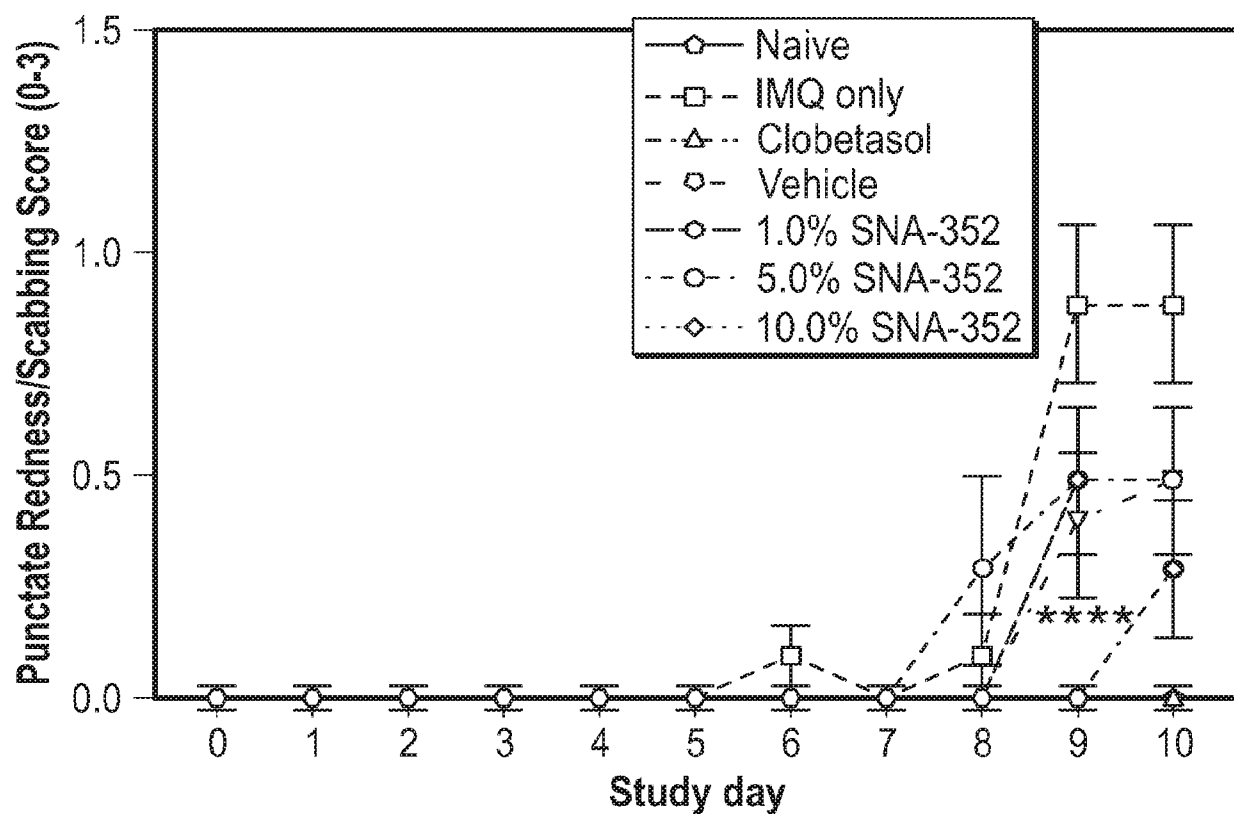


FIG. 102D

159/207

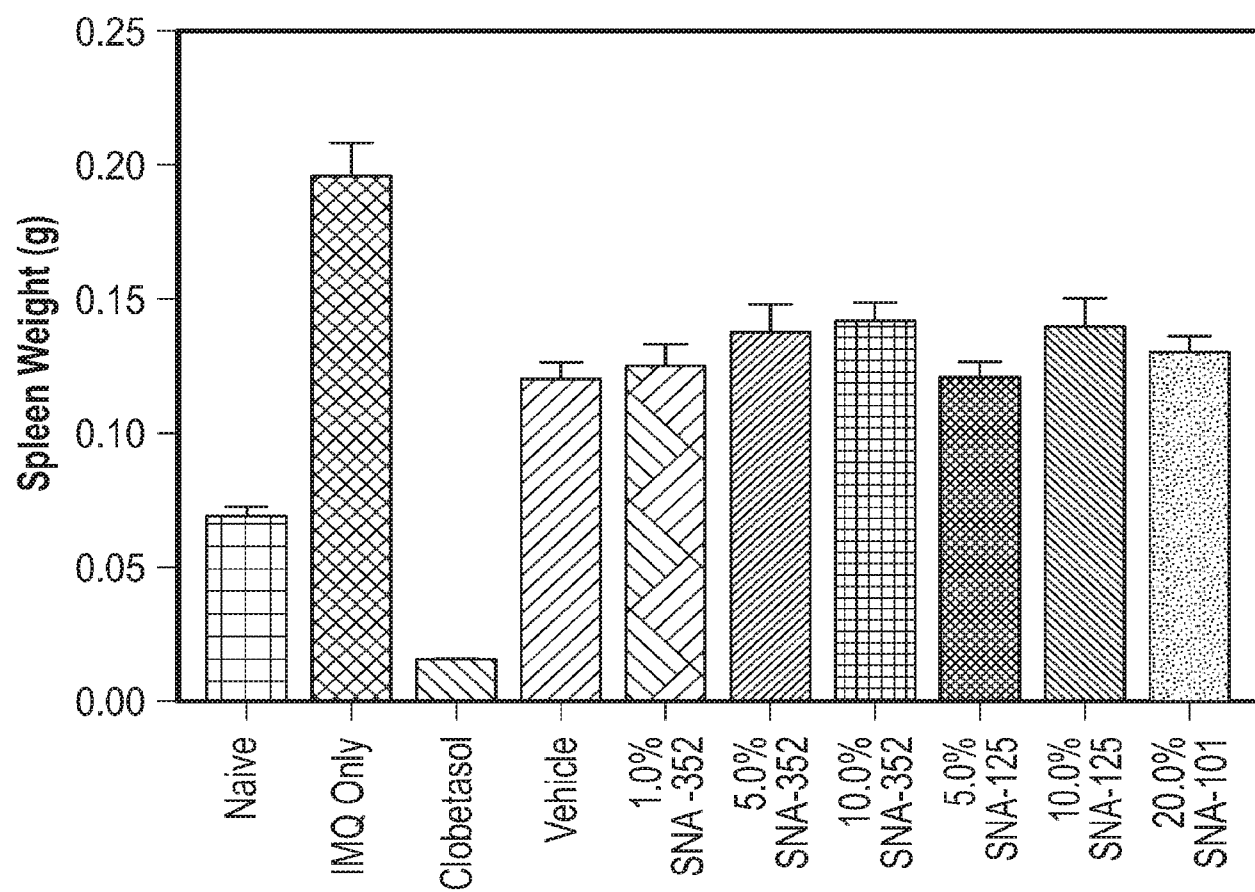


FIG. 103A

160/207

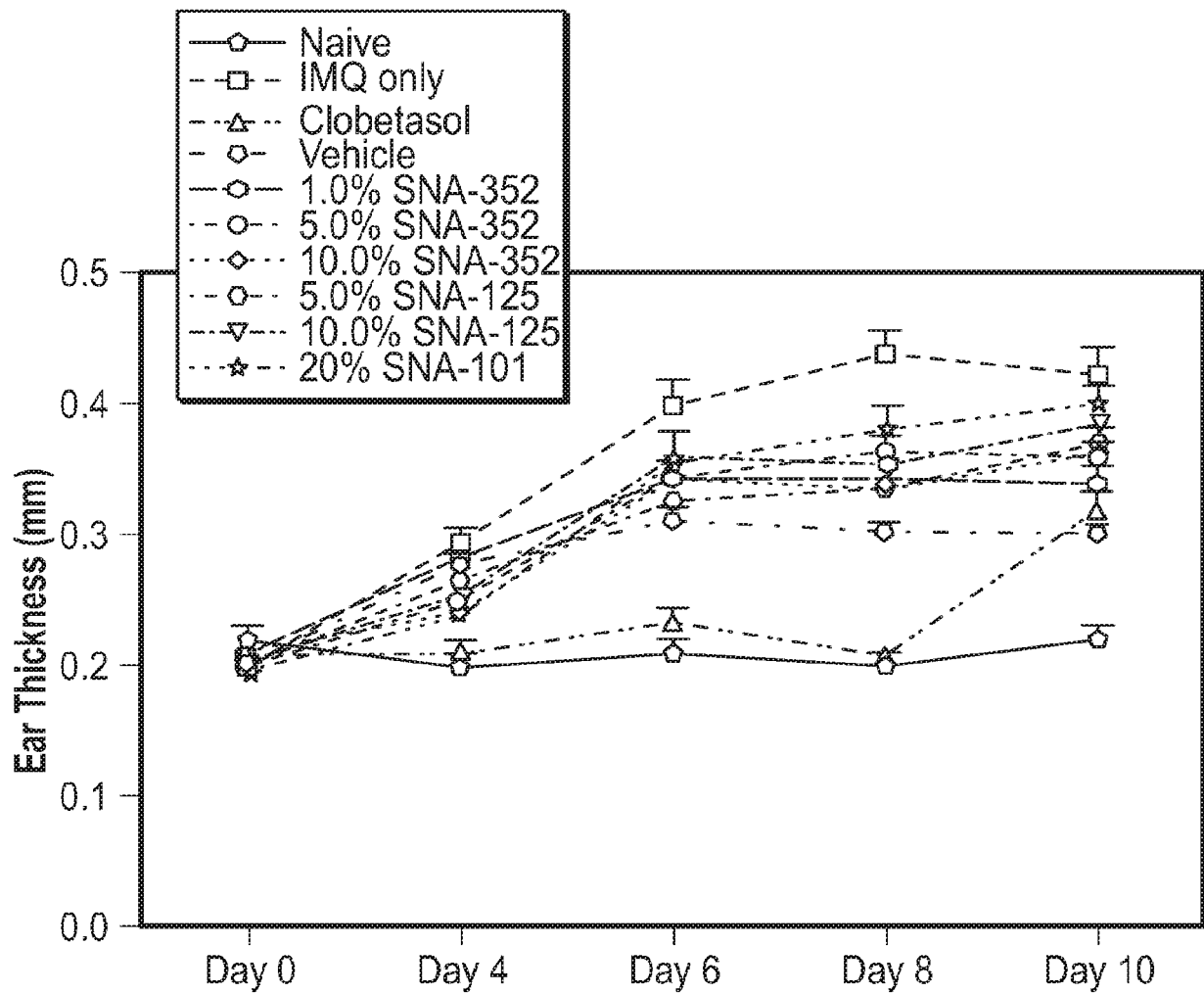


FIG. 103B

161/207

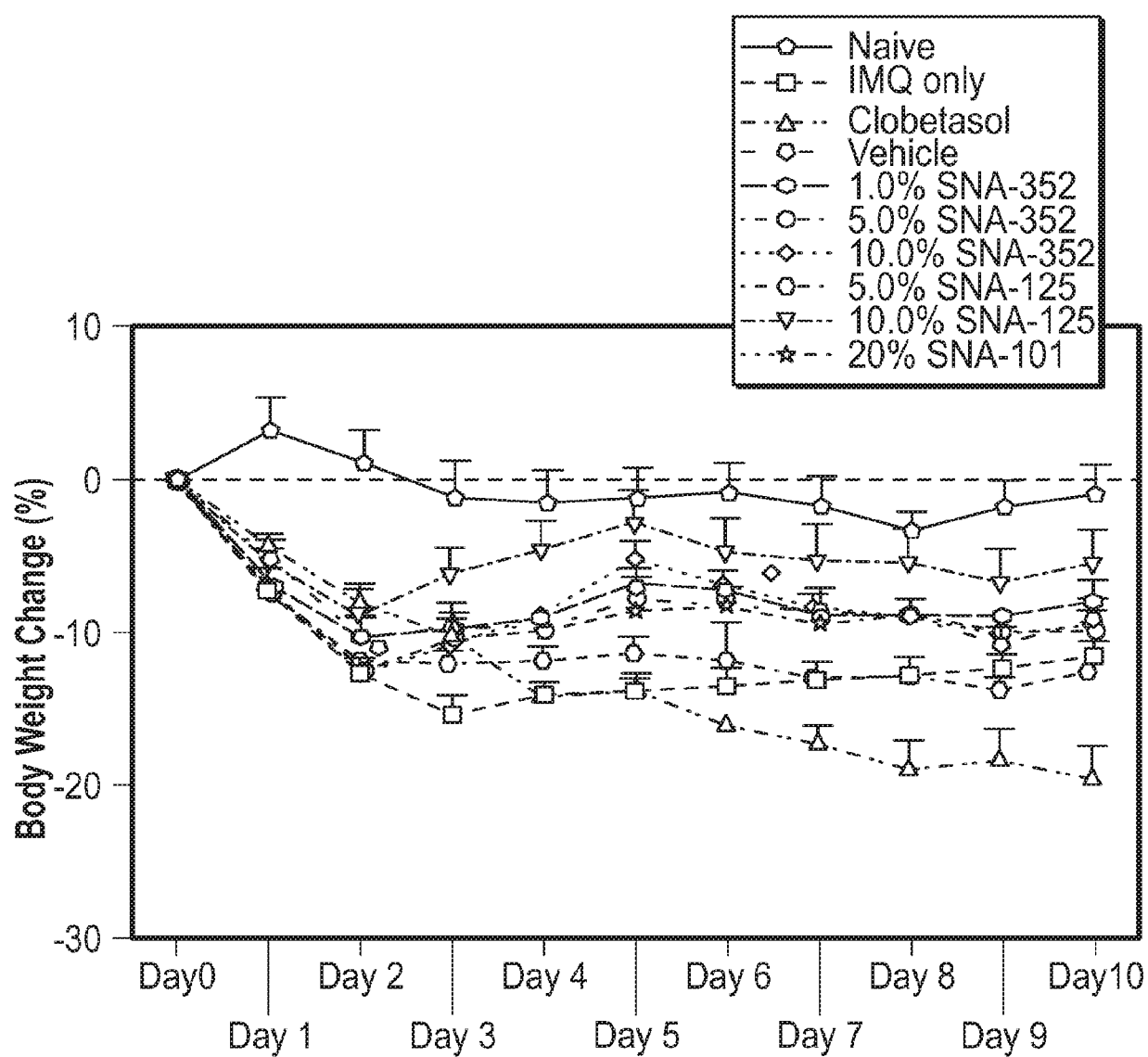


FIG. 103C

162/207

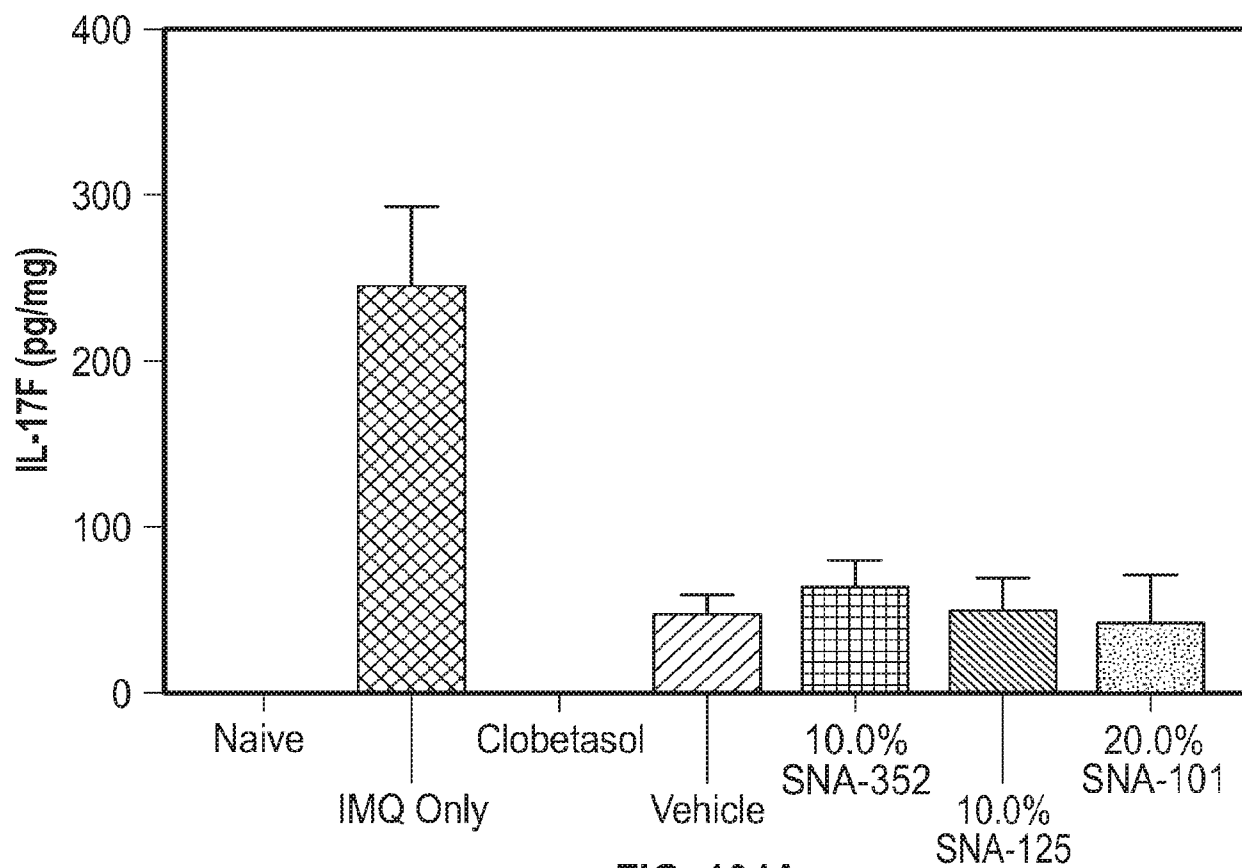


FIG. 104A

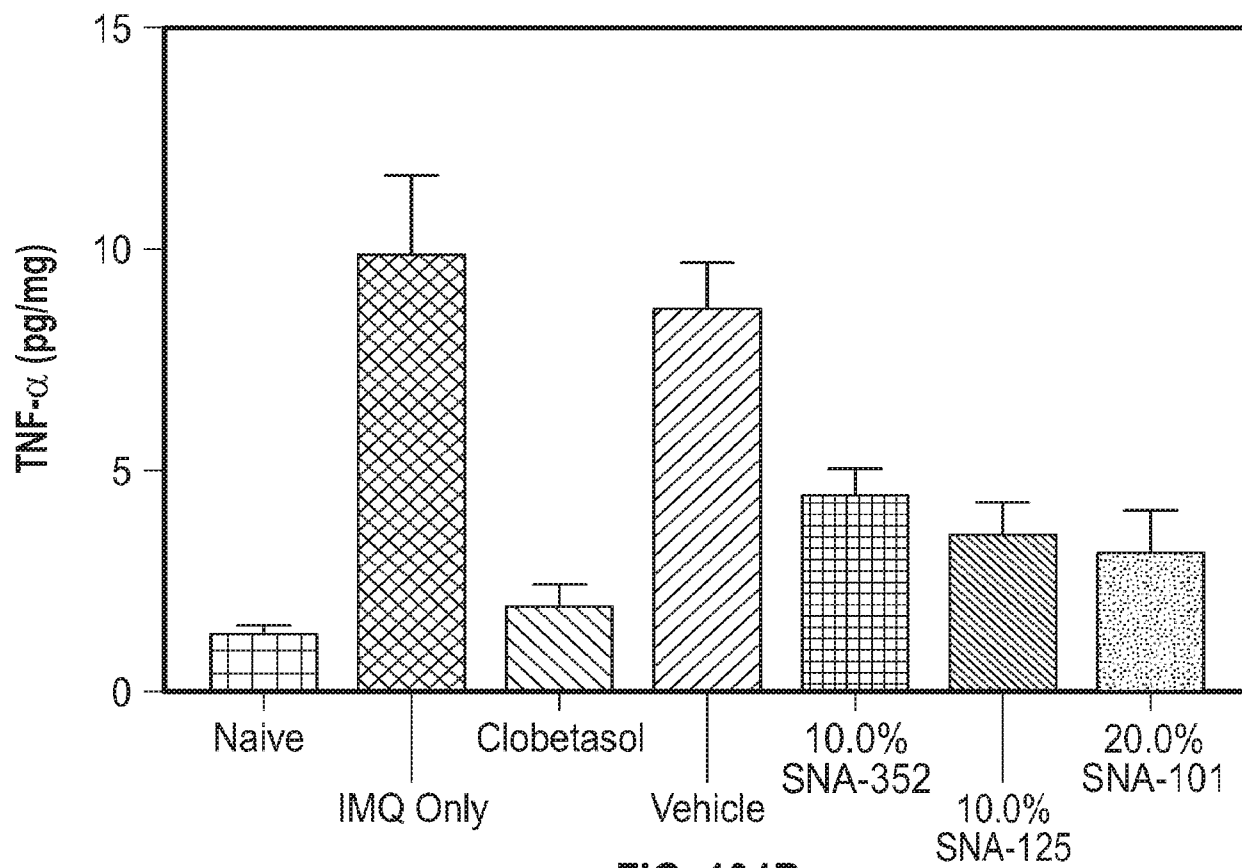


FIG. 104B

163/207

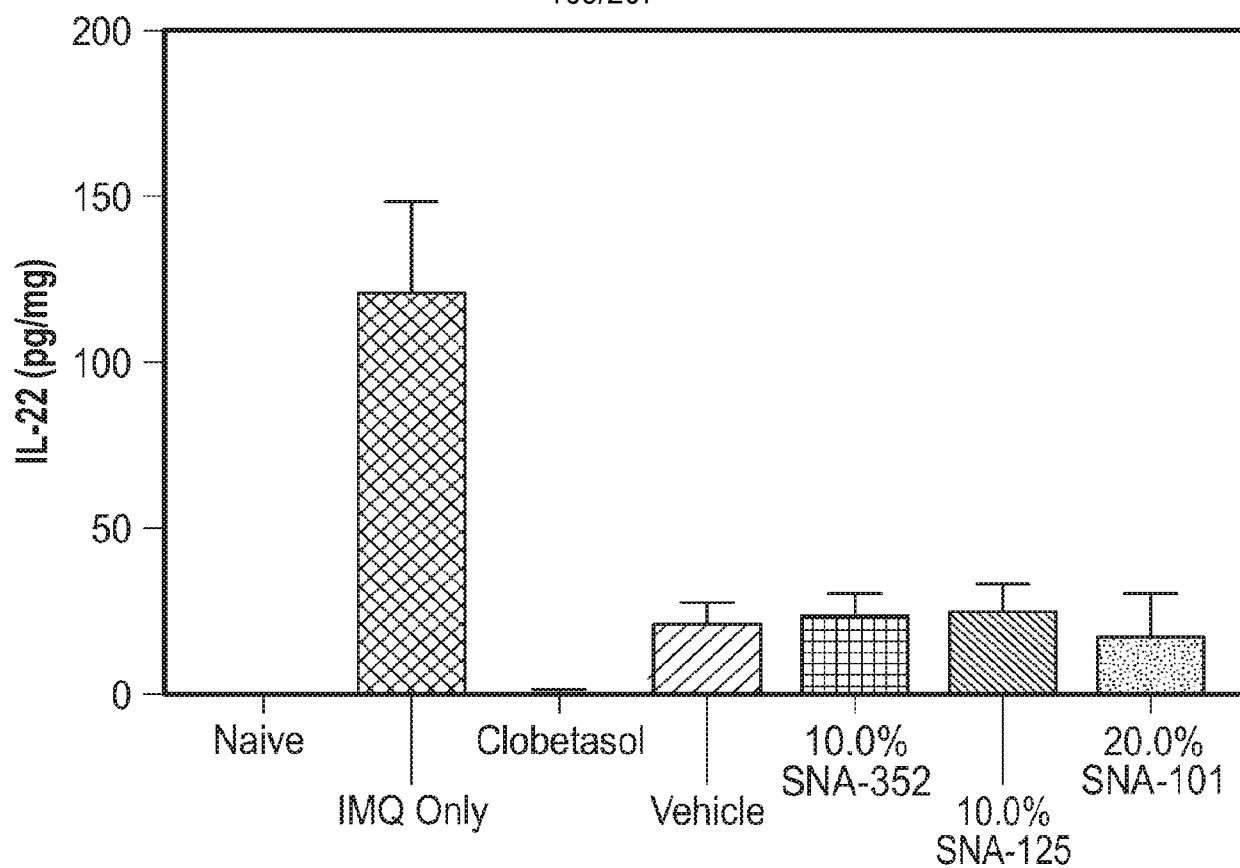


FIG. 104C

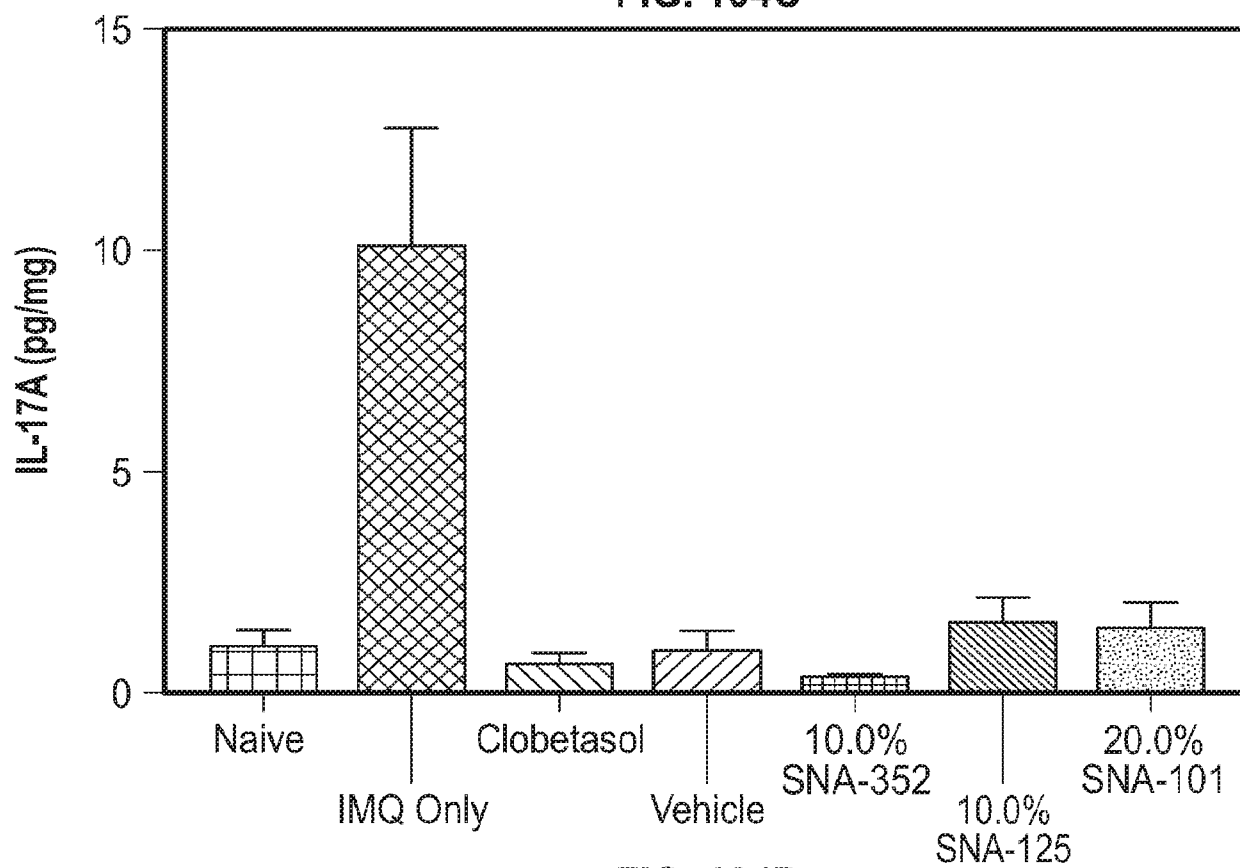
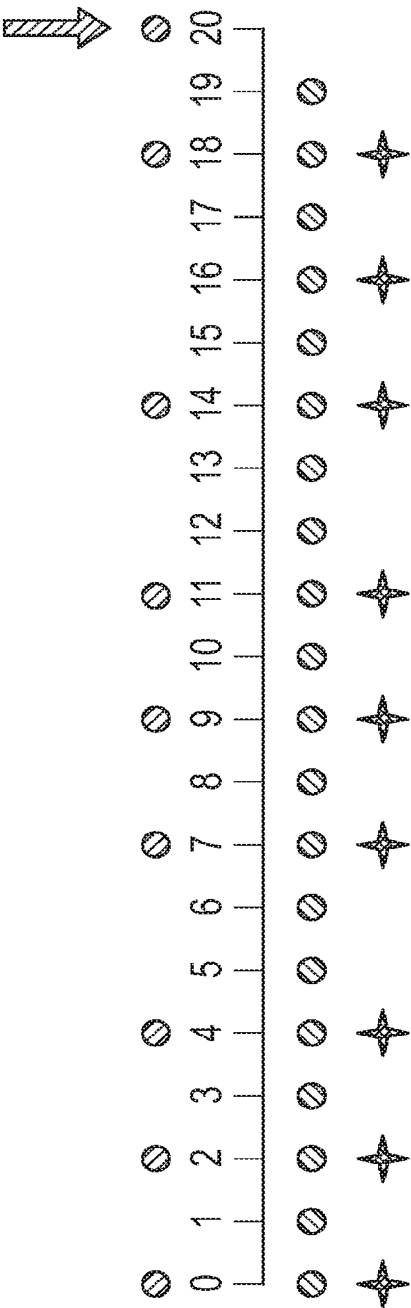


FIG. 104D



<u>Treatment</u>	
T1s Administration. Positive Control	
Clobetasol was Administered Topically	
Daily	
<u>Termination</u>	
Animals were Terminated and Ears	
were collected	
<u>Body Weight, Clinical Score</u>	
<u>and Ear Thickness</u>	
<u>IL 23 Administration:</u>	
IL 23 or PBS i.d. on Ears	

FIG. 105

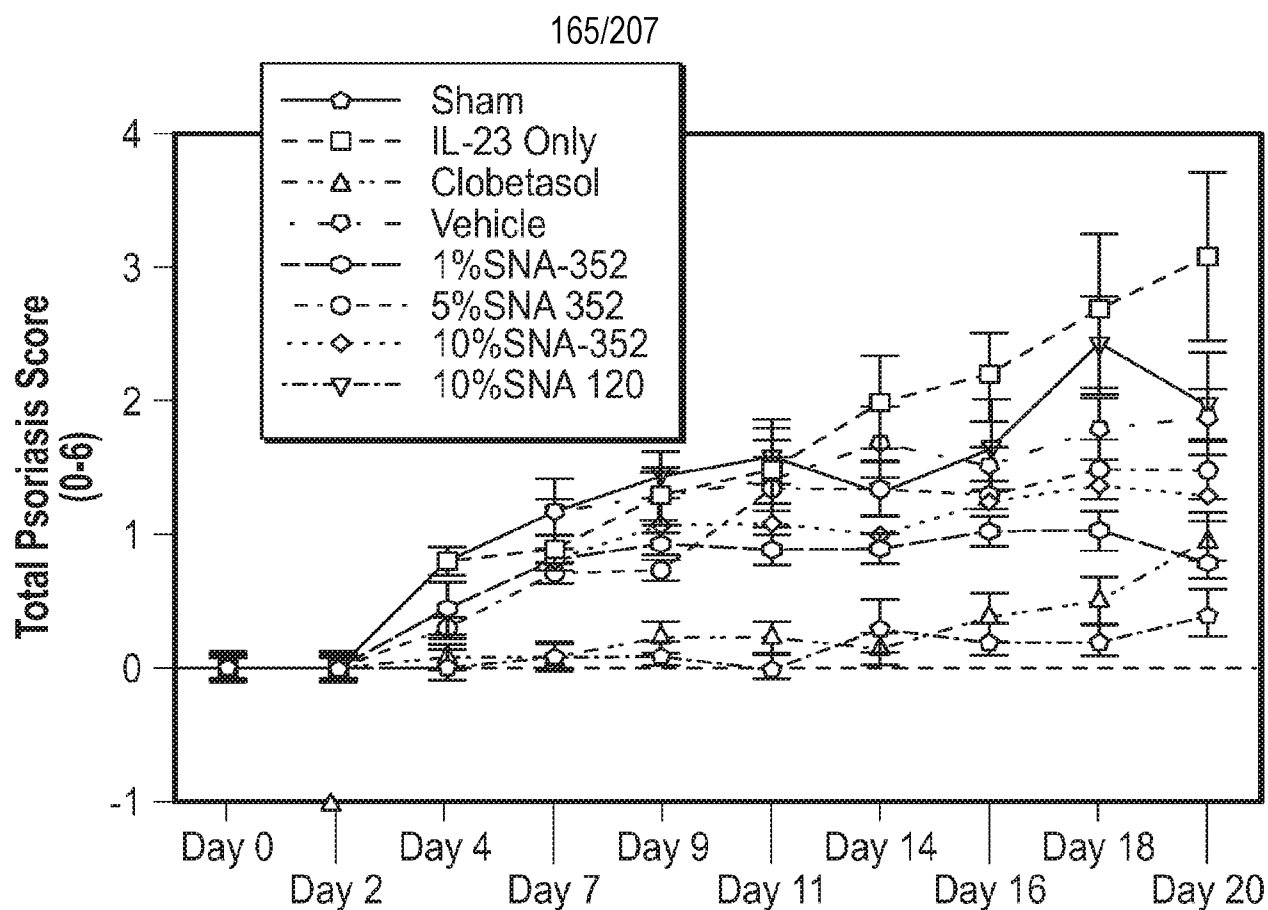


FIG. 106A

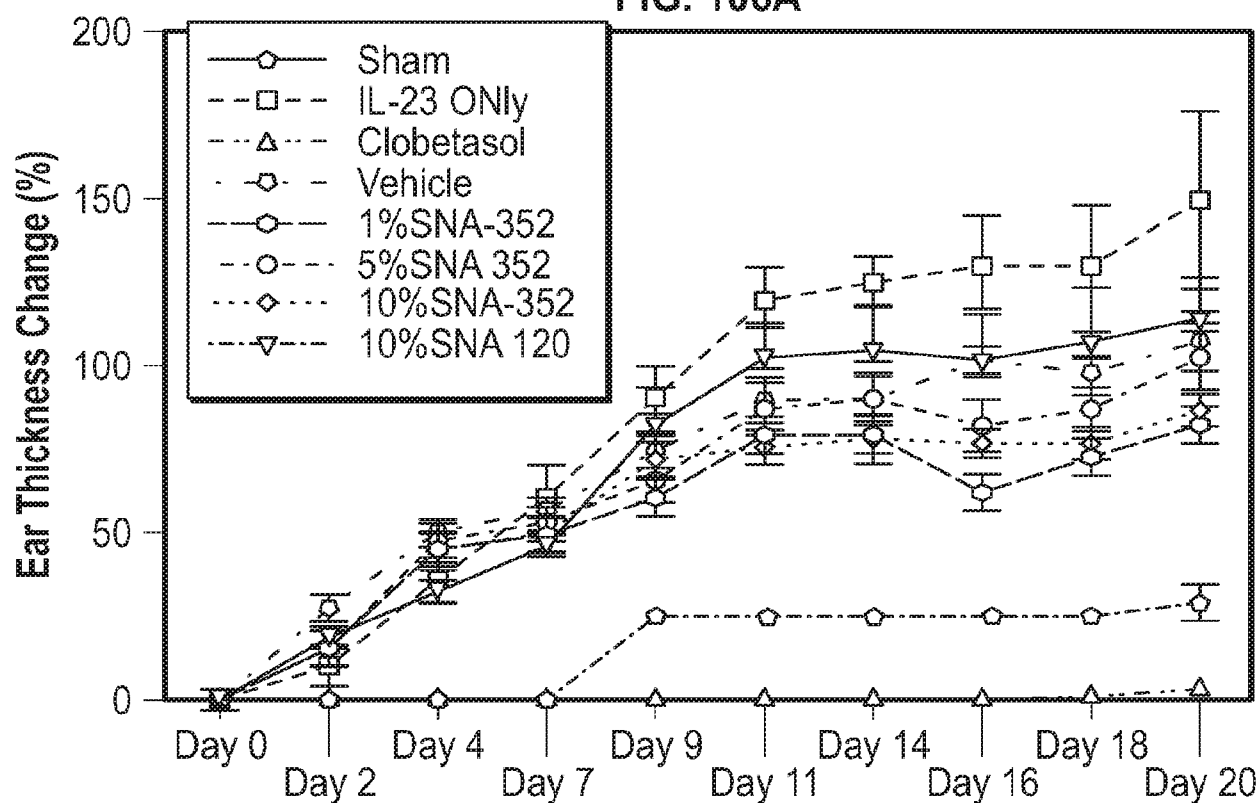


FIG. 106B

166/207

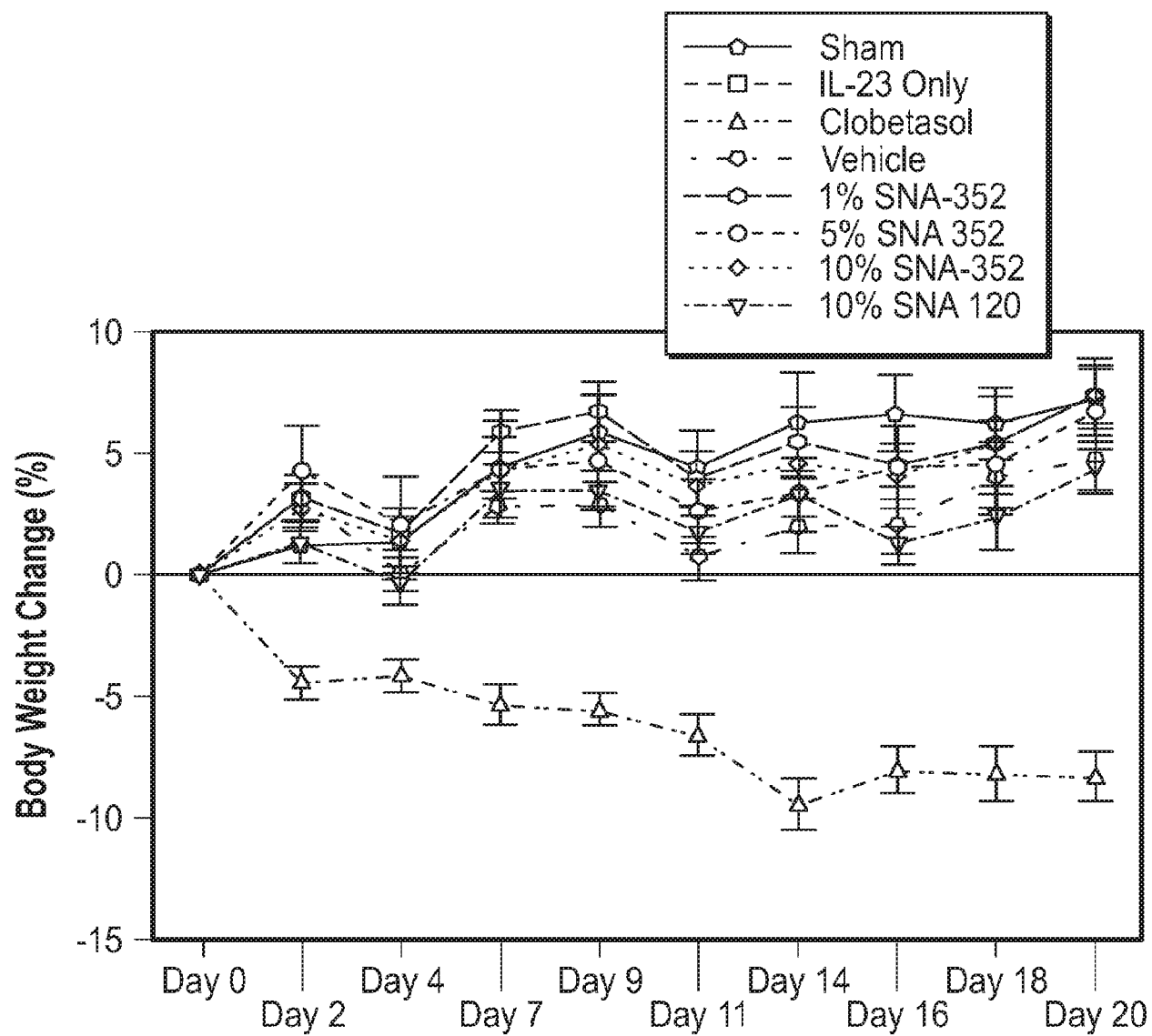


FIG. 106C

167/207

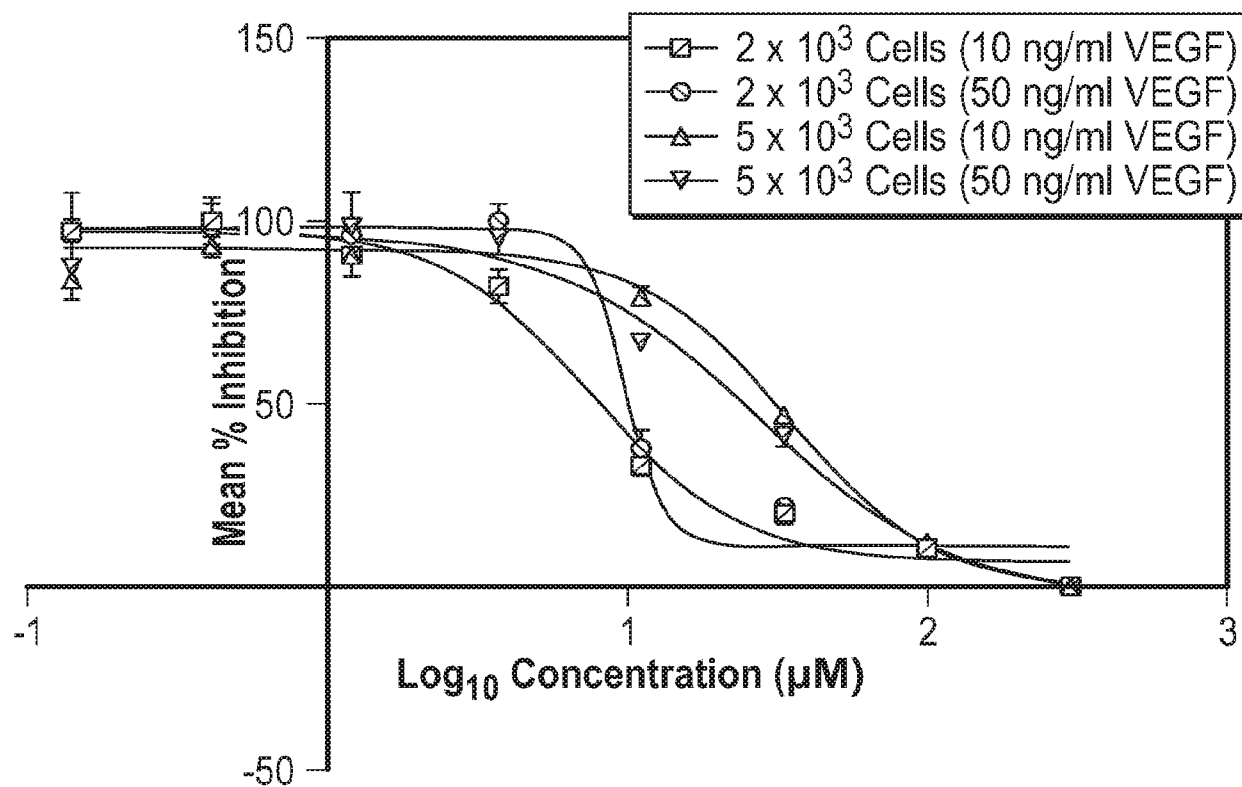


FIG. 107A

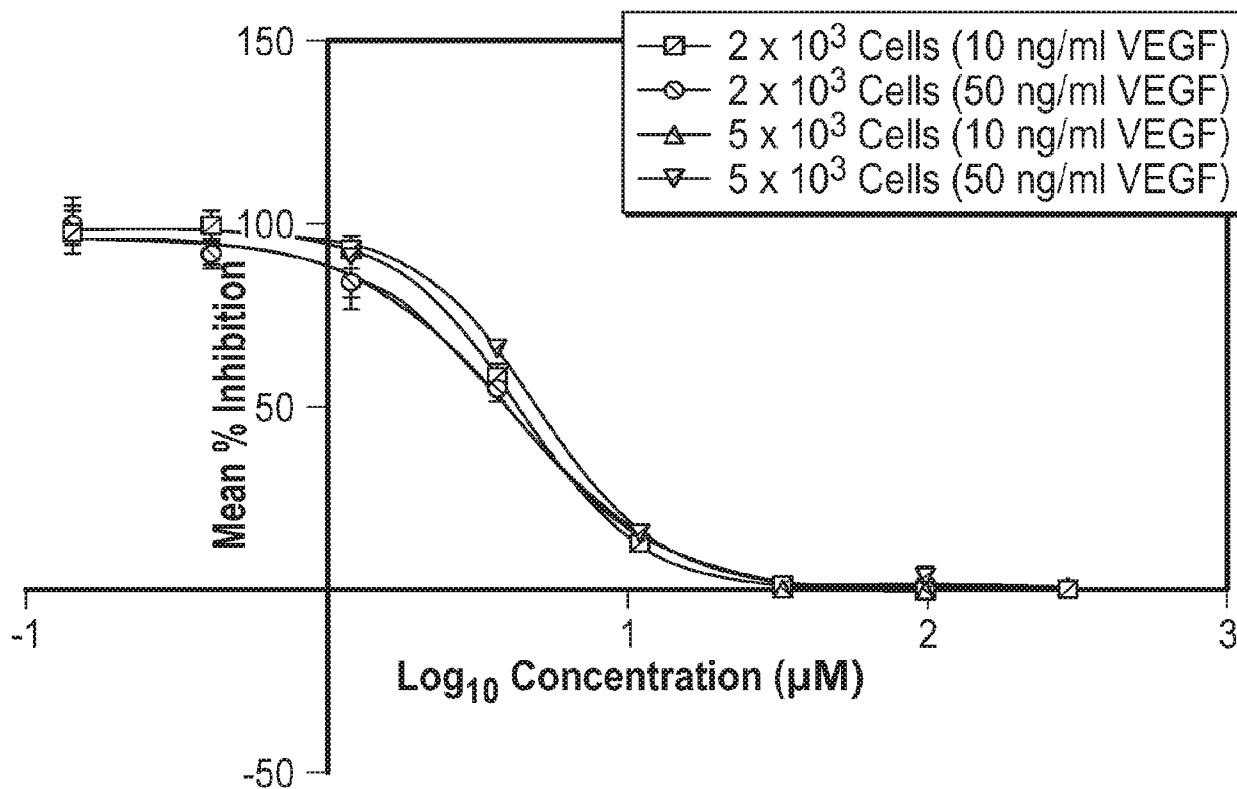


FIG. 107B

168/207

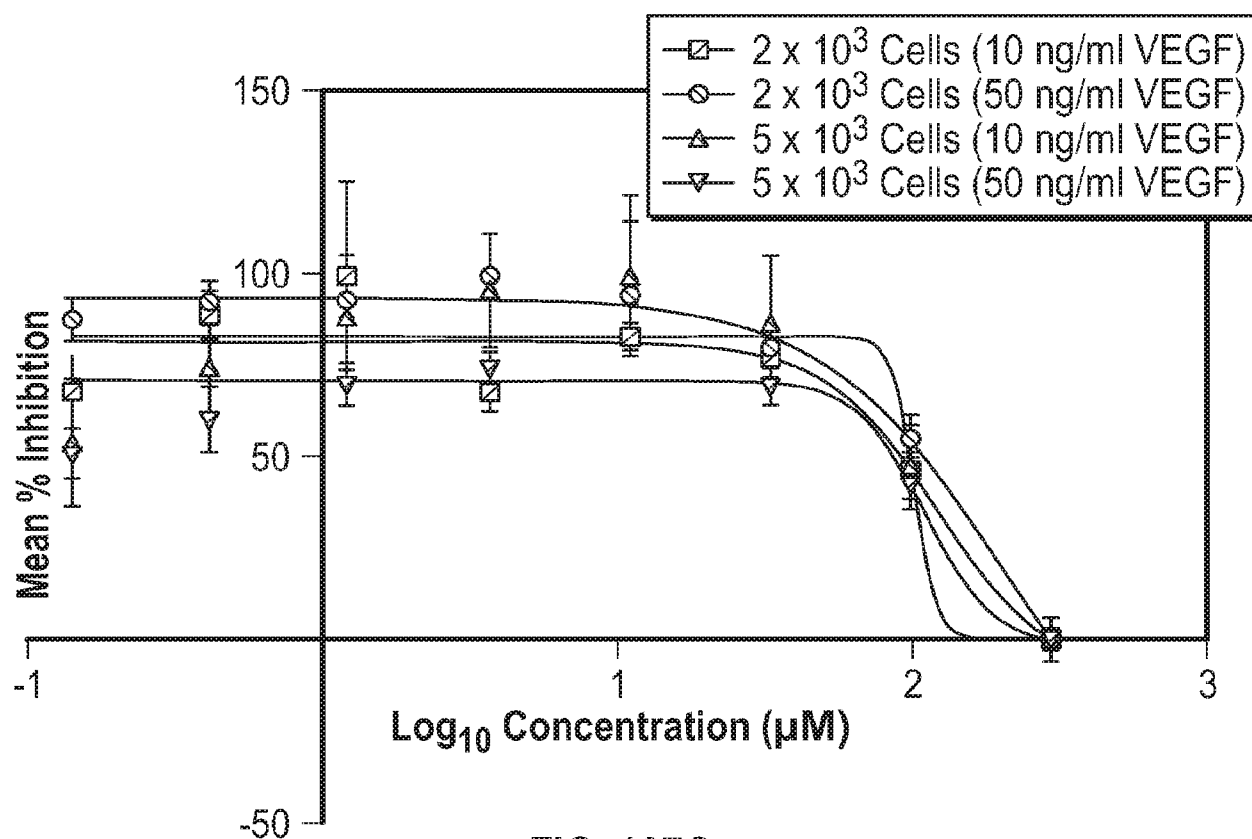


FIG. 107C

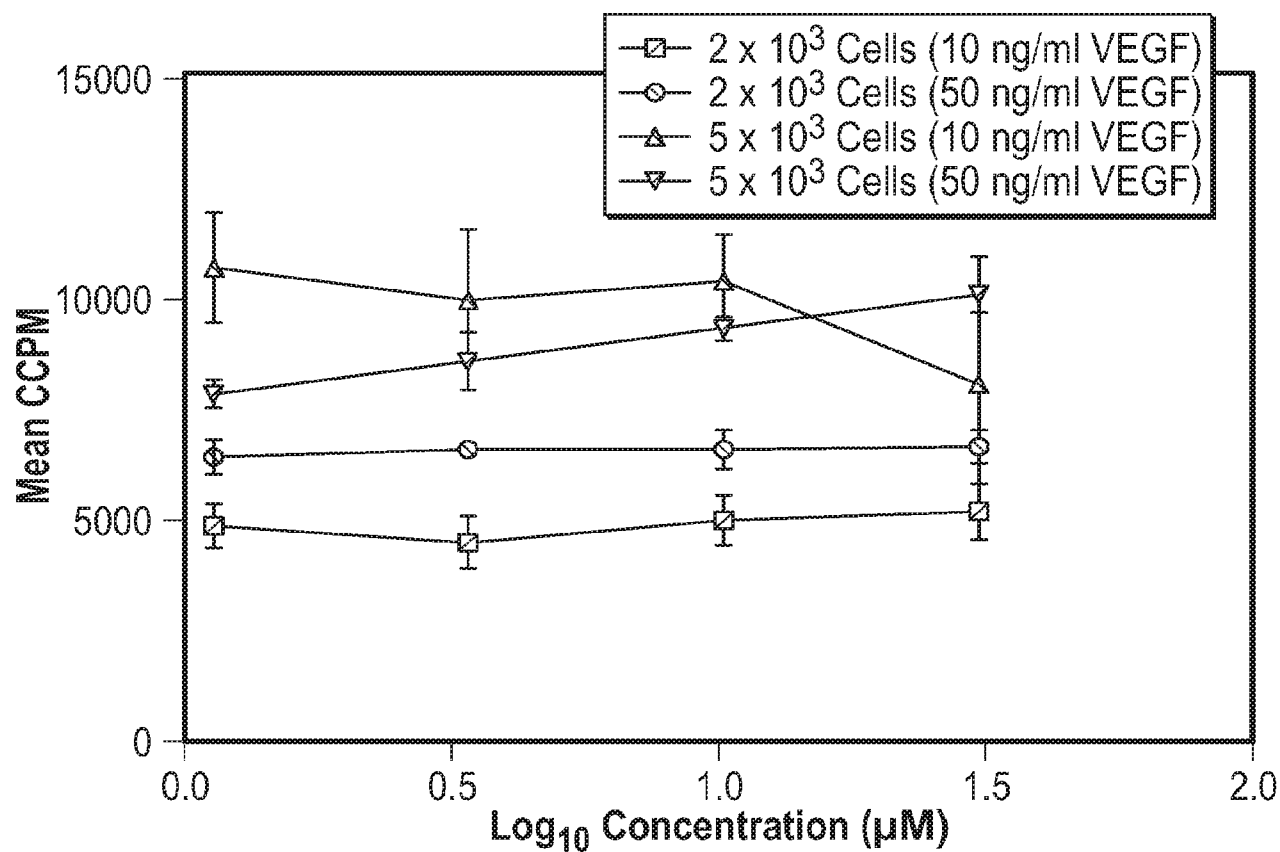
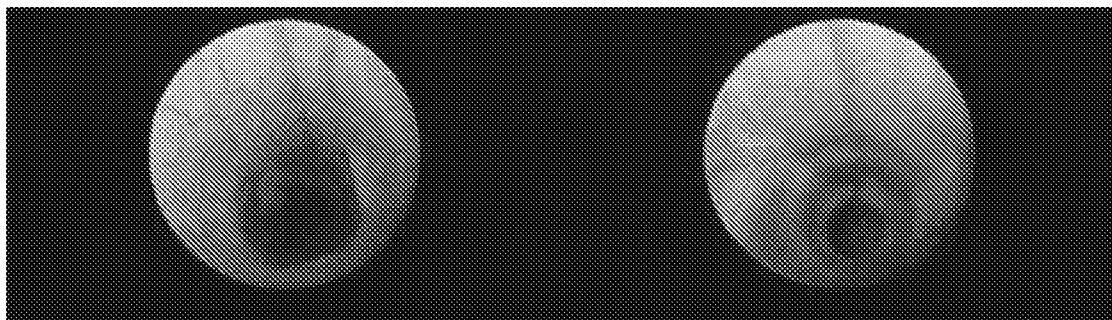
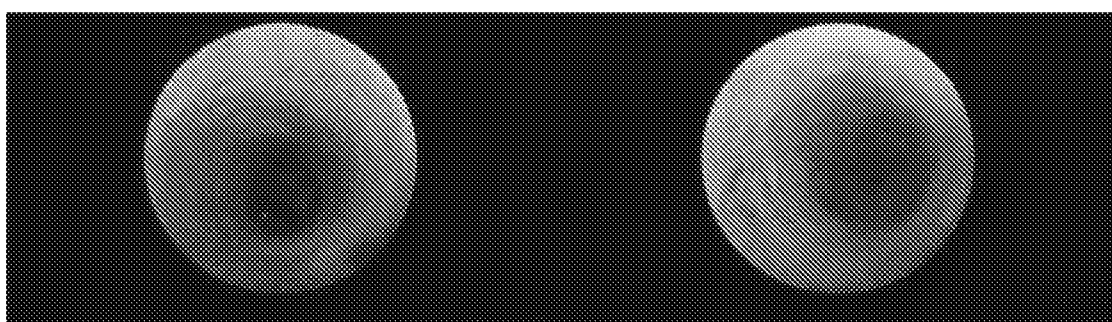


FIG. 107D

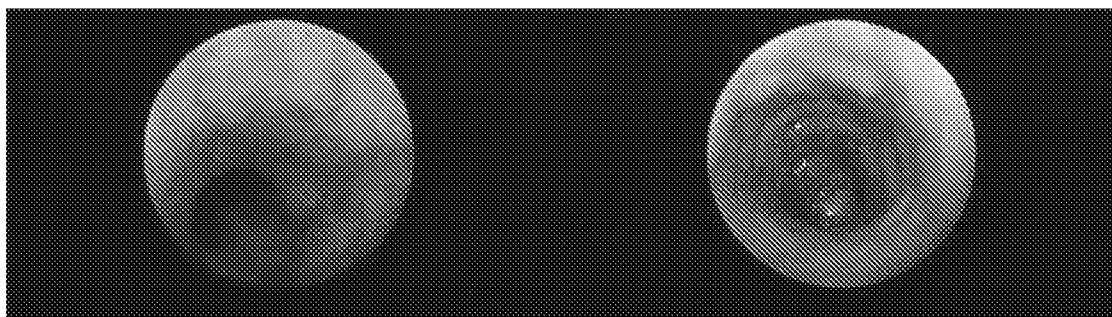
169/207



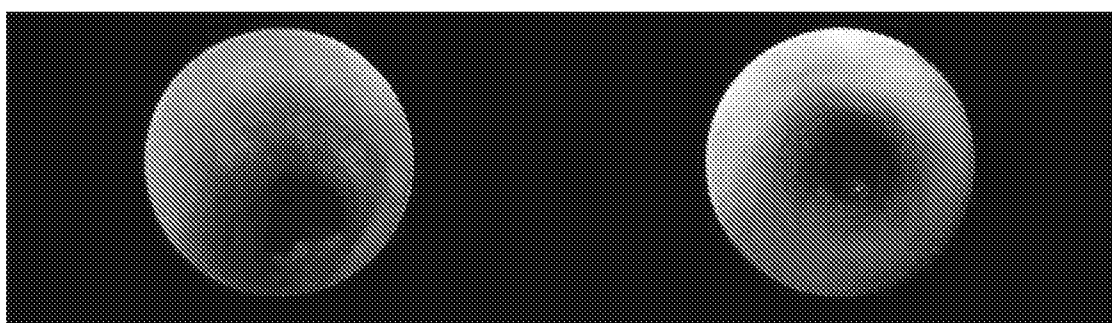
Naive Control (Average Score=0.00)



Vehicle Control P.O. (Average Score=2.13)



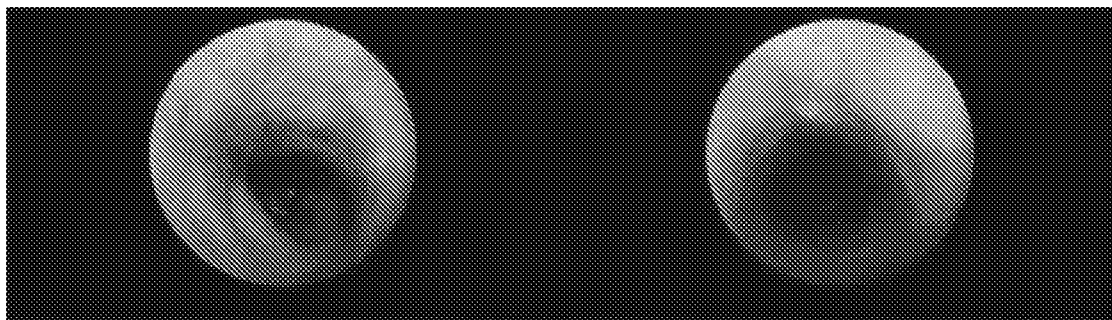
Vehicle Control I.C. (Average Score=1.92)



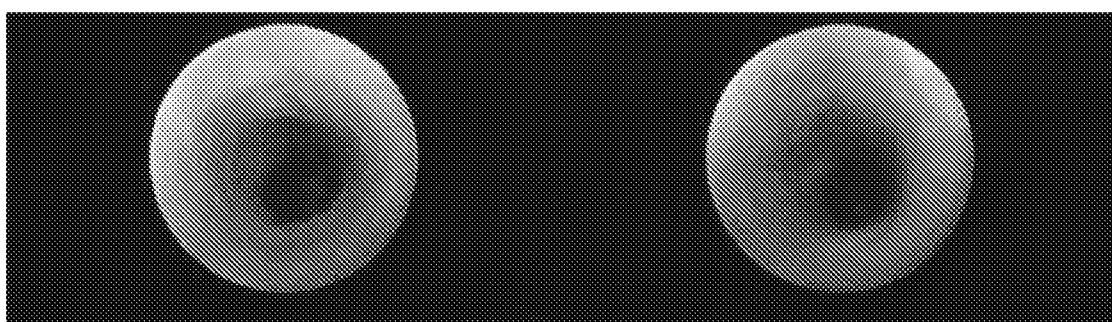
Tofacitnib 15mg/kg P.O. (Average Score=1.92)

FIG. 108

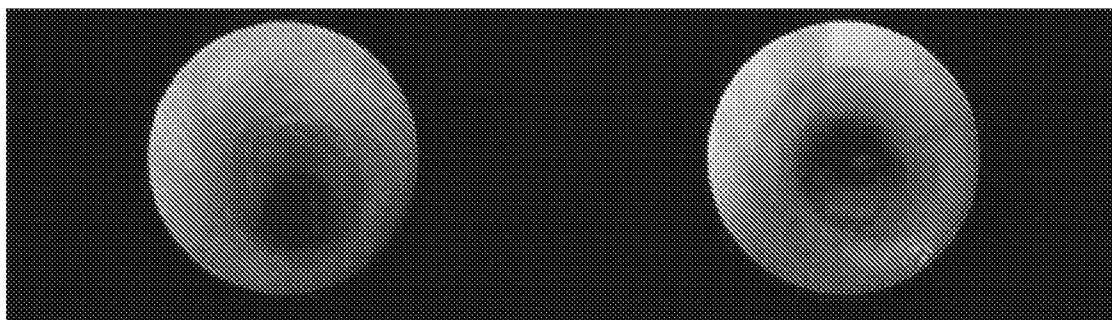
170/207



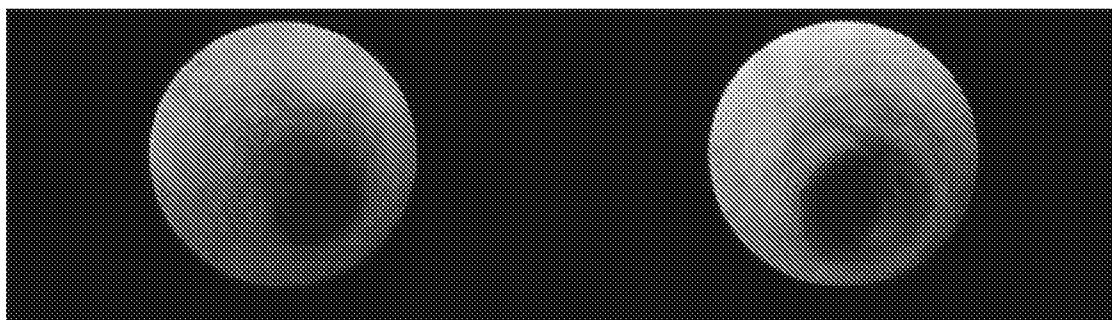
Tofacitnib 15mg/kg I.C (Average Score=1.73)



Prednisolone 1.0mg/kg P.O. (Average Score=1.83)



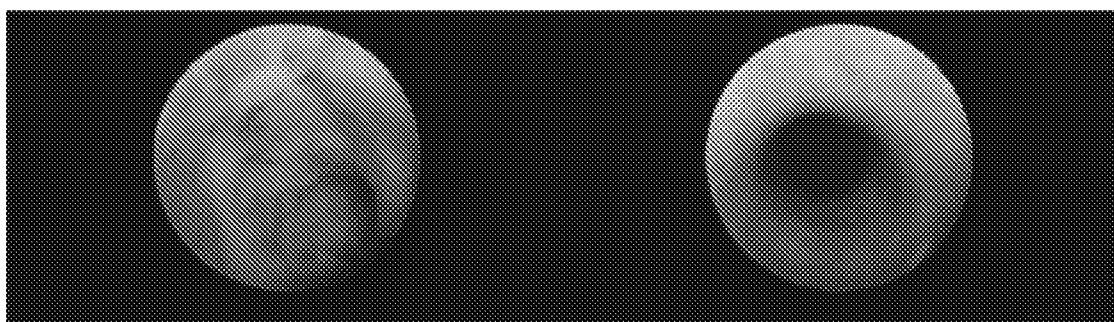
SNA-125 400mg/kg P.O. (Average Score=1.67)



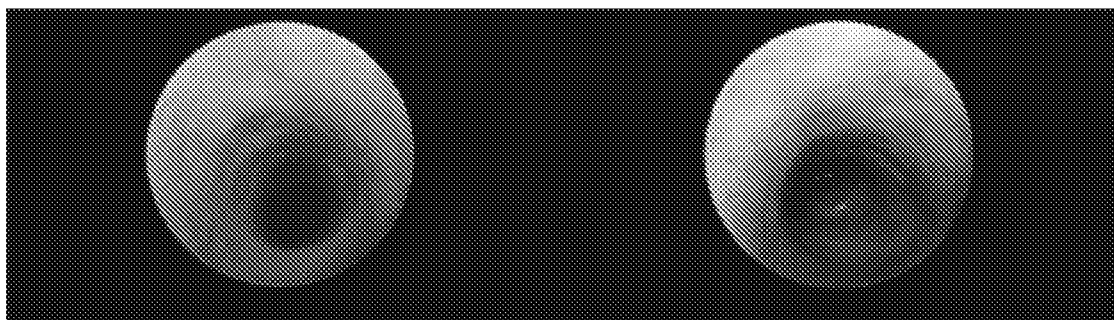
SNA-352 400mg/kg P.O. (Average Score=1.82)

FIG. 109

171/207



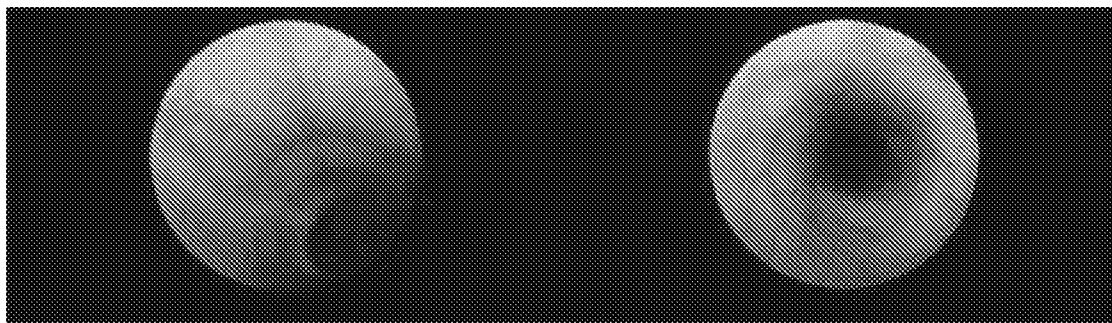
SNA-125 400mg/kg I.C. (Average Score=2.10)



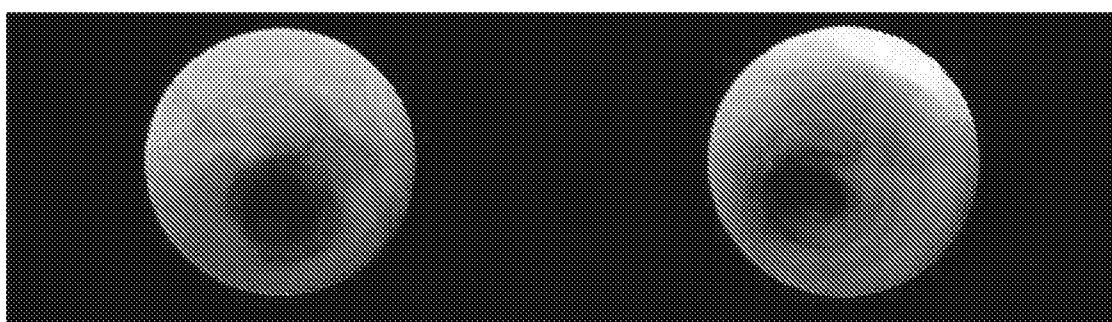
SNA-352 400mg/kg I.C. (Average Score=2.00)

FIG. 110

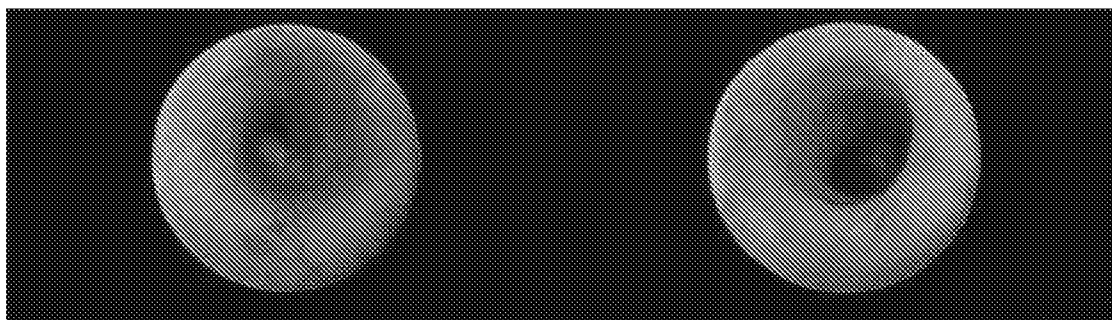
172/207



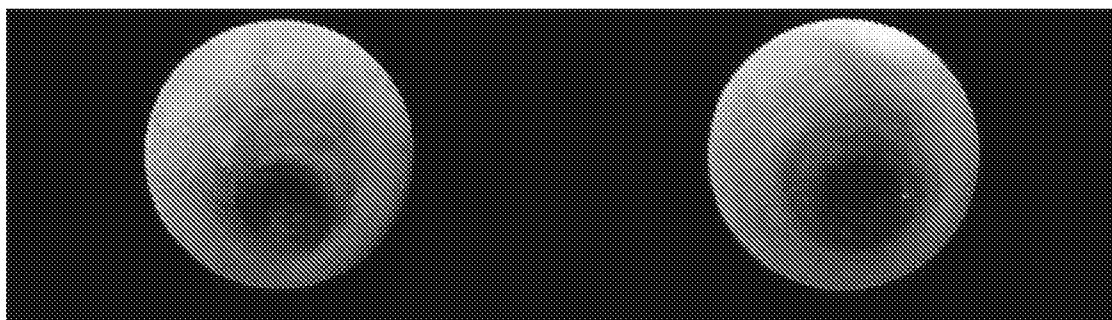
Naive Control (Average Score=0.00)



Vehicle Control P.O. (Average Score=1.92)



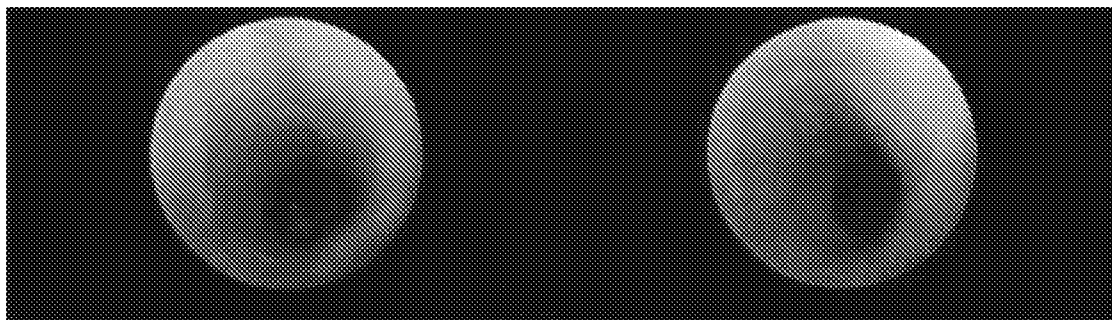
Vehicle Control I.C. (Average Score=2.00)



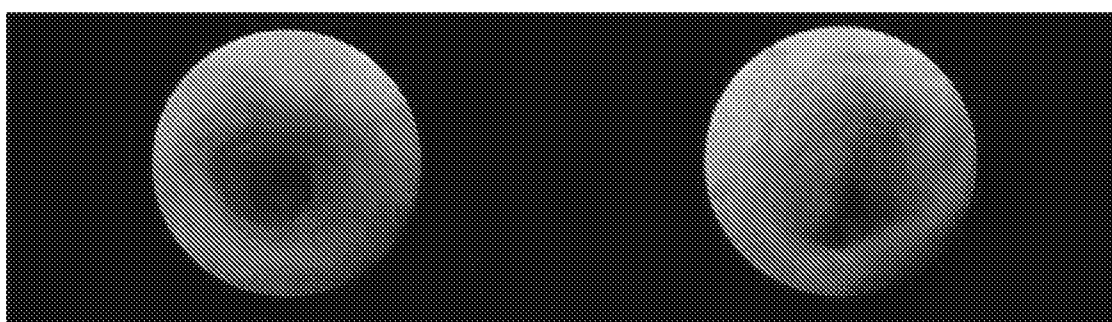
Tofacitnib 15mg/kg P.O. (Average Score=1.82)

FIG. 111

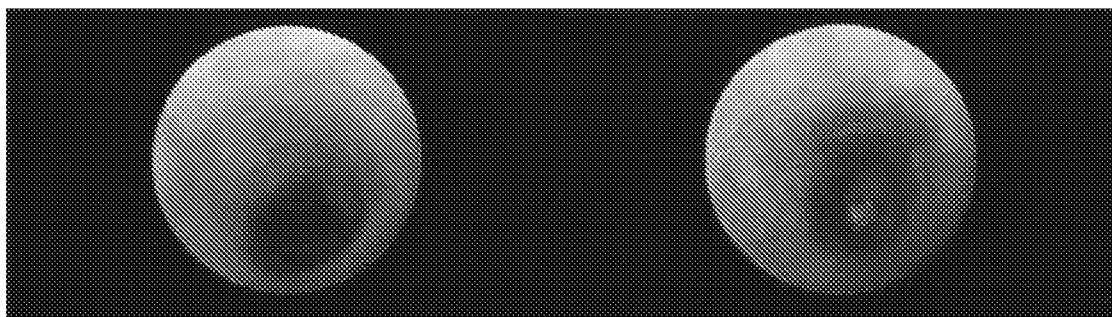
173/207



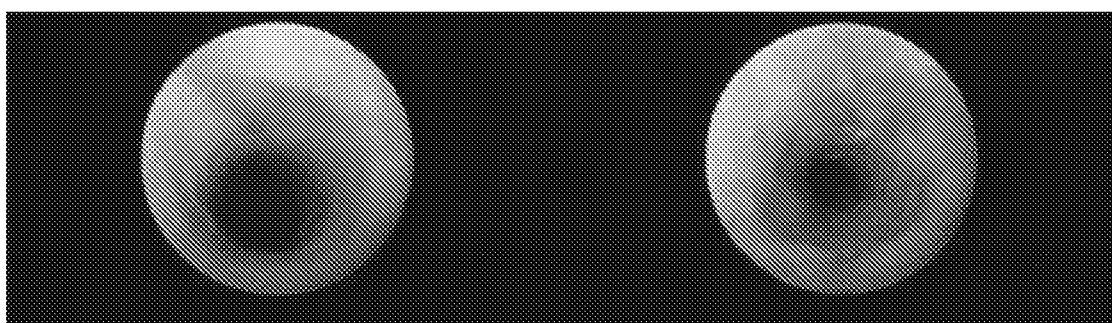
Tofacitnib 15mg/kg I.C (Average Score=1.63)



Prednisolone 1.0mg/kg P.O. (Average Score=1.83)



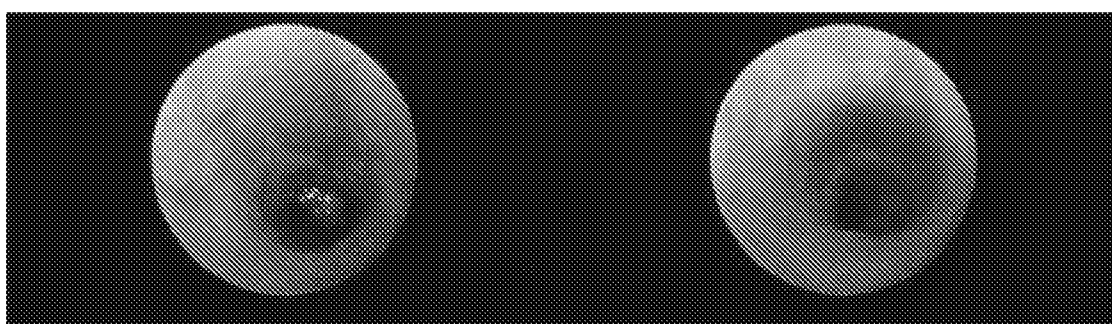
SNA-125 400mg/kg P.O. (Average Score=1.64)



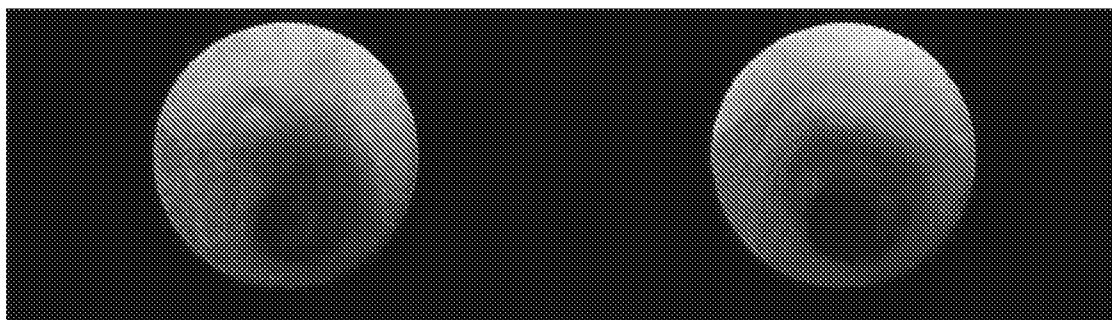
SNA-352 400mg/kg P.O. (Average Score=1.55)

FIG. 112

174/207



SNA-125 400mg/kg I.C. (Average Score=1.40)



SNA-352 400mg/kg I.C. (Average Score=2.13)

FIG. 113

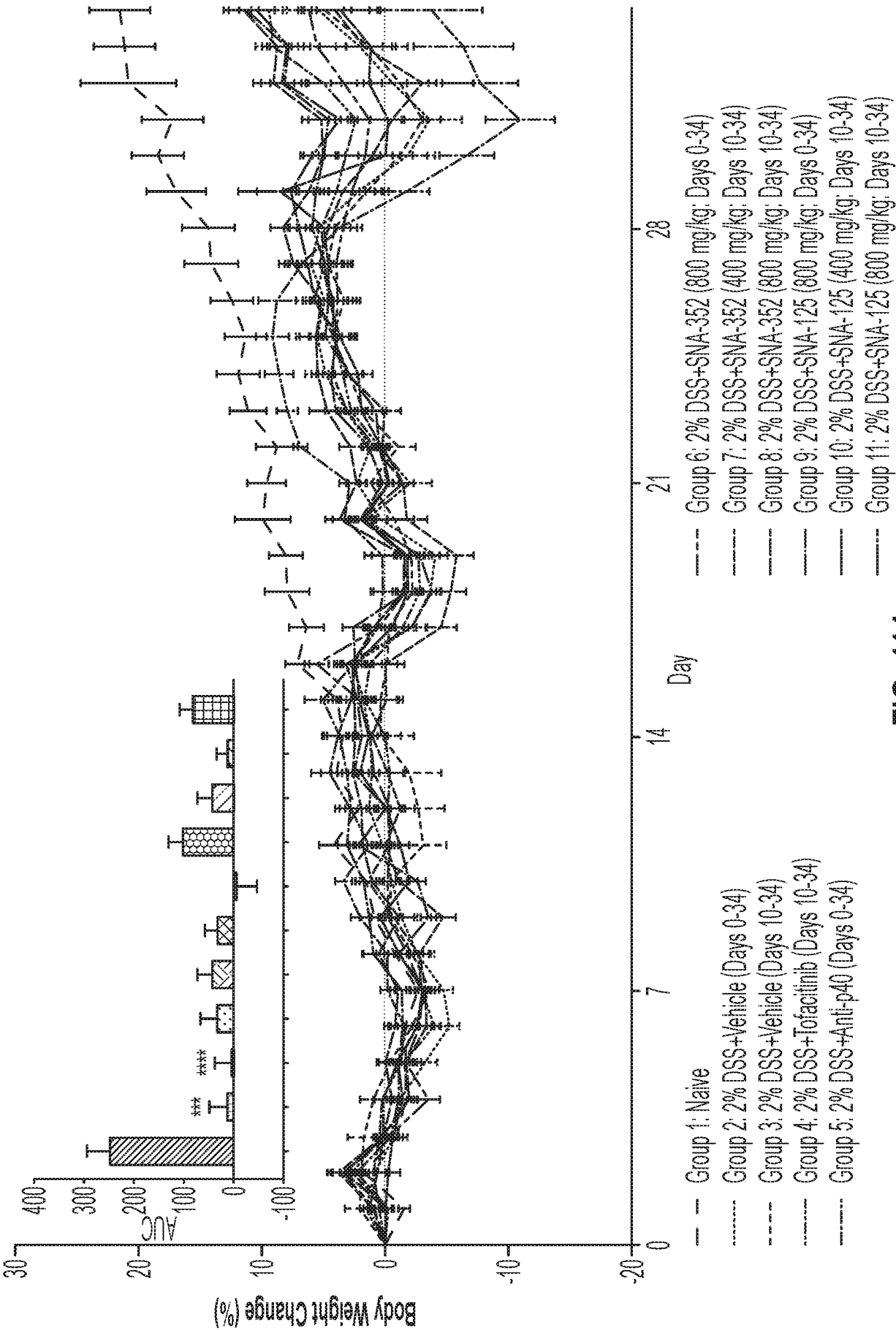


FIG. 114

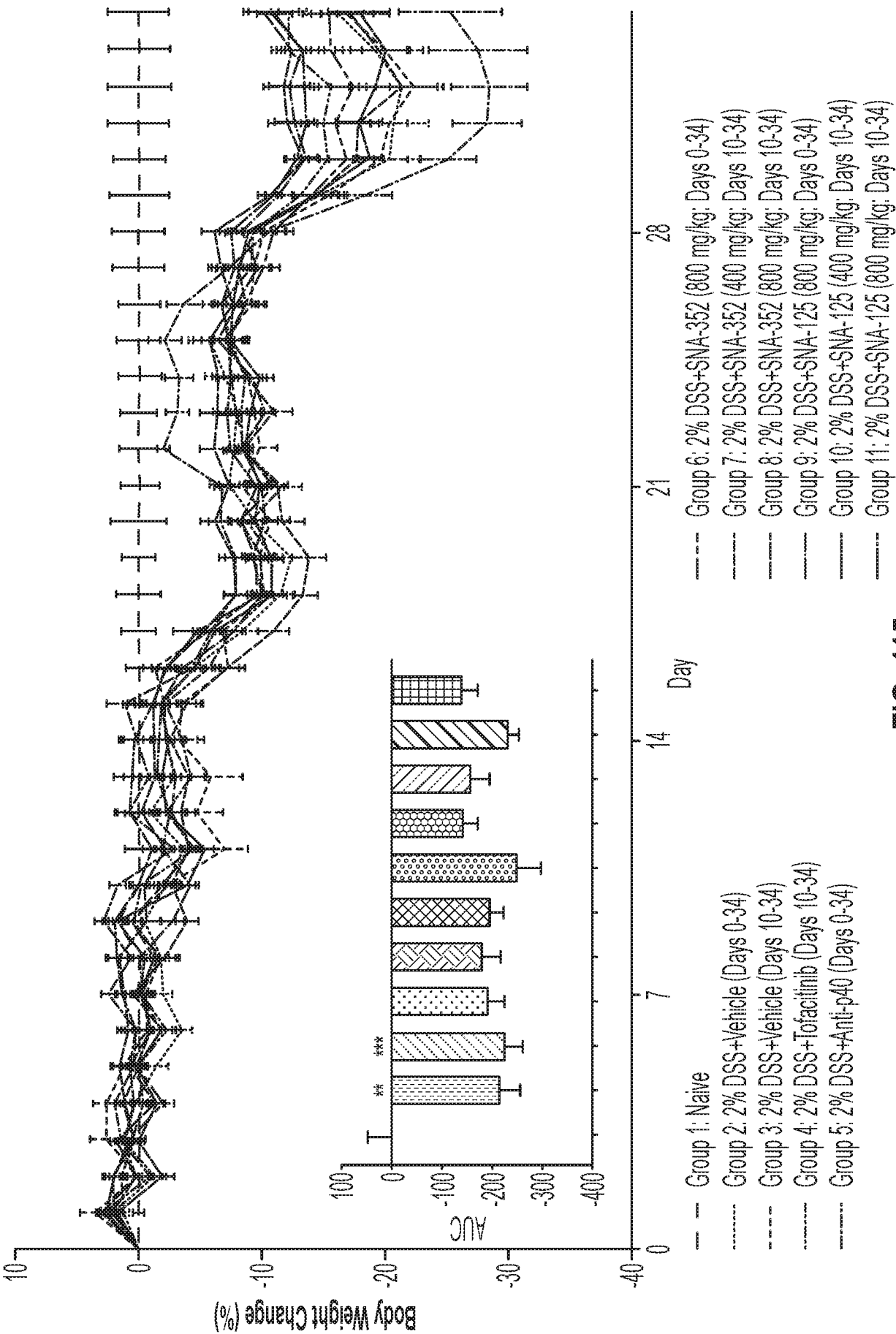


FIG. 115

177/207

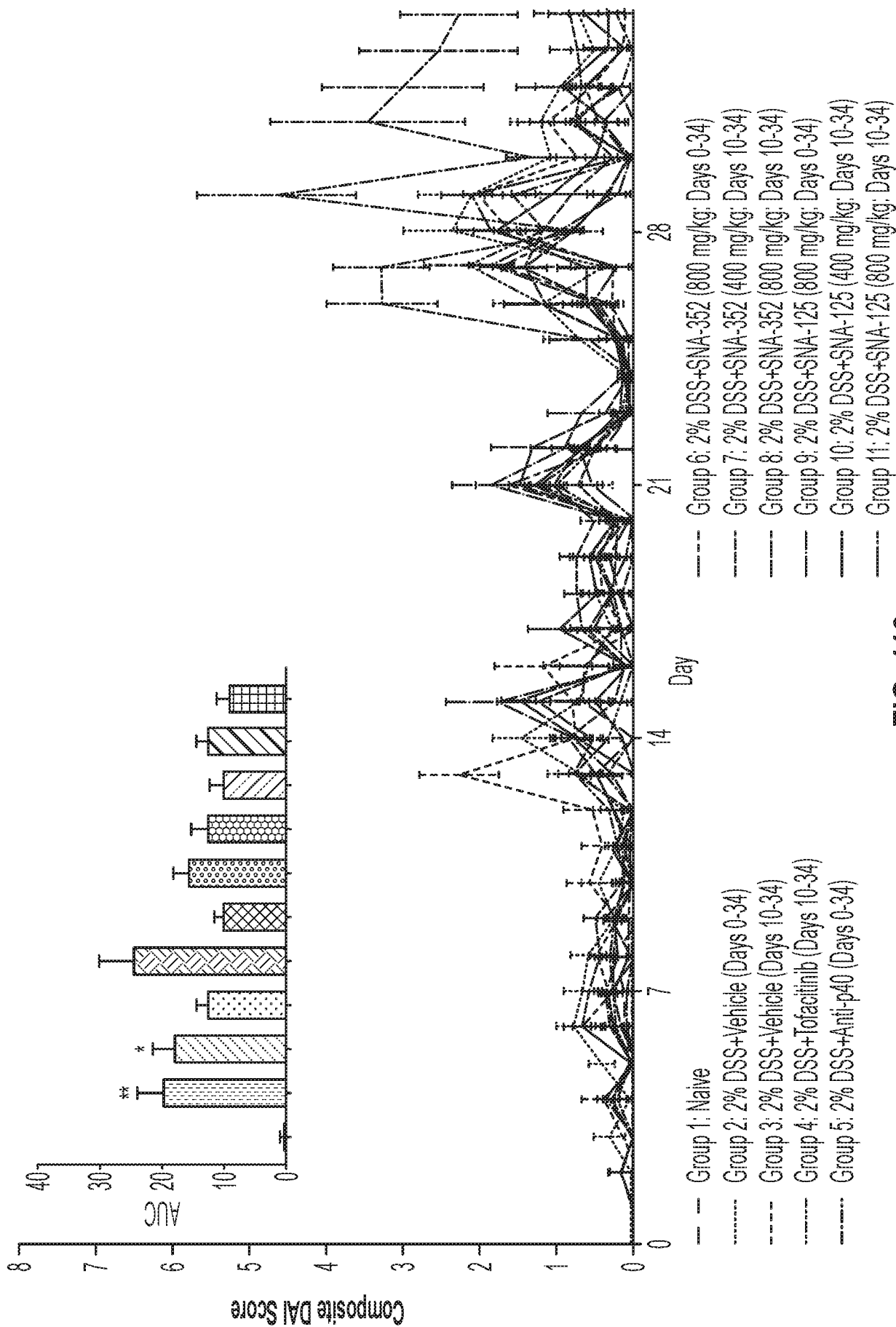


FIG. 116

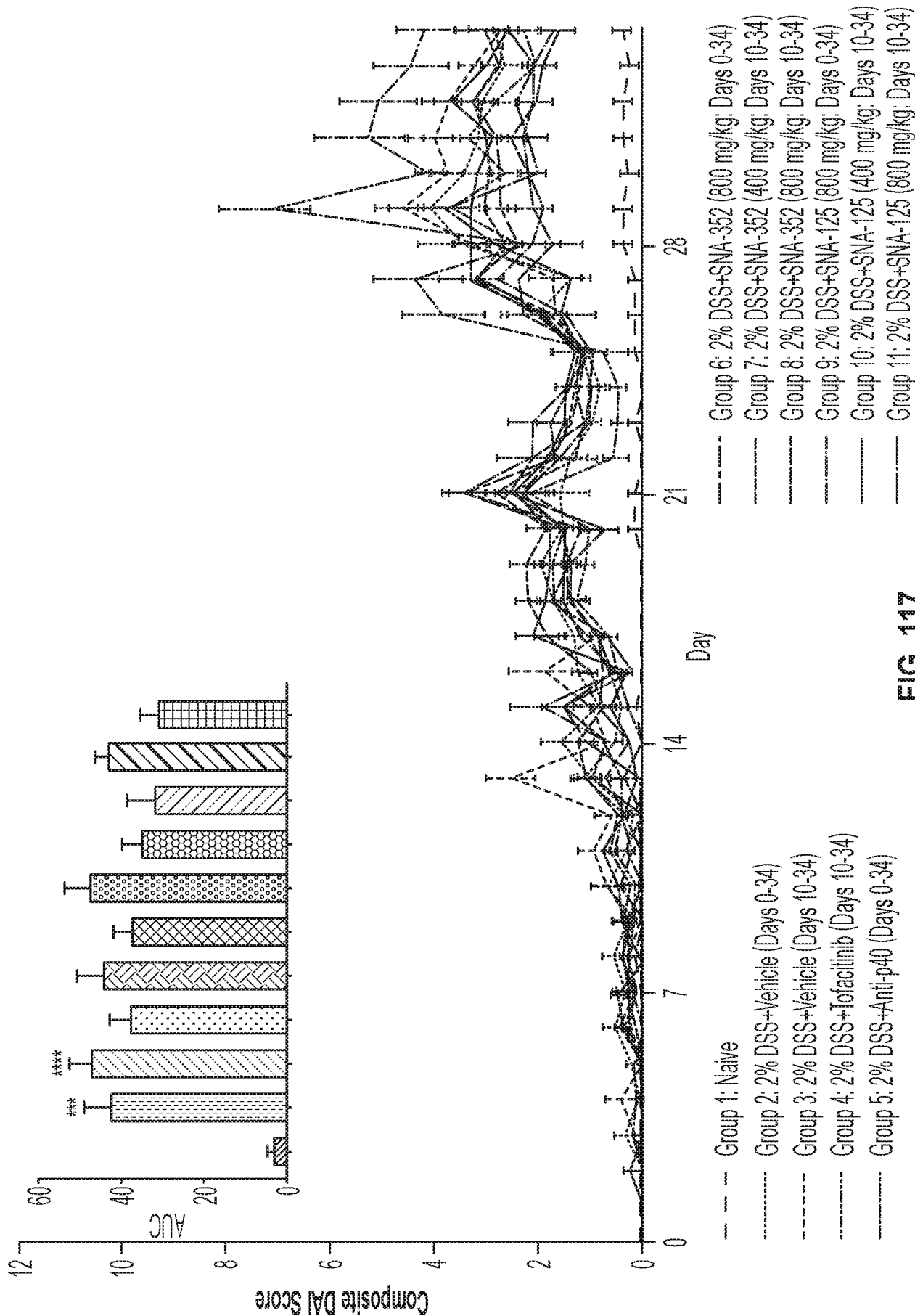


FIG. 117

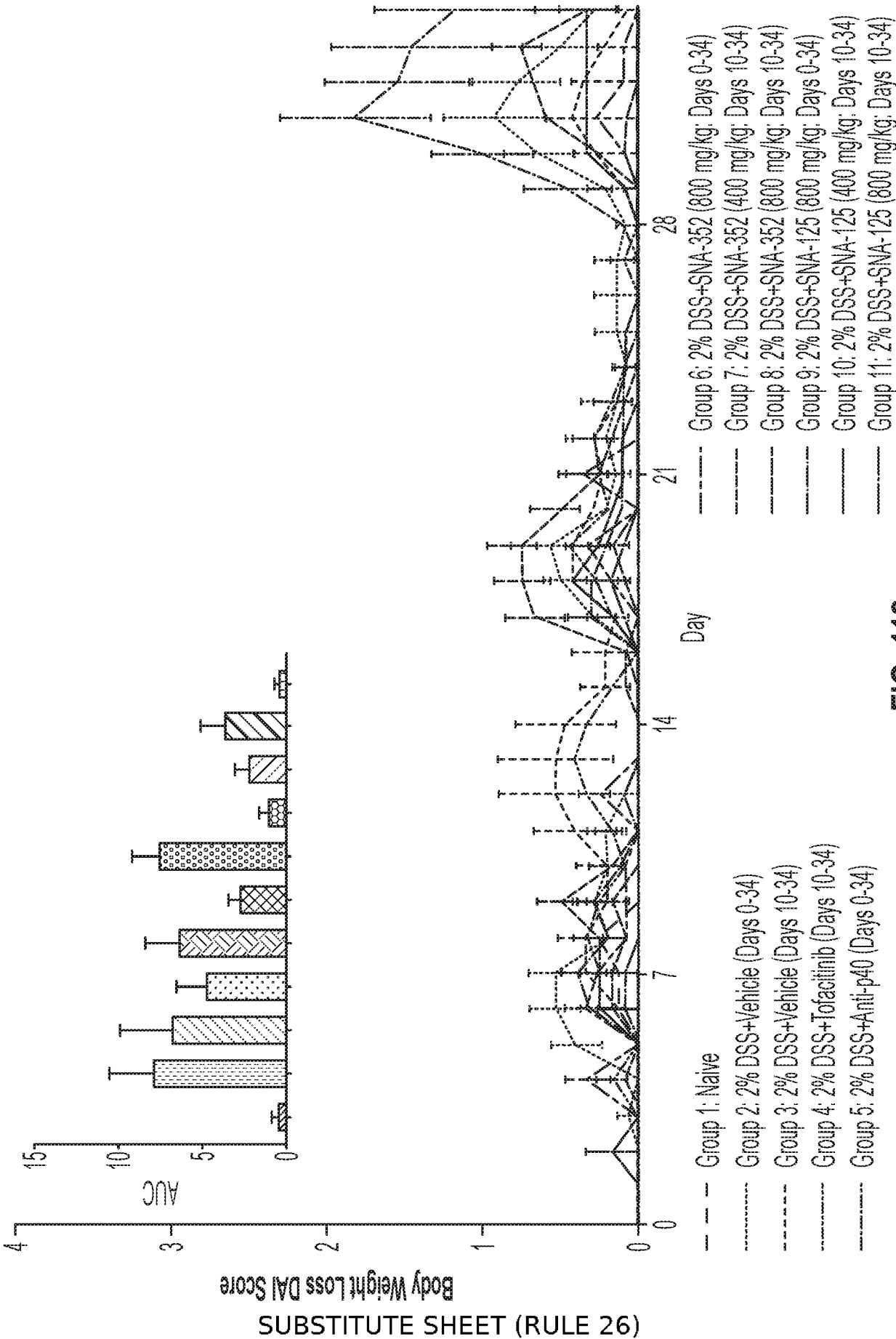


FIG. 118

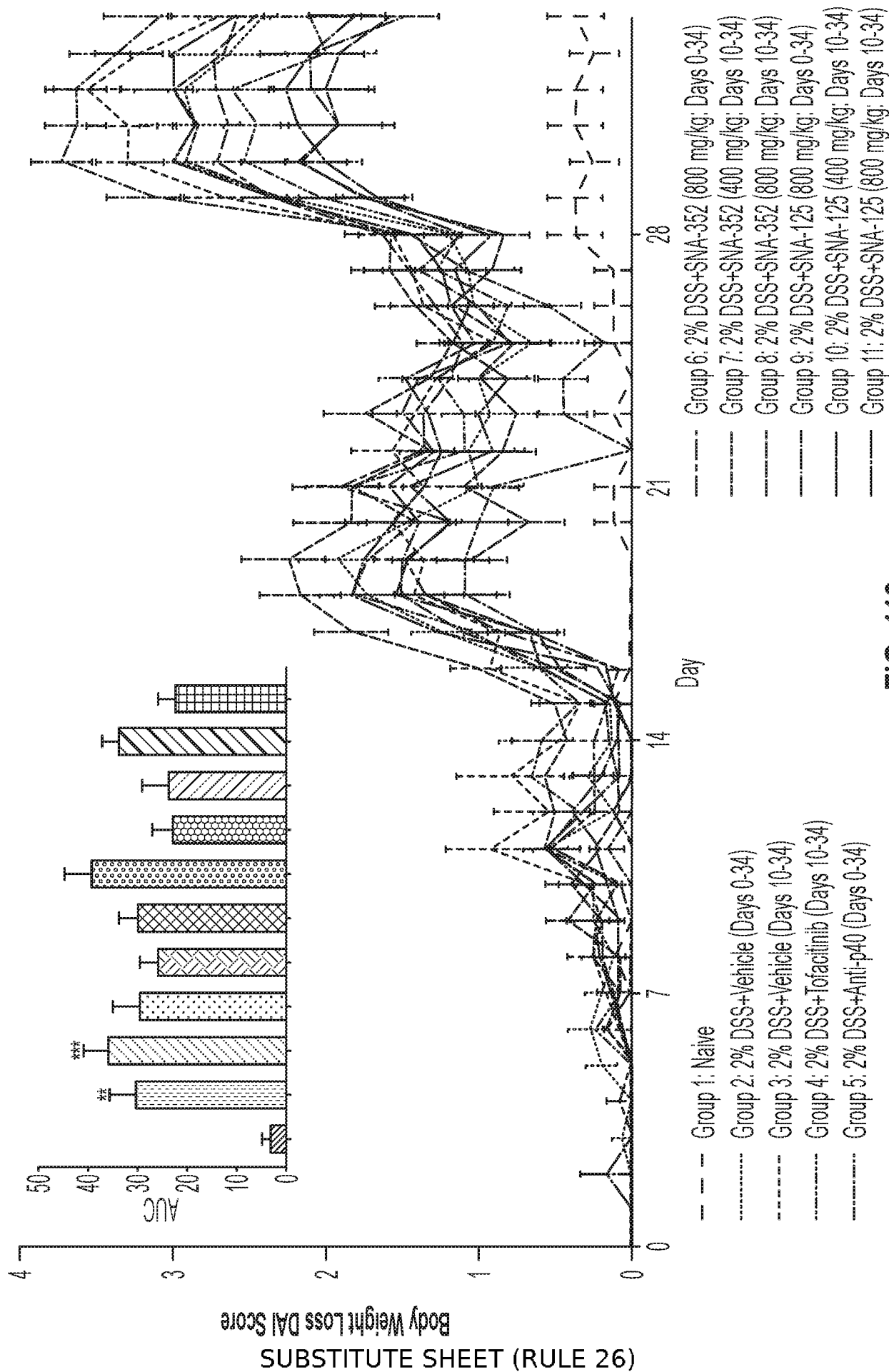


FIG. 119

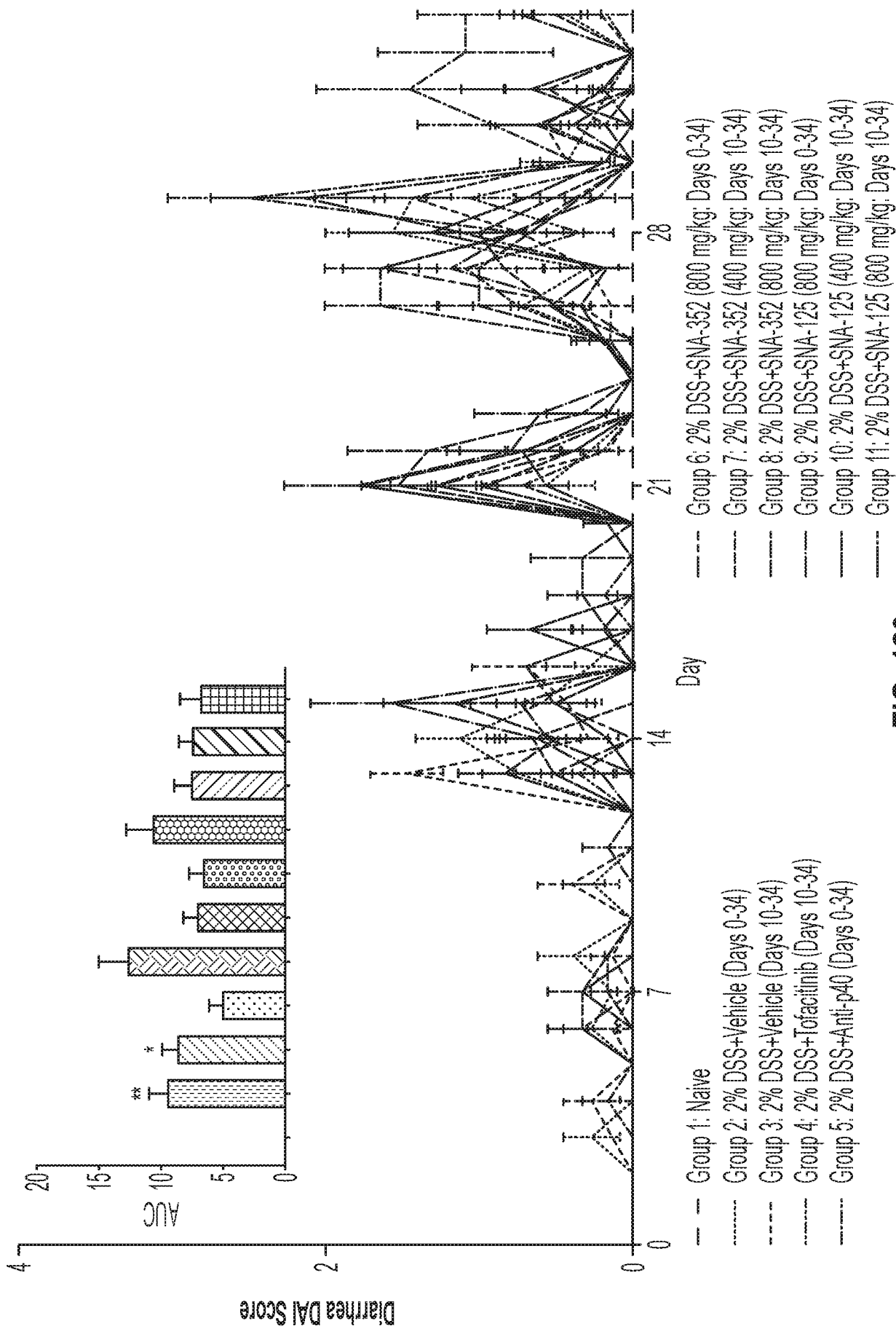


FIG. 120

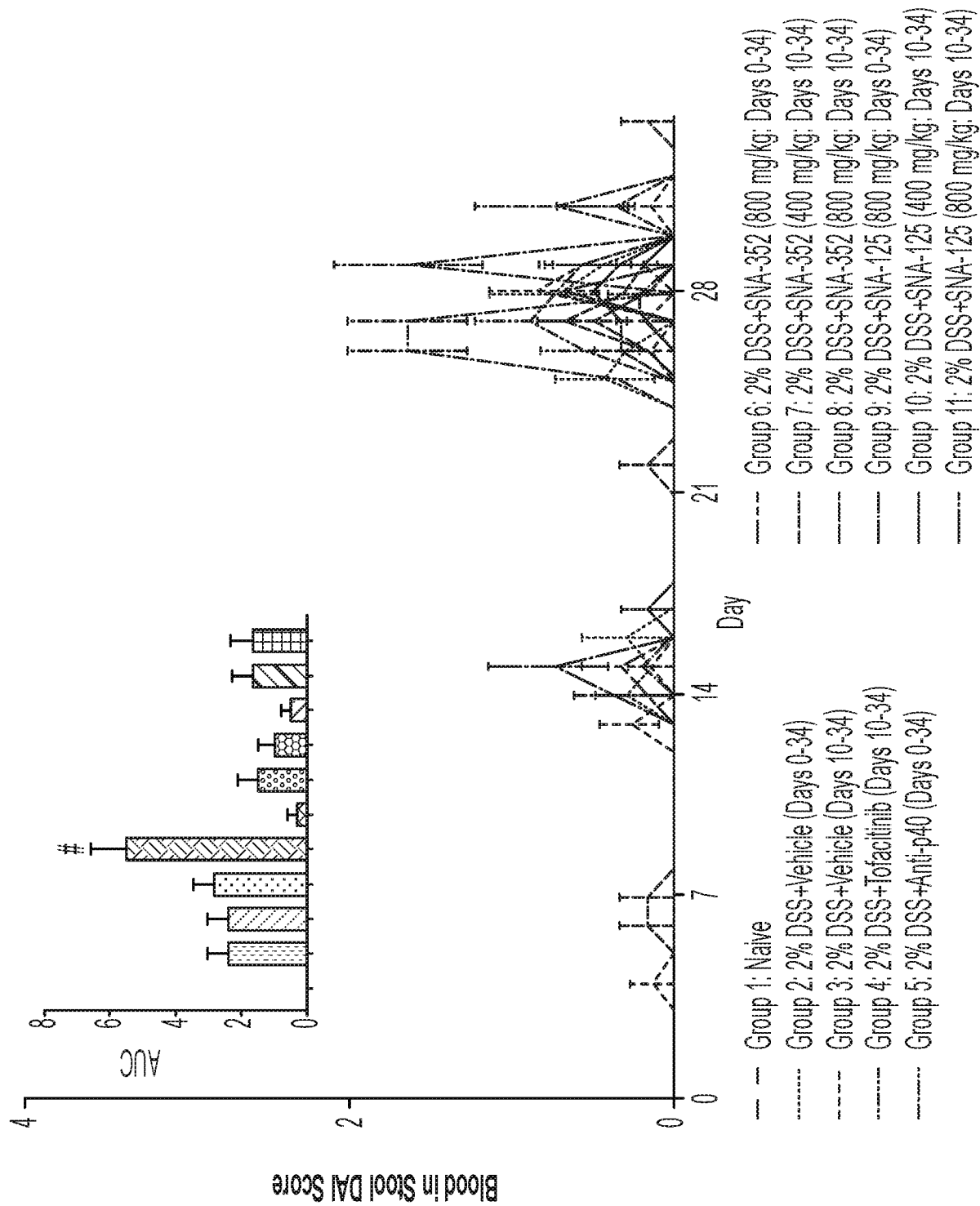


FIG. 121

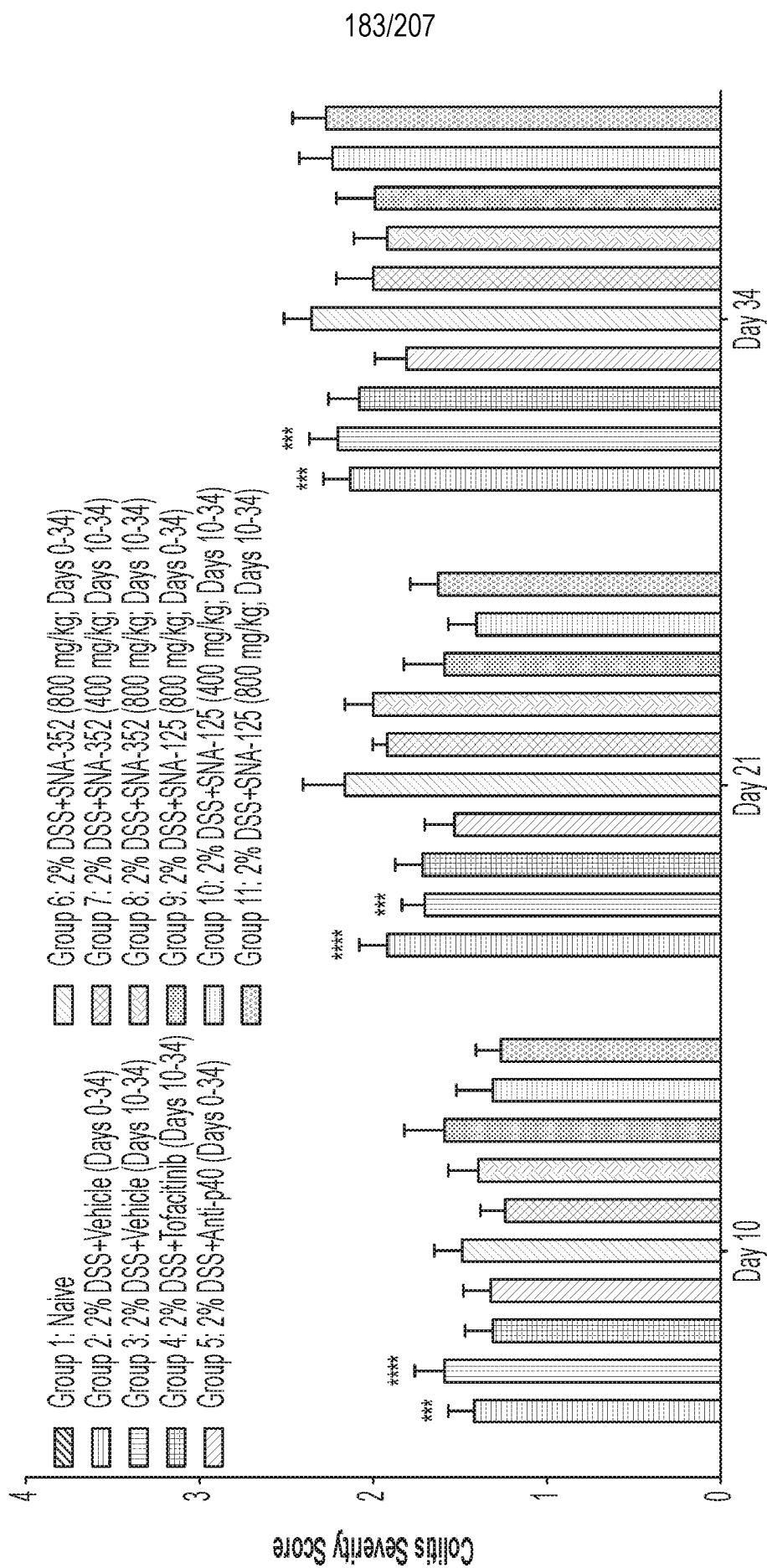


FIG. 122

184/207

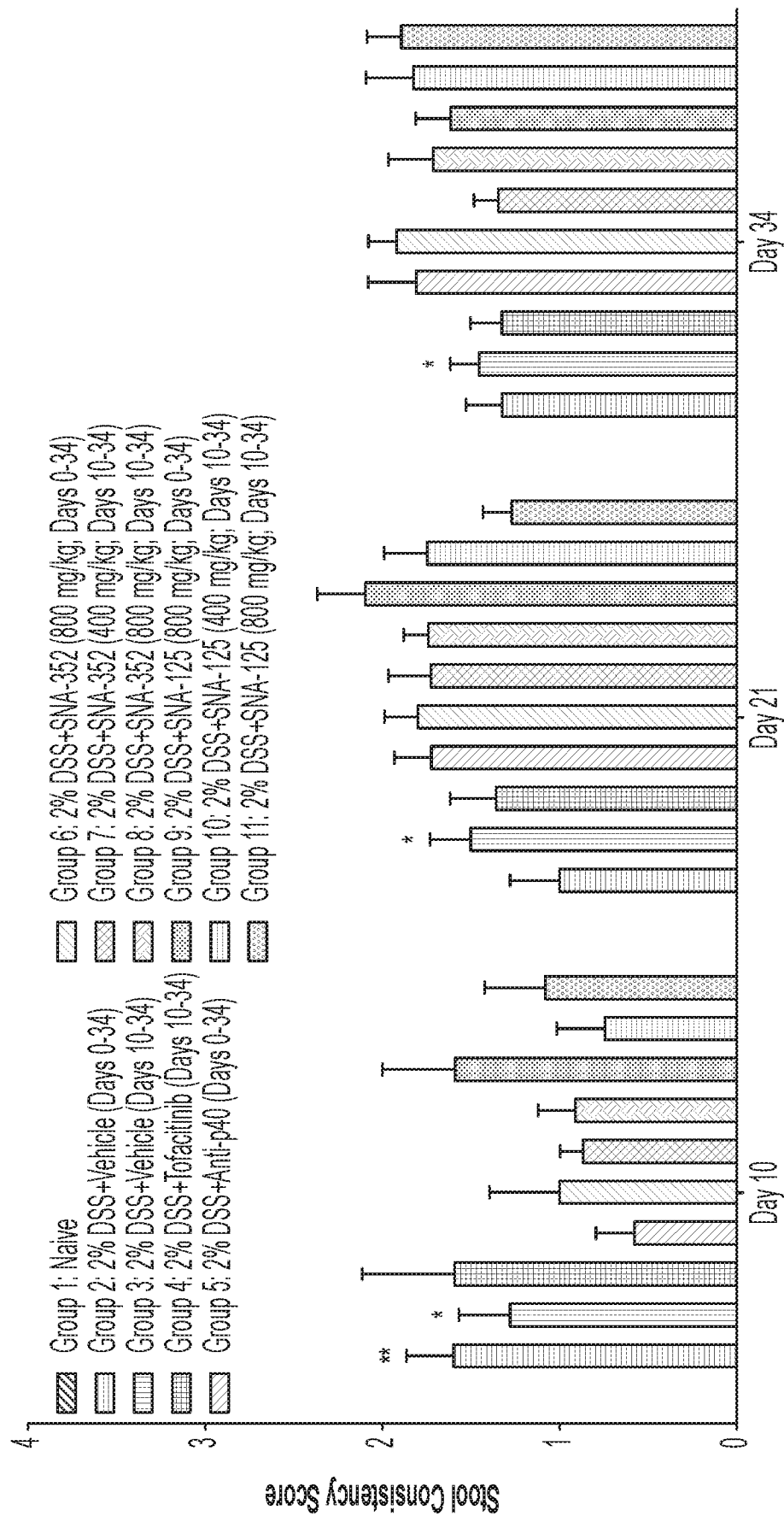


FIG. 123

185/207

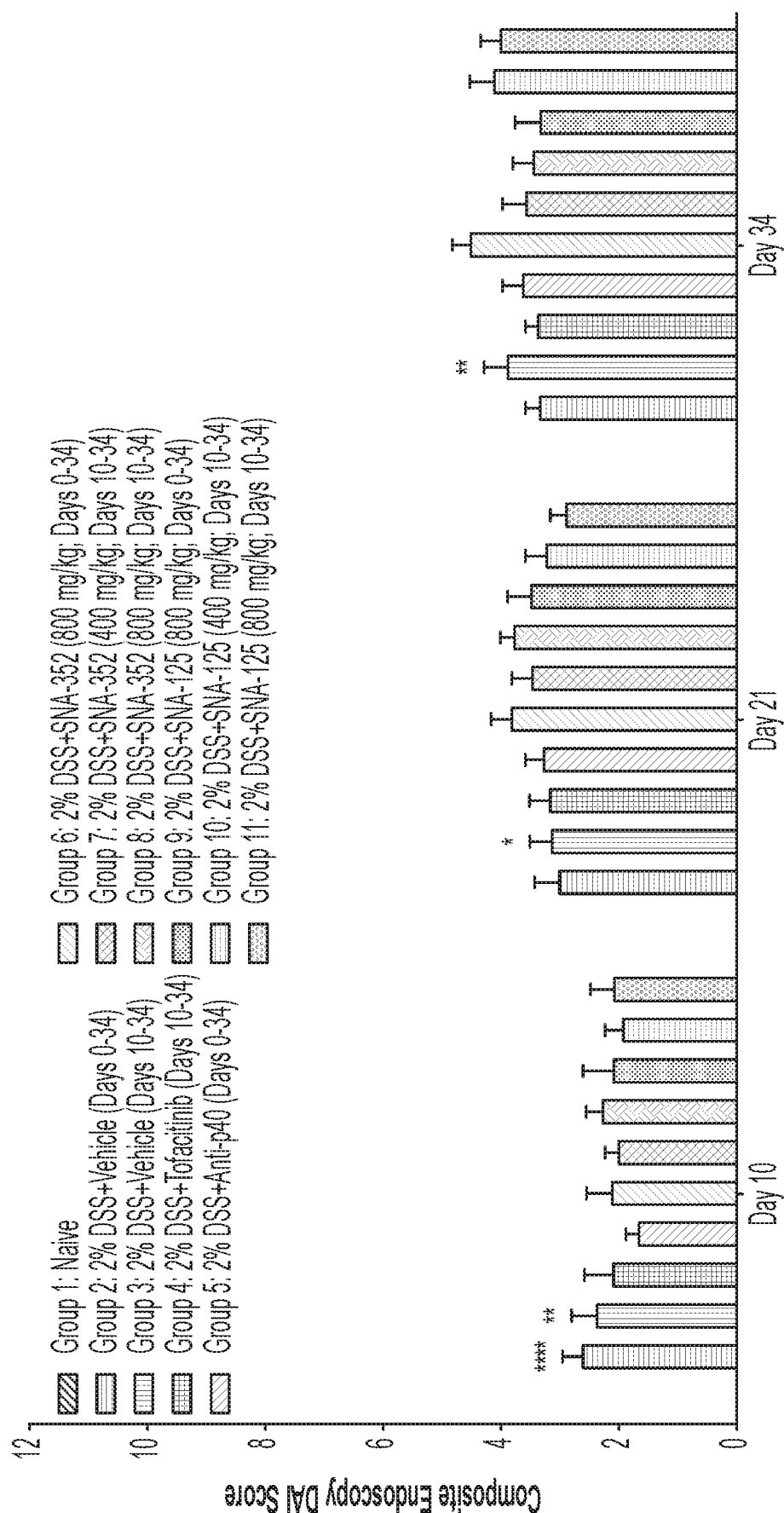


FIG. 124

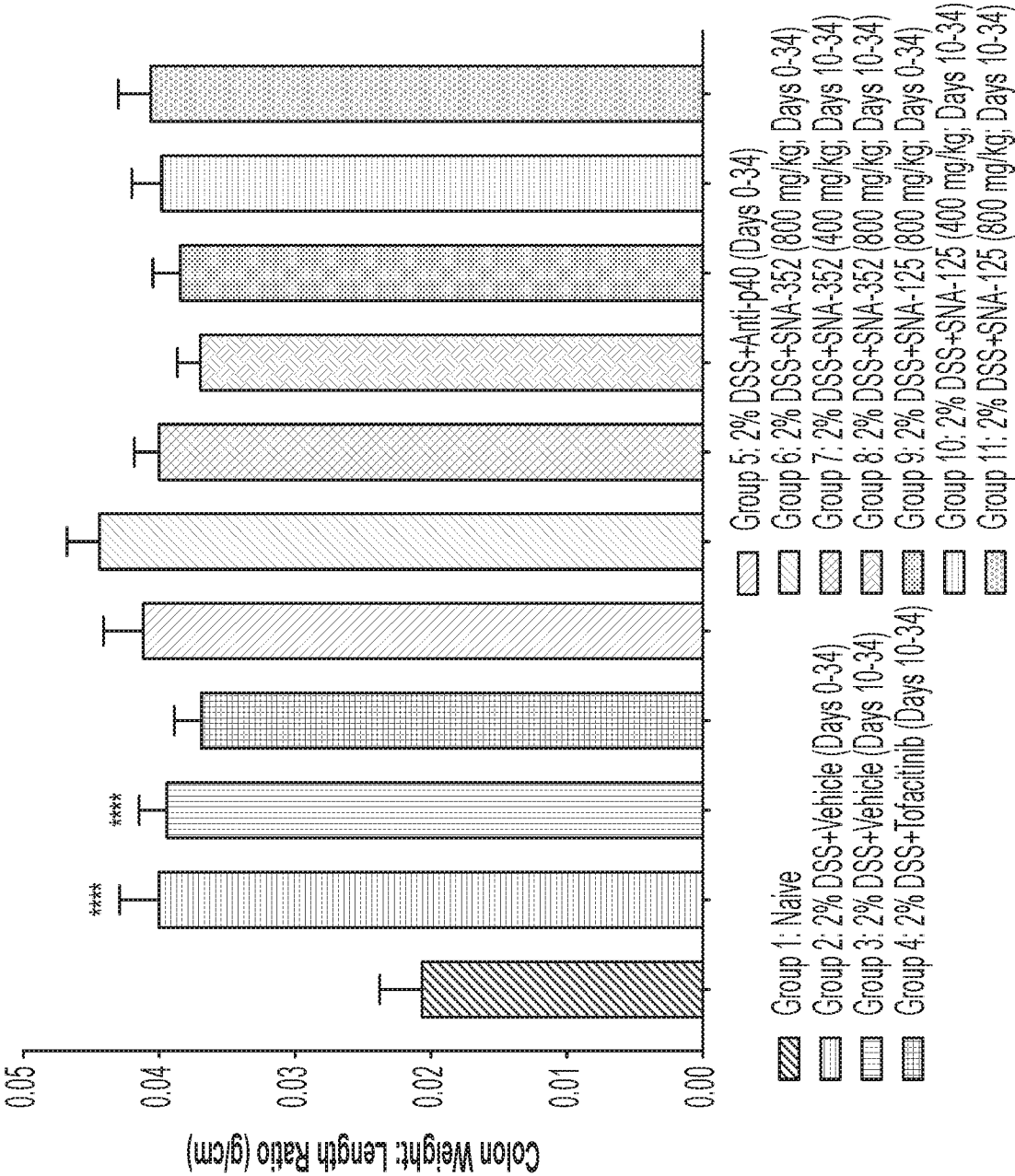


FIG. 125

187/207

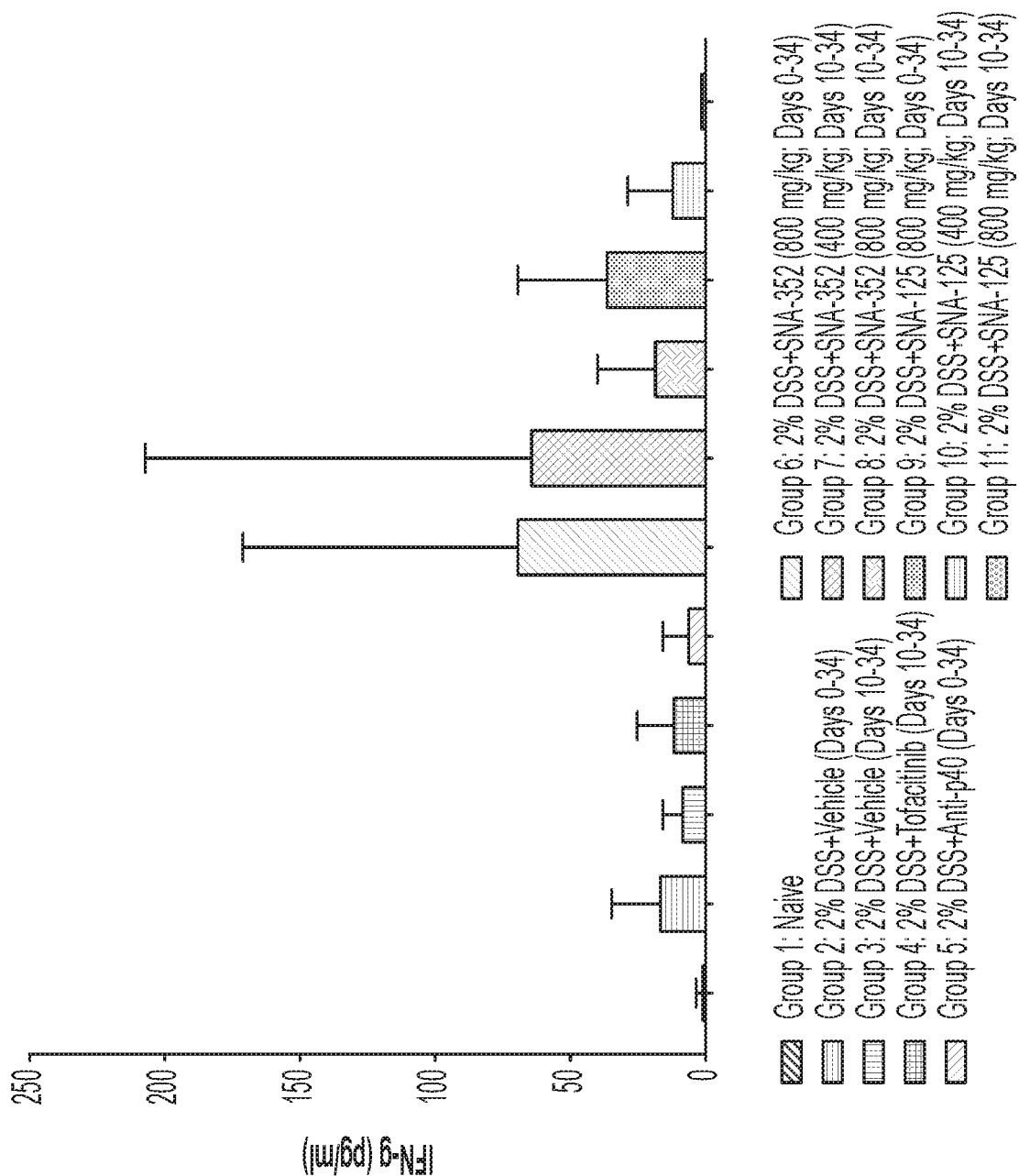


FIG. 126

188/207

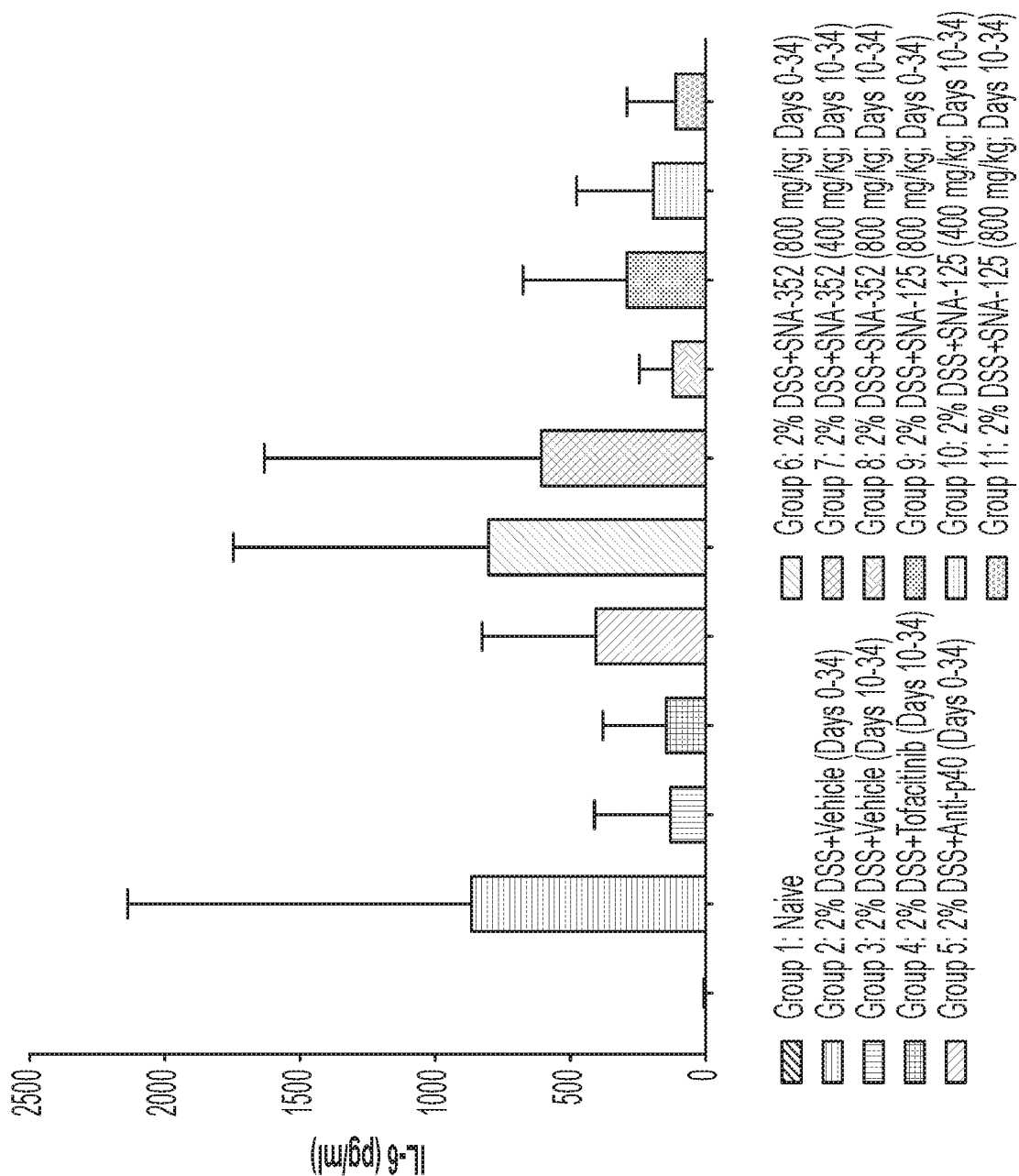


FIG. 127

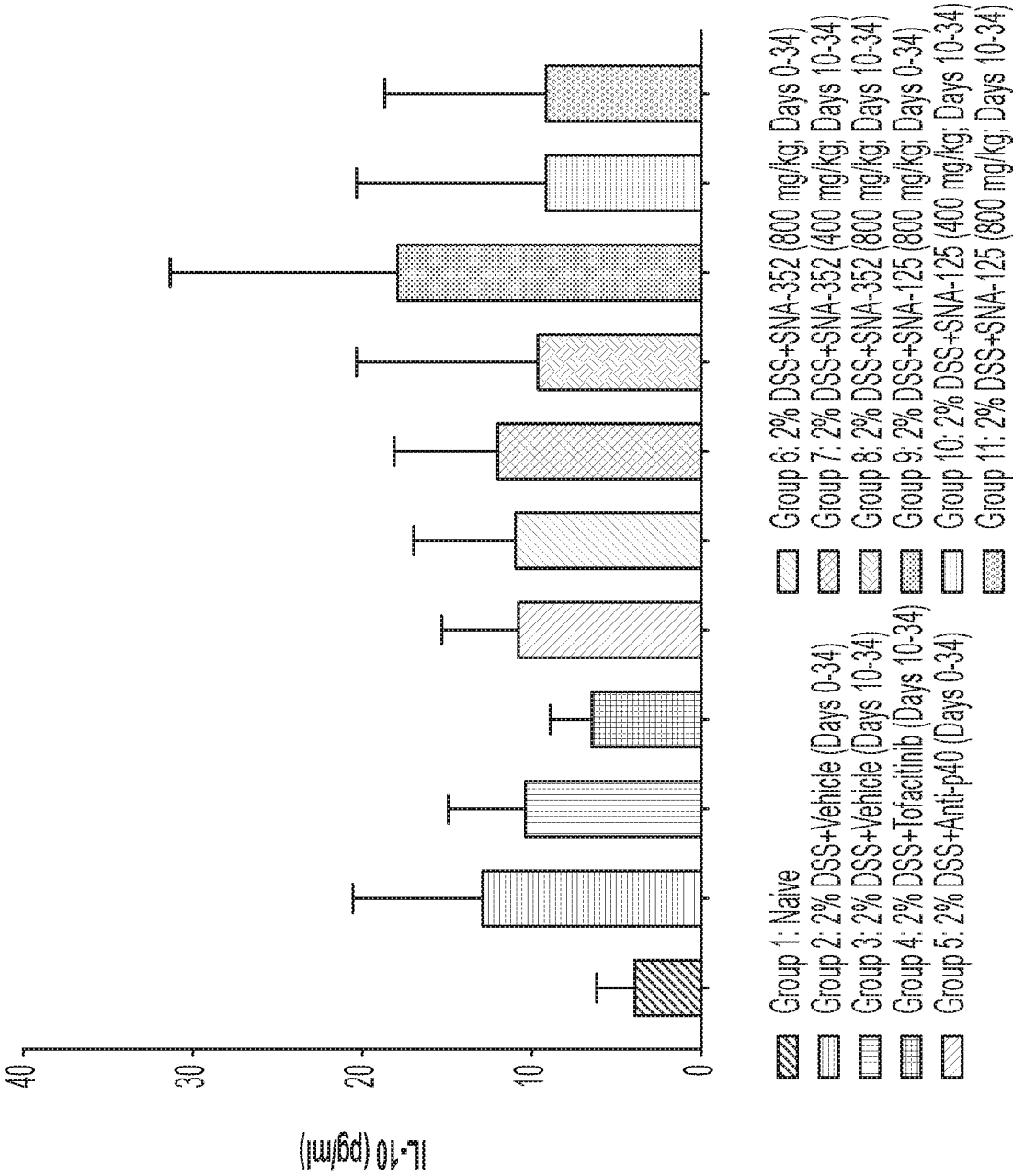


FIG. 128

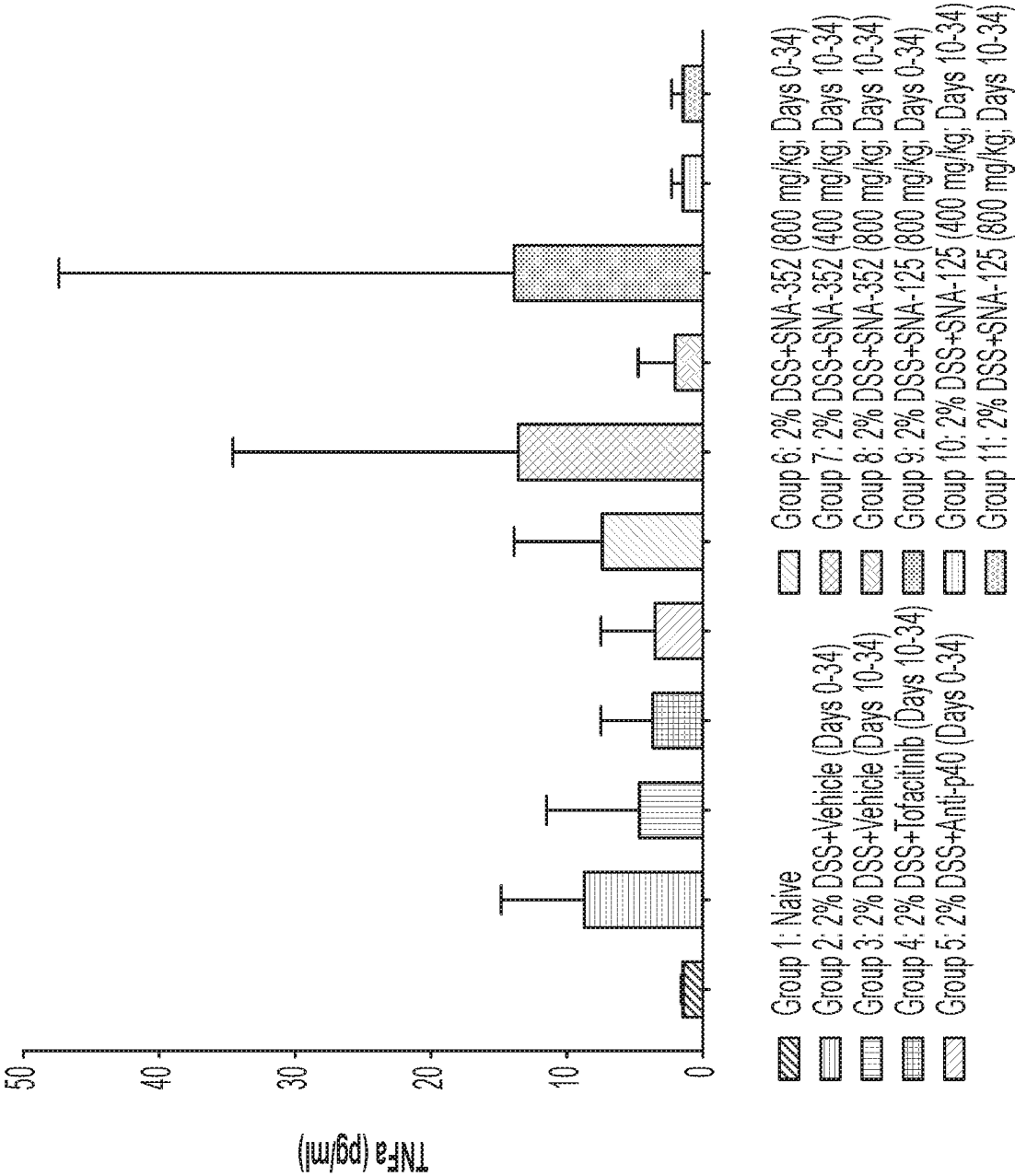


FIG. 129

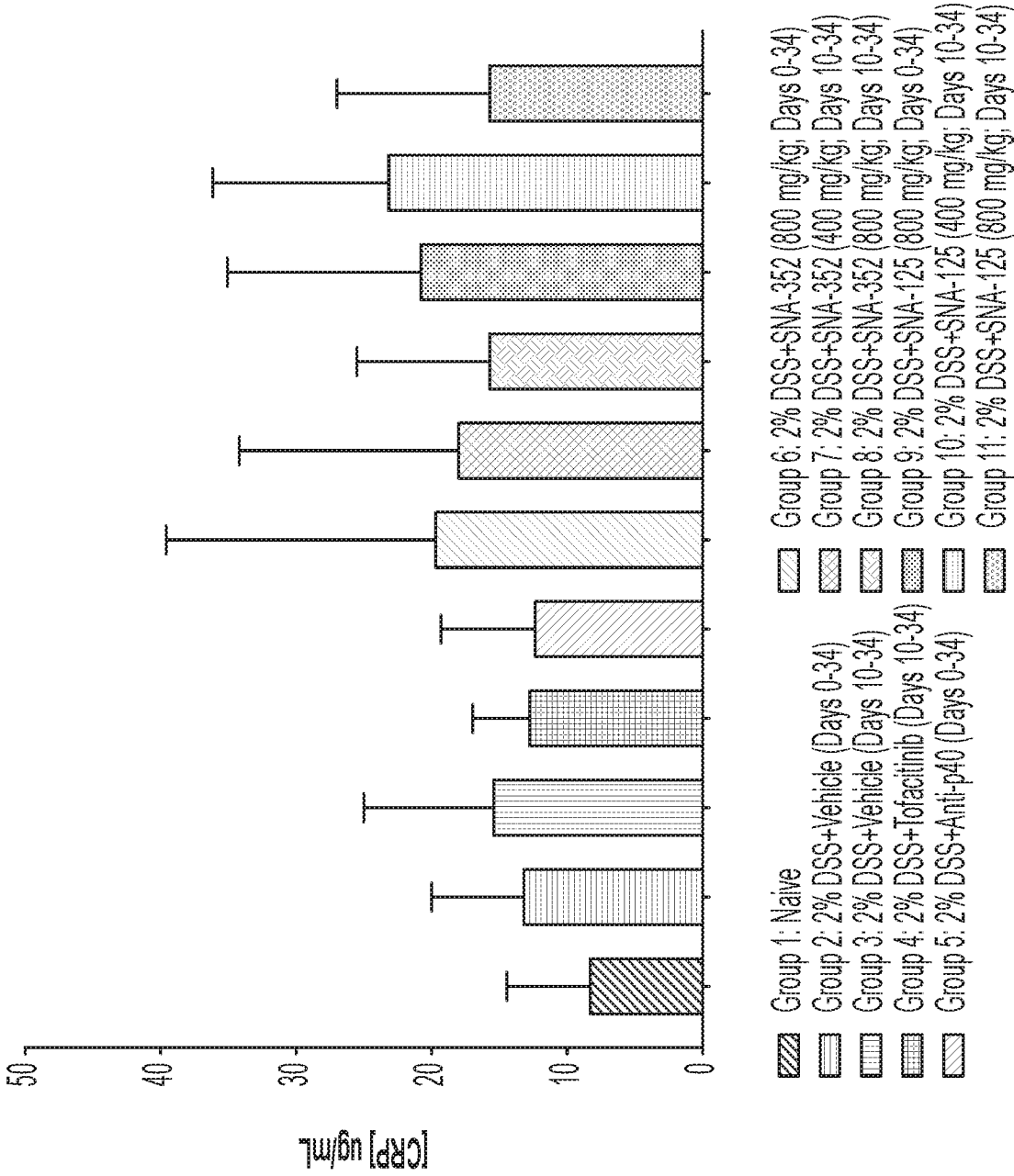


FIG. 130

192/207

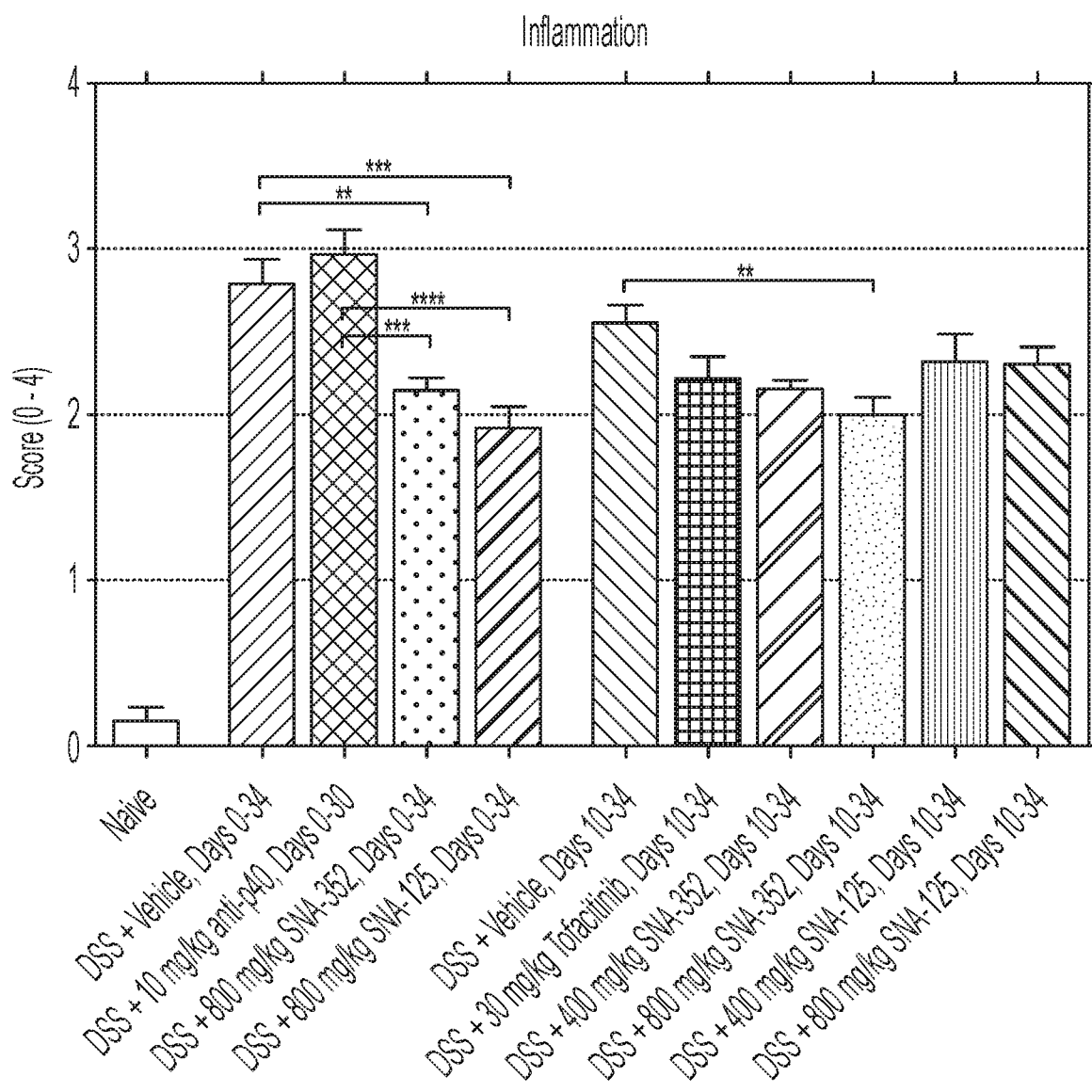


FIG. 131

193/207

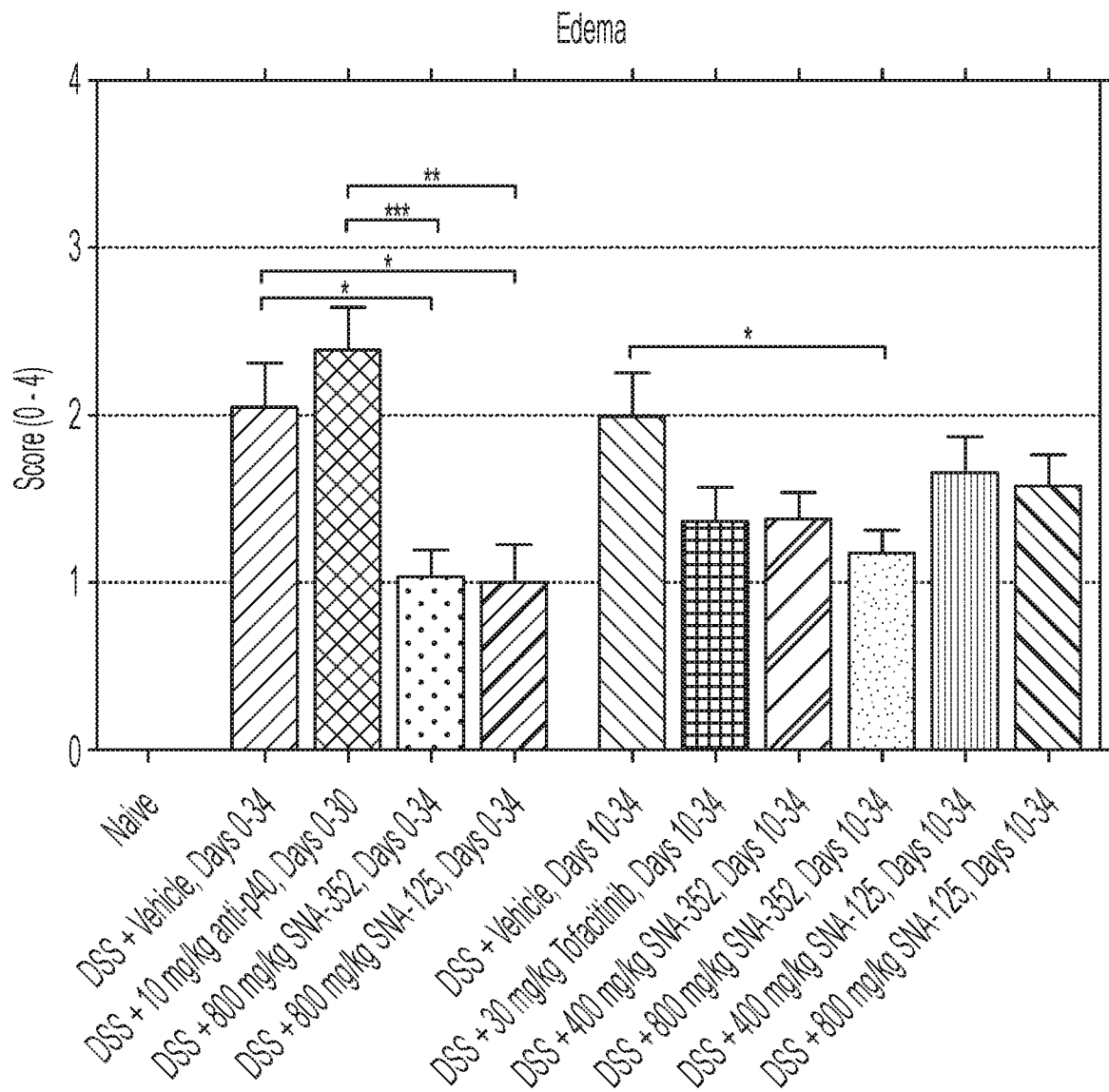


FIG. 132

194/207

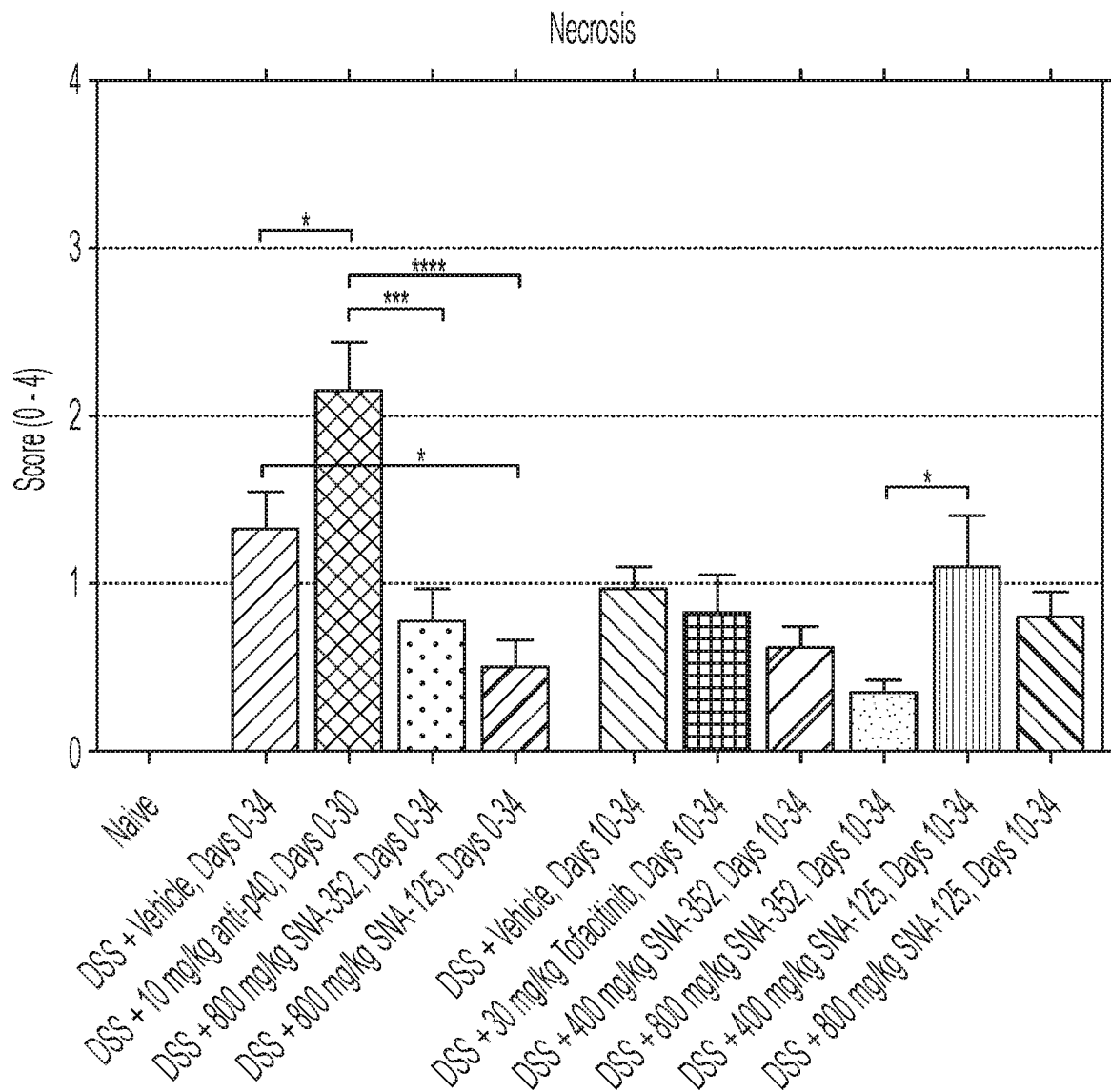


FIG. 133

195/207

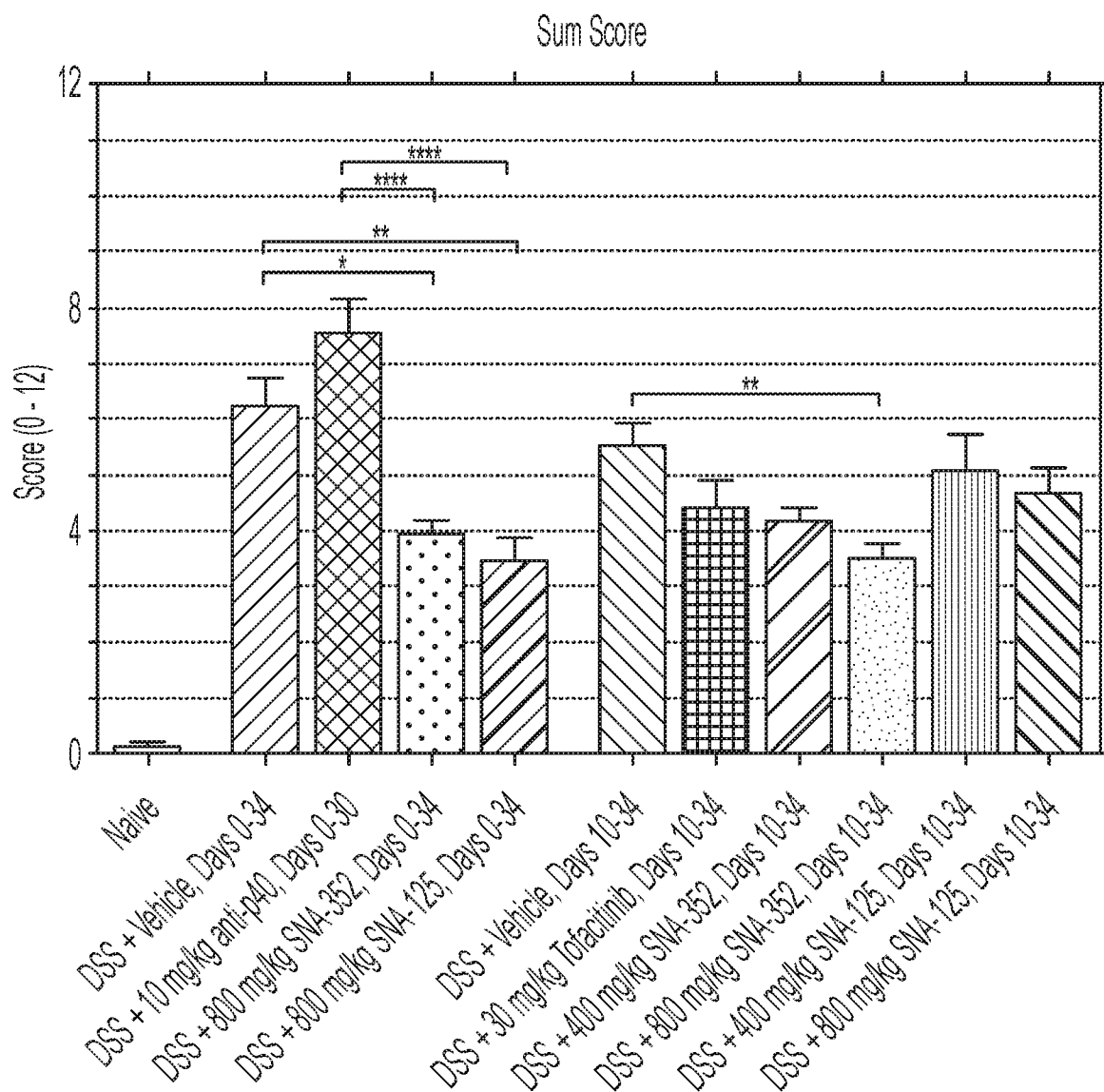


FIG. 134

196/207

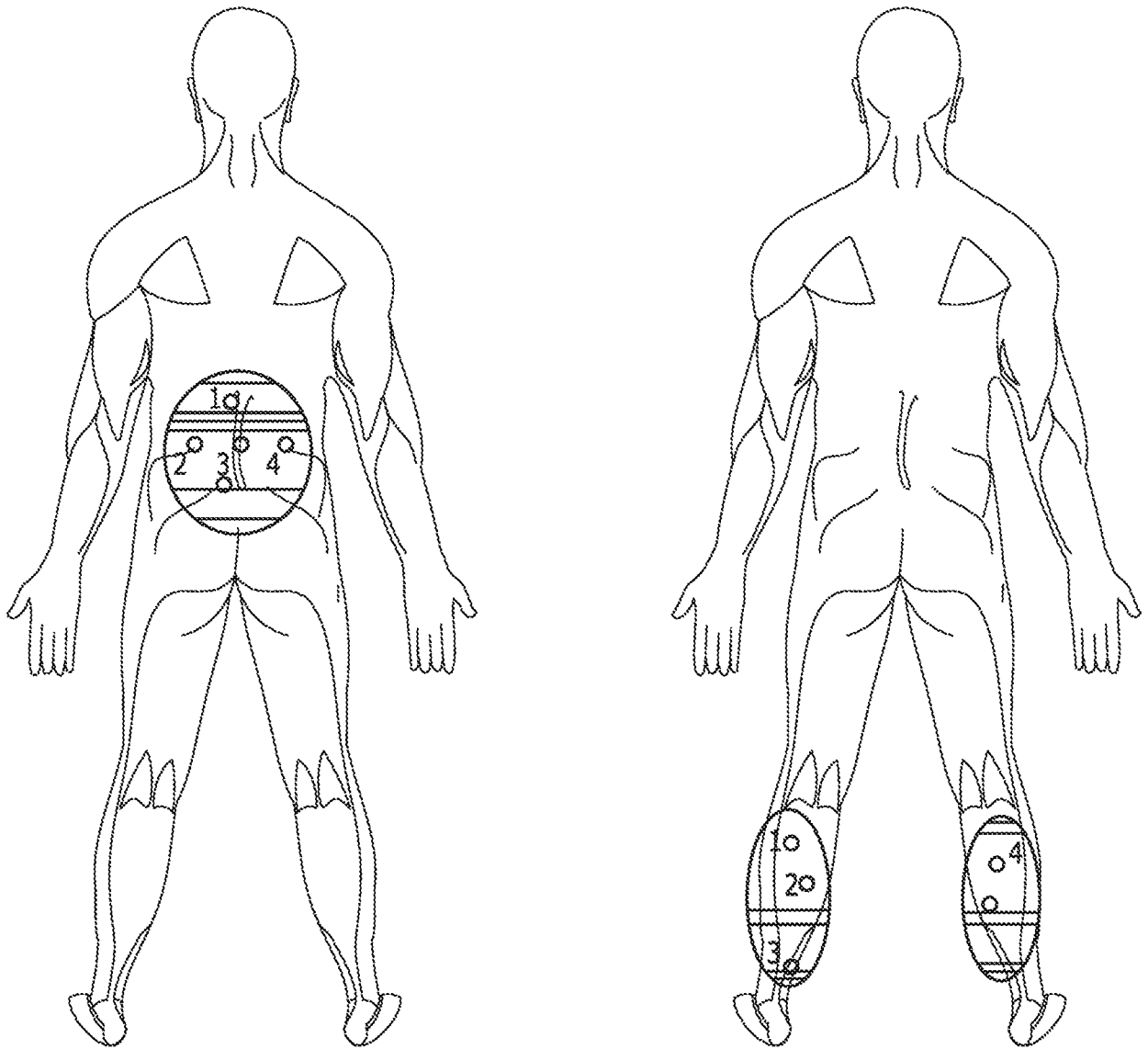


FIG. 135

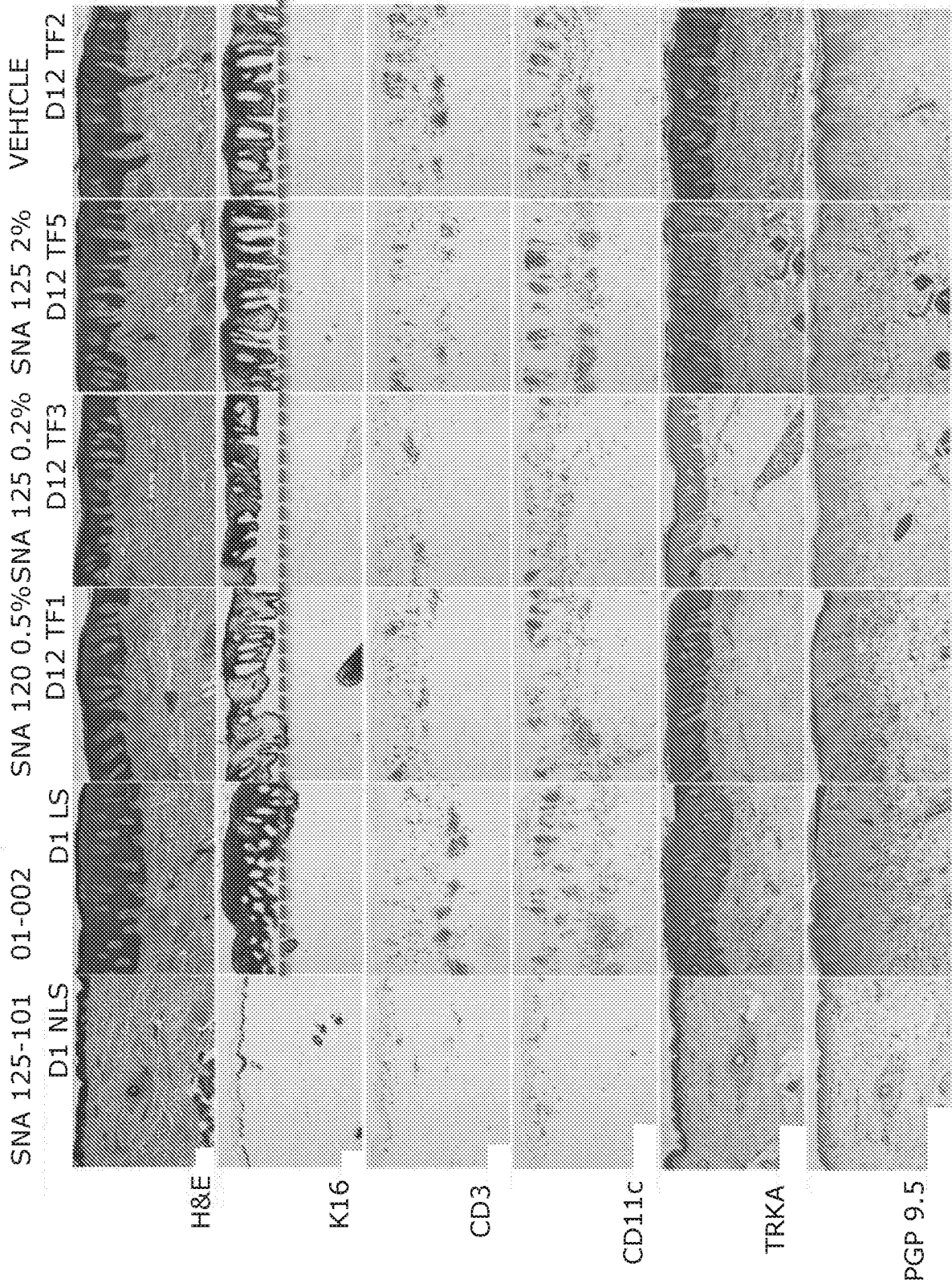


FIG. 136

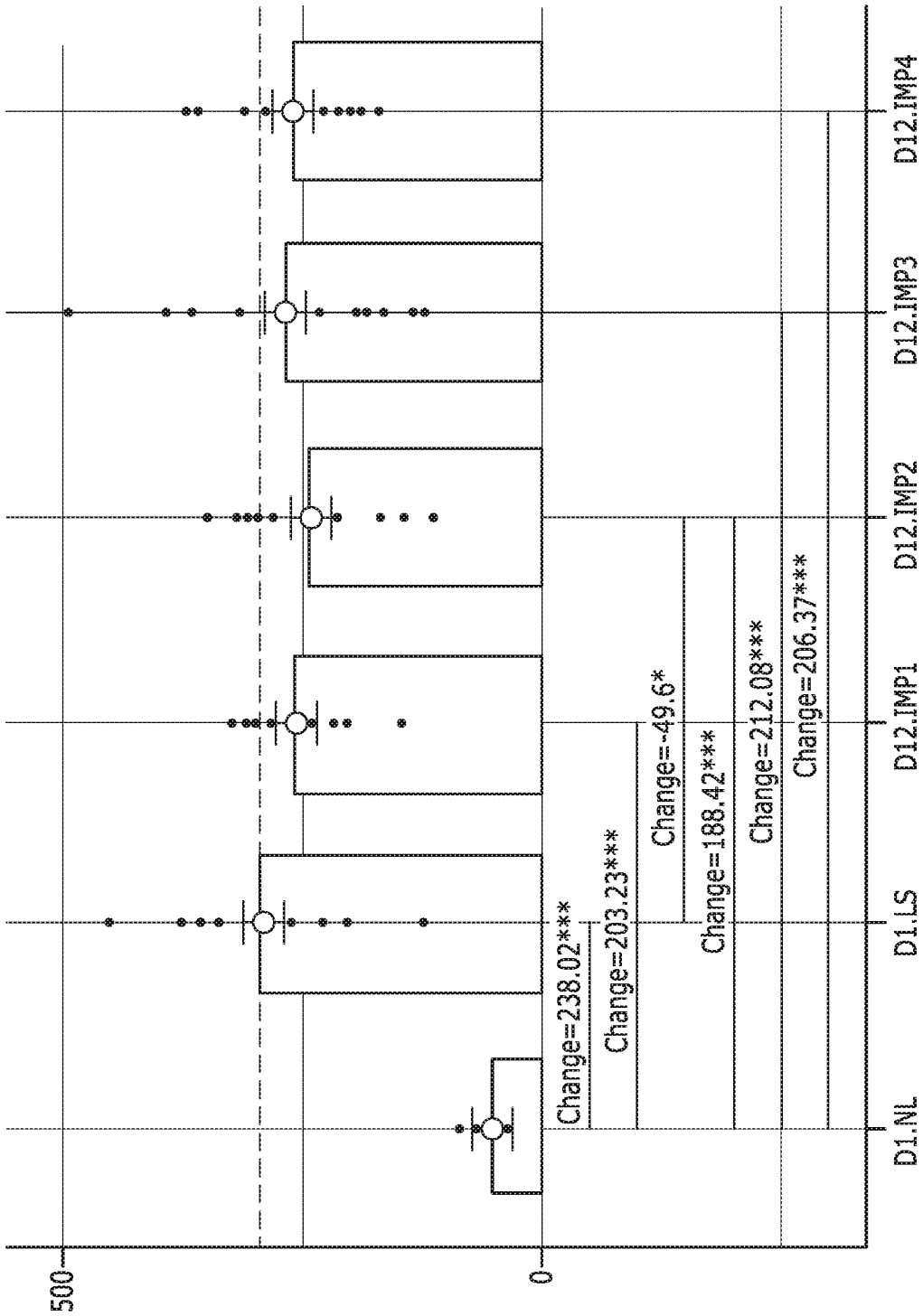


FIG. 137

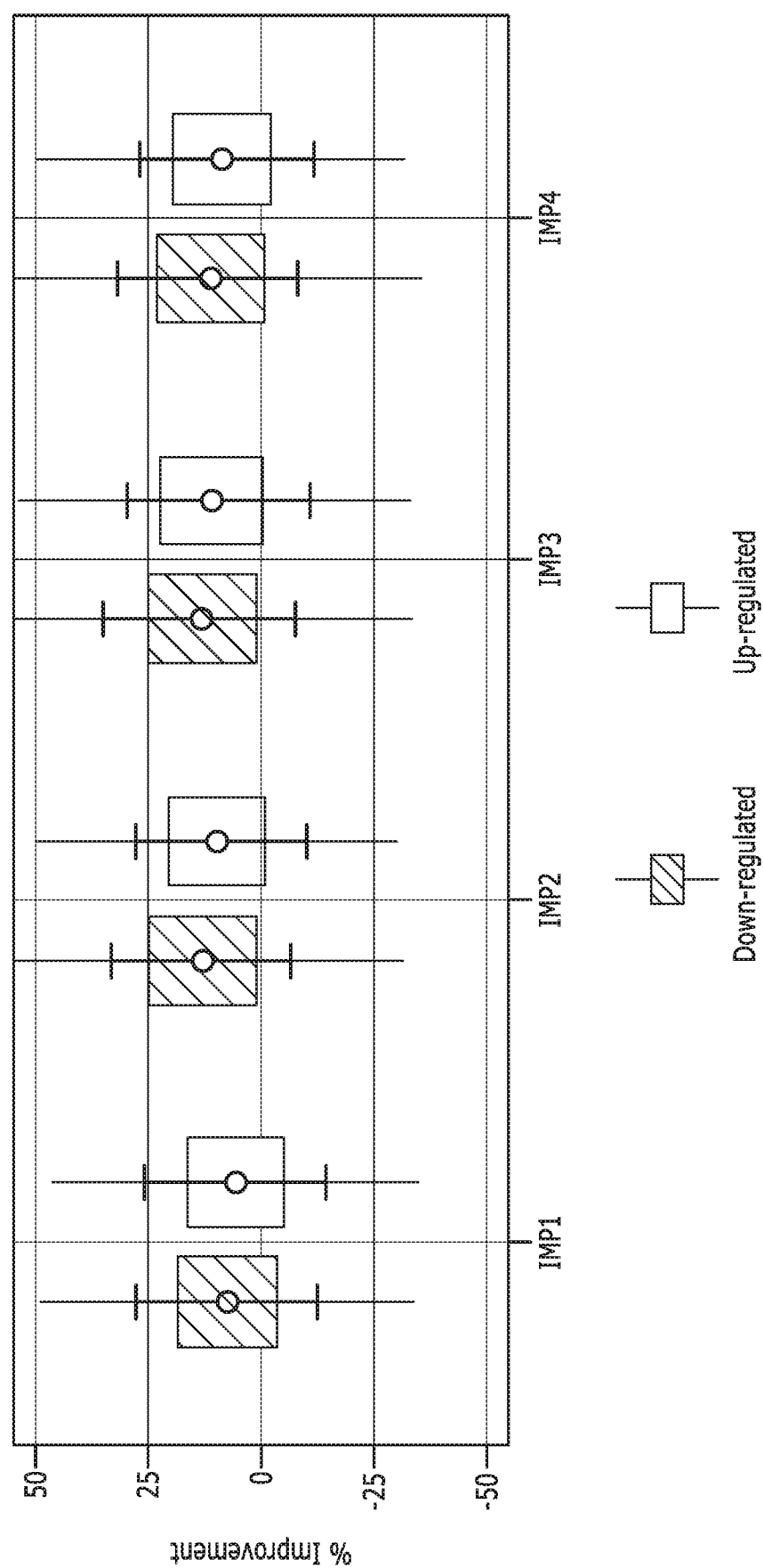


FIG. 138

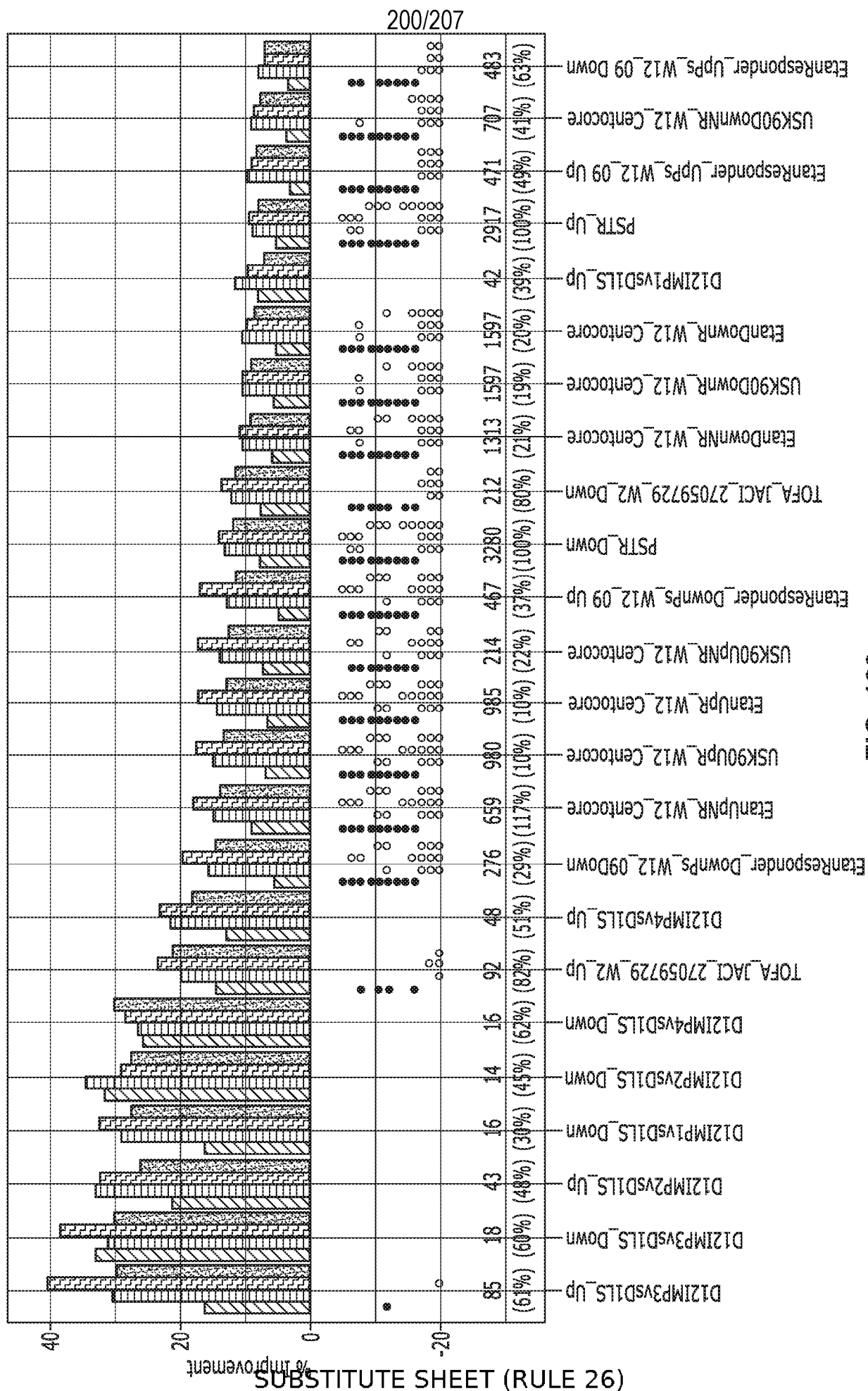


FIG. 139

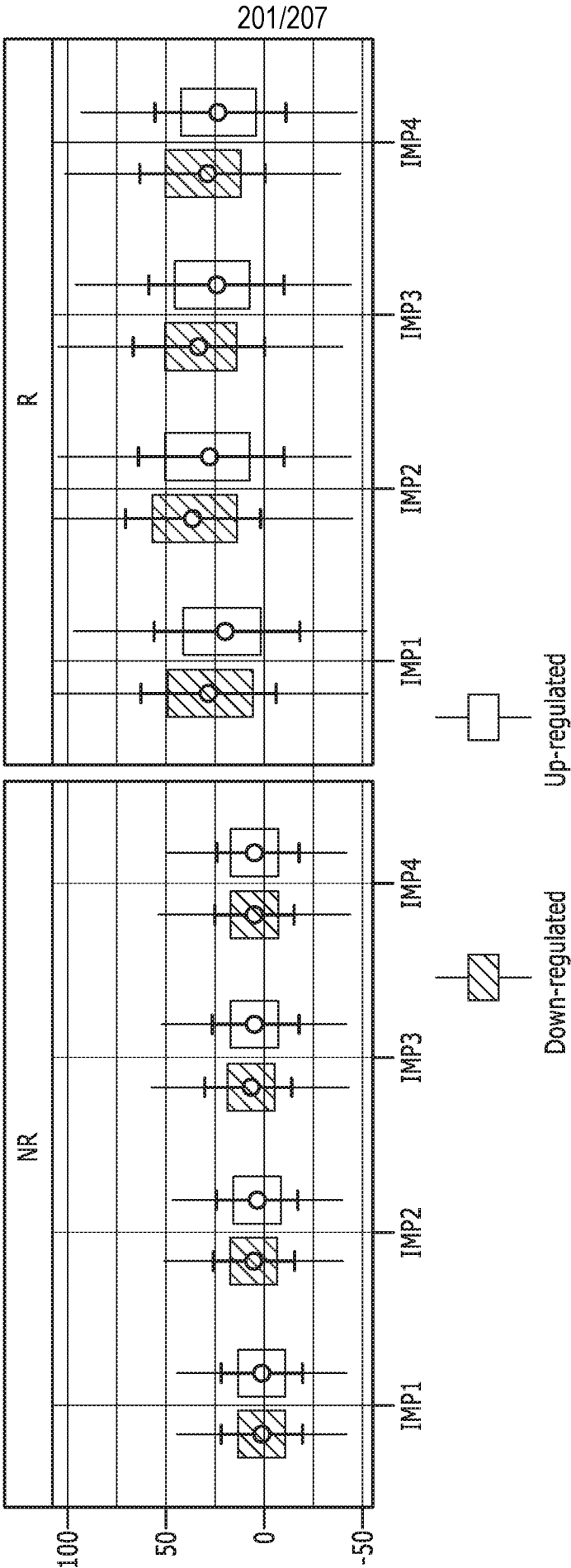


FIG. 140

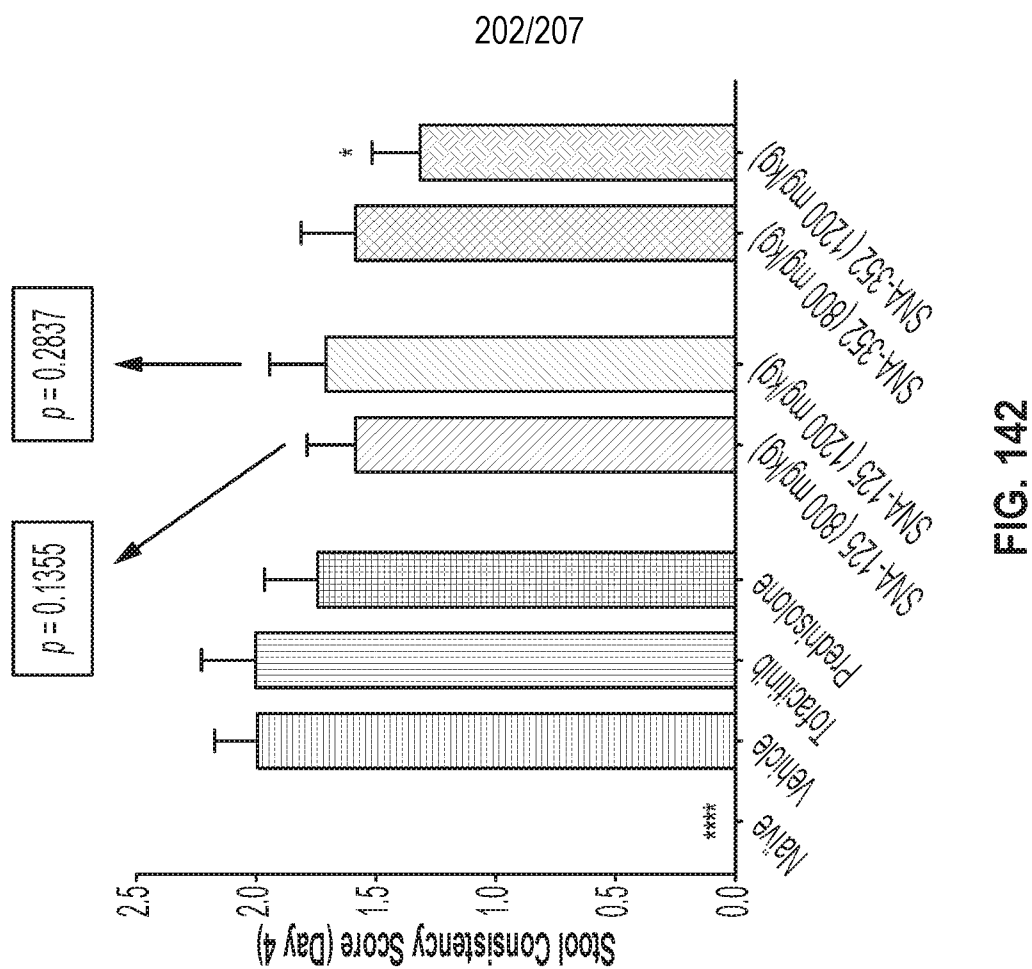


FIG. 142

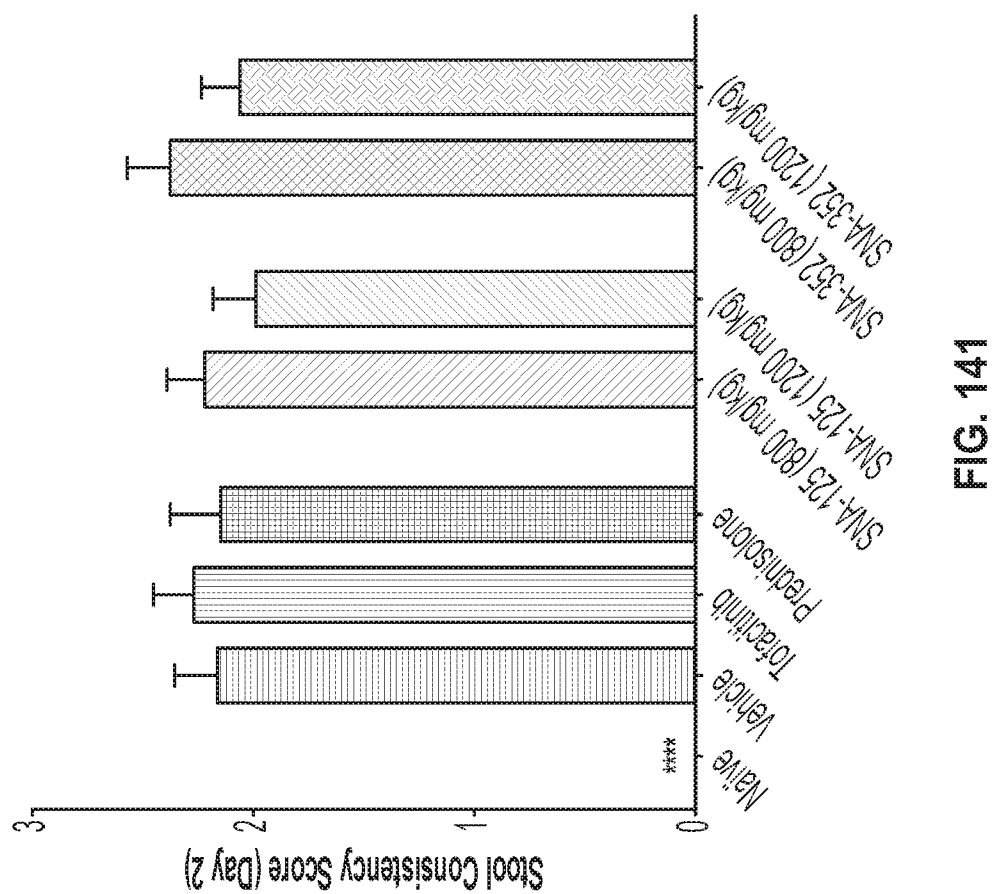


FIG. 141

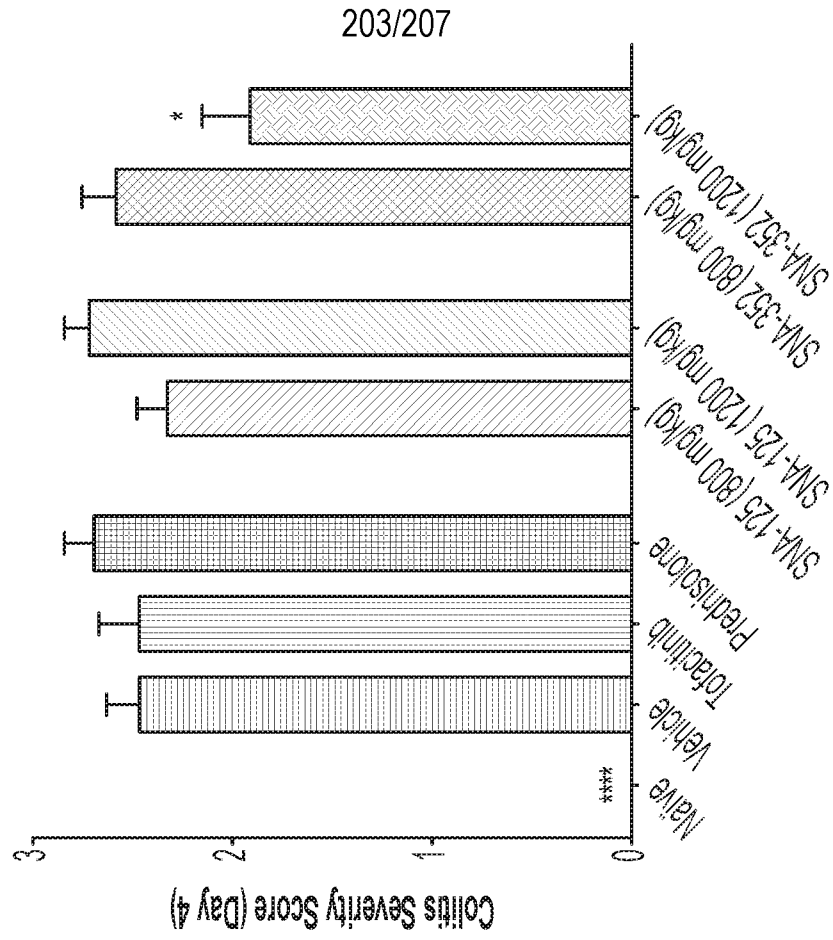


FIG. 144

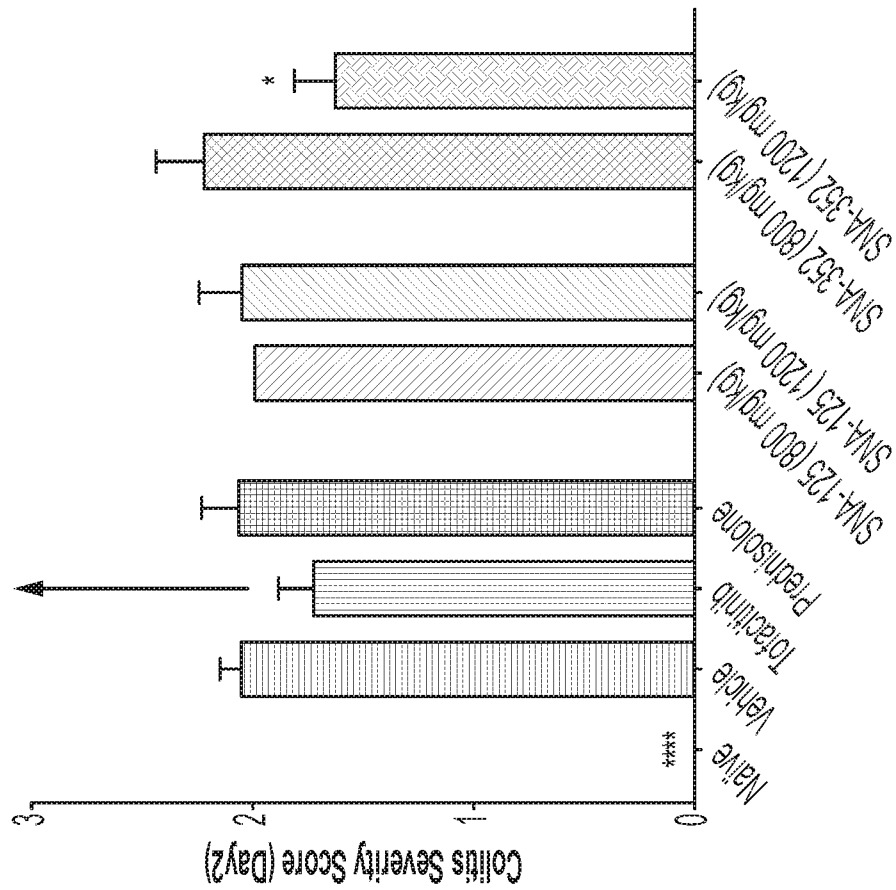
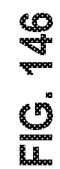
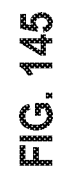


FIG. 143

$p = 0.0670$



205/207

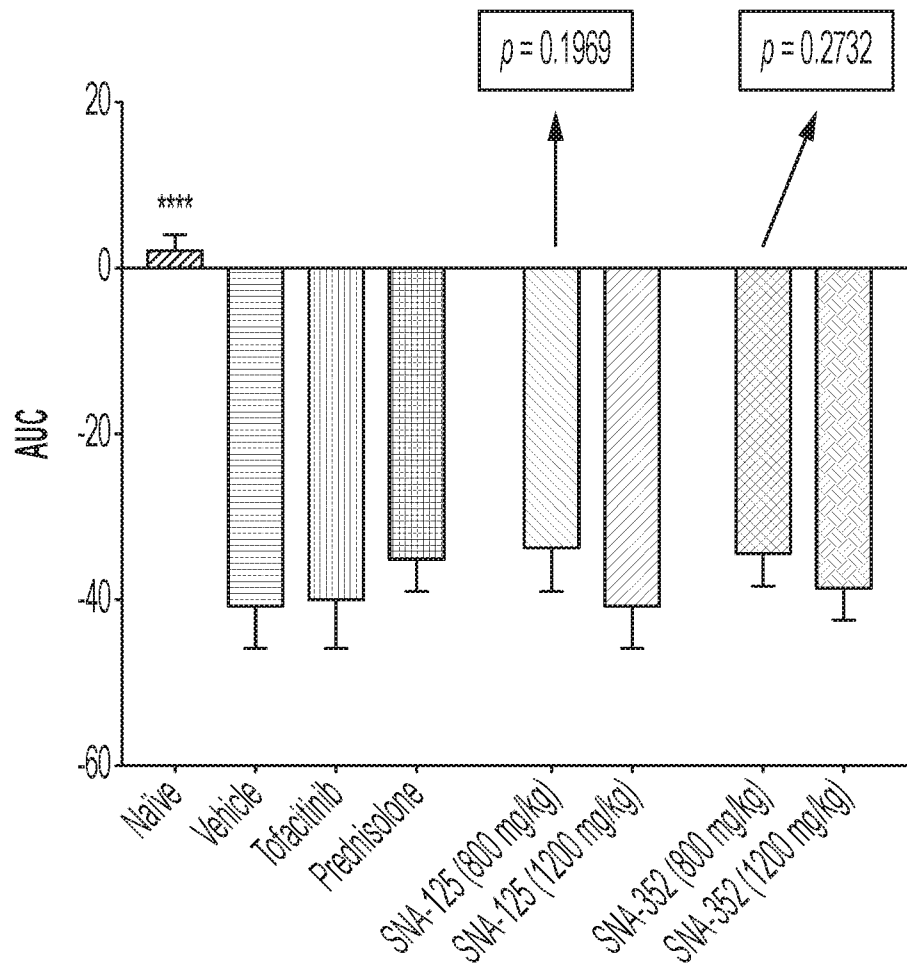


FIG. 147

206/207

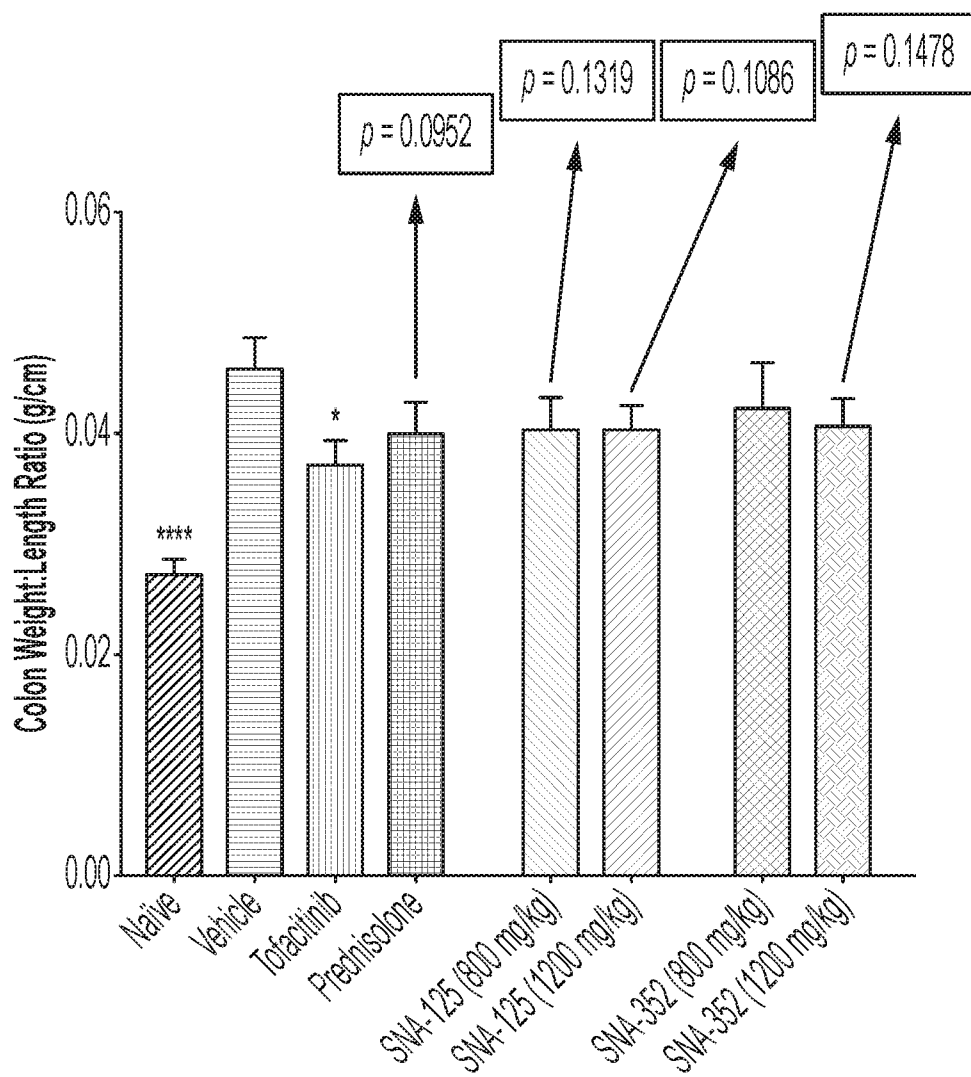


FIG. 148

207/207

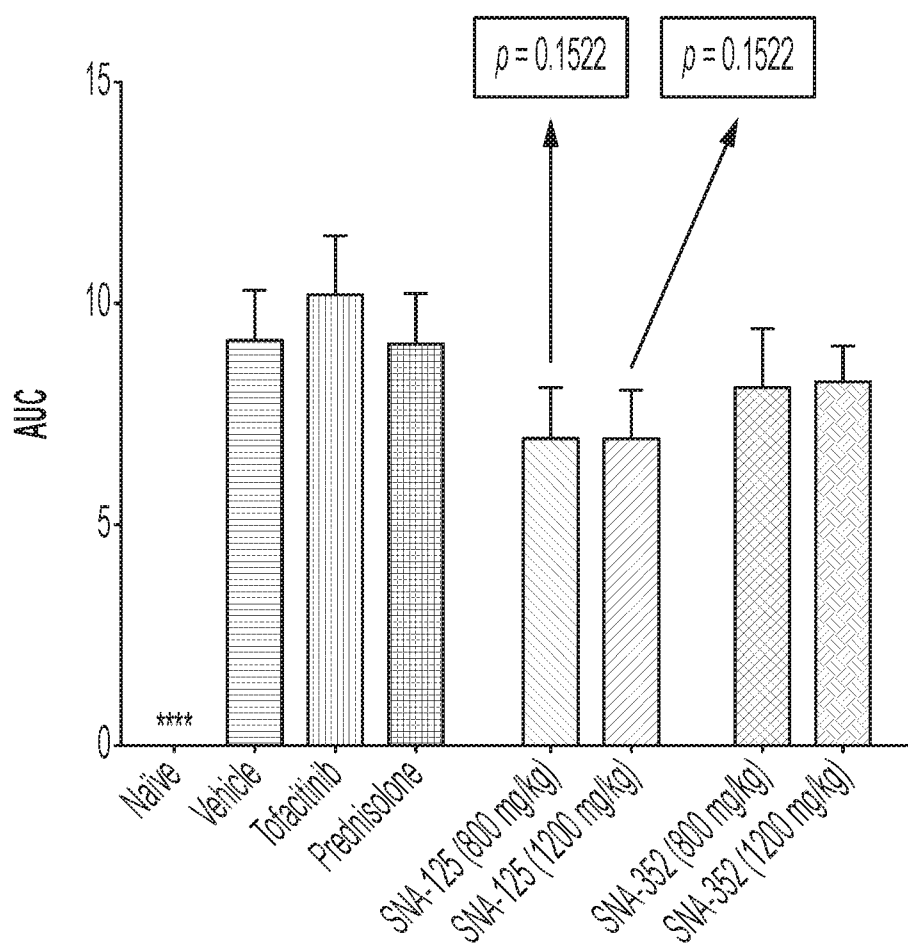


FIG. 149

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/42823

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61 K 47/60; A61 P 1/00; A61 P 11/00 (2019.01)
CPC - A61 K 31/553; A61 K 31/765; A61 K 45/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y -- A	US 201 1/0172140 A1 (Ley et al.) 14 July 201 1 (14.07.201 1); para [0003], [0006], [0057], [0059], [0067]-[0068], [0237], [0239]-[0240]	1-3, 5-8, (24-27)/(1-3, 5-8) ----- 4, (24-27)/(4) ----- 14-23/(1-8)
Y	US 2008/0318931 A1 (Arkin et al.) 25 December 2008 (25.12.2008); para [0033], [0151]	4, (24-27)/(4)
A	US 201 1/031 1451 A1 (Bagnod et al.) 22 December 201 1 (22.12.201 1); Figure 1	14-23/(1-8)
A	WO 2014/177510 A2 (CREABILIS SA) 06 November 2014 (06.11.2014); pg. 16, ln 1-3	14-23/(1-8)
A	US 2008/0193517 A1 (Traversa et al.) 14 August 2008 (14.08.2008); entire document	1-8, (14-23)/(1-8), 24-27

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

“A” document defining the general state of the art which is not considered to be of particular relevance

“E” earlier application or patent but published on or after the international filing date

“L” document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

“O” document referring to an oral disclosure, use, exhibition or other means

“P” document published prior to the international filing date but later than the priority date claimed

“T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

“X” document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

“Y” document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

“&” document member of the same patent family

Date of the actual completion of the international search

04 November 2019

Date of mailing of the international search report

22 NOV 2019

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/42823

Box No. **II** Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 28-35, 40-47, 49, 52-53
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. **III** Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
(see supplemental page)

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-8, (14-23)/(1-8) and 24-27

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐

The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 19/42823

--continued from Box No. III--

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I: Claims 1-8, 14-23 (in part) and 24-27, directed to a method of treating a gastrointestinal system condition comprising a compound comprising an active entity linked to at least one polymer.

Group II: Claims 9-13, 14-23 (in part), 36-39, 48, 50-51 and 54-62, directed to a method of treating a dermal condition comprising a compound comprising an active entity linked to at least one polymer.

Group III+: Claims 63-73, directed to a method of a condition comprising administering a compound of formula I or II. The compound of claim 63 may be searched, for example, to the extent that it encompasses the first species of claim 63, represented by a compound according to Formula I wherein R1 and R2 are the same and are hydrogen; R3 is hydrogen; W1 and W2 are hydrogen; X is a polymer moiety which is linear; for payment of additional fee and election as such. It is believed that claims 63-73 read on this first named invention of Group III+. Applicant is invited to elect additional compounds of claim 63, wherein each additional compound elected will require one additional invention fee. Applicants must specify the claims that encompass any additionally elected compound. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the '+' group(s) will result in only the first claimed invention to be searched. Another exemplary election would be a compound of claim 63, represented by a compound according to Formula I wherein R1 and R2 are the same and are hydrogen; R3 is hydrogen; W1 and W2 are hydrogen; X is a polymer moiety which is branched (i.e., claims 63-73).

The group of inventions listed above do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features:

Group I includes the technical feature of a method of treating a gastrointestinal system condition comprising a compound comprising an active entity linked to at least one polymer, which is not required by any other invention of Group II or III+.

Group II includes the technical feature of a method of treating a dermal condition comprising a compound comprising an active entity linked to at least one polymer, which is not required by any other invention of Group I or III+.

Group III+ includes the technical feature of a method of treating a condition comprising a unique compound of formula I or II, which is not required by any other invention of Group I or II.

Common technical features:

The inventions of Groups I, II and III+ share the technical feature of treating a condition in a subject in need thereof comprising administering a compound comprising an active entity linked to at least one polymer.

The inventions of Group III+ share the technical feature of a method of treating a condition comprising a compound of formula I or II.

These shared technical features, however, do not provide a contribution over the prior art, as being anticipated by US 2008/0193517 A1 to Traversa et al. (hereinafter Traversa). Traversa discloses a method of treating a condition in a subject in need thereof (claim 36), comprising administering to the subject (claim 36) an effective amount of a compound of formula II (a compound comprising an active entity linked to at least one polymer) wherein R1 and R2 are hydrogen; R3 is hydrogen; W1 and W2 are hydrogen; A represents -L1-X' and B represents -L2-Y', wherein at least one of X' and Y' is a polymer moiety, either linear or branched, which is bound by L1 and/or L2 to the tetrahydrofuran ring of the compound of formula (II); L2 is a linker group; Y' is a polymer moiety; X' is not a polymer moiety; L1 is covalent bond; X' is lower alkoxy carbonyl (abstract: 2nd compound listed).

As said compound was known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the inventions of Groups I, II or III+. The inventions of Group I, II and III+ thus lack unity under PCT Rule 13.

Item 4 (contd.): Claims 28-35, 40-47, 49, 52-53 have been found to be unsearchable because they are not drafted in accordance with the second and third sentences of Rule 6.4(a).