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METHODS OF TREATMENT WITH DLL4 ANTAGONISTS

Sequence Listing

[000] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on February 16, 2015, is named DLL4-400WO1_SL.txt and is 8,551 bytes in size.

DESCRIPTION

Field

[001] Methods of treatment with DLL4 antagonists.

Background

[002] Inhibition of angiogenesis is a well-recognized mechanism for targeting tumor vasculature in solid tumors. Recent studies have shown that Delta-like Ligand 4 (DLL4), which is normally induced by the Vascular Endothelial Growth Factor (VEGF), provides negative-feedback regulation through its receptor, called the Notch receptor, restraining vascular sprouting and branching. Blockade of the DLL4 signaling promotes excessive and non-productive angiogenesis resulting in decreased tumor growth. Thurston and Kitajewski, VEGF and Delta-Notch: interacting signalling pathways in tumour angiogenesis, British Journal of Cancer, 2008, 99, 1204-1209. DLL4 antagonists, such as monoclonal antibodies, can therefore be useful treatments against solid tumors.

[003] Yet, as with other therapeutic agents, it is important to determine the dose that will achieve therapeutic efficacy without undue side effects. This need exists both to determine the best dosage for entire patient populations, as well as for individual patients, especially in light of a projected small therapeutic window and delayed safety signals. Data from studies in

cynomolgus monkeys as well as data from Phase I clinical studies in humans reveal the complexity in providing an efficacious dose while minimizing side effects.

[004] In considering the level of efficacious drug to provide, assays were performed indicating that concentration of the anti-DLL4 antibody 21H3RK that would exhibit a 90% effect was approximately 14 µg/mL. A pharmacokinetics (PK) model simulation predicted that in humans a dose of 200 mg of 21H3RK, when administered intravenously (IV) every three weeks (Q3W), would maintain a trough concentration of 21H3RK at steady state above 14 µg/mL and full target occupancy throughout the dosing interval.

[005] From cynomolgus studies, from a safety perspective, the highest non-severely toxic dose (HNSTD) was determined to be 1mg/kg Q2W. In a phase I study, the exposure of 21H3RK in humans following 100 mg 21H3RK administration IV Q3W reached the HNSTD in monkeys based on model prediction and first time in human (FTIH or FIH) trial results. In a first time in human (FTIH) clinical trial, anti-tumor activity has been noted at dose of 30 mg Q3W while abnormal ECHO has been observed for a patient administered with 60 mg Q3W. Delayed safety signals have been observed in both monkeys and humans. Animal mortality occurred during the recovery period of a one-month GLP safety study and clinical safety signs appeared after week seven in a three-month GLP safety study. In the FTIH study, abnormal ECHO was detected eight weeks after the first dose for one patient in the 60 mg dose group and four weeks after the first dose for another patient in the 100 mg dose group. The potential problematic side effects related to liver and heart.

[006] Thus, in view of the above, an appropriate dose needs to be considered in view of various factors. Physicians and patients would benefit from additional techniques to determine the best dosages for DLL4 antagonists.

SUMMARY

[007] In accordance with the description, the present invention is directed to a method of reducing side effects due to the administration of a DLL4 antagonist to a patient comprising: administering an initial dose of the DLL4 antagonist to the patient; determining a justified DLL4 antagonist dose where the justified dose is increased relative to the initial dose if the plasma level of sVEGFR-2 and/or sVEGFR-3 is at or below a first reference standard and/or the justified dose is decreased relative to the initial dose if the plasma level of sVEGFR-2 and/or sVEGFR-3 is at or above a second reference standard; and administering to the patient a justified DLL4 antagonist dose calibrated to reduce side effects to the patient.

[008] The invention is further directed to a method of treating a patient with the minimal effective dose of a DLL4 antagonist comprising: administering an initial dose of the DLL4 antagonist to the patient; determining a justified DLL4 antagonist dose where the justified dose is increased relative to the initial dose if the plasma level of sVEGFR-2 and/or sVEGFR-3 is at or below a first reference standard and/or the justified dose is decreased relative to the initial dose if the plasma level of sVEGFR-2 and/or sVEGFR-3 is at or above a second reference standard; and administering to the patient a justified DLL4 antagonist dose calibrated to achieve efficacy with the minimal dose to the patient.

[009] The invention is also directed to a method of administering a DLL4 antagonist to a patient comprising administering an initial dose of the DLL4 antagonist to the patient; determining a justified DLL4 antagonist dose where the justified dose is increased relative to the initial dose if the plasma level of sVEGFR-2 and/or sVEGFR-3 is at or below a first reference standard and/or the justified dose is decreased relative to the initial dose if the plasma level of

sVEGFR-2 and/or sVEGFR-3 is at or above a second reference standard; and administering to the patient a justified DLL4 antagonist dose.

[010] In certain embodiments, where both the first reference standard and the second reference standard are used and the first reference standard and the second reference standard are different. In additional embodiments only the first reference standard is used or only the second reference standard is used. In particular embodiments, the first reference standard is a baseline sVEGFR-2 and/or sVEGFR-3 level without treatment with a DLL4 antagonist.

[011] In further embodiments, the patient has had a prior DLL4 antagonist dose that is no longer affecting the levels of sVEGFR-2 and/or sVEGFR-3 or the patient has not had a DLL4 antagonist dose for at least 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, or longer.

[012] In certain other embodiments, the first reference standard is a baseline sVEGFR-2 and/or sVEGFR-3 level and the justified dose produces a sVEGFR-2 plasma level that is at least 125%, 150%, 200%, or 250% of the first reference standard of sVEGFR-2 and/or the justified dose may be the dose that produces a sVEGFR-3 plasma level that is 150%, 200%, 250%, 300%, 400%, or 450% of the first reference standard of sVEGFR-3.

[013] In other embodiments, the second reference standard is the level of sVEGFR-2 and/or sVEGFR-3 yielding efficacy with minimal side effects or the second reference standard is the maximal response on a dose response curve.

[014] In certain embodiments, the plasma level of sVEGFR-2 used to calculate the justified dose based on a dose-response curve is 25 ng/L, 30 ng/L, 35 ng/L, 40 ng/L, 45 ng/L, or 50 ng/L and/or the sVEGFR-3 plasma level used to calculate the justified dose based on a dose-response curve is 25 ng/L, 30 ng/L, 40 ng/L, 50 ng/L, 75 ng/L, 100 ng/L, 125 ng/L, or 150 ng/L.

[015] In other embodiments, the first reference standard and/or the second reference standard are historical controls based on testing with a pool of subjects. In particular embodiments, the first reference standard and the second reference standard are obtained by administering to the patient a range of DLL4 antagonist doses over time and assigning the lowest plasma level of sVEGFR-2 and/or sVEGFR-3 as the first reference standard and the highest plasma level of sVEGFR-2 and/or sVEGFR-3 as the second reference standard.

[016] In certain embodiments of the methods of the invention, the initial dose of the DLL4 antagonist is from 10 mg to 100 mg, and in particular 10 mg, 30 mg, 60 mg, or 100 mg. In particular embodiments, the justified dose is from 10 mg to 30 mg.

[017] In methods of the invention, the sVEGFR-2 and/or sVEGFR-3 response to the initial dose is low and the dose is increased in 10% intervals until the patient achieves 85% of the sVEGFR-2 and/or sVEGFR-3 peak on the standard dose-response curve. In methods of the invention, alternatively, the sVEGFR-2 and/or sVEGFR-3 response to the initial dose is at or near the peak of the sVEGFR-2 and/or sVEGFR-3 standard dose-response curve and the dose increased in 10% intervals until the patient achieves 75% of the sVEGFR-2 and/or sVEGFR-3 peak on the standard dose-response curve.

[018] In certain embodiments, the plasma level of sVEGFR-2 and/or sVEGFR-3 is measured 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 10 weeks, 12 weeks, or 16 weeks after administration of the DLL4 antagonist. In further embodiments, the plasma level of sVEGFR-2 and/or sVEGFR-3 is measured at one time point, two, three, four, five, or more time points after administration of the DLL4 antagonist.

[019] In other embodiments, the method of treatment includes administering a justified dose at a justified dosing interval. In particular embodiments, the justified dosing interval is determined by evaluating the sVEGFR-2 and/or sVEGFR-3 levels for at least one time point and choosing a justified dosing interval in an effort to avoid the sVEGFR-2 and/or sVEGFR-3 level dropping below 50% of the peak on the standard dose-response curve. In further embodiments, the justified dosing interval may be every 7 days.

[020] In other embodiments of the invention, the method evaluates the level of both sVEGFR-2 and sVEGFR-3. In further embodiments, the justified DLL4 antagonist dose is determined by averaging the justified DLL4 antagonist dose that would have been determined by measuring sVEGFR-2 alone and the justified DLL4 antagonist dose that would have been determined by measuring sVEGFR-3 alone.

[021] In certain embodiments, the goal of the method is to maximize efficacy and the justified DLL4 antagonist dose is determined by selecting the higher of: the justified DLL4 antagonist dose that would have been determined by measuring sVEGFR-2 alone and the justified DLL4 antagonist dose that would have been determined by measuring sVEGFR-3 alone.

[022] In other embodiments, the goal of the method is to minimize side effects while achieving moderate efficacy and the justified DLL4 antagonist dose is determined by selecting the lower of: the justified DLL4 antagonist dose that would have been determined by measuring sVEGFR-2 alone and the justified DLL4 antagonist dose that would have been determined by measuring sVEGFR-3 alone.

[023] The present invention is also directed to a method for detecting efficacy of DLL4 antagonist comprising administering a DLL4 antagonist to a patient; detecting the level of

at least one pharmacodynamic biomarker chosen from sVEGFR-2 and sVEGFR-3 in the patient for at least one time point after administration of the DLL4 antagonist; evaluating the level of the pharmacodynamic biomarker in the patient. In further embodiments, the levels of both sVEGFR-2 and sVEGFR-3 are detected.

[024] In certain embodiments of the methods of the invention, the DLL4 antagonist is an anti-DLL4 antibody. In particular embodiments, the antibody has the same heavy chain CDRs and the same light chain CDRs as 21H3RK, the antibody has a heavy chain sequence and a light chain sequence of 21H3RK, the antibody has the same heavy chain CDRs and the same light chain CDRs as YW152F, the antibody has a heavy chain sequence and light chain sequence of YW152F, the antibody has the same heavy chain CDRs and the same light chain CDRs as 21M18, the antibody has a heavy chain sequence and a light chain sequence of 21M18, the antibody has the same heavy chain CDRs and light chain CDRs as REG421 or the antibody has a heavy chain sequence and a light chain sequence of REG421.

[025] In certain embodiments of the methods of the invention, the DLL4 antagonist blocks a Notch receptor signaling pathway or the DLL4 antagonist is administered to promote excessive and unproductive angiogenesis.

[026] In other embodiments of the methods of the invention, the patient has cancer that can be lung cancer, colon cancer, clear-cell renal cell carcinoma, glioblastoma, breast cancer, or bladder cancer.

[027] In further embodiments of the methods of the invention, the method reduces tumor volume by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% or the method reduces tumor growth by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%

compared to the rate of prior growth or predicted growth based on historical controls. In further embodiments, the patient's tumor does not grow in size or is reduced in size.

[028] In additional embodiments, the level of the pharmacodynamic biomarker is compared to a control level, the level of the pharmacodynamic biomarker is compared to the patient's own level for at least one time point prior to administration of the DLL4 antagonist, the level of the pharmacodynamic biomarker is measured in a plasma sample, or the increase in the level of the pharmacodynamic biomarker predicts a decrease in tumor volume.

[029] The invention is also directed to a method of evaluating the effectiveness of a DLL4 antagonist comprising administering a DLL4 antagonist to a subject, detecting the level of sVEGFR-2 and/or sVEGFR-3 in the subject for at least one time point after administration of the DLL4 antagonist, and comparing the level of sVEGFR-2 and/or sVEGFR-3 to a reference standard.

[030] The invention is also directed to a method of comparing the effectiveness of DLL4 antagonists comprising administering a first DLL4 antagonist to a subject, detecting the level of at least one pharmacodynamics biomarker chosen from sVEGFR-2 and sVEGFR-3 in the subject for at least one time point after administration of the first DLL4 antagonist; evaluating the level of the pharmacodynamics biomarker in the subject, administering a second DLL4 antagonist to the subject, detecting the level of at least one pharmacodynamics biomarker chosen from sVEGFR-2 and sVEGFR-3 in the subject for at least one time point after administration of the second DLL4 antagonist; evaluating the level of the pharmacodynamics biomarker in the subject, comparing the response of the at least one pharmacodynamic biomarker after the administration of the first DLL4 antagonist to the level after the administration of the second DLL4 antagonist; and selecting the more effective DLL4 antagonist. In further

embodiments, the first DLL4 antagonist and the second DLL4 antagonist are administered to the same subject. the first DLL4 antagonist and the second DLL4 antagonist are administered to different individuals in a pool of subjects or the pool of subjects is a group of laboratory animals.

[031] Additional objects and advantages will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice. The objects and advantages will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims.

[032] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the claims.

[033] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments and together with the description, serve to explain the principles described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[034] Figure 1 shows the relationship between DLL4 activation and VEGFR-2 and VEGFR-3 based on the present research. The figure shows the relationship before addition of a DLL4 antagonist.

[035] Figure 2 shows the upregulation of mouse sVEGFR-2 by anti-DLL4 treatment (YW152F and 2A5) in Calu-6 xenografts in nude mice and the inverse correlation between tumor volume and sVEGFR-2 levels.

[036] Figure 3 shows the upregulation of mouse sVEGFR-2 by anti-DLL4 treatment (2A5) in Calu-6 xenografts in nude mice and an inverse correlation between tumor volume and sVEGFR-2 levels.

[037] Figures 4A-B evaluate sVEGFR-2 and tumor volume in a Calu-6 xenograft model with an inverse correlation to tumor volume.

[038] Figures 5A-B shows mouse sVEGFR-1 response to anti-DLL4 treatment (2A5) in Calu-6 xenografts; no correlation was observed between sVEGFR-1 response and tumor volume. Data for sVEGFR-1 levels after treatment with Comparator YW152F are also shown.

[039] Figures 6A-C show the upregulation of mouse sVEGFR-2 by anti-DLL4 treatment (21H3RK, 2A5, Comparator 21M18, and Comparator REG421) in Calu-6 xenografts in hDLL4 KIKO mice.

[040] Figures 7A-E show that dosing level (2A5) and frequency determines sVEGFR-2 accumulation following treatment in Calu-6 xenografts.

[041] Figures 8A-D show that mouse sVEGFR-2 correlates to drug exposure (2A5) in Calu-6 xenografts and also shows the inverse correlation between sVEGFR-2 levels and tumor volume.

[042] Figure 9 shows sVEGFR-1 response after treatment with Comperater YW152F in a Calu-6 bearing nude mouse model.

[043] Figures 10A-B show that sVEGFR-2 levels increase in a dose-dependent manner in COLO205 xenografts after treatment with DLL4 antagonists (2A5YW152F). These figures also show an inverse correlation between sVEGFR-2 levels and tumor volume for 2A5.

[044] Figures 11A-B shows the upregulation of mouse sVEGFR-2 by anti-DLL4 treatment (2A5) with an inverse correlation to tumor volume in a COLO205 xenograft model. Data for sVEGFR-2 levels after treatment with Comparator YW152F are also shown.

[045] Figures 12A-B evaluate mouse sVEGFR-1 and tumor volume after treatment with anti-DLL4 treatment (2A5) in a COLO205 xenograft model. Data for sVEGFR-1 levels after treatment with comparator YW152F are also shown.

[046] Figures 13A-B evaluates mouse sVEGFR-1 and tumor volume response to anti-DLL4 treatment (2A5) in COLO205 xenografts. No correlation was observed between sVEGFR-1 and tumor volume. Data for sVEGFR-1 levels after treatment with Comparator YW152F are also shown.

[047] Figure 14 evaluates sVEGFR-1 levels in COLO205 xenograft nude mice after treatment with Comparator YW152F.

[048] Figures 15A-B show sVEGFR-2 levels increasing in a dose-dependent manner following treatment with 2A5 and Comparator YW152F in Calu-6 and COLO205 mouse xenograft models.

[049] Figures 16A-B evaluate sVEGFR-1 levels in two mouse xenograft models, Calu-6 and COLO205, following treatment with 2A5 and Comparator YW152F

[050] Figures 17A-B plot sVEGFR-1 levels against tumor volume in two mouse xenograft models, Calu-6 and COLO205, following treatment with 2A5/YW152F. No correlation was found between sVEGFR-1 levels and tumor volume.

[051] Figure 18 shows the upregulation of mouse sVEGFR-2 by anti-DLL4 treatment (2A5) in AsPC-1 xenografts with an inverse correlation to tumor volume.

[052] Figures 19A-B show the upregulation of mouse sVEGFR-2 by anti-DLL4 treatment (2A5) in AsPC-1 xenografts with an inverse correlation to tumor volume.

[053] Figure 20 evaluates mouse sVEGFR-1 levels after treatment with anti-DLL4 2A5 in AsPC-1 xenografts. No correlation was observed between sVEGFR-1 and tumor volume.

[054] Figure 21 shows sVEGFR-1 levels in non-tumor bearing mice treated with Comparator YW152F or R347 (isotype control). Levels of sVEGFR-1 after treatment fell within the range of the control samples, suggesting no treatment effect.

[055] Figure 22 shows sVEGR-1 levels in an immunotoxicity study of non-tumor bearing mice treated with Comparator YW152F or R347 (isotype control).

[056] Figures 23A-F show results across 6 experiments in which mouse sVEGFR-1 response to Comparator YW152F and 2A5 in several xenograft models are inconsistent.

[057] Figures 24A-E show cynomolgus monkey sVEGFR-2 and sVEGFR-1 levels after treatment with 21H3RK. Soluble VEGFR-2 levels increased in a dose-dependent manner in all the studies. During the recovery period, a dose-dependent recovery of sVEGFR-2 levels towards baseline was observed. In contrast, no treatment response was observed for sVEGFR-1.

[058] Figure 25 shows sVEGFR-1 levels in a 24-day intravenous study of 21H3RK in female cynomolgus monkeys. No treatment response was observed.

[059] Figure 26 shows sVEGFR-2 levels in a one-month repeat-dose intravenous bolus injection study in cynomolgus monkeys following treatment with 21H3RK. Soluble VEGFR-2 levels increased for all treatment groups, reaching maximal response for all dose levels. Levels decreased to near baseline levels during the recovery phase.

[060] Figures 27A-B show the results of a one-month study of toxicokinetics and pharmacodynamics in cynomolgus monkeys following treatment with 21H3RK. A dose

proportional PK was observed between 3 and 100 mg/kg with maximal PD effect in blood achieved at 3 mg/kg QW. PK and sVEGFR-2 levels returned to baseline after recovery.

[061] Figure 28 shows the sVEGFR-2 results of a three-month repeat-dose study in cynomolgus monkeys following treatment with 21H3RK. Maximal increase in sVEGFR-2 levels was observed in all dose groups with a dose-dependent recovery to baseline.

[062] Figure 29 shows the PK and sVEGFR-2 results of a three-month study in cynomolgus monkeys following treatment with 21H3RK (MEDI0639). A dose-proportional PK between 3 and 30 mg/kg was observed; however, a non-linear PK was observed between 1 and 3 mg/kg. Maximal PD effect in blood was achieved at 1 mg/kg Q2W.

[063] Figure 30 shows the sVEGFR-2 results of a 4-week single dose study by intravenous bolus injection in cynomolgus monkeys following treatment with 21H3RK. Levels of sVEGFR-2 increased after 10 mg/kg treatment; however no effect was observed at doses of up to 1 mg/kg.

[064] Figure 31 shows the 21H3RK (MEDI0639) concentration over time in a concentration-time profile study in cynomolgus monkeys. A nonlinear PK was observed indicating the presence of an antigen sink saturated at 10 mg/kg.

[065] Figure 32 shows upregulation of sVEGFR-2 levels in monkeys following 21H3RK treatment.

[066] Figure 33 shows the sVEGFR-2 levels in male cynomolgus monkeys in a 3-month repeat-dose cardiovascular safety pharmacology study of 21H3RK by intravenous bolus injection in a male telemetered cynomolgus monkey study. Dose-dependent increase and dose-dependent recoveries were observed in sVEGFR-2 levels with maximal response in 3 and 10 mg/kg dose groups.

[067] Figure 34 shows the mean serum concentrations of 21H3RK and the plasma sVEGFR-2 levels over time in a telemetered cynomolgus monkey study. A nonlinear PK was observed between 1 and 10 mg/kg, reaching maximal PD response at 3 and 10 mg/kg. Partial PD response was observed at 1mg/kg. Soluble VEGFR-2 levels returned to baseline levels during the recovery phase.

[068] Figures 35A-B show steep upregulation of sVEGFR-2 and sVEGFR-3 responses in human subjects after treatment with 21H3RK with maximal response achieved in the 30, 60, and 100 mg dose groups; whereas no changes were observed in the 10 mg dose group. The following time points were not plotted to scale: S, screening; EOT, end of treatment, 30D, 30 day post treatment; 3M, 3 months post treatment; cycle 1 and 2, full profile plotted; cycles ≥ 3 , only predose are plotted.

[069] Figures 36A-B show sVEGFR-2 and sVEGFR-3 responses in human subjects following treatment with 21H3RK at 10, 30, 60, and 100 mgs. Median levels increased for sVEGFR-2, and to a lesser extent for sVEGFR-3, in subjects with clinical response (1009 with clinical benefit and 1013 with partial response). The following time points were not plotted to scale: S, screening; EOT, end of treatment, 30D, 30 day post treatment; 3M, 3 months post treatment; cycle 1 and 2, full profile plotted; cycles ≥ 3 , only predose are plotted.

[070] Figures 37A-C show sVEGR-2 concentrations in individual human subjects for the 30 mg, 60 mg and 100 mg dose groups throughout their treatment period. Subjects with clinical benefit is noted for subjects# 1009 (clinical benefit) and 1013 (partical response).

[071] Figure 38 shows a visualization of 21H3RK exposure and selected safety events in a human study.

[072] Figure 39 shows sVEGFR-2 concentrations in individual subjects for the 60 mg (panel A) and 100 mg (panel B) dose groups, following treatment with 21H3RK, throughout their treatment period. Similar concentrations of sVEGFR-2 and sVEGFR-3 were observed in subjects with BNP levels greater than 100 (plotted in red) and subject with no BNP (plotted in black). The following time points were not plotted to scale: S, screening; EOT, end of treatment, 30D, 30 day post treatment; 3M, 3 months post treatment; cycle 1 and 2, full profile plotted; cycles ≥ 3 , only predose are plotted.

[073] Figure 40 shows sVEGFR-2 levels relative to BNP concentration in a human study.

[074] Figure 41 shows theoretical DLL-4 occupancy by 21H3RK based on PK/PD model simulation.

[075] Figure 42, as part of the assay qualification for sVEGFR-2, shows that recoveries of sVEGFR-2 spiked at 4 ng/ml and 1 ng/ml were acceptable, indicating no significant matrix interference.

[076] Figure 43, as part of the assay qualification for sVEGFR-2, shows parallelism between the endogenous sVEGFR-2 (sample) compared to the recombinant sVEGFR-2 (standard curve) for sample dilution of up to 600-fold.

[077] Figure 44, as part of the assay qualification for sVEGFR-2, shows that baseline levels of sVEGFR-2 in 28 individual samples (10 from normal donors, 8 from donors with pancreatic cancer and 10 from donors with colon cancer) were all measurable using this assay

[078] Figure 45 shows that detection of endogenous sVEGFR-2 in 3 individual samples (1 each from a normal donor and from donors with pancreatic cancer and colon cancer)

were significantly reduced following immuno-depletion with a commercially available anti-VEGFR-2 antibody indicating that the measurement of sVEGR-2 in this assay is specific.

DESCRIPTION OF THE EMBODIMENTS

I. Methods of Treatment with DLL4 Antagonists

A. Methods of Treatment

[079] With the present research, it has been determined that antagonizing DLL4 increases the levels of sVEGFR-2 and sVEGFR-3, but not sVEGFR-1. Figure 1. One embodiment includes a method of reducing side effects due to the administration of a DLL4 antagonist to a patient comprising: administering an initial dose of the DLL4 antagonist to the patient; increasing the dose of the DLL4 antagonist if the plasma level of sVEGFR-2 and/or sVEGFR-3 is at or below a first reference standard and/or decreasing the dose of the DLL4 antagonist if the plasma level of sVEGFR-2 and/or sVEGFR-3 is at or above a second reference standard; and administering to the patient a justified DLL4 antagonist dose calibrated to reduce side effects to the patient.

[080] Another embodiment includes a method of treating a patient with the minimal effective dose of a DLL4 antagonist comprising administering an initial dose of the DLL4 antagonist to the patient; increasing the dose of the DLL4 antagonist if the plasma level of sVEGFR-2 and/or sVEGFR-3 is at or below a first reference standard and/or decreasing the dose of the DLL4 antagonist if the plasma level of sVEGFR-2 and/or sVEGFR-3 is at or above a second reference standard; and administering to the patient a justified DLL4 antagonist dose calibrated to achieve efficacy with the minimal dose to the patient.

[081] A further embodiment includes a method of administering a DLL4 antagonist to a patient comprising administering an initial dose of the DLL4 antagonist to the patient;

increasing the dose of the DLL4 antagonist if the plasma level of sVEGFR-2 and/or sVEGFR-3 is at or below a first reference standard and/or decreasing the dose of the DLL4 antagonist if the plasma level of sVEGFR-2 and/or sVEGFR-3 is at or above a second reference standard; and administering to the patient a justified DLL4 antagonist dose.

[082] In one embodiment, both the first reference standard and the second reference standard are used and the first reference standard and the second reference standard are different. In another embodiment, only the first reference standard is used. In another embodiment, wherein only the second reference standard is used. Thus, having two reference standards is optional.

B. DLL4 Antagonists

[083] In one embodiment, the DLL4 antagonist blocks a Notch receptor signaling pathway. In one embodiment, the DLL4 antagonist may be an anti-DLL4 antibody. In another embodiment, the DLL4 antagonist may be a chimeric, humanized, or fully human antibody. In one embodiment, it may be a monoclonal antibody. In another embodiment, the DLL antagonist is an anti-DLL4 human antibody 21H3RK, described in US Patent No. 8,192,738 or WO 2010/032060, the contents of each of which are herein incorporated by reference. 2A5 is a mouse surrogate monoclonal antibody to 21H3RK. Since 21H3RK has a low affinity to muDLL4, a monoclonal antibody, 2A5, which is cross-reactive to both huDLL4 and muDLL4, was generated by affinity-optimization of 21H3RK. Both 21H3RK and 2A5 bind to competing epitope of human DLL4 with comparable affinity. In another embodiment, the DLL4 antagonist may be Comparator YW152F, Comparator 21M18, or Comparator REG421. In another embodiment, the DLL4 antagonist may have the same heavy and light chain CDRs as any of the

Comparator Antibodies, for example, REGN-421, disclosed in U.S. Patent No. 7,488,806 (SAR153192; Regeneron, Sanofi-Aventis; WO2008076379) and OPM-21 M18, disclosed in U.S. Patent No. 7,750,124 (OncoMed) (Hoey et al., Cell Stem Cell. 2009 Aug 7; 5(2):168-77), both fully human DLL4 antibodies; YW152F, disclosed in U.S. Patent No. 7,803,377 (Genentech), a humanized DII4 antibody (Ridgway et al., Nature. 2006 Dec 21;444(7122):1083-7). The contents of each of the above U.S. patents, patent documents and references describing and disclosing DLL4 antagonists are incorporated by reference herein. In another embodiment, the DLL4 antagonist may be a small molecule.

[084] Antibodies may be polyclonal, monoclonal, chimeric, humanized, or fully human. The term YW152F and the term DLL4 antagonist also include a binding fragment of a fully human monoclonal antibody. For example, the targeted binding agent can be a full-length antibody (e.g., having an intact human Fc region) or an 21M18 binding fragment (e.g., a Fab, Fab' or F(ab')₂, FV or dAb). In addition, the antibodies can be single-domain antibodies such as camelid or human single VH or VL domains that bind to DLL4, such as a dAb fragment. Exemplary DLL4 antibody sequences include the human antibody 21H3RK, described in US Patent No. 8,192,738 or WO 2010/032060, the contents of each of which are herein incorporated by reference. 21H3RK is a human anti-human DLL4 antibody that demonstrates minimal binding to human Jagged-1 or human DLL1. The amino acid sequence of the variable region of the heavy chain (VH) of 21H3RK is set forth in US Patent No. 8,192,738, and the amino acid sequence of the variable region of the light chain (VL) of 21H3RK is set forth in of US Patent No. 8,192,738. Complementarity determining regions (CDR) of 21H3RK are reproduced as follows: the variable heavy (VH) CDR1 corresponds to the amino acid sequence of NYGIT (SEQ ID NO:1), VH CDR2 corresponds to the amino acid sequence of

WISAYNGNTNYAQLQD (SEQ ID NO:2), and VH CDR3 corresponds to the amino acid sequence of DRVPRIPVTTEAFDI (SEQ ID NO:3) ; the variable light (VL) CDR1 corresponds to the amino acid sequence of SGSSSNIGSYFVY (SEQ ID NO:4), the VL CDR2 corresponds to the amino acid sequence of RNNQRPS (SEQ ID NO:5), and the VL CDR3 corresponds to the amino acid sequence of AAWDDSLSGHWV (SEQ ID NO:6).

[085] The corresponding VH and VL sequences of 21H3RK are also reproduced below: 21H3RK VH: Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr Gly Ile Thr Trp Val Arg Gln Ala Pro Gly Gln Gly Pro Glu Trp Met Gly Trp Ile Ser Ala Tyr Asn Gly Asn Thr Asn Tyr Ala Gln Lys Leu Gln Asp Arg Val Thr Val Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp Arg Val Pro Arg Ile Pro Val Thr Thr Glu Ala Phe Asp Ile Trp Gly Gln Gly Thr Met Val Thr Val Ser Ser (SEQ ID NO:7).

[086] 21H3RK VL: Gln Ser Val Leu Thr Gln Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Tyr Phe Val Tyr Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu Ile Tyr Arg Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Glu Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Ala Ala Trp Asp Asp Ser Leu Ser Gly His Trp Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu (SEQ ID NO:8).

[087] Additional anti-DLL antibodies sequences are as follows:

[088] REGN421 VH: Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Phe Leu Trp Tyr Asp Gly Thr Asn Lys Asn Tyr Val Glu Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys

Asn Met Leu Tyr Leu Glu Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
Ala Arg Asp His Asp Phe Arg Ser Gly Tyr Glu Gly Trp Phe Asp Pro Trp Gly Gln Gly
Thr Leu Val Thr Val Ser Ser (SEQ ID NO: 9).

[089] REGN VL: Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Tyr Leu Ala Trp Tyr Gln Gln Lys
Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Asp Ala Ser Asn Arg Ala Thr Gly Ile Pro Ala Arg Phe
Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu Asp Phe Ala Val
Tyr Tyr Cys Gln His Arg Ser Asn Trp Pro Pro Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
(SEQ ID NO:10).

[090] 21M18 VH: Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Ala Tyr Tyr Ile His Trp Val Lys Gln
Ala Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Ser Ser Tyr Asn Gly Ala Thr Asn Tyr Asn Gln
Lys Phe Lys Gly Arg Val Thr Phe Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr Met Glu Leu Arg
Ser Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp Tyr Asp Tyr Asp Val Gly Met
Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser (SEQ ID NO: 11).

21M18 VL: Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly Glu Arg Ala Thr
Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Asn Tyr Gly Ile Ser Phe Met Lys Trp Phe Gln Gln Lys
Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Gln Gly Ser Gly Val Pro Asp Arg Phe
Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val
Tyr Tyr Cys Gln Gln Ser Lys Glu Val Pro Trp Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
(SEQ ID NO:12).

[091] In one embodiment the DLL4 antagonist specifically binds to DLL4 and inhibits
binding to a Notch receptor, e. g., Notch 1. In one embodiment the DLL4 antagonist inhibits at

least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% of DLL4 binding to a Notch receptor, (e.g., Notch 1), compared to binding that would occur in the absence of the DLL4 antagonist. In some embodiments, the DLL4 antagonist binds DLL4 with a binding affinity (KB) of less than 5 nM, 4 nM, 3 nM, 2 nM or 1 nM, 950 pM, 900 pM, 800 pM, 700 pM, 600 pM, 500 pM, 400 pM, 300 pM, 200 pM, 150 pM, 100 pM, 50 pM, 10 pM, or 1 pM. The KD may be assessed using a method known to one of skill in the art (e.g., a BIAcoreTM assay (label-free interaction analysis), ELISA, FACS) (Biacore International AB, Uppsala, Sweden).

[092] The binding properties of the DLL4 antagonist may also be measured by reference to the dissociation or association rates (k_{on} and k_{off} respectively).

[093] In one embodiment, a DLL4 antagonist may have an k_{on} rate of at least $10^4 \text{ M}^{-1}\text{s}^{-1}$, at least $5 \times 10^4 \text{ M}^{-1}\text{s}^{-1}$, at least $10^5 \text{ M}^{-1}\text{s}^{-1}$, at least $2 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$, at least $5 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$, at least $10^6 \text{ M}^{-1}\text{s}^{-1}$, at least $5 \times 10^6 \text{ M}^{-1}\text{s}^{-1}$, at least $10^7 \text{ M}^{-1}\text{s}^{-1}$, at least $5 \times 10^7 \text{ M}^{-1}\text{s}^{-1}$, or at least $10^8 \text{ M}^{-1}\text{s}^{-1}$.

[094] In another embodiment, DLL4 antagonist may have a k_{off} rate of less than $5 \times 10^{-1} \text{ s}^{-1}$, less than 10^{-1} s^{-1} , less than $5 \times 10^{-2} \text{ s}^{-1}$, less than 10^{-2} s^{-1} , less than $5 \times 10^{-3} \text{ s}^{-1}$, less than 10^{-3} s^{-1} , less than $5 \times 10^{-4} \text{ s}^{-1}$, less than $4 \times 10^{-4} \text{ s}^{-1}$, less than $3 \times 10^{-4} \text{ s}^{-1}$, less than $2 \times 10^{-4} \text{ s}^{-1}$, less than 10^{-4} s^{-1} , less than $5 \times 10^{-5} \text{ s}^{-1}$, less than 10^{-5} s^{-1} , less than $5 \times 10^{-6} \text{ s}^{-1}$, less than 10^{-6} s^{-1} , less than $5 \times 10^{-7} \text{ s}^{-1}$, less than 10^{-7} s^{-1} , less than $5 \times 10^{-8} \text{ s}^{-1}$, less than 10^{-8} s^{-1} , less than $5 \times 10^{-9} \text{ s}^{-1}$, less than 10^{-9} s^{-1} , or less than 10^{-10} s^{-1} .

[095] In yet another embodiment, the DLL4 antagonist inhibits DLL4-Notch1 receptor-ligand binding. In one example, activity possessed by the targeted binding agent can be demonstrated at an IC50 concentration (a concentration to achieve 50% inhibition of) below

10 pM. In another example, the DLL4 antagonist can have an IC₅₀ concentration of less than 50, 40, 30, 20, 10, 5, 4, 2, 1, 0.8, 0.7, 0.6, 0.5 or 0.4 nM.

[096] In yet another aspect, the DLL4 antagonist may be conjugated to another agent, such as a toxin, a radioisotope, or another substance that will kill a cancer cell. In another embodiment, the DLL4 antagonist can be administered alone or can be administered in combination with other agents, such as antibodies, chemotherapeutic drugs, and/or radiation therapy.

[097] In one embodiment, the initial dose of a DLL4 antagonist may be 0.0001 mg/kg, 0.001 mg/kg, 0.01 mg/kg, 0.1 mg/kg, 1 mg/kg, 3 mg/kg, 10 mg/kg, 30 mg/kg, or 100 mg/kg. In another embodiment, the initial dosage may be between 0.0001 mg/kg and 20 mg/kg, 0.0001 mg/kg and 10 mg/kg, 0.0001 mg/kg and 5 mg/kg, 0.0001 and 2 mg/kg, 0.0001 and 1 mg/kg, 0.0001 mg/kg and 0.75 mg/kg, 0.0001 mg/kg and 0.5 mg/kg, 0.0001 mg/kg to 0.25 mg/kg, 0.0001 to 0.15 mg/kg, 0.0001 to 0.10 mg/kg, 0.001 to 0.5 mg/kg, 0.01 to 0.25 mg/kg, or 0.01 to 0.10 mg/kg. In one embodiment, the initial dose of a DLL4 antagonist may be 1 mg, 5 mg, 10 mg, 15 mg, 20 mg, 30 mg, 40 mg, 50 mg, 60 mg, 100 mg, 125 mg, or 150 mg. In one embodiment, the justified dose of a DLL4 antagonist may be at a single dosage from 1 mg to 150 mg, from 1 mg to 50 mg, from 5 mg to 30 mg, from 10 mg to 30 mg, from 20 mg to 60 mg.

C. Types of Cancer

[098] The present method may be used to treat patients with cancer. In one embodiment, the present method may be used to treat patients with a solid tumor. In another embodiment, the present method may be used to treat patients with lung cancer, colon cancer, clear-cell renal cell carcinoma, glioblastoma, breast cancer, or bladder cancer.

[0099] In yet another embodiment, the method may be used to treat patients with sarcomas, carcinomas, and/or lymphomas. In another embodiment, the method may be used to treat patients with malignant tumors such as melanoma, small cell lung cancer, non-small cell lung cancer, glioma, hepatocellular (liver) carcinoma, thyroid tumor, gastric (stomach) cancer, prostate cancer, breast cancer, ovarian cancer, bladder cancer, lung cancer, glioblastoma, endometrial cancer, kidney cancer, colon cancer, pancreatic cancer, esophageal carcinoma, head and neck cancers, mesothelioma, sarcomas, biliary (cholangiocarcinoma), small bowel adenocarcinoma, pediatric malignancies and epidermoid carcinoma. In another embodiment, treatable proliferative or angiogenic diseases include neoplastic diseases, such as, melanoma, small cell lung cancer, non-small cell lung cancer, glioma, hepatocellular (liver) carcinoma, thyroid tumor, gastric (stomach) cancer, gallbladder cancer, prostate cancer, breast cancer, ovarian cancer, bladder cancer, lung cancer, glioblastoma, endometrial cancer, kidney cancer, colon cancer, pancreatic cancer, ovarian, esophageal carcinoma, head and neck cancers, mesothelioma, sarcomas, biliary (cholangiocarcinoma), small bowel adenocarcinoma, pediatric malignancies, and epidermoid carcinoma.

[0100] In one embodiment, the method is beneficial in reducing tumor volume. In certain embodiments, tumor volume may be reduced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%. In another embodiment, the method is beneficial in reducing tumor growth. In one embodiment, tumor growth may be reduced by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%. Reducing tumor growth includes reducing growth as compared to the rate of prior growth or compared to a predicted rate of growth based on historical controls. In another embodiment, the method promotes excessive and uncontrolled vascularization, leading to unproductive angiogenesis due to poor perfusion and increased hypoxia resulting in

decreased tumor growth (Noguera-Troisem Blockade of dll4 inhibits tumor growth by promoting non-productive angiogenesis, Nature, 2006, 444, 1032-1037. ProQinase studies of 21H3RK in mice provided the efficacy data of 21H3RK. In these studies, animals were administered 21H3RK intraperitoneally two times a week. A dose-dependent increase in number of blood vessels and decrease of mural cell coverage were observed.

II. Determining sVEGFR-2 and sVEGFR-3 Levels

[0101] The present method operates regardless of the techniques used to determine sVEGFR-2 and/or sVEGFR-3 levels. A number of commercially available assays are available to measure sVEGFR-2 and sVEGFR-3 in serum and plasma. These include traditional ELISA from multiple vendors such as Bender Medical Systems, Biovendor, Abcam, R&D Systems, electrochemiluminescence assay from MesoScale Discovery and bead-based assay from Millipore. Assay sensitivity for these platforms are similar and comparable. Soluble receptor levels reported for these assays are within levels reported in the literature. Moreover, a large number of antibodies to sVEGFR-2 and sVEGFR-3 are commercially available from multiple vendors, allowing the development of assays for measuring these receptors. Based on literature, both serum and plasma levels of sVEGFR2 and sVEGFR3 are similar. Thus, determining either serum or plasma levels would support the current invention. In one embodiment, for human or cynomolgus monkey testing the Human soluble Vascular Endothelial Growth Factor Receptor-2 (sVEGFR-2) may be measured using the MSD® 96-Well MULTI-ARRAY® Human KDR Assay kit (MesoScale Discovery, Cat# K151BOC-3) according to the manufacturer's recommendations. In another embodiment, mouse soluble Vascular Endothelial Growth Factor Receptor-2 (sVEGFR-2) may be measured in mouse K2-EDTA plasma using the Quantikine

Immunoassay Assay Kit from R&D Systems (Cat# MVR200B) according to the manufacturer's recommendations.

[0102] In one embodiment, the sVEGFR-2 and/or sVEGFR-3 levels are determined from a plasma sample. In one embodiment, the sVEGFR-2 and/or sVEGFR-3 levels may be determined 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 8 hours, 12 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 10 weeks, 12 weeks, or 16 weeks after administration of the DLL4 antagonist. In one embodiment, the levels may be determined at one time point, two, three, four, five, or more time points after administration of the DLL4 antagonist. In certain embodiments, the levels are determined during the time the patient is being screened for treatment. Determination can be performed within several days (one, two, three, four, five, six, or seven) or within 1 week, 2 weeks, 3 weeks or within 1 month.

[0103] In one embodiment, the levels of sVEGFR-2 and/or sVEGFR-3 may be compared to the patient's own level for at least one time point prior to administration of a DLL4 antagonist. In another embodiment, a baseline level may be measured at one or more time points prior to administration of a DLL4 antagonist. In another embodiment, prior to administration of a DLL4 antagonist does not exclude the scenario where the patient may have received an earlier DLL4 antagonist dose that is no longer affecting the levels of sVEGFR-2 and/or sVEGFR-3. In one embodiment, the patient has not had a DLL4 antagonist dose for at least 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, or longer.

[0104] In another embodiment, the level of sVEGFR-2 and/or sVEGFR-3 may be compared to a control level. In one embodiment, this is a historical control value based on at least one baseline level prior to administration of a DLL4 antagonist (negative control). In

another embodiment, this is a historical control value based on at least one level after administration of a DLL4 antagonist (positive control). In one embodiment, at least one control value is determined using one or more healthy subjects. In another embodiment, at least one control value is determined using one or more patients with cancer or the same type of cancer as the patient receiving treatment.

[0105] In one embodiment, the first reference standard is a negative control, either in the patient or a historical control. For example, one example of a negative control is the baseline level of sVEGFR-2 and/or sVEGFR-3 in the patient. In one embodiment, the first reference standard is obtained by administering a DLL4 antagonist at a dose that did not produce clinical efficacy in the patient. In another embodiment, the second reference standard is a positive control, either in the patient or a historical control.

[0106] In one embodiment where the first reference standard is a negative control, the justified dose may be the dose that produces a sVEGFR-2 plasma level that is at least 125%, 150%, 200%, or 250% of the first reference standard. In one embodiment where the first reference standard is a negative control, the justified dose may be the dose that produces a sVEGFR-3 plasma level that is 150%, 200%, 250%, 300%, 400%, or 450% of the first reference standard. In one embodiment, the sVEGFR-2 plasma level produced from administration of the justified dose or used to calculate the justified dose based on a dose-response curve is at least 25 ng/L, 30 ng/L, 35 ng/L, 40 ng/L, 45 ng/L, or 50 ng/L. In one embodiment, the sVEGFR-3 plasma level produced from administration of the justified dose or used to calculate the justified dose based on a dose-response curve is at least 25 ng/L, 30 ng/L, 40 ng/L, 50 ng/L, 75 ng/L, 100 ng/L, 125 ng/L, or 150 ng/L.

[0107] In another embodiment, the first reference standard and the second reference standard are obtained by administering to the patient a range of DLL4 antagonist doses over time and assigning the lowest plasma level of sVEGFR-2 and/or sVEGFR-3 as the first reference standard and the highest plasma level of sVEGFR-2 and/or sVEGFR-3 as the second reference standard. In one embodiment, if increasing levels of a DLL4 antagonist does not produce an increasing plasma level of sVEGFR-2 and/or sVEGFR-3, then the DLL4 antagonist level is decreased. In one embodiment, the justified dose of the DLL4 antagonist is the lowest dose that provides the maximal sVEGFR-2 and/or sVEGFR-3 response. In another embodiment, the justified DLL4 antagonist dose is at the peak of the dose response curve, wherein the response is measured as the level of sVEGFR-2 and/or sVEGFR-3.

[0108] In one, the first reference standard and the second reference standard are the same. In this embodiment, the single reference standard may be based on clinical data from a pool of cancer patients receiving DLL4 antagonist treatment that was effective in reducing tumor volume and promoting excessive and unproductive angiogenesis. Depending on the value of the reference standard, different approaches may be taken. In one embodiment, the justified dose is increased from the initial dose if the patient's sVEGFR-2 and/or sVEGFR-3 levels are below the single reference standard. In the case where the single reference standard is lower than the peak of the dose response curve, the justified dose is decreased from the initial dose if the patient's sVEGFR-2 and/or sVEGFR-3 levels are above the reference standard. This may be done to reduce side effects but achieve a reasonable degree of efficacy, such as when administering a DLL4 antagonist with troublesome side effects. In one embodiment, the initial dose is maintained as the justified dose if the patient's sVEGFR-2 and/or sVEGFR-3 levels are at the reference standard. In the embodiment where the reference standard is at the peak of the dose

response curve, the justified dosage is decreased from the initial dose and in one embodiment the sVEGFR-2 and/or sVEGFR-3 levels retested to determine if they decrease. In one embodiment, the justified dosage is the lowest dose of the DLL4 antagonist that produces the maximal level of sVEGFR-2 and/or sVEGFR-3 response.

[0109] In one embodiment, when the sVEGFR-2 and/or sVEGFR-3 response to the initial dose was lacking, the dose may be increased in 10% intervals based on the initial dose until the patient achieves 75%, 80%, 85%, 90%, 95%, 99%, 100%, or exceeds the sVEGFR-2 and/or sVEGFR-3 peak on the standard dose response curve, unless the patient's own sVEGFR-2 and/or sVEGFR-3 levels peak earlier. In other words, the patient may receive a series of doses starting with an initial dose, 110% of the initial dose, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200%, and so on, until the desired result is achieved and the justified dose established. Such an approach may be used when achieving maximal efficacy is the treatment goal. In another embodiment, when the sVEGFR-2 and/or sVEGFR-3 response to the initial dose is sufficient (for example, and depending on the antagonist chosen, within 75%, 80%, 85%, 90%, 95%, 99%, 100%, or exceeding the sVEGFR-2 and/or sVEGFR-3 peak on the standard dose-response curve), the dosage may be lowered in 10% intervals based on the initial dose until the patient's sVEGFR-2 and/or sVEGFR-3 levels drop below 75%, 80%, 85%, 90%, 95% of the sVEGFR-2 and/or sVEGFR-3 peak on the standard dose-response curve). The justified dose is chosen from the lowest dosage able to produce the desired result. Such an approach may be used when avoiding side effects and achieving reasonable efficacy is the treatment goal. In some embodiments, reasonable efficacy is less than maximal efficacy.

[0110] Additionally, methods of treatment also include justified dosing intervals. In one embodiment, sVEGFR-2 and/or sVEGFR-3 levels are used to determine the justified dosing

interval. In one embodiment, an initial DLL4 antagonist dose is provided and the level of sVEGFR-2 and/or sVEGFR-3 is measured for at least one and optionally 2, 3, 4, 5, or 6 time points after the initial DLL4 antagonist dose. A justified dosing interval may be chosen in an effort to avoid the sVEGFR-2 and/or sVEGFR-3 level dropping below a desired level, such as 40%, 50%, 60%, 70%, 80%, or 90% of the peak on the standard dose-response curve. In an embodiment where the DLL4 antagonist has problematic side effects, a longer dosing interval may be chosen to avoid side effects. In one embodiment, a justified dosing interval may be twice a day, daily, every 2 days, every 4 days, every 7 days, every 10 days, every 2 weeks, every 3 weeks, or every month.

III. Quantifying Justified Dosages for DLL4 Antagonists Based on sVEGFR-2 and sVEGFR-3 Response

[0111] In one embodiment, the method of treatment administers a justified dose based on the level of both sVEGFR-2 and sVEGFR-3. There are a variety of modes for selecting a justified dose based on the levels of both sVEGFR-2 and sVEGFR-3, optionally incorporating the attributes described above.

[0112] In one embodiment, the justified DLL4 antagonist dose is determined by averaging the justified DLL4 antagonist dose that would have been determined by measuring sVEGFR-2 alone and the justified DLL4 antagonist dose that would have been determined by measuring sVEGFR-3 alone.

[0113] In one embodiment, wherein the justified DLL4 antagonist dose is determined by selecting the higher of the justified DLL4 antagonist dose that would have been determined by measuring sVEGFR-2 alone and the justified DLL4 antagonist dose that would have been determined by measuring sVEGFR-3 alone. In one embodiment, selecting the higher dose may

be useful in situations where a maximal response is desired and/or where side effects of the DLL4 antagonist are less problematic.

[0114] In one embodiment, the justified DLL4 antagonist dose is determined by selecting the lower of the justified DLL4 antagonist dose that would have been determined by measuring sVEGFR-2 alone and the justified DLL4 antagonist dose that would have been determined by measuring sVEGFR-3 alone. In one embodiment, selecting the lower dose may be useful in situations where side effects of the DLL4 antagonist are more problematic and/or when the justified dose is approaching the peak of the dose-response curve for either sVEGFR-2 and/or sVEGFR-3 and where only incremental benefit is achieved from the higher dose, such as when the dose is nearing the peak of a dose-response curve.

IV. Evaluating the Effectiveness of DLL4 Antagonists

[0115] The effectiveness of DLL4 antagonists can be evaluated by assessing the response of sVEGFR-2 and sVEGFR-3 to the candidate DLL4 antagonist. The effectiveness of a single DLL4 candidate may be evaluated or multiple candidates may be compared to each other.

[0116] In one embodiment, a method for detecting efficacy of a DLL4 antagonist comprises administering a DLL4 antagonist to a patient; detecting the level sVEGFR-2 and/or sVEGFR-3 in the patient for at least one time point after administration of the DLL4 antagonist; and evaluating the level of sVEGFR-2 and/or sVEGFR-3 in the patient, as described herein.

[0117] In one embodiment, a method of evaluating the effectiveness of a DLL4 antagonist comprises administering a DLL4 antagonist to a subject, detecting the level of sVEGFR-2 and/or sVEGFR-3 in the subject for at least one time point after administration of the DLL4 antagonist, and comparing the level of sVEGFR-2 and/or sVEGFR-3 to a reference

standard. In one embodiment, the reference standard is a level produced by administering a positive control compound. In another embodiment, the reference standard is a positive control based on administering a different subject or a different individual within a pool of subjects a control compound. In one embodiment, the reference standard is a historical control value. In one embodiment, at least two candidate DLL4 antagonists are administered and each serves as the other's reference standard. In this way, the DLL4 antagonist with the desired profile is selected from among the plurality of candidate compounds and no previously-standardized positive control is used.

In another embodiment, a method of comparing the effectiveness of DLL4 antagonists comprises (a) administering a first DLL4 antagonist to a subject, (b) detecting the level of sVEGFR-2 and/or sVEGFR-3 in the subject for at least one time point after administration of the first DLL4 antagonist; (c) evaluating the level of the sVEGFR-2 and/or sVEGFR-3 in the subject, (d) administering a second DLL4 antagonist to a subject, (e) detecting the level of sVEGFR-2 and/or sVEGFR-3 in the subject for at least one time point after administration of the second DLL4 antagonist; (f) evaluating the level of sVEGFR-2 and/or sVEGFR-3 in the subject, (g) comparing the response of sVEGFR-2 and/or sVEGFR-3 after the administration of the first DLL4 antagonist to the level after the administration of the second DLL4 antagonist; and (h) selecting the more effective DLL4 antagonist. In one embodiment, the first DLL4 antagonist and the second DLL4 antagonist are administered to the same subject. In another embodiment, the first DLL4 antagonist and the second DLL4 antagonist are administered to different individuals in a pool of subjects.

[0118] In one embodiment, the subject is a healthy human volunteer. In another embodiment, the subject is a human patient with a solid tumor. In another embodiment, the

subject is a laboratory animal. In another embodiment, the pool of subjects is a group of patients enrolled in a clinical study or a group of laboratory animals. In one embodiment, the same sVEGFR-2 and sVEGFR-3 assays described above in section II, entitled “Determining sVEGFR-2 and sVEGFR-3 Levels” may be used to evaluate or compare the effectiveness of DLL4 antagonists.

EXAMPLES

Example 1. Mouse sVEGFR-2 Assay

[0119] Mouse soluble Vascular Endothelial Growth Factor Receptor-2 (sVEGFR-2) was measured in mouse K2-EDTA plasma using the Quantikine Immunoassay Assay Kit from R&D Systems (Cat# MVR200B) according to the manufacturer’s recommendations. All necessary reagents were provided in the assay kit and all incubation steps were performed on a rotating platform at room temperature.

[0120] Briefly, 50 µl/well of “Assay Diluent” was added to an assay plate pre-coated anti-mouse VEGFR-2 antibody. Reference standards, quality controls, and negative controls prepared in “Calibrator Diluent”, and plasma test samples, diluted to the minimal required dilution of 1:15 into “Calibrator Diluent”, were added at 50 µl/well. The plate was incubated for 2 hours and unbound analyte was removed by washing the plate with ELISA wash buffer. To detect bound analyte, “Mouse VEGF R2 Conjugate” (horseradish peroxidase-conjugated anti-mouse VEGF-R2 antibody) was added at 100µl/well and the plate was incubated for an additional 2 hours. Unbound detection antibody was removed by washing the plate with ELISA wash buffer and “Substrate Solution” was added at 100 µl/well. Following a 30 minutes incubation step, “Stop Solution” was added to the wells at 100 µl/well and the plate was read within 30 minutes on a spectrophotometer. In order to correct for optical imperfections in the

assay plate, wavelength correction is applied to subtract the optical density values at 540 nm from the readings at 450 nm. The concentration of sVEGFR-2 was extrapolated from the standard curve plotted with the SoftMax Pro GxP v5.2 Software (Molecular Devices). The detection range for sVEGFR-2 was 1.2 ng/ml to 150 ng/ml in 100% mouse plasma.

Example 2. Mouse sVEGFR-1 Assay

[0121] Mouse soluble Vascular Endothelial Growth Factor Receptor-1 (sVEGFR-1) was measured in mouse K2-EDTA plasma using the Quantikine Immunoassay Assay Kit from R&D Systems (Cat# MVR100) according to the manufacturer's recommendations. All necessary reagents were provided in the assay kit and all incubation steps were performed on a rotating platform at room temperature.

[0122] Briefly, 50 µl/well of "Assay Diluent" was added to an assay plate pre-coated anti-mouse VEGFR-1 antibody. Reference standards, quality controls, and negative controls prepared in "Calibrator Diluent", and plasma test samples, diluted to the minimal required dilution of 1:2 into "Calibrator Diluent", were added at 50 µl/well. The plate was incubated for 2 hours and unbound analyte was removed by washing the plate with ELISA wash buffer. To detect bound analyte, "Mouse VEGF R1 Conjugate" (horseradish peroxidase-conjugated anti-mouse VEGFR-1 antibody) was added at 100 µl/well and the plate was incubated for an additional 2 hours. Unbound detection antibody was removed by washing the plate with ELISA wash buffer and "Substrate Solution" was added at 100 µl/well. Following a 30 minutes incubation step, "Stop Solution" was added to the wells at 100 µl/well and the plate was read within 30 minutes on a spectrophotometer. In order to correct for optical imperfections in the assay plate, wavelength correction is applied to subtract the optical density values at 540 nm from the readings at 450 nm. The concentration of sVEGFR-1 was extrapolated from the

standard curve plotted with the SoftMax Pro GxP v5.2 Software (Molecular Devices). The detection range for sVEGFR-1 was 125 pg/ml to 16,000 pg/ml in 100% mouse plasma.

Example 3. Cynomolgus Monkey and Human sVEGFR-2 Assay

[0123] Cynomolgus monkey and human soluble Vascular Endothelial Growth Factor Receptor-2 (sVEGFR-2) were measured using the MSD® 96-Well MULTI-ARRAY® Human KDR Assay kit (MesoScale Discovery, Cat# K151BOC-3) according to the manufacturer's recommendations. This assay kit was qualified for measuring sVEGFR-2 in human K2-EDTA plasma; the assay detection range was 1.15 ng/ml to 250 ng/ml in 100% human plasma. The same assay kit was qualified for measuring sVEGFR-2 in cynomolgus monkey K2-EDTA plasma; the assay detection range was 1.05 ng/ml to 750 ng/ml in 100% cynomolgus monkey plasma. All necessary reagents were provided in the assay kit.

[0124] Briefly, an assay plate pre-coated with an anti-human VEGFR-2 antibody was blocked with "Blocker A" reagent overnight at 4°C. Reference standards, quality controls, negative controls, prepared in "Calibrator and Sample Diluent", and plasma test samples, diluted to the minimal required dilution of 1:50 into "Calibrator and Sample Diluent", were added at 50 µl/well. The plate was incubated for 2 hours and unbound analyte was removed by washing the plate with ELISA wash buffer. To detect bound analyte, "SULFO-TAG™ anti-hKDR Detection Antibody" (Sulfo-Tag-conjugated anti-human VEGFR-2 antibody) was added at 30 µl/well and the plate was incubated for an additional 2 hours. Unbound detection antibody was removed by washing the plate with ELISA wash buffer. "Read Buffer" was added at 150 µl/well and the plate was read on the MSD Sector Imager within 20 minutes. The concentration of sVEGFR-2 was extrapolated from the standard curve plotted with the SoftMax Pro GxP v5.2 Software (Molecular Devices).

Example 4. Cynomolgus Monkey and Human sVEGFR-1 Assay

[0125] Cynomolgus monkey and human soluble Vascular Endothelial Growth Factor Receptor-1 (sVEGFR-1) were measured using the MSD® 96-Well MULTI-ARRAY® Human KDR Assay kit (MesoScale Discovery, Cat# K15029C-3) according to the manufacturer's recommendations. This assay kit was qualified for measuring sVEGFR-1 in human K2-EDTA plasma; the assay detection range was 80 pg/ml to 1280 pg/ml in 100% human plasma. The same assay kit was qualified for measuring sVEGFR-1 in cynomolgus monkey K2-EDTA plasma; the assay detection range was 37 pg/ml to 3000 pg/ml in 100% cynomolgus monkey plasma. All necessary reagents were provided in the assay kit.

[0126] Briefly, an assay plate pre-coated with anti-VEGFR-1 antibody was blocked with "Blocker C" overnight at 4°C. Reference standards, quality controls, negative controls, prepared in "Calibrator Diluent" and undiluted neat plasma test samples, were added at 25 µl/well. The plate was incubated for 2 hours and unbound analyte was removed by washing the plate with ELISA wash buffer. To detect bound analyte, "SULFO-TAG™ anti-hKDR Detection Antibody" (Sulfo-Tag-conjugated anti-human VEGFR-1 antibody) was added at 25 µl/well and the plate was incubated for an additional 2 hours. Unbound detection antibody was removed by washing the plate with ELISA wash buffer. "Read Buffer" was added at 150 µl/well and the plate was read on the MSD Sector Imager within 20 minutes. The concentration of sVEGFR-1 was extrapolated from the standard curve plotted with the SoftMax Pro GxP v5.2 Software (Molecular Devices).

Example 5. Human sVEGFR-3 Assay

[0127] Human soluble Vascular Endothelial Growth Factor Receptor-3 (sVEGFR-3) was measured using an electrochemiluminescence (ECL) assay. This assay kit (R&D Systems, Cat# DY349) was qualified for measuring sVEGFR-3 in human K2-EDTA plasma. The assay detection range was 0.275 ng/ml to 200 ng/ml in 100% human plasma.

[0128] Briefly, an assay plate was coated with 4 µg/ml of “Capture Antibody” to human sVEGFR-3 overnight. The plate was blocked with I-Block Buffer (IBB, MedImmune). Reference standards, quality controls and negative control, prepared in IBB, and plasma test samples, diluted at the minimum required dilution of 1:5 in IBB, were added to the plate at 30 µl/well. The plate was incubated for approximately 2 hours and unbound analyte was removed by washing the plate with ELISA wash buffer. To detect bound analyte, biotinylated “Detection Antibody” was added at 30 µl/well and the plate was incubated for an additional 2 hours. Following a wash step, “Streptavidin SULFO-TAG™” detection dye was added to the wells and the plate was incubated for 30 minutes. Excess detection dye was removed with a wash step. MSD “Read Buffer” was added and the plate was read on the MSD Sector Imager within 20 minutes. All incubation steps were performed at room temperature with gentle shaking on a rotating platform. The concentration of sVEGFR-3 was extrapolated from the standard curve plotted with the SoftMax Pro GxP v5.2 Software (Molecular Devices).

Example 6. Mouse Xenograft Models

A) Calu-6 Xenograft Mouse Model

[0129] A Calu-6 xenograft mouse model was developed to assess the impact of DLL4 antagonists. The xenografts in this model are derived from a pulmonary carcinoma.

1. Experiments with Nude Mice

[0130] In a first experiment, Calu-6 xenograft mice were dosed at 0.5 mg/kg, 1mg/kg and 5mg/kg by intraperitoneal injections twice weekly for six doses with 2A5 and Comparator YW152F and vehicle control. Soluble VEGFR-2 levels responded in a dose-dependent manner after 2A5 treatment. sVEGFR-2 levels were also correlated with tumor volume at terminal 4 weeks, as shown in Figure 2. Similar results were shown for the 2A5 and Comparator YW152F.

[0131] In a second experiment, Calu-6 xenograft mice were dosed at 0.1 mg/kg, 0.5mg/kg and 2.5mg/kg by intraperitoneal injections twice weekly with 2A5 and vehicle control. Soluble VEGFR-2 levels were determined 96-hours post-dose. Similar results were shown, see Figure 3. A further depiction of the data from this study shows that sVEGFR-2 increases with higher dose treatment and results in a concurrent decrease in tumor volume. Results are shown in Figure 4.

[0132] In these two studies, mouse sVEGFR-1 response to anti-DLL4 treatment in Calu-6 xenografts was not consistent across studies. In one study, reported in Figure 5A, mouse sVEGFR-1 levels were moderately suppressed after treatment with 2A5 and high levels of sVEGFR-1 was weakly associated with increased tumor volume, if any. In another study, reported in Figure 5B, no significant modulation of mouse sVEGFR-1 levels were observed after treatment with 2A5; however, no correlation to tumor volume was observed. Results are shown in Figures 5A-B, with the data from Figure 5A from the same experiment as the data in Figure 2 and the data from Figure 5B from the same experiment as the data in Figures 3.

2. Experiments with KIKO Mice

[0133] In one study, Calu-6 xenografts were used in a female KIKO hDLL4 mouse model. Animals received 21H3RK, 2A5, or Comparator 21M18 at a dose of 2.5 mg/kg with

twice weekly intraperitoneal injections. There were 8-10 animals/group, with a total of 38/40 animals tested for sVEGFR-2 levels. sVEGFR-2 levels were evaluated after treatment with 21H3RK, 2A5, and Comparator 21M18. Results are shown in Figure 6A. There was no significant difference in mouse sVEGFR-2 levels between treatment groups.

[0134] In another study, Calu-6 xenografts were used in a female KIKO hDLL4 mouse model. Animals received 21H3RK, 2A5, or Comparator REG421 at a dose of 2.5 mg/kg with twice weekly intraperitoneal injections. There were 9 animals/group, with a total of 36/40 animals tested for sVEGFR-2 levels. Soluble VEGFR-2 levels were evaluated after treatment with 21H3RK, 2A5, and Comparator REG421. Results are shown in Figure 6B.

[0135] In a further study, Calu-6 xenografts were used in a female KIKO hDLL4 mouse model. Animals received 21H3RK, Comparator 21M18, or Comparator REG421 at a dose of 1.0 mg/kg with twice weekly intraperitoneal injections. There were 9 animals/group. Soluble VEGFR-2 levels were evaluated after treatment with 21H3RK, Comparator 21M18, and Comparator REG421. A significantly better response was observed after treatment with 1 mg/kg with 21H3RK compared to the same dose of Comparator REG421. Results are shown in Figure 6C.

3. Evaluating sVEGFR-2 Levels After Treatment

[0136] In one study Calu-6 xenograft mice were dosed at 2.5 mg/kg, 5.0 mg/kg and 7.5 mg/kg by intraperitoneal injections twice weekly, once weekly, once every other week, or with a single dose with 2A5 and vehicle control. For the MOA study, sVEGFR-2 levels were determined at Day 4 (for group 1 animals) and Day 8, Day 15, Day 22 (for all groups) . For the efficacy study, sVEGFR-2 levels were determined at the terminal time point for all groups. Soluble VEGFR-2 levels were evaluated after treatment with 2A5. Dosing two times per week

with 2A5 in Calu-6 xenografts resulted in the highest accumulation of mouse sVEGFR-2 and accumulation was dose-responsive. For the same dosing level, sVEGFR-2 concentrations correlated to frequency of treatment. At 5.0 mg/kg at a frequency of one time every two weeks, an increase of mouse sVEGFR-2 was observed; however the increase was not sustained. At a dose of 7.5 mg/kg (single dose) a minimal effect, if any, on sVEGFR-2 levels was observed and the pharmacokinetic profile showed a concurrent drop in the level of sVEGFR-2 compared to 5 mg/kg treatment after day 8. Mouse sVEGFR-2 levels correlated with drug exposure, frequency of treatment, and pharmacokinetic profile. Results are shown in Figure 7A-E.

[0137] In one study sVEGFR-2 levels were evaluated after treatment with 2A5. When dosing with 2.5 mg/kg 2A5, the highest accumulation of mouse sVEGFR-2 was observed when dosing at 2 times/week, while dosing at 1 time/week resulted in minimal effect, if any. sVEGFR-2 levels were also inversely proportional to tumor volume and dose. Results are shown in Figures 8A-D.

4. Evaluation of sVEGFR-1 Levels in Nude Mice

[0138] In another study, sVEGFR-1 levels were evaluated in Calu-6 bearing nude mice that received either 1 mg/kg or 5 mg/kg of Comparator YW152F. Samples were tested from 48 hours post 1st dose and 1 day and 5 days post second dose. Plasma was evaluated in 5 animals per group for sVEGFR-1 levels. Figure 9 shows that treatment did not significantly affect expression of sVEGFR-1.

B) COLO205 Xenograft Mouse Model

[0139] A COLO205 xenograft model was developed to assess the impact of DLL4 antagonists. The xenografts in this model are derived from colorectal cancer.

1. Mouse sVEGFR-2 Upregulated by Anti-DLL4 Treatment in COLO205 Xenografts in Nude Mice

[0140] In another set of studies, COLO205 xenograft mice were dosed at 0.5 mg/kg, 1mg/kg and 5mg/kg by intraperitoneal injections twice weekly for six doses with 2A5 and Comparator YW152F and vehicle control. Mouse sVEGFR-2 levels increased in a dose-dependent manner after treatment with 2A5. A similar response was shown for 2A5 and Comparator YW152F. An increase of mouse sVEGFR-2 was also concurrent with a decrease in tumor volume. Results are shown in Figures 10A-B.

[0141] In one of the studies, COLO205 xenograft mice were dosed at 0.2 mg/kg, 1.0 mg/kg, 2.5mg/kg, 5.0 mg/kg, and 12.5 mg/kg by intraperitoneal injections twice weekly with 2A5, and 12.5 mg/kg with Comparator YW152F and control. Soluble VEGFR-2 levels were determined 24-hours post-dose. sVEGFR-2 levels were evaluated and plotted against tumor volume. Mouse sVEGFR-2 increases with higher dose levels and is concurrent with a decrease in tumor volume. Results are shown in Figures 11A-B. sVEGFR-1 levels were also evaluated in this study. No significant modulation of sVEGFR-1 levels were observed; additionally, sVEGFR-1 levels did not correlate with tumor volumes. Results are shown in Figures 12A-B.

[0142] In these same two studies, mouse sVEGFR-1 response to anti-DLL4 treatment in COLO205 xenografts was inconsistent across studies. As shown in Figure 13A, sVEGFR-1 levels were lightly suppressed following treatment with 2A5. As shown in Figure 13B, sVEGFR-1 levels were slightly elevated after treatment; however, this data set had smaller sample set and more scatter. In both Figures 13A and 13B, mouse sVEGFR-1 response was not correlated to tumor volume. Mean responses were typically less than 2-fold of the controls. Results are shown

in Figures 13A-B, with the data from Figure 13A from the same experiment as the data in Figure 10 and the data from Figure 13B from the same experiment as the data in Figures 12A-B.

2. Further Evaluating sVEGFR-1 Levels in COLO205 Xenograft Model

[0143] In another study sVEGFR-1 levels were evaluated in COLO205 xenograft nude mice after treatment with Comparator YW152F at a dose of 5 mg/kg twice weekly through an intraperitoneal route. Plasma samples were taken from 6 animals per group. Human and mouse sVEGFR-1 and sVEGFR-2 were determined in order to determine whether sVEGF-receptors were produced in the tumor or the host cells. Additionally, since VEGF A indirectly upregulates DLL4 in human and mouse, sVEGF A was also evaluated as a potential biomarker. Mouse sVEGFR-1 levels decreased following treatment with Comparator YW152F compared to vehicle control. No treatment modulations were observed for other mouse protein markers tested (data not shown). Since baseline VEGF A levels were undetectable, VEGF was not pursued as a potential biomarker for anti-DLL4 treatment. Results are shown in Figure 14.

C) Comparison of Calu-6 and COLO205 Xenograft Model Results

[0144] sVEGFR-2 results of the Calu-6 study shown in Figure 2 and COLO205 study shown in Figure 10 are presented side-by-side with results of a study performed in Calu-6 mice (Figure 15). Similar effects were shown in both xenograft models.

[0145] sVEGFR-1 results of the same Calu-6 and COLO205 studies were also presented side-by-side (Figure 16). Mouse sVEGFR-1 levels decreased in a dose-dependent manner following treatment with 2A5 and Comparator YW152F. The sVEGFR-1 effect was more evident in the Calu-6 xenograft mice compared to the COLO205 xenograft mice. Results are shown in Figure 16.

[0146] sVEGFR-1 results were compared to tumor volume in the two studies. No correlation was observed for sVEGFR-1 and tumor volume in Calu-6 and inCOLO205 xenograft models. Results are shown in Figure 17.

D) AsPC-1 Xenograft Mouse Model

[0147] An AsPC-1 xenograft model was developed to assess the impact of DLL4 antagonists. The xenografts in this model are derived from pancreatic cancer.

1. Mouse sVEGFR-2 Upregulated by Anti-DLL4 Treatment in AsPC-1 Xenografts in Nude Mice

[0148] Another study, AsPC-1 xenograft mice were dosed at 1.0 mg/kg, 5.0 mg/kg, and 12.5 mg/kg by intraperitoneal injections twice weekly with 2A5 and vehicle control. Soluble VEGFR-2 levels were determined 24-hours post-dose. Mouse sVEGFR-2 levels increased after treatment with 2A5. sVEGFR-2 levels, while nearly background at 1 mg/kg, reached maximal levels at the next dose of 5 mg/kg. A biphasic response was observed where a very sharp increase of circulating sVEGFR-2 corresponded to low tumor volumes. Results are shown in Figure 18. A further representation of the data in this study is provided in Figure 19, showing mouse sVEGFR-2 increasing with higher dose levels and concurrent with a sharp decrease in tumor volume.

[0149] Mouse sVEGFR-1 and tumor volume were also assessed after anti-DLL4 treatment in AsPC-1 xenografts in nude mice. Mouse sVEGFR-1 was slightly elevated by 2A5 treatment in AsPC-1 xenograft tumor models, however there was a small response window and a low sample population. Additionally sVEGFR-1 levels did not correlate with tumor volume. Figure 20 shows that the treatment effect on mouse sVEGFR-1 was not significant and does not correlate well with tumor volume.

Example 7. Non-Tumor Bearing Mouse Models**A) Immunotoxicity Study of Non-Tumor Bearing Mice**

[0150] In one study mouse sVEGFR-1 levels were observed after treatment with Comparator YW152F. Compared to an untreated group Comparator YW152F and R347 (isotype control) did not show significant response in sVEGFR-1 levels, though the sample size was small. Results are shown in Figure 21.

[0151] In another study sVEGFR-1 levels were evaluated in untreated mice, mice receiving R347 (isotype control), 3 mg/kg of Comparator YW152F, or 30 mg/kg of Comparator YW152F. Animals were dosed twice weekly for 4 weeks and plasma was analyzed from 5 animals per group for sVEGFR-1. sVEGFR-1 expression in non-tumor bearing mice was not significantly modulated by treatment. Results are shown in Figure 22.

Example 8. sVEGFR-1 Conclusions

[0152] As shown in the some of the preceding examples, sVEGFR-1 levels are not consistently altered by the administration or dose of DLL4 antagonists, nor is there a correlation with tumor volume in patients. Finding that sVEGFR-2 and sVEGFR-3 are useful in justifying the DLL4 antagonist dose is surprising in view of the fact that sVEGFR-1 levels are not.

[0153] Side-by-side sVEGFR-1 results in Examples 6.A.4 (panel A), 7 (panel B), 6.B.2 (panel C), 6.A.1 (panel D), 6.B.1 (panel E), and 6.B.1 (panel F) are shown in Figures 23A-F. Results demonstrated that mouse sVEGFR-1 response to Comparator YW152F was inconsistent. Suppression of mouse sVEGFR-1 in response to Comparator YW152F in one study was significant (see Figure 23C); however, it was not supported in other studies. Excluding the results in Figure 23C, both suppression and enhancement of sVEGFR-1 were observed; however,

since the responses were conflicting and generally less than 2-fold of their controls, the treatment response was not conclusive.

Example 9. Evaluation of sVEGFR-1, sVEGFR-2, and VEGF A in SCID Mice

[0154] In two further studies, used a human MatriGel in SCID mice. Animals were dosed with 5 and 10 mg/kg in the first study. Ten animals were placed into each group and received one of three fully human anti-DLL4 antibodies: 4B4, 21H3R, or 21H3WT. Sampling for sVEGFR-1 (mouse and human), sVEGFR-2 (mouse and human), and VEGF A (mouse and human) was performed. All samples tested were below the limits of quantitation.

[0155] In the second study, animals were dosed with 1, 2.5, and 5 mg/kg in the second study. Ten animals were placed into each group and received one of three fully human anti-DLL4 antibodies: 4B4, 21H3R, or 21H3WT. The study also included two arms for Comparator YW152F (doses of 1 and 2.5 mg/kg). Sampling for sVEGFR-1 (mouse and human), sVEGFR-2 (mouse and human), and VEGF A (mouse and human) was performed. All samples tested were below the limits of quantitation.

Example 10. Cynomolgus Monkey Models

[0156] A variety of studies were conducted to evaluate the response of sVEGFR-2, sVEGFR-1, and other parameters in cynomolgus monkeys after dosing with DLL4 antagonists. Figures 24A-E show the response of sVEGFR-2 and sVEGFR-1 levels after treatment with 21H3RK. sVEGFR-2 levels increased in response to treatment. A maximal response was achieved by ~3 mg/kg 21H3RK; response following treatment with 1 mg/kg was moderate, if any. Dose-response recovery of sVEGFR-2 levels was observed in the 3-month repeat-dose studies. No response was observed in sVEGFR-1 levels. Results are shown in Figures 24A-E.

A) A 24-Day Intravenous Toxicity, Toxicokinetics, and Immunogenicity Study of DLL4 Antagonist in Cynomolgus Monkeys

[0157] A 24-day intravenous toxicity, toxicokinetic, and immunogenicity study of 21H3RK was performed in female cynomolgus monkeys. Female cynomolgus monkeys were administered 21H3RK by an intravenous route, each receiving four injections at a rate of one injection per week. Animals were dosed with 0, 1, 10, and 30 mg/kg of the antibody, with 4 animals per group. Various protein markers were measured in EDTA-buffered plasma at various time points (sVEGFR-1, VEGF, bFGF, PIGF). Treatment did not affect the levels of the soluble proteins tested compared to the control groups (only data for sVEGFR-1 shown). Results are shown in Figure 25.

B) A One-Month Repeat-Dose Toxicity, Toxicokinetic and Immunogenicity Study of DLL4 Antagonist in Cynomolgus Monkeys

[0158] A one-month repeat-dose toxicity, toxicokinetic, and immunogenicity study of 21H3RK in cynomolgus monkeys with a three month recovery period was performed. A total of 12 animals were dosed with 0, 3, 30, 100 mg/kg by IV injections once per week for five weeks. Six animals were assessed in the main study and the remaining six animals were assessed in the recovery phase. Protein biomarkers were assessed on Days 1, 8, 15, 22, and 29.

Table 1						
Group	Treatment	Dose Route & Regimen	Dose (mpk)	Volume (mL/kg)	Terminal Necropsy (Day 31)	Recovery Necropsy (Day 141)
1	Control	IV weekly	0	5	3/sex	3/sex
2	21H3RK	IV weekly	3	5	3/sex	3/sex
3	21H3RK	IV weekly	30	5	3/sex	3/sex
4	21H3RK	IV weekly	100	5	3/sex	3/sex

[0159] sVEGFR-2 levels increased following treatment with 21H3RK. No response was observed in control animals and a maximal effect was observed for all dose levels. Results

are provided in Figure 26. The study did not detect significant modulation in other soluble proteins detected (data not shown).

[0160] Two animals were humanely euthanized on day 63 during recovery for moribund condition attributed to blood loss and heart failure. The target organs of toxicity were heart, liver, and thymus. The highest non-severely toxic dose (HNSTD) was found to be 3 mg/kg (no lethality, no life-threatening toxicities, or irreversible findings).

Table 2									
T= Terminal Necropsy / R= Recovery Necropsy									
Parameter	Group	1		2		3		4	
	21H3RK (mg/kg/week)	0		3		30		100	
	Necropsy	T (N=6)	R (N=6)	T (N=6)	R (N=6)	T (N=6)	R (N=5)	T (N=6)	R (N=5)
In-life	Mortality	0	0	0	0	0	1	0	1
	Clinical Observation	0	0	0	0	0	1	0	1
Gross	Prominent lobules (liver)	0	0	0	0	2	0	4	0
	Organ weights (% thymus ↓)	0	0	-66%	0	-77%	0	-74%	0
Histopathology	Heart	2	0	6	0	6	0	6	0
	Liver	0	0	6	2	6	1	6	1
	Thymus	0	0	6	1	6	1	6	1

[0161] Following once-weekly IV administrations of 21H3RK in cynomolgus monkeys, the mean AUC(0-7d) values of 21H3RK were 211, 2180, and 8190 d·µg/mL in Week 1 and 718, 5870, and 14200 d·µg/mL in Week 5 for the 3, 30, and 100 mg/kg dose levels, respectively. The mean TK profiles of 21H3RK were linear in the dose range from 3 to 100 mg/kg. Based on sample time points taken throughout the duration of the study, steady state was reached at approximately Week 5. The apparent $t_{1/2}$ of 21H3RK, calculated from recovery animals only, was approximately 5 to 8 days. The CL of 21H3RK was 4.23, 5.16, and

7.18 mL/kg/d for the 3, 30, and 100 mg/kg dose levels, respectively. The ARs of 21H3RK, as assessed by AUC values, ranged from 1.73 to 3.40, consistent with $t_{1/2}$ and dosing frequency of 21H3RK. Similar exposure was observed in males and females. Six of 48 animals developed ADA response. The PK profile was only impacted by ADA in one animal.

[0162] Figures 27A-B shows that there was dose-proportional pharmacokinetics between 3 and 100 mg/kg with a maximal pharmacodynamic effect in blood achieved at 3 mg/kg once weekly dosing. Animals with anti-drug-antibody (ADA+) were excluded from the 21H3RK time-concentration profile (Figure 27A) since ADA may impact the PK profile and the assessment of PK evaluation such as drug half-life. No apparent gender differences were observed in toxicokinetics in 21H3RK (data not shown). Soluble VEGFR-2 levels return to baseline after recovery.

C) A Three-Month Repeat-Dose Toxicity, Toxicokinetic, and Immunogenicity Study of DLL4 Antagonist in Cynomolgus Monkeys

[0163] A three-month repeat-dose toxicity, toxicokinetic, and immunogenicity study of 21H3RK was conducted by intravenous bolus injection in cynomolgus monkeys with a four-month recovery period. There were six animals in each study group for the treatment phase and six in each group for the recovery phase with five dose groups (0 mg/kg, 1 mg/kg, 3 mg/kg, 10 mg/kg, and 30 mg/kg). Each group received an IV injection one time every two weeks (on days 1, 15, 29, 43, 57, 71, and 85). sVEGFR-2 levels were evaluated at recovery phase time points (on days 87, 127, 135, 155, 163, and 197). At the testing times, VEGF, sVEGFR-1, sVEGFR-2, CEACAM-5, and AFP were measured. The sVEGFR-2 maximally increases in all groups receiving treatment and the sVEGFR-2 recovery is dose dependent. There was no modulation of other proteins (data not shown). Results are presented in Figure 28.

Table 3						
Study Design						
Group	Treatment	Dose Route & Regimen	Dose (mpk)	Volume (mL/kg)	Terminal Necropsy (Day 87)	Recovery Necropsy (Day 197)
1	Control	IV q2wks	0	5	3/sex	3/sex
2	21H3RK	IV q2wks	1	5	3/sex	3/sex
3	21H3RK	IV q2wks	3	5	3/sex	3/sex
4	21H3RK	IV q2wks	10	5	3/sex	3/sex
5	21H3RK	IV q2wks	30	5	3/sex	3/sex

[0164] In general, clinical signs appeared after ~week 7 within 1 to 4 weeks of euthanasia. Signs included emesis, prostrate with low body temperature, body weight loss, low food consumption, hunched, pale, decreased activity. Consistent with the one-month study, clinical pathology indicated blood loss and liver toxicity (low red blood cells, low protein, and increased liver enzymes). Necropsy findings frequently included red material in GI tract, pale cobblestoned liver, and edema in many tissues.

Table 4				
Mortalities in 3 month GLP Study				
Group	Treatment	Dose (mg/kg)	Dosing Phase (N=6/group)	Recovery Phase (N=6/group)
1	Control	0	0	0
2	21H3RK	1	0	0
3	21H3RK	3	0	0
4	21H3RK	10	1	2
5	21H3RK	30	4	1

[0165] Following seven IV administrations every 2 weeks of 1, 3, 10, and 30 mg/kg 21H3RK in cynomolgus monkeys, an interim analysis of the TK data up to Day 87 was available. Similar concentration-time profiles of 21H3RK were observed in males and females

suggesting similar systemic exposure. The mean AUC(0-336h) values, pooled between males and females, were 93.9, 333, 1370, and 3640 d·µg/mL in Week 1 for the 1, 3, 10, and 30 mg/kg dose levels of 21H3RK, respectively. They reflected a slightly more than dose-proportional increase in AUC from a dose of 1 mg/kg 21H3RK to 3, 10, and 30 mg/kg. This dose nonlinearity at 1 mg/kg was attributed to an increase in CL of 21H3RK. Mean CL ranged from 5.22 to 8.15 mL/kg/d for the higher doses of 21H3RK as compared to 10.7 mL/kg/d for the 1 mg/kg dose. Based on predose concentrations of the higher doses (3 to 30 mg/kg 21H3RK) measured throughout the treatment period from Day 1 to Day 85, the steady state was generally attained between Weeks 4 to 6 (Days 29 to 43). The time to steady state was consistent with the corresponding mean $t_{1/2}$ for these doses. The mean $t_{1/2}$, calculated from concentration of 21H3RK on Day 1, ranged from 6.24 to 8.72 days for the higher dose levels of 21H3RK, and was 2.74 days for the 1 mg/kg dose level. The ARs of 21H3RK were assessed using trough concentrations and ranged from 1.34 to 1.70.

[0166] This study evaluated 21H3RK concentration over time and sVEGFR-2 levels in plasma over time. The study found dose proportional pharmacokinetics from 3 to 30 mg/kg. It also showed nonlinear pharmacokinetics from 1 to 3 mg/kg. No apparent gender differences were shown in 21H3RK toxicokinetics (data not shown). The maximal pharmacodynamic effect in blood was achieved at 1 mg/kg one time every two weeks. sVEGFR-2 levels returned to baseline in a dose-dependent manner. Results are shown in Figure 29.

D) A Four-Week Single Dose Study of DLL4 Antagonist in Cynomolgus Monkeys

[0167] A four-week single dose study of 21H3RK by intravenous bolus injection in cynomolgus monkeys was performed. The study included 5 dose groups (0 mg/kg, 0.1 mg/kg,

0.3 mg/kg, 1 mg/kg, and 10 mg/kg), with three animals in each group. The monkeys received an IV injection once on day 1. Protein markers (VEGF, sVEGFR-1, sVEGFR-2, CEACAM-5, and AFP) were measured at various time points. sVEGFR-2 levels increased after 10 mg/kg treatment with 21H3RK. No effect on sVEGFR-2 levels was observed at doses of 1 mg/kg or lower. Results are shown in Figure 30. There was also no modulation of other protein levels (data not shown).

[0168] Figure 31 shows 21H3RK concentrations over time. Following a single intravenous (IV) dose of 21H3RK in cynomolgus monkeys, the area under the concentration-time curve at infinity (AUC_{inf}) values were 1.78, 11.6, 66.8, and 1220 d·μg/mL for the 0.1, 0.3, 1, and 10 mg/kg dose levels, respectively; the increase in AUC_{inf} values was more than dose proportional in the tested dose levels of 21H3RK. A dose-proportional increase in maximal concentration (C_{max}) of 21H3RK was observed for all animals in all dose levels tested. The clearance (CL) was 59.6, 26.4, 15.1, and 9.95 mL/kg/d, and the half-life (t_{1/2}) was 0.577, 1.24, 2.71, and 6.85 days for the 0.1, 0.3, 1 and 10 mg/kg dose levels, respectively. A decrease in CL with increasing dose of 21H3RK was paralleled by an increase in t_{1/2}. The results were consistent with nonlinear PK and the presence of an antigen sink that becomes saturated between single IV doses of 1 and 10 mg/kg 21H3RK. Five of 15 animals have developed ADA response. However, ADA did not affect PK exposures. Plasma levels of sVEGFR-2 increased in animals following administration of 10 mg/kg 21H3RK, but no significant changes were observed in the other dose groups. The results suggested that circulating sVEGFR-2 could serve as a potential PD marker for treatment with 21H3RK. Data is mean plus standard error. ADA+ data was not excluded. This demonstrates nonlinear pharmacokinetics and the presence of an antigen sink saturated at 10 mg/kg.

[0169] Figure 32 shows sVEGFR-2 levels in the monkeys following 21H3RK treatment (mean plus standard error). The data show that sVEGFR-2 levels in plasma were up-regulated by 21H3RK.

E) A Three-Month Repeat-Dose Cardiovascular Safety Pharmacology Study of DLL4 Antagonist by Intravenous Bolus Injection in Male Cynomolgus Monkeys

[0170] A three-month repeat-dose cardiovascular safety pharmacology study of 21H3RK by intravenous bolus injection in male cynomolgus monkeys was conducted. The study design placed 4-5 animals per group in the treatment phase and 3-5 animals in the recovery phase per group. The study included four dose groups (0 mg/kg, 1 mg/kg, 3 mg/kg, and 10 mg/kg). The animals received 21H3RK by intravenous injection (as a slow bolus) one time every two weeks (on days 1, 15, 29, 43, 57, 71, and 85). Recovery phase time points were on days 114 and 141. sVEGFR-2 was measured at each time point. This showed a dose-dependent increase in sVEGFR-2, maximal in 3 and 10 mg/kg only. The sVEGFR-2 response was recovery dose-dependent. Results are shown in Figure 33.

Table 5				
Group	Treatment	Dose (mg/kg IV q2wk)	Volume (mL/kg)	Animals Males
1	Control	0	5	5
2	21H3RK	1	5	5
3	21H3RK	3	5	5
4	21H3RK	10	5	5

[0171] It was one objective of this study to identify any potential adverse functional effects on the cardiovascular system associated with repeat dosing of 21H3RK in animals surgically implanted with a radiotelemetry transmitter. The study monitored cardiovascular

safety endpoints (ECG, blood pressure, heart rate, etc.) continuously via telemetry in conscious animals; TK, ADA, PD by sVEGFR2, cardiac biomarkers (e.g. Troponin I, CK isozymes, NT-pro-BNP), and clinical pathology at various time points. The study also continued monitoring over additional 8-week dose-free recovery phase.

[0172] Adverse effects were seen at doses ≥ 3 mg/kg including mortality (2 animals at 3 mg/kg; 1 animal at 10 mg/kg).

[0173] This study also assessed serum concentrations of 21H3RK over time and sVEGFR-2 levels over time. Following seven twice weekly IV administrations of 1, 3, and 10 mg/kg of 21H3RK in cynomolgus monkeys, all serum samples collected from animals treated with control article contained no quantifiable concentrations of 21H3RK and confirmed that these animals were not exposed to 21H3RK. Concentrations of 21H3RK in predose samples collected on Days 1, 15, 29, 43, 57, and 71 from animals in the 1 mg/kg dose level were also below the assay's lower limit of quantification (LLOQ, 0.078 $\mu\text{g/mL}$). However, samples collected 5 hours after administration of 21H3RK on Days 1 and 85 from these animals had mean concentrations of 21H3RK of 16.9 and 17.6 $\mu\text{g/mL}$, respectively. Higher doses of 21H3RK (3 and 10 mg/kg) gave quantifiable predose levels of 21H3RK at the time of administration except for the first dose. Serum levels of 21H3RK decreased to below level of quantification (BLQ) during recovery (Days 114 and 141) for the 1 and 3 mg/kg 21H3RK dose groups. Mean residual concentration of 21H3RK was 8.24 mg/mL on Day 114 and was BLQ on Day 141 for the 10 mg/kg 21H3RK dose group. The predose concentrations of 21H3RK determined at repeat twice weekly dosing suggested a steady state between Day 29 to Day 43. Comparison of the trough concentrations collected on Days 29, 43, 57, and 71 to that of Day 15 gave ARs ranging from 1.1 to 1.5 for animals in 3 mg/kg 21H3RK dose group and from 1.5 to

2.0 for 10 mg/kg 21H3RK dose group. The extent of accumulation observed was consistent with a $t_{1/2}$ of 6 to 10 days determined for 21H3RK administered twice weekly in the 3-month toxicity study conducted in male and female cynomolgus monkeys. Comparison of the mean trough concentrations at steady state between the 1, 3, and 10 mg/kg dose levels of 21H3RK further suggested that the increase in dose from 1 to 3 mg/kg, and from 3 to 10 mg/kg 21H3RK resulted in greater than proportional increases in concentrations of 21H3RK. Nonlinear PK with a low dose of 1 mg/kg 21H3RK had been reported previously. In this study, nonlinearity was observed with the 1 and 3 mg/kg dose levels of 21H3RK. Four of 20 animals were positive for ADA.

[0174] The study showed nonlinear pharmacokinetics from 1 to 10 mg/kg and a maximum pharmacodynamic response at 3 and 10 mg/kg. There was only a partial pharmacodynamic response at 1 mg/kg. sVEGFR-2 levels returned to baseline during the recovery phase. Results are shown in Figure 34.

Example 11. Testing and Treatment of Human Subjects with DLL4 Antagonists

[0175] A first-time-in-human, Phase 1, Multicenter, Open-label, single-arm, dose-escalation study to evaluate the safety, tolerability, and antitumor activity, PK, and immunogenicity of 21H3RK in adult subjects with advanced solid tumors refractory to standard therapy or for which no standard therapy exists was conducted. A number of the planned 45 subjects for the trial were administered with 21H3RK at fixed doses, starting at 10mg and escalating to 30, 60, 100, 150, and 200 mg once every 3 weeks or until the MTD is reached. Plasma samples were collected at multiple timepoints and their sVEGFR-2 and sVEGFR-3 responses were assessed. Both sVEGFR-2 and sVEGFR-3 levels increased following 21H3RK treatment. A step dose-response was observed with no changes in the 10 mg dose group and the maximum response observed in the 30, 60, and 100 mg dose groups. This observation was

consistent with anti-tumor activity at 30 and 60 mg doses. sVEGFR-2 and sVEGFR-3 are pharmacodynamic markers downstream to DLL4, demonstrate the mechanism of action, demonstrate dose-response, and can be used to evaluate duration of action of anti-DLL4 antibodies and other DLL4 antagonists. Results are provided in Figures 35A-B. (The following time points were not plotted to scale: S = screening; EOT = end of treatment; 30D = 30 day post tx; 3M = 3 months post tx; Cycle 1 and 2 - full profiles; Cycle 3 and beyond – only predose; Cycles were plotted on log scale.)

[0176] Subjects with clinical response (patient 1009, clinical benefit; and 1013, partial response, PR) had increased levels of sVEGFR-2 and to a lesser extent sVEGFR-3, as compared to the median (Figures 39A and B). Patient 1013 had only 2-fold increases but a very high baseline. Not all subjects with increased levels of sVEGFR-2 and sVEGFR-3 had clinical benefit (Figures 37A-C). Similar observations were made for sVEGFR-3 (data not shown): subjects with clinical response had an increase of sVEGFR-3 compared to the median; patient 1013 had approximately a 3-fold increase but a very high baseline; and not all subjects with increased sVEGFR-3 had clinical benefit.

[0177] Figure 38 shows a visualization of exposure and selected safety events. The data shows an increasing incidence of BNP > 100 with increasing dose. One of three subjects (33%) in 10 mg and 30 mg dosage group; 2 of 3 (67%) in the 60 mg dose group, and 4 of 6 (67%) in 100 mg dose group had an incident of BNP > 100. The increase is transient at 10 mg (one patient, single time point) and it occurs earlier and is sustained at higher doses. This patient received a dose of 150 mg/kg Q3W.

[0178] Figure 39 shows individual sVEGFR-2 concentrations versus time relative to BNP concentrations. sVEGFR-2 concentrations are similar in subjects with and without BNP > 100. Similar observations were made for sVEGFR-3 (data not shown).

[0179] Figure 40 plots sVEGFR-2 levels against BNP concentration. At this sample size, no correlation was demonstrated. Similar observations were made for sVEGFR-3 (data not shown).

[0180] Figure 41 illustrates theoretical receptor occupancy based on human pharmacokinetics. This figure shows that full predicted receptor occupancy over the entire dosing interval is not required for maximum downstream pharmacodynamic effect.

Example 12. sVEGFR-2 Assay Qualification

[0181] An ECL assay was qualified to measure sVEGFR-2 in human K2-EDTA plasma derived from normal and cancer patients. Parameters included the determination of the assay dynamic range and the limits of quantitation; evaluation of the inter- and intra-assay accuracy and precision; evaluation of the stability of sVEGFR-2 in plasma under various sample storage and handling conditions; evaluate parallelism of endogenous analyte following multiple dilution; evaluate the potential matrix effect; and determine the baseline levels of sVEGFR-2 in samples from normal and disease individuals. Data is shown as follows:

Table 6			
Standard Curve Level	Nominal Concentration (ng/ml)	Mean %R	Mean %CV
RS01	10	100	4.0
RS02	5	100	2.5
RS03	2.5	101	1.5
RS04	1.25	98	2.6
RS05	0.625	101	2.0

RS06	0.208	102	3.6
RS07	0.069	104	3.5
RS08	0.023	102	3.6
RS09	0.012	90	8.7

Table 7					
QC, nominal concentration, ng/ml	QC01 (5.000)	QC02 (4.000)	QC03 (1.000)	QC04 (0.100)	QC05 (0.023)
Inter-assay accuracy (Mean % R)	98	100	97	95	96
Inter-assay precision (% CV)	7.7	8.5	8.2	10	16.8
Intra-assay accuracy (Mean % R, Range)	97-105	98-118	97-105	82-105	69-112
Intra-assay precision (% CV, Range)	1.0-4.9	0.4-5.9	0.1-6.8	0.0-7.3	0.0-10.9

%R, percent recovery based on nominal concentrations

[0182] Nine standard curve calibrators (RS01 - RS09) were prepared in assay buffer from the reference standard, sVEGFR-2, to determine the assay's dynamic range. The standard curve levels ranged from 10 ng/ml (RS01) to 0.012 ng/ml (RS09). RS01 and RS09 were the upper and lower anchor points, respectfully. RS02 and RS08 were the tentative upper limit of quantitation (ULOQ) and lower limit of quantitation (LLOQ), respectfully. The mean % recoveries of the standard levels within the assay range (RS02 - RS08) was from 98% - 104% which met the acceptance criteria of $100 \pm 30\%$ ($100\% \pm 35\%$ for the LLOQ). The % CV of the standard levels within the assay range was from 1.5% - 3.6% which met the acceptance criteria of $\leq 30\%$ ($\leq 35\%$ for the LLOQ). The assay dynamic range was determined to be 0.023 ng/ml – 5.0 ng/ml in assay buffer.

[0183] Five spiked QC samples (QC01 - QC05) were prepared with the sVEGFR-2 standards to assess inter- and intra-assay accuracy and precision. QC01 and QC05 are the high and low QC's corresponding to the concentrations for the ULOQ and LLOQ, respectively. Inter- and intra-assay accuracy and precision were demonstrated with the observed over-all % recoveries ranging from 69% - 118% and meeting the acceptable criteria of $100 \pm 30\%$ ($100\% \pm 35\%$ for the LLOQ). The observed over-all % CVs ranged from 0% to 16.8% and met the acceptance criteria of $\leq 30\%$ ($\leq 35\%$ for the LLOQ). Thus the sVEGFR-2 assay is sensitive and has acceptable accuracy and precision.

[0184] As part of assay qualification, twelve individual plasma samples, diluted at the minimal required dilution (MRD) of 1:50, were spiked with 4 ng/ml and 1 ng/ml of the reference standard, recombinant human sVEGFR-2. Percent recovery of the spiked reference standard was determined relative to the unspiked sample. If an unexceptable level of matrix interference is present at the MRD, recovery of the spiked sample will fall outside of the acceptable range of recovery of $100\% \pm 30\%$. Figure 42 shows that sVEGFR-2 recovery was acceptable and matrix interference was not significant.

[0185] Assay qualification also included an evaluation of parallelism. Four individual plasma samples, with endogenous levels of sVEGFR-2 ranging from 18 ng/ml - 26 ng/ml were diluted to the MRD of 1:50 and then serially diluted into assay buffer. Soluble VEGFR-2 concentrations were determined and adjusted for the dilution factor. The observed %CV of the sVEGFR-2 concentrations derived from the dilution of each sample from the MRD to 1:600 dilution ranged from 1.8% - 2.5%, which met the acceptance criteria of $\leq 30\%$. The dilution-adjusted concentration of sVEGFR-2 remained constant up to 600-fold dilution demonstrating parallelism at these concentrations. Results are shown in Figure 43.

[0186] Four individual plasma samples, with endogenous levels of sVEGFR-2 ranging from 14 ng/ml to 22 ng/ml, were evaluated for stability following overnight storage at room temperature (RT OVN), 3 cycles of freeze/thaw cycles from -80°C (3 F/T (-80°C)), and refrigerated for overnight (4°C OVN). The % recoveries were determined from the corresponding fresh thaw samples. Results indicate that sVEGFR-2 was stable in plasma following all storage conditions tested.

Table 8				
Stability Study				
Storage Conditions	BRH536208 Mean % R Pancreatic Cancer	BRH536209 Mean % R Pancreatic Cancer	BRH536210 Mean % R Pancreatic Cancer	BRH536211 Mean % R Pancreatic Cancer
RT OVN	102	103	100	98
3 F/T (-80°C)	103, 91, 95	111, 105, 101	96, 101, 104	92, 95, 90
4°C OVN	88	88	90	115

[0187] The assay qualification also sought to determine whether baseline levels of sVEGFR-2 could be detected. A total of 28 individual plasma samples were tested and the mean with a 95% confidence interval was plotted. Tested samples included those from normal subjects, pancreatic cancer patients, and colon cancer patients. The results indicate that baseline levels of sVEGFR-2 was measurable in all the samples tested. Results are shown in Figure 44.

[0188] Specificity of detection was also evaluated. Three individual plasma samples, with endogenous levels of sVEGFR-2 ranging from 010 ng/ml to 25 ng/ml were tested before and after immunodepletion with a commercially available antibody to sVEGFR-2. Percent recovery was based on endogenous sVEGFR-2 concentration prior to immunodepletion. This assay was performed during assay development and was not part of qualification. Results are shown in Figure 45 and show that sVEGFR-2 measurement is specific.

Example 13. Method of Treating a Patient with a Justified Dose

[0189] A patient with cancer may be administered an initial dosage of 10 mg of 21H3RK. On day 3, the patient's plasma level of sVEGFR-2 may be determined according to the method set forth in Example 3 above discussing sVEGFR-2 assay performed using the MSD® 96-Well MULTI-ARRAY® Human KDR Assay kit (MesoScale Discovery, Cat# K151BOC-3) according to the manufacturer's recommendations.

[0190] The sVEGFR-2 level may be plotted against a dose-response curve for 21H3RK effect on sVEGFR-2 levels. The patient's dose may be increased by 10% and sVEGFR-2 samples taken in repeating cycles until the patient achieves 90% of the sVEGFR-2 peak on the standard dose response curve, unless the patient's own sVEGFR-2 levels peak earlier.

INCORPORATION BY REFERENCE

[0191] All references cited herein, including patents, patent applications, papers, text books, and the like, and the references cited therein, to the extent that they are not already, are hereby incorporated herein by reference in their entirety for all purposes.

EQUIVALENTS

[0192] The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the technology. The foregoing description and Examples detail certain preferred embodiments and describes the best mode contemplated. It will be appreciated, however, that no matter how detailed the foregoing may appear in text, the embodiments may be practiced in many ways and the embodiments should be construed in accordance with the appended claims and any equivalents thereof.

WHAT IS CLAIMED IS:

1. A method of reducing side effects due to the administration of a DLL4 antagonist to a patient comprising:
 - a. administering an initial dose of the DLL4 antagonist to the patient;
 - b. determining a justified DLL4 antagonist dose where the justified dose is increased relative to the initial dose if the plasma level of sVEGFR-2 and/or sVEGFR-3 is at or below a first reference standard and/or the justified dose is decreased relative to the initial dose if the plasma level of sVEGFR-2 and/or sVEGFR-3 is at or above a second reference standard; and
 - c. administering to the patient the justified DLL4 antagonist dose calibrated to reduce side effects to the patient.
2. A method of treating a patient with the minimal effective dose of a DLL4 antagonist comprising:
 - a. administering an initial dose of the DLL4 antagonist to the patient;
 - b. determining a justified DLL4 antagonist dose where the justified dose is increased relative to the initial dose if the plasma level of sVEGFR-2 and/or sVEGFR-3 is at or below a first reference standard and/or the justified dose is decreased relative to the initial dose if the plasma level of sVEGFR-2 and/or sVEGFR-3 is at or above a second reference standard; and
 - c. administering to the patient a justified DLL4 antagonist dose calibrated to achieve efficacy with the minimal dose to the patient.
3. A method of administering a DLL4 antagonist to a patient comprising
 - a. administering an initial dose of the DLL4 antagonist to the patient;
 - b. determining a justified DLL4 antagonist dose where the justified dose is increased relative to the initial dose if the plasma level of sVEGFR-2 and/or sVEGFR-3 is at or below a first reference standard and/or the justified dose is decreased relative to the initial dose if the plasma level of sVEGFR-2 and/or sVEGFR-3 is at or above a second reference standard; and
 - c. administering to the patient a justified DLL4 antagonist dose.
4. The method of any one of claims 1-3, wherein both the first reference standard and the second reference standard are used and the first reference standard and the second reference standard are different.
5. The method of any one of claims 1-3, wherein only the first reference standard is used.

6. The method of any one of claims 1-3, wherein only the second reference standard is used.
7. The method of any one of claims 1-5, wherein the first reference standard is a baseline sVEGFR-2 and/or sVEGFR-3 level without treatment with a DLL4 antagonist.
8. The method of claim 7, wherein the patient had a prior DLL4 antagonist dose that is no longer affecting the levels of sVEGFR-2 and/or sVEGFR-3.
9. The method of claim 8, wherein the patient has not had a DLL4 antagonist dose for at least 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, or longer.
10. The method of any one of claims 1-5 and 7-9, wherein the first reference standard is a baseline sVEGFR-2 and/or sVEGFR-3 level and
 - a. the justified dose produces a sVEGFR-2 plasma level that is at least 125%, 150%, 200%, or 250% of the first reference standard of sVEGFR-2 and/or
 - b. the justified dose may be the dose that produces a sVEGFR-3 plasma level that is 150%, 200%, 250%, 300%, 400%, or 450% of the first reference standard of sVEGFR-3.
11. The method of any one of claims 1-4 and 6-10 wherein the second reference standard is the level of sVEGFR-2 and/or sVEGFR-3 yielding efficacy with minimal side effects.
12. The method of any one of claims 1-4 and 6-11, wherein the second reference standard is the maximal safe response on a dose response curve.
13. The method of any one of claims 1-12, wherein the plasma level of sVEGFR-2 used to calculate the justified dose based on a dose-response curve is 25 ng/L, 30 ng/L, 35 ng/L, 40 ng/L, 45 ng/L, or 50 ng/L and/or the sVEGFR-3 plasma level used to calculate the justified dose based on a dose-response curve is 25 ng/L, 30 ng/L, 40 ng/L, 50 ng/L, 75 ng/L, 100 ng/L, 125 ng/L, or 150 ng/L.
14. The method of any one of claims 1-13, wherein the first reference standard and/or the second reference standard are historical controls based on testing with a pool of subjects.
15. The method of any one of claims 1-4 and 7-14, wherein the first reference standard and the second reference standard are obtained by administering to the patient a range of DLL4 antagonist doses over time and assigning the lowest plasma level of sVEGFR-2 and/or sVEGFR-3 as the first reference standard and the highest plasma level of sVEGFR-2 and/or sVEGFR-3 as the second reference standard.
16. The method of any one of claims 1-15, wherein the initial dose of the DLL4 antagonist is from 10 mg to 100 mg.
17. The method of claim 16, wherein the initial dose of the DLL4 antagonist is 10 mg, 30 mg, 60 mg, or 100 mg.

18. The method of any one of claims 1-17, wherein the justified dose is from 10 mg to 30 mg.
19. The method of any one of claims 1-18 wherein the sVEGFR-2 and/or sVEGFR-3 response to the initial dose was low and the dose is increased in 10% intervals until the patient achieves 85% of the sVEGFR-2 and/or sVEGFR-3 peak on the standard dose-response curve.
20. The method of any one of claims 1-19, wherein the sVEGFR-2 and/or sVEGFR-3 response to the initial dose was at or near the peak of the sVEGFR-2 and/or sVEGFR-3 standard dose-response curve and the dose increased in 10% intervals until the patient achieves 75% of the sVEGFR-2 and/or sVEGFR-3 peak on the standard dose-response curve.
21. The method of any one of claims 1-20, wherein the plasma level of sVEGFR-2 and/or sVEGFR-3 is measured 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 10 weeks, 12 weeks, or 16 weeks after administration of the DLL4 antagonist.
22. The method of any one of claims 1-21, wherein the plasma level of sVEGFR-2 and/or sVEGFR-3 is measured at one time point, two, three, four, five, or more time points after administration of the DLL4 antagonist.
23. The method of any one of claims 1-22, wherein the method of treatment includes a administering a justified dose at a justified dosing interval.
24. The method of claim 23, wherein the justified dosing interval is determined by evaluating the sVEGFR-2 and/or sVEGFR-3 levels for at least one time point and choosing a justified dosing interval in an effort to avoid the sVEGFR-2 and/or sVEGFR-3 level dropping below 50% of the peak on the standard dose-response curve.
25. The method of any one of claims 23-24, wherein the justified dosing interval may be every 7 days.
26. The method of any one of claims 1-25, wherein the method evaluates the level of both sVEGFR-2 and sVEGFR-3.
27. The method of claim 26, wherein the justified DLL4 antagonist dose is determined by averaging:
 - a. the justified DLL4 antagonist dose that would have been determined by measuring sVEGFR-2 alone and
 - b. the justified DLL4 antagonist dose that would have been determined by measuring sVEGFR-3 alone.

28. The method of claim 26, wherein the goal of the method is to maximize efficacy and the justified DLL4 antagonist dose is determined by selecting the higher of:
 - a. the justified DLL4 antagonist dose that would have been determined by measuring sVEGFR-2 alone and
 - b. the justified DLL4 antagonist dose that would have been determined by measuring sVEGFR-3 alone.
29. The method of claim 26, wherein the goal of the method is to minimize side effects while achieving moderate efficacy and the justified DLL4 antagonist dose is determined by selecting the lower of:
 - a. the justified DLL4 antagonist dose that would have been determined by measuring sVEGFR-2 alone and
 - b. the justified DLL4 antagonist dose that would have been determined by measuring sVEGFR-3 alone.
30. A method for detecting efficacy of DLL4 antagonist comprising
 - a. administering a DLL4 antagonist to a patient;
 - b. detecting the level of at least one pharmacodynamic biomarker chosen from sVEGFR-2 and sVEGFR-3 in the patient for at least one time point after administration of the DLL4 antagonist; and
 - c. evaluating the level of the pharmacodynamic biomarker in the patient.
31. The method of claim 30, wherein the levels of both sVEGFR-2 and sVEGFR-3 are detected.
32. The method of any one of claims 1-31, wherein the DLL4 antagonist is an anti-DLL4 antibody.
33. The method of claim 32, wherein the antibody has the same heavy chain CDRs and the same light chain CDRs as 21H3RK.
34. The method of claim 32, wherein the antibody has a heavy chain sequence and a light chain sequence of 21H3RK.
35. The method of claim 32, wherein the antibody has the same heavy chain CDRs and the same light chain CDRs as YW152F. The method of claim 32, wherein the antibody has a heavy chain sequence and light chain sequence of YW152F.
36. The method of claim 32, wherein the antibody has the same heavy chain CDRs and the same light chain CDRs as 21M18.

37. The method of claim 32, wherein the antibody has a heavy chain sequence and a light chain sequence of 21M18.
38. The method of claim 32, wherein the antibody has the same heavy chain CDRs and light chain CDRs as REG421.
39. The method of claim 32, wherein the antibody has a heavy chain sequence and a light chain sequence of REG421.
40. The method of any one of claims 1-40, wherein the DLL4 antagonist blocks a Notch receptor signaling pathway.
41. The method of any one of claims 1-41, wherein the DLL4 antagonist is administered to promote excessive and unproductive angiogenesis.
42. The method of any one of claims 1-42, wherein the patient has cancer.
43. The method of claim 43, wherein the patient has lung cancer, colon cancer, clear-cell renal cell carcinoma, glioblastoma, breast cancer, or bladder cancer.
44. The method of any one of claims 43-44, wherein the method reduces tumor volume by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90%.
45. The method of any one of claims 43-45, wherein the method reduces tumor growth by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% compared to the rate of prior growth or predicted growth based on historical controls.
46. The method of any one of claims 43-45, wherein the patient's tumor does not grow in size or is reduced in size.
47. The method of any one of claims 30-47, wherein the level of the pharmacodynamic biomarker is compared to a control level.
48. The method of any one of claims 30-48, wherein the level of the pharmacodynamic biomarker is compared to the patient's own level for at least one time point prior to administration of the DLL4 antagonist.
49. The method of any one of claims 30-49, wherein the level of the pharmacodynamic biomarker is measured in a plasma sample.
50. The method of any one of claims 30-50, wherein the increase in the level of the pharmacodynamic biomarker predicts a decrease in tumor volume.
51. A method of evaluating the effectiveness of a DLL4 antagonist comprising
 - a. administering a DLL4 antagonist to a subject,

- b. detecting the level of sVEGFR-2 and/or sVEGFR-3 in the subject for at least one time point after administration of the DLL4 antagonist,
 - c. comparing the level of sVEGFR-2 and/or sVEGFR-3 to a reference standard, and
 - d. evaluating the effectiveness of the DLL4 antagonist based on the level of sVEGFR-2 and or sVEGFR-3 in the subject
52. A method of comparing the effectiveness of DLL4 antagonists comprising
- a. administering a first DLL4 antagonist to a subject,
 - b. detecting the level of at least one pharmacodynamics biomarker chosen from sVEGFR-2 and sVEGFR-3 in the subject for at least one time point after administration of the first DLL4 antagonist;
 - c. evaluating the level of the pharmacodynamics biomarker in the subject,
 - d. administering a second DLL4 antagonist to the subject,
 - e. detecting the level of at least one pharmacodynamics biomarker chosen from sVEGFR-2 and sVEGFR-3 in the subject for at least one time point after administration of the second DLL4 antagonist;
 - f. evaluating the level of the pharmacodynamics biomarker in the subject,
 - g. comparing the response of the at least one pharmacodynamic biomarker after the administration of the first DLL4 antagonist to the level after the administration of the second DLL4 antagonist;
 - h. selecting the more effective DLL4 antagonist, and
 - i. evaluating the effectiveness of the DLL4 antagonist based on the level of sVEGFR-2 and or sVEGFR-3 in the subject
- .
53. The method of claim 53, wherein the first DLL4 antagonist and the second DLL4 antagonist are administered to the same subject.
54. The method of claim 53, wherein the first DLL4 antagonist and the second DLL4 antagonist are administered to different individuals in a pool of subjects.
55. The method of claim 55, wherein the pool of subjects is a group of laboratory animals.

56. The method of claim 33 or 34, wherein the heavy chain sequence corresponds to SEQ ID NO: 7 and the light chain sequence corresponds to SEQ ID NO: 8.
57. The method of claim 36 or 37, wherein the heavy chain sequence corresponds to SEQ ID NO: 11 and the light chain sequence corresponds to SEQ ID NO:12.
58. The method of claim 38 or 39, wherein the heavy chain sequence corresponds to SEQ ID NO: 9 and the light chain sequence corresponds to SEQ ID NO:10.

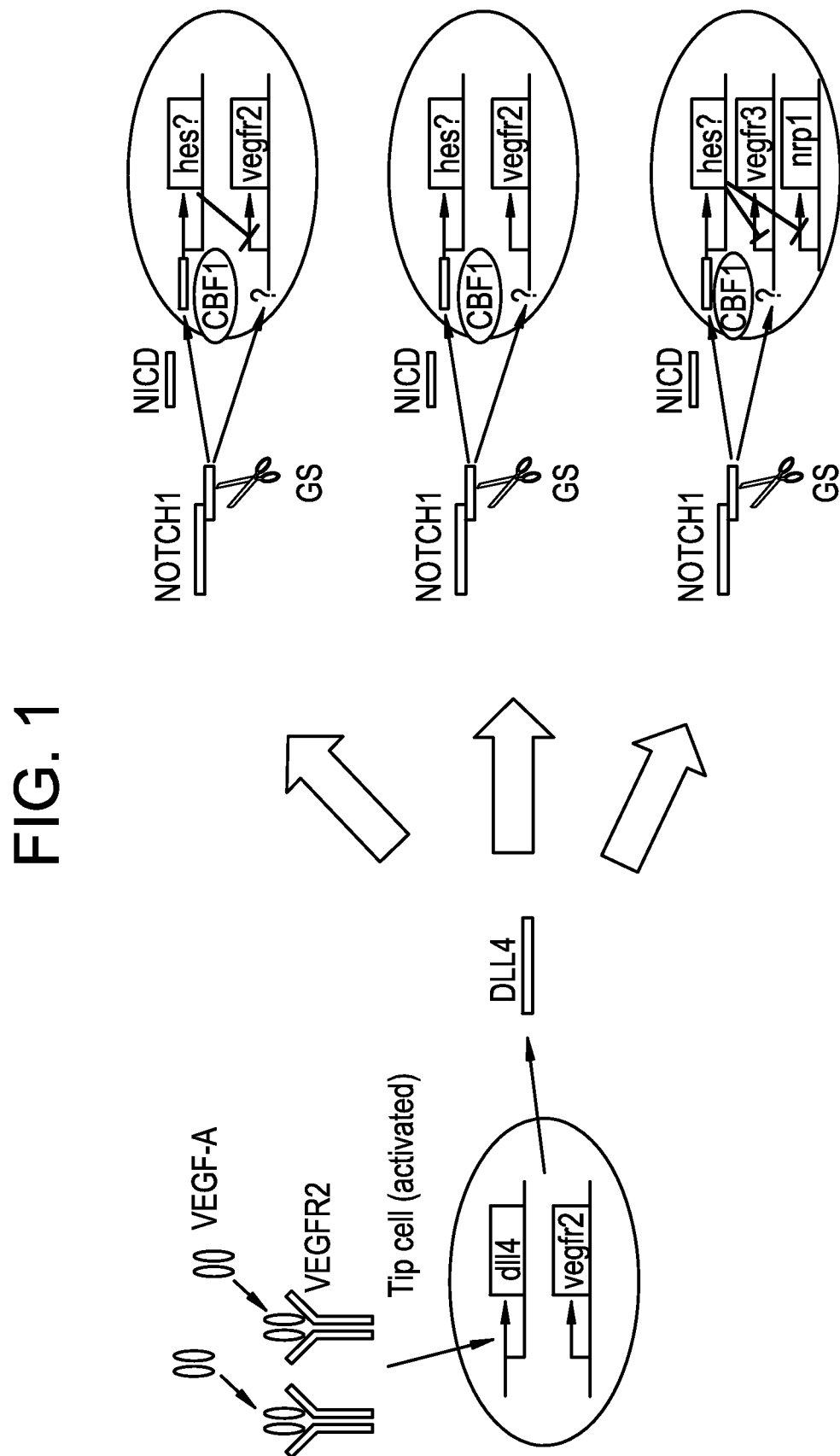
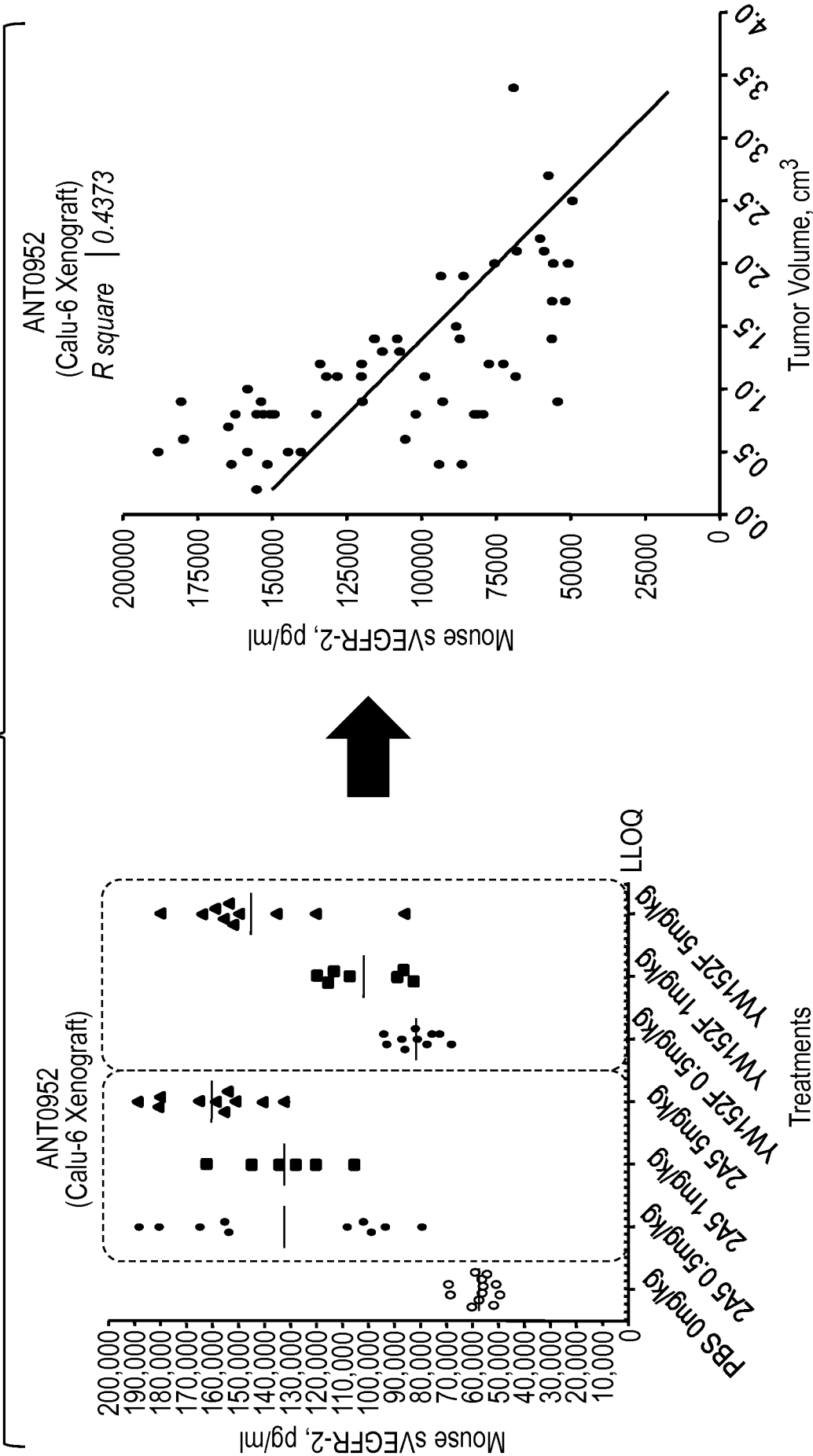


FIG. 2



F/G/3

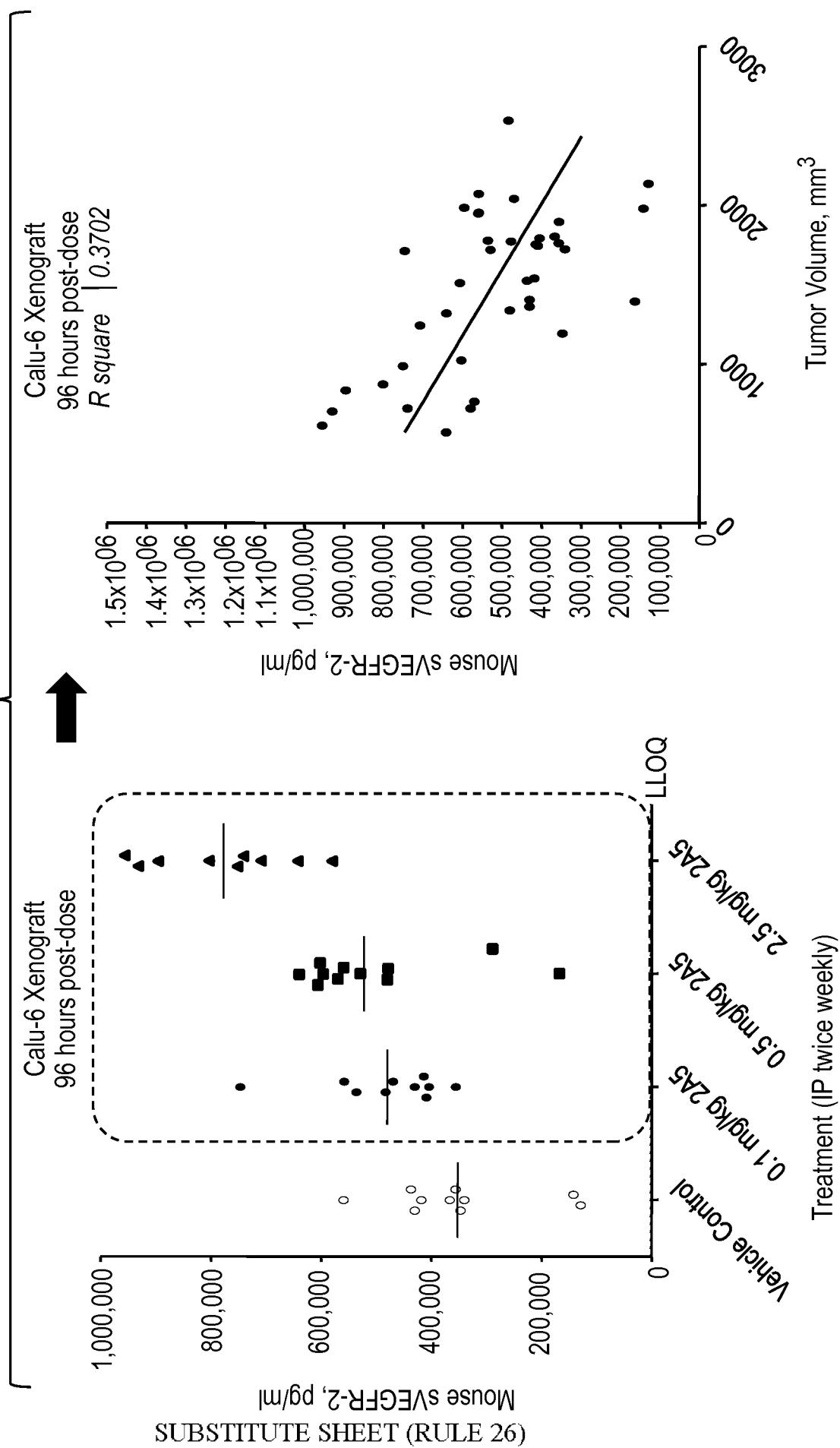


FIG. 4A

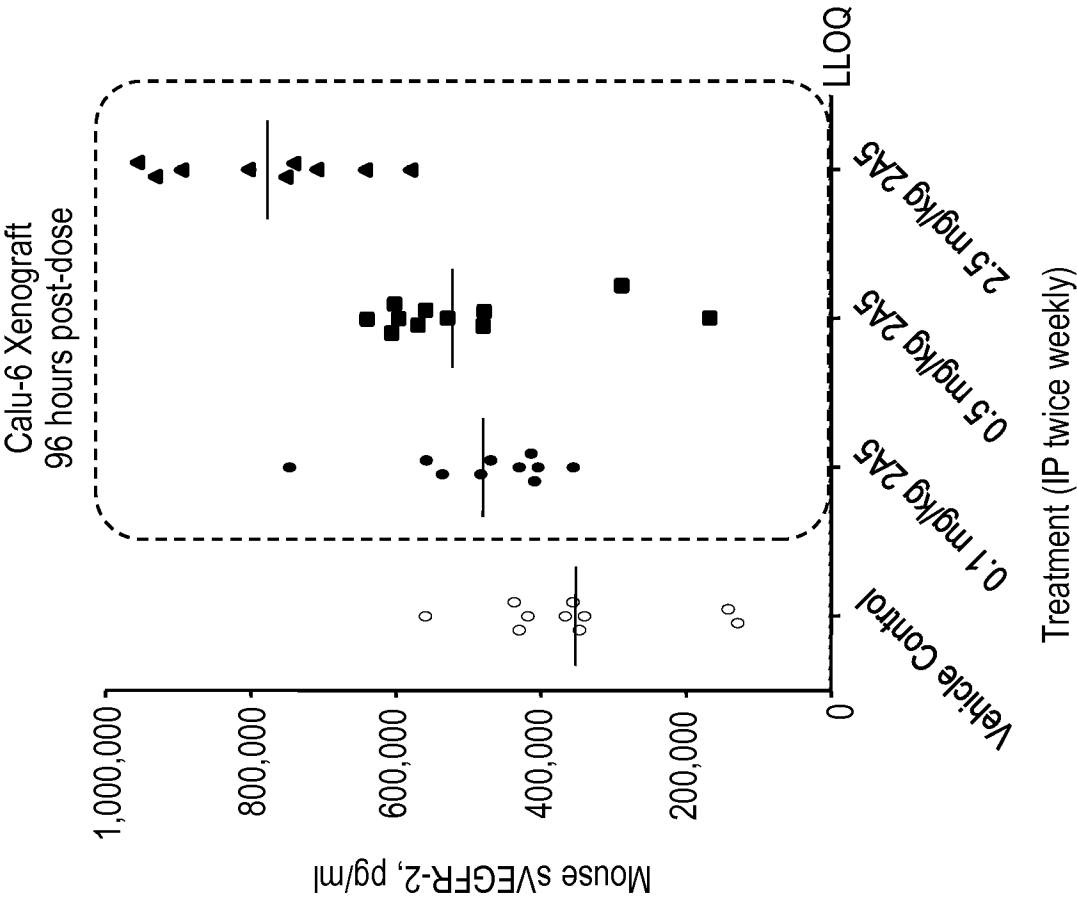


FIG. 4B

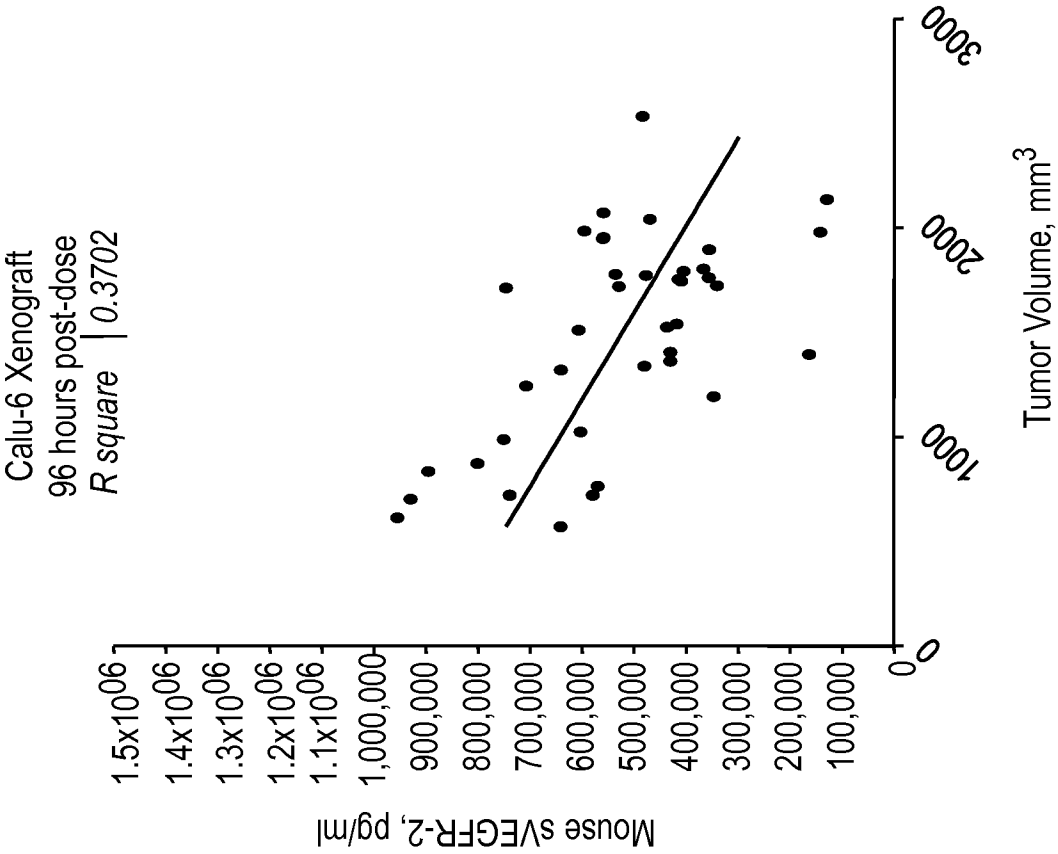


FIG. 5A

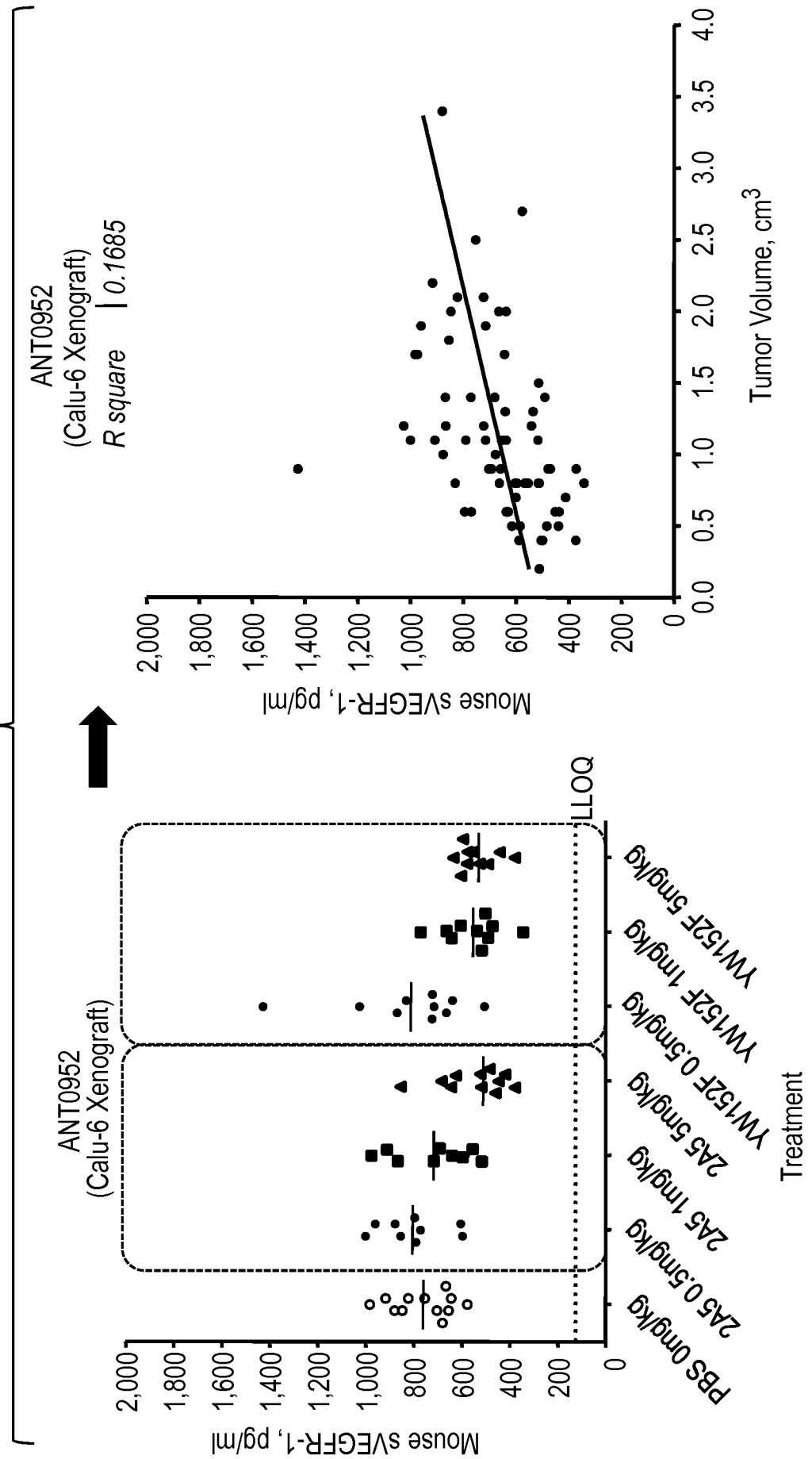


FIG. 5B

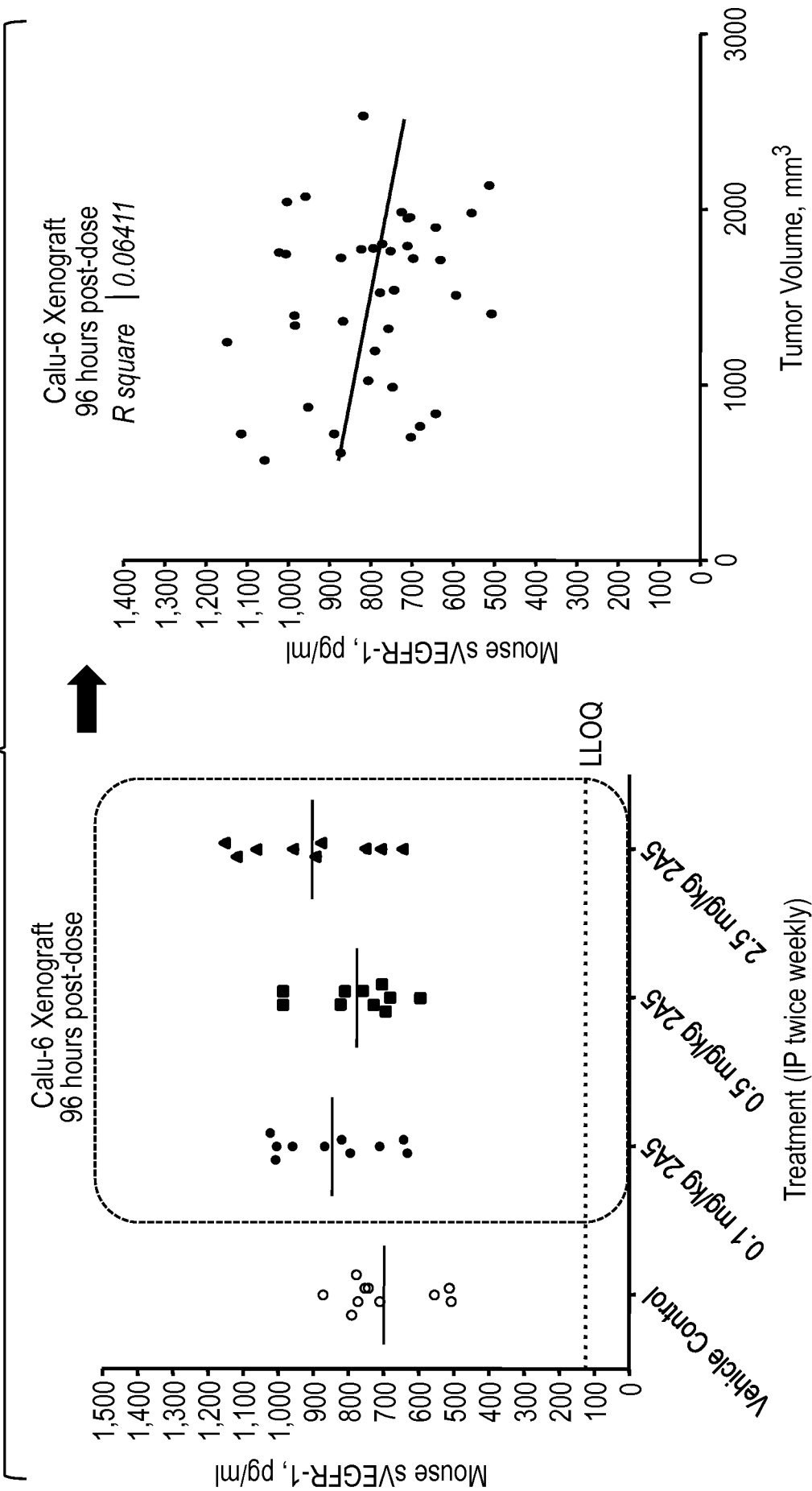


FIG. 6A

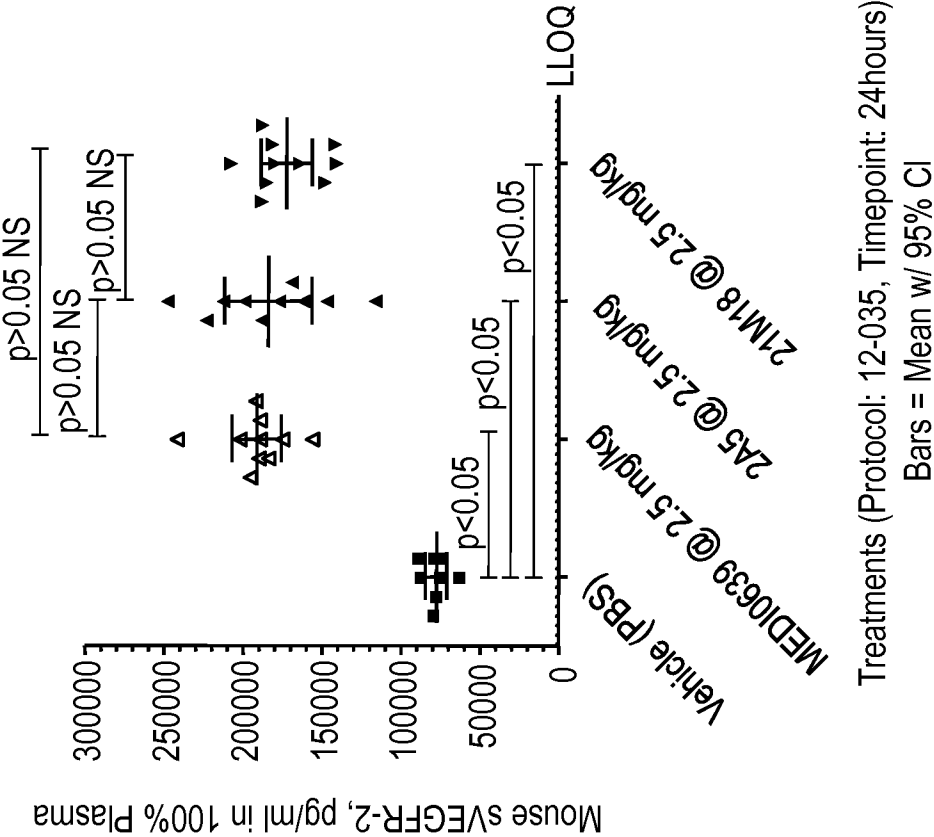
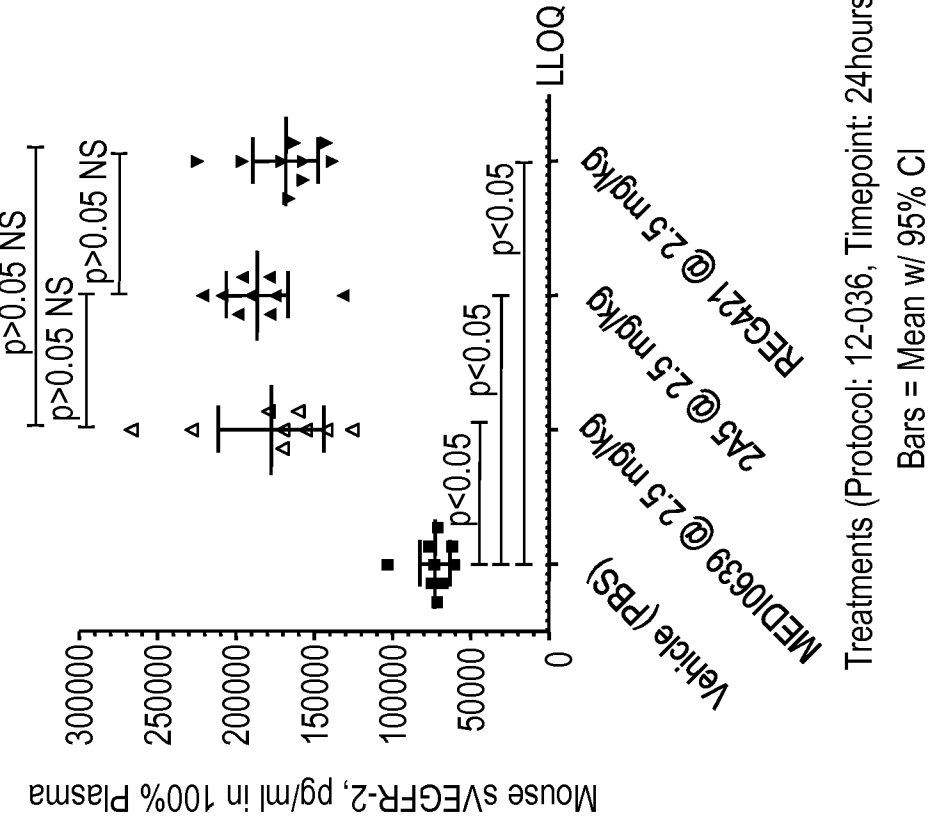


FIG. 6B



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FIG. 6C

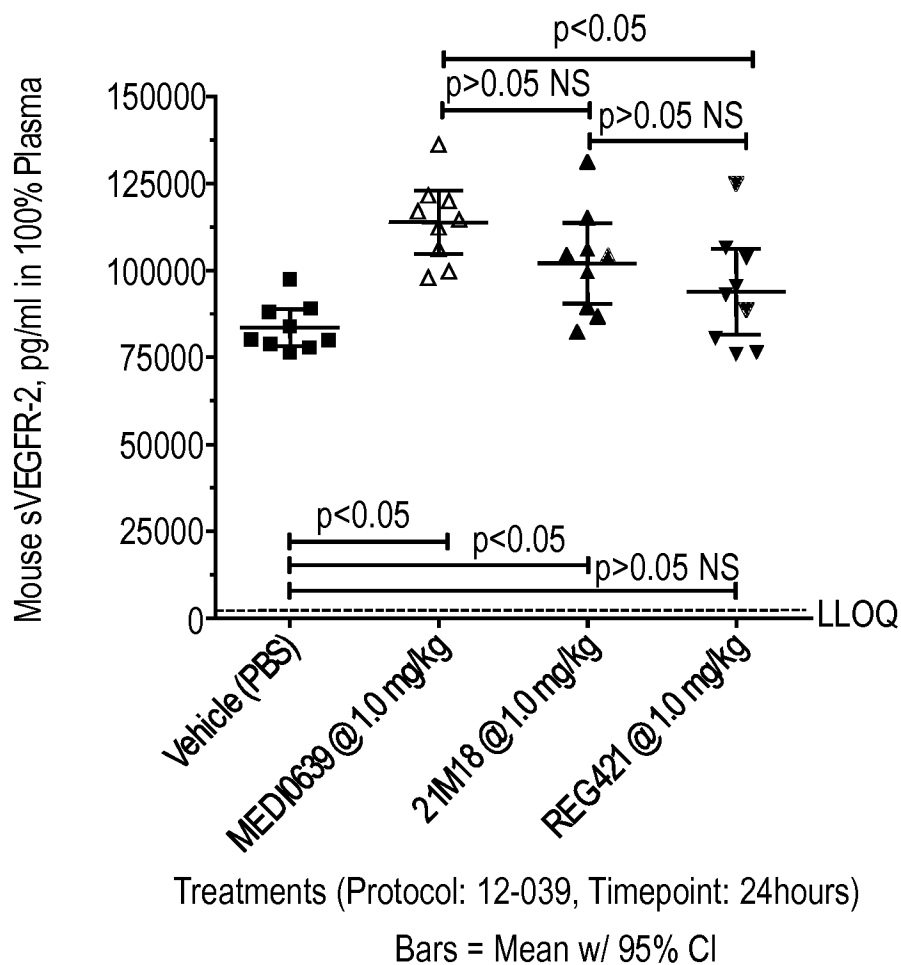


FIG. 7A

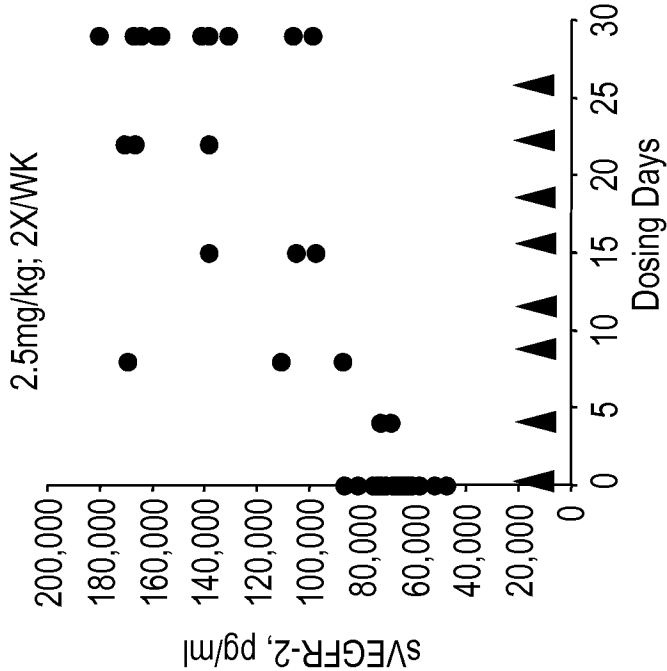
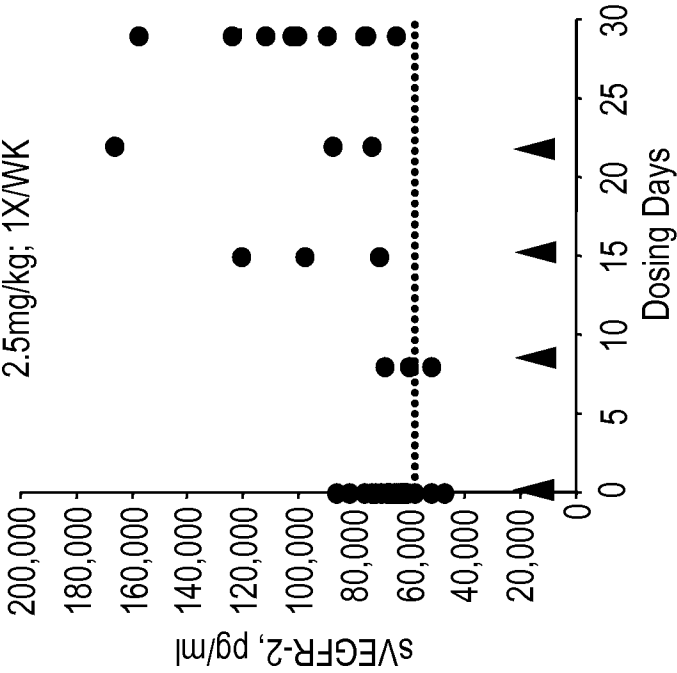
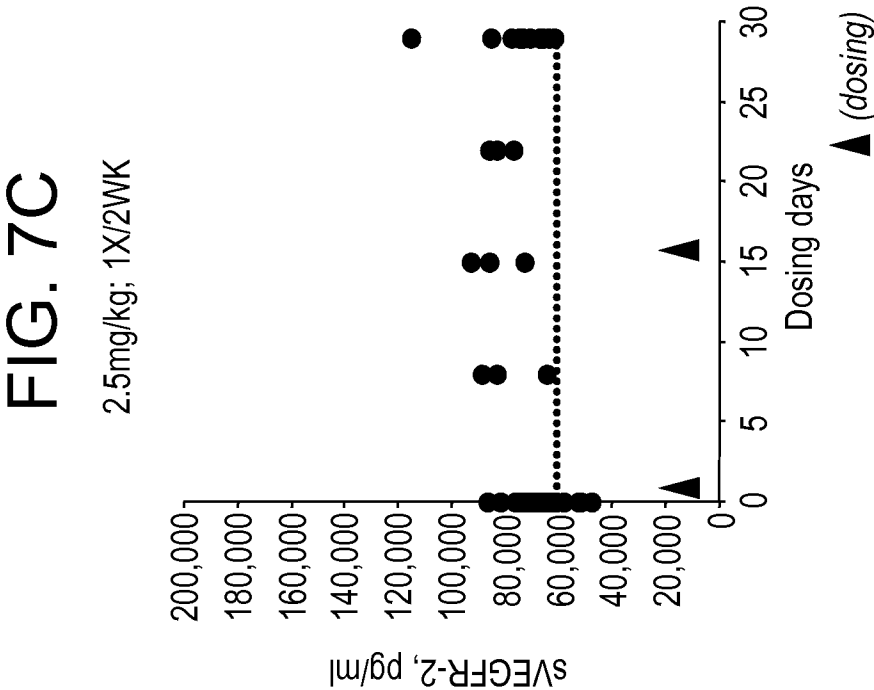
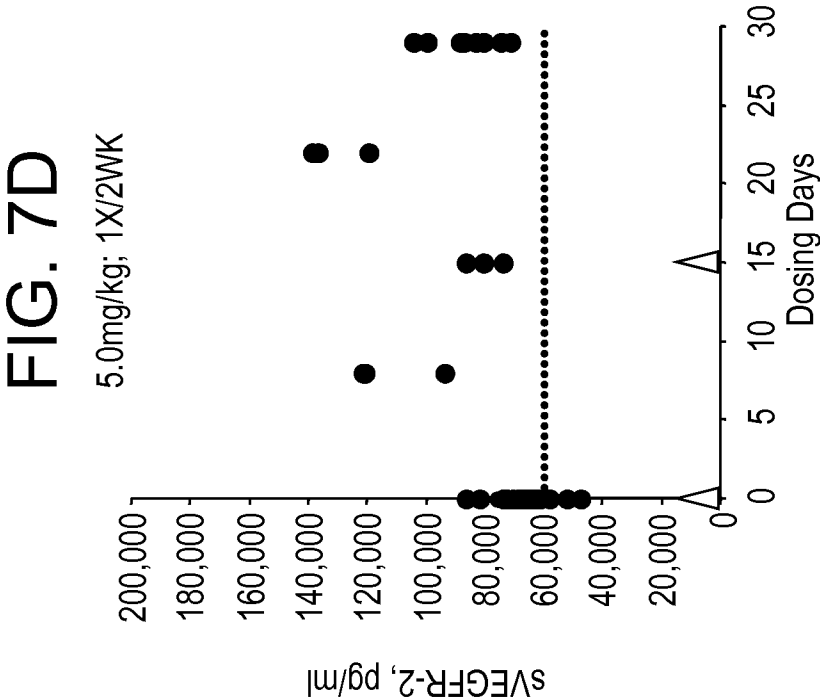


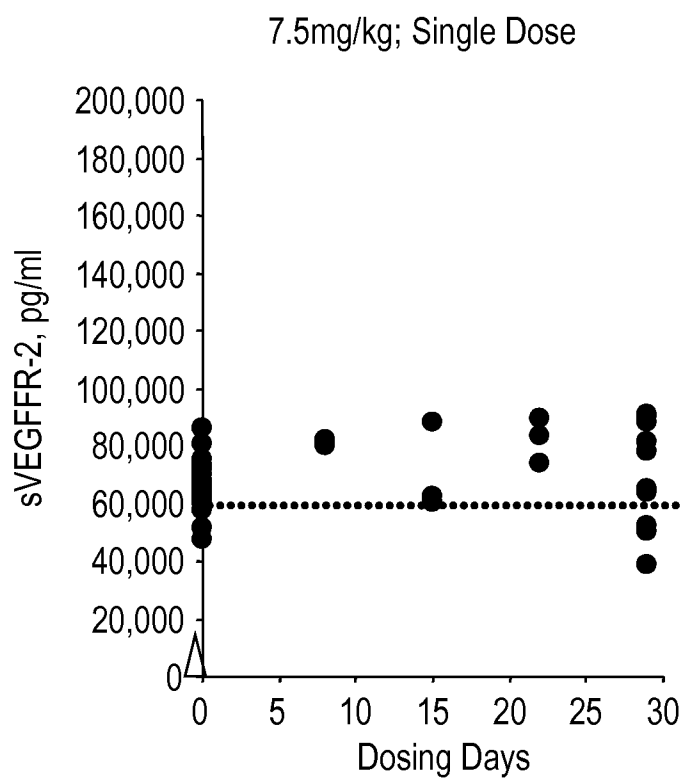
FIG. 7B





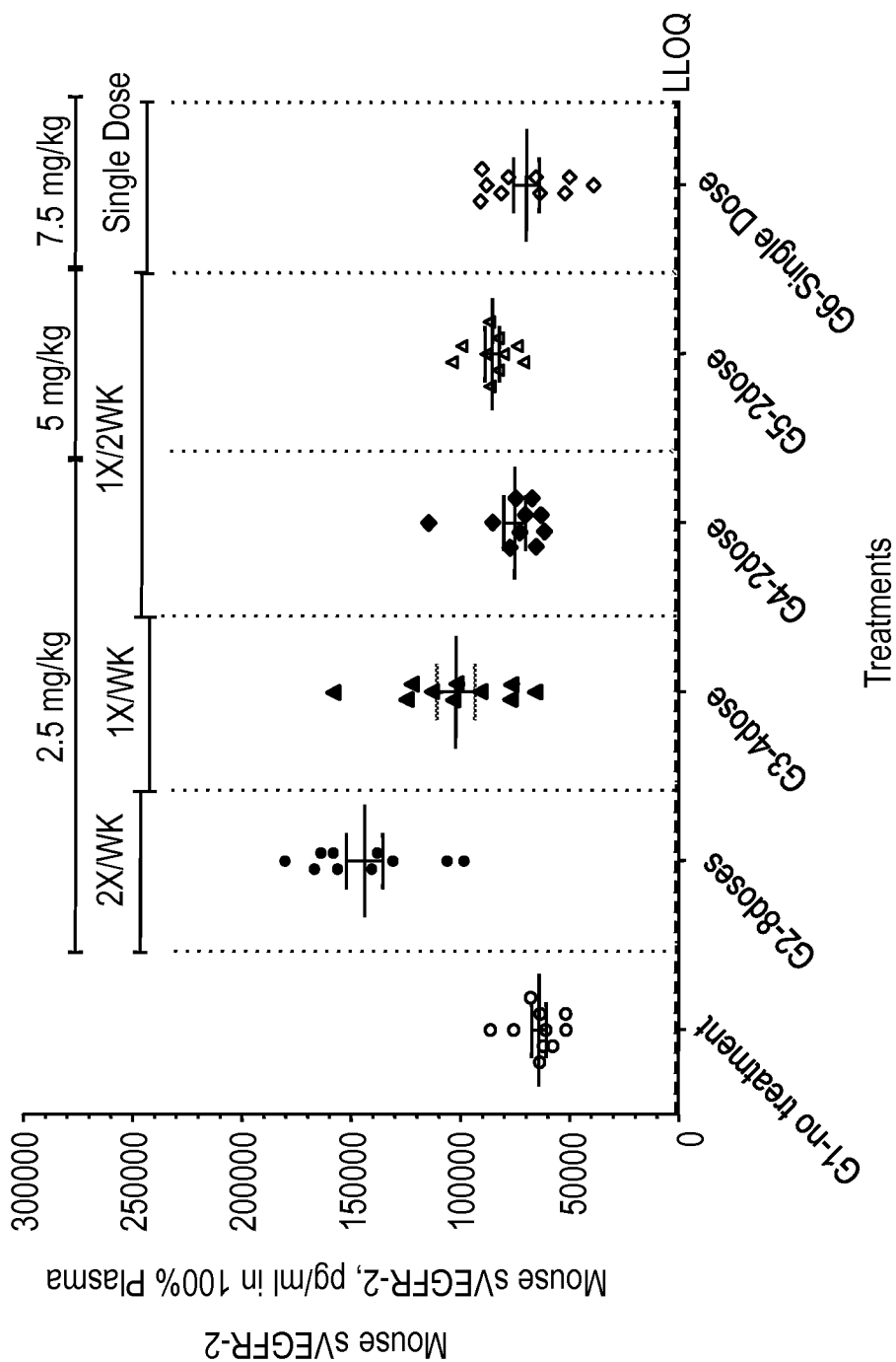
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FIG. 7E



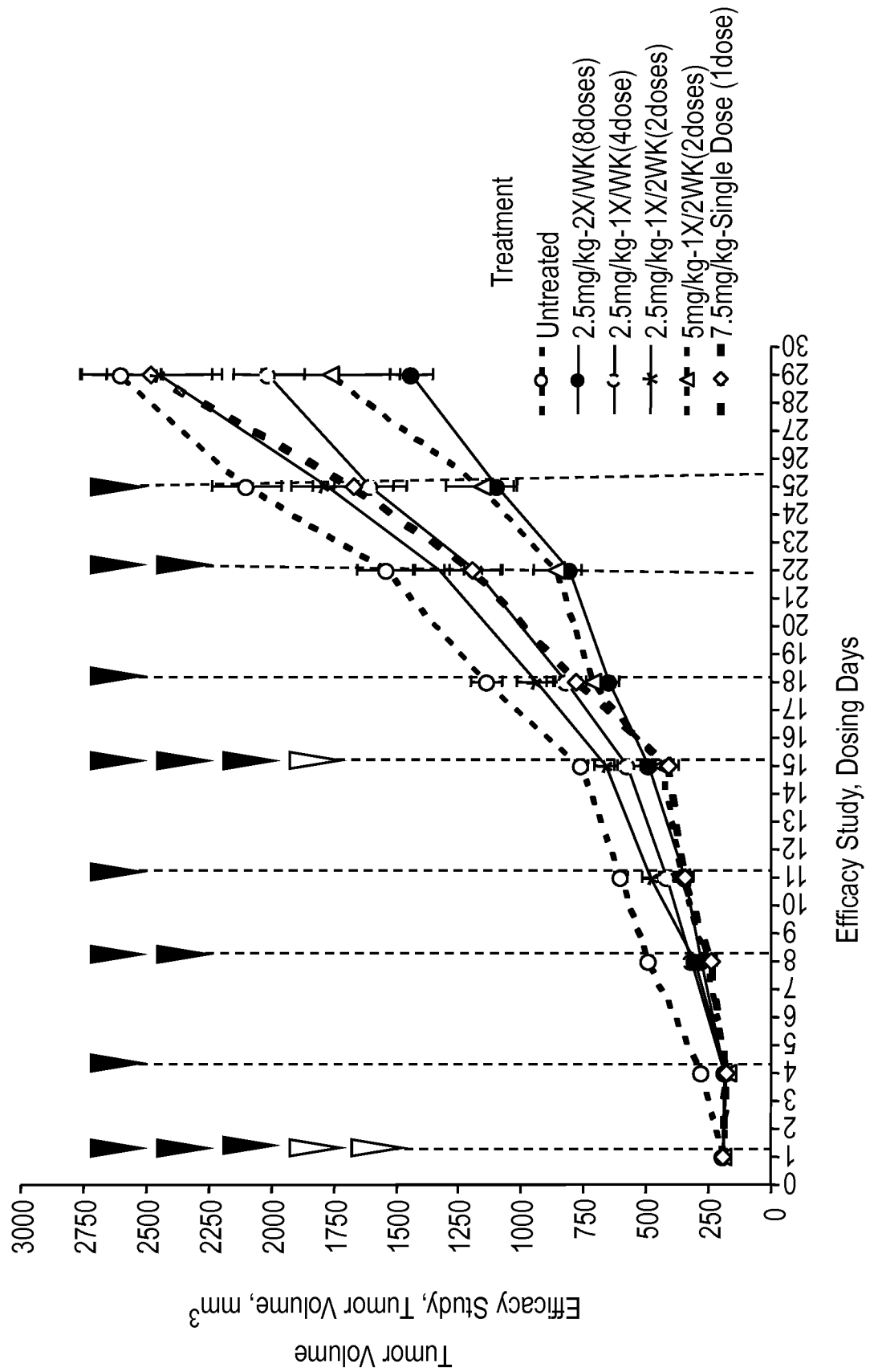
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FIG. 8A



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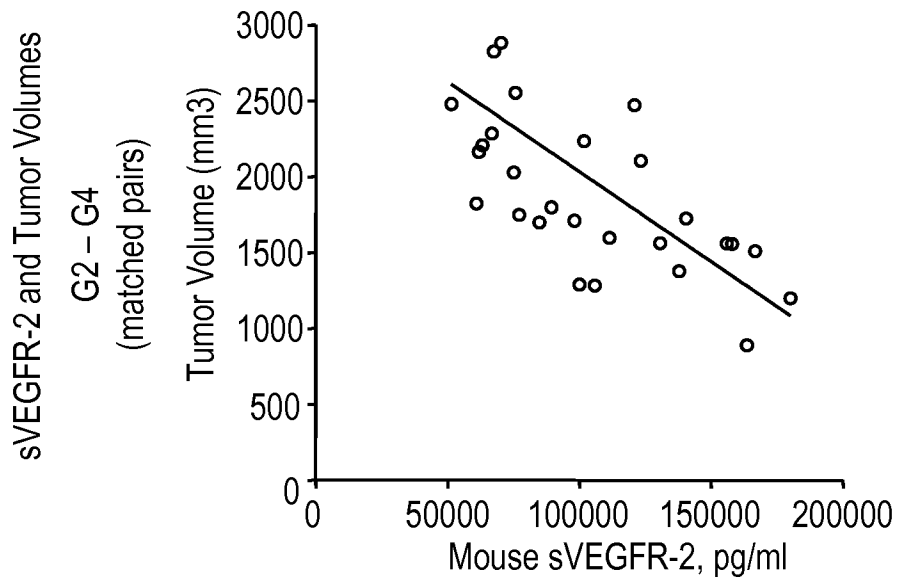
FIG. 8B



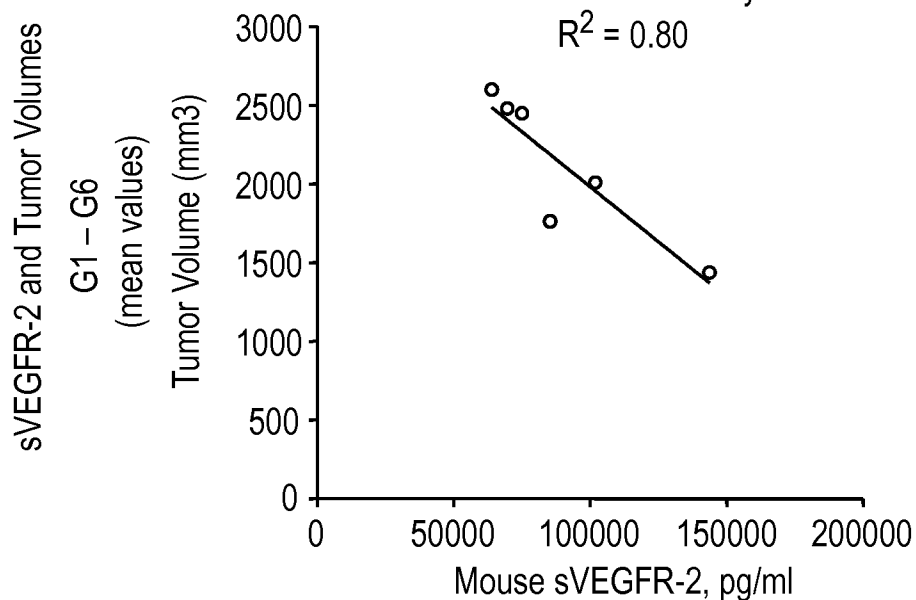
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FIG. 8C

EFFICACY STUDY
Correlate Individuals from G1 - G4 with
Tumor Volume on D29
 $R^2 = 0.50$

**FIG. 8D**

EFFICACY STUDY
Correlate sVEGFR-2 Concentration with
Tumor Volume at Day 29
 $R^2 = 0.80$



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FIG. 9

Calu6/Nude Mouse

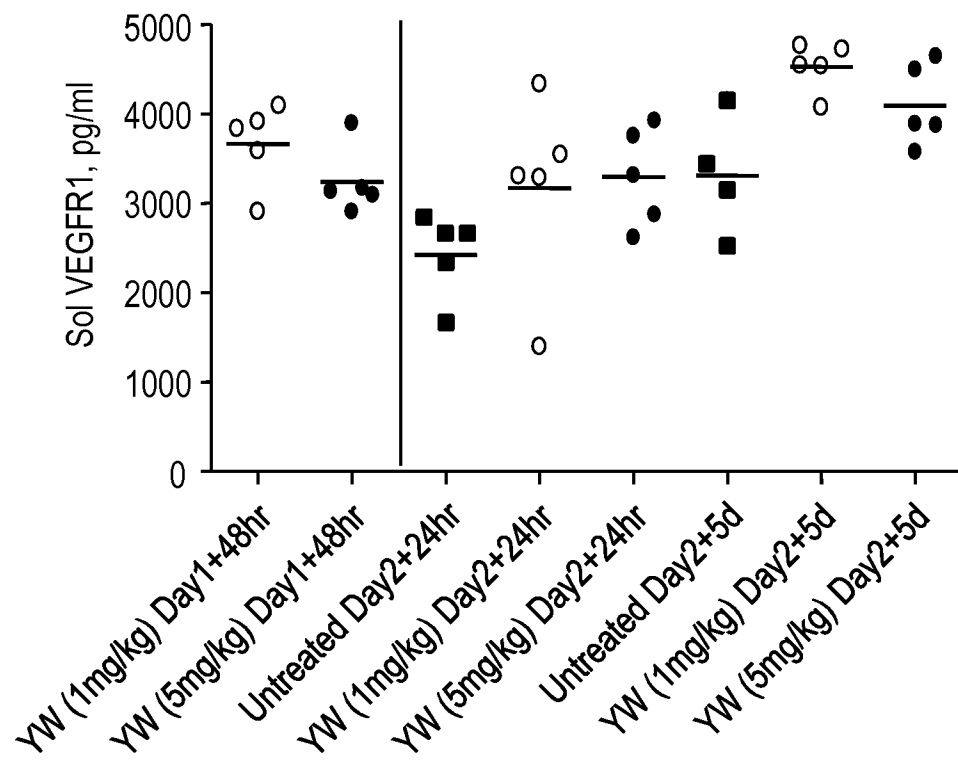


FIG. 10A

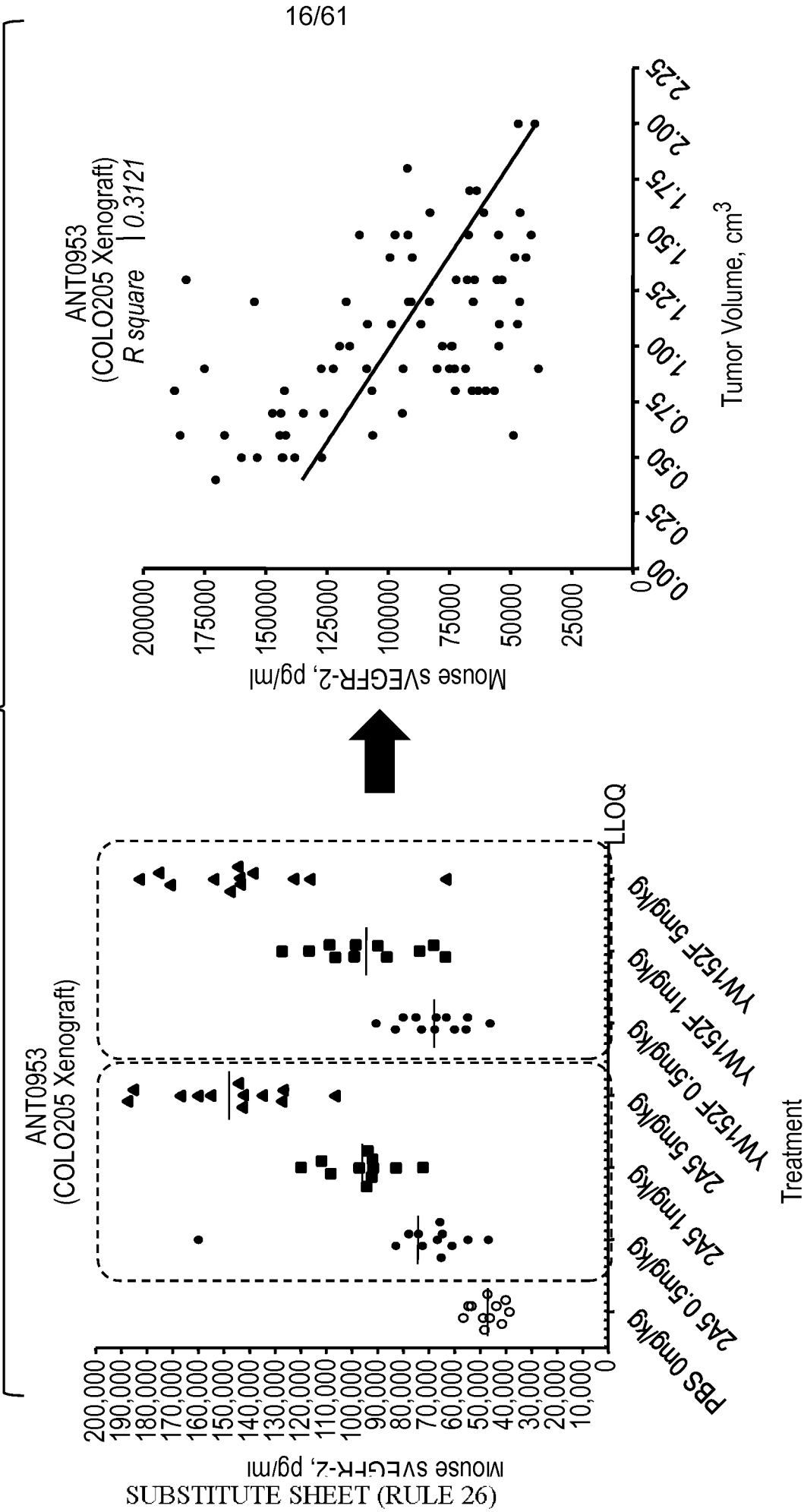


FIG. 10B

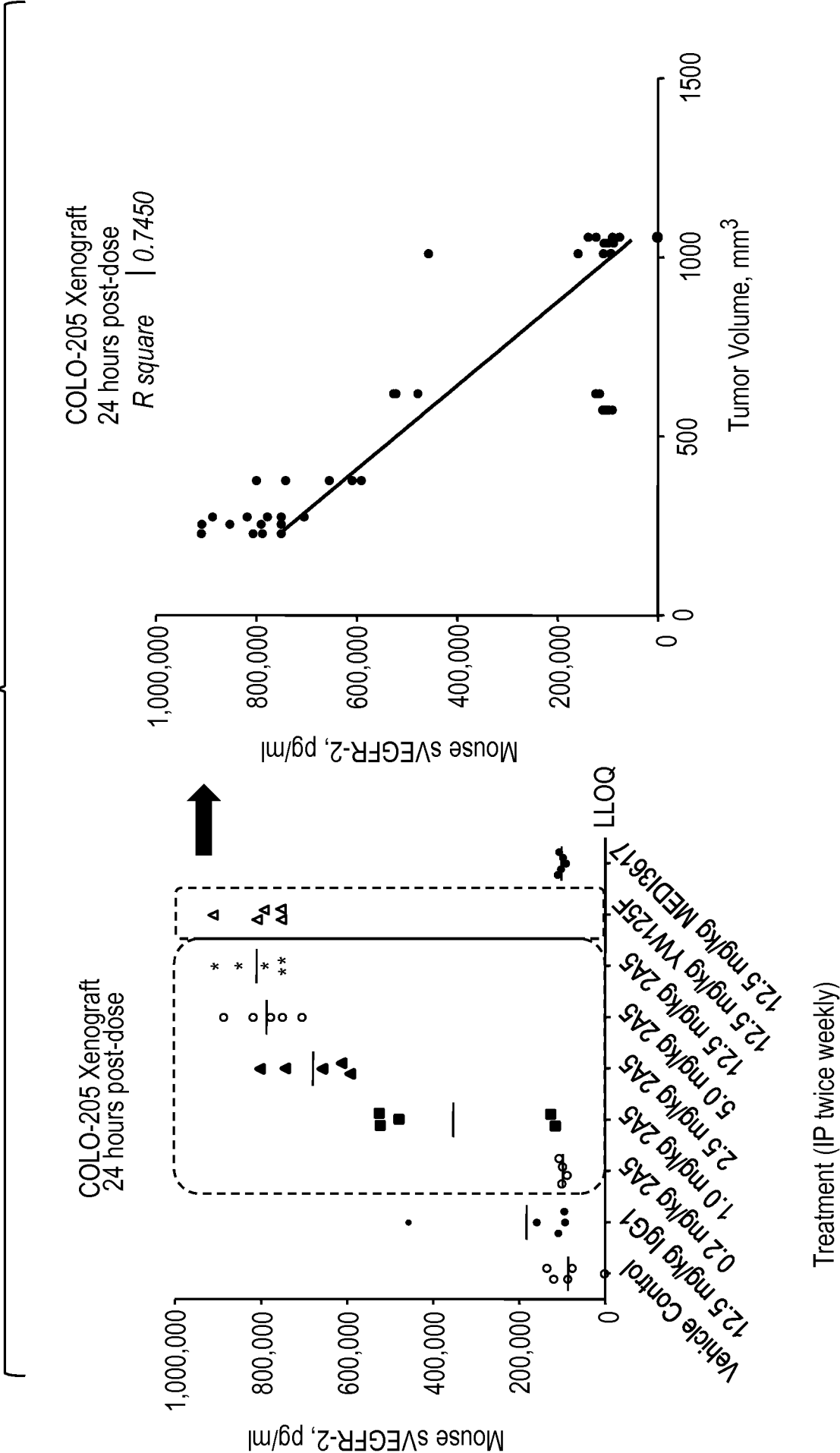


FIG. 11A

COLO-205 Xenograft
24 hours post-dose

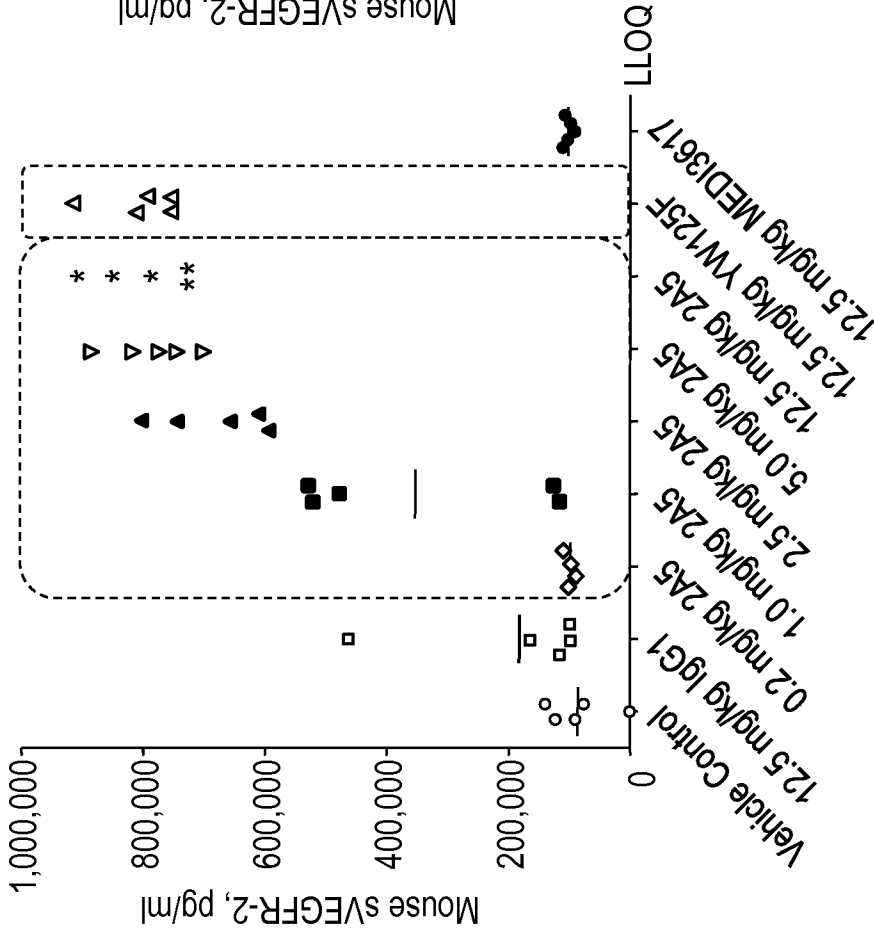


FIG. 11B

COLO-205 Xenograft
24 hours post-dose

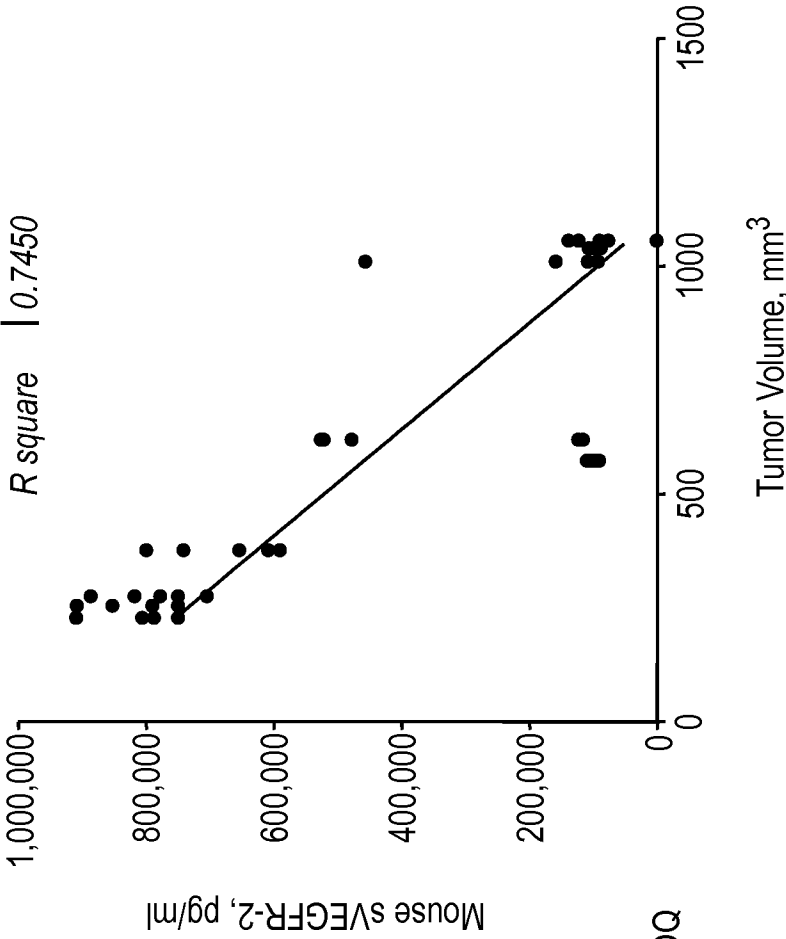


FIG. 12A

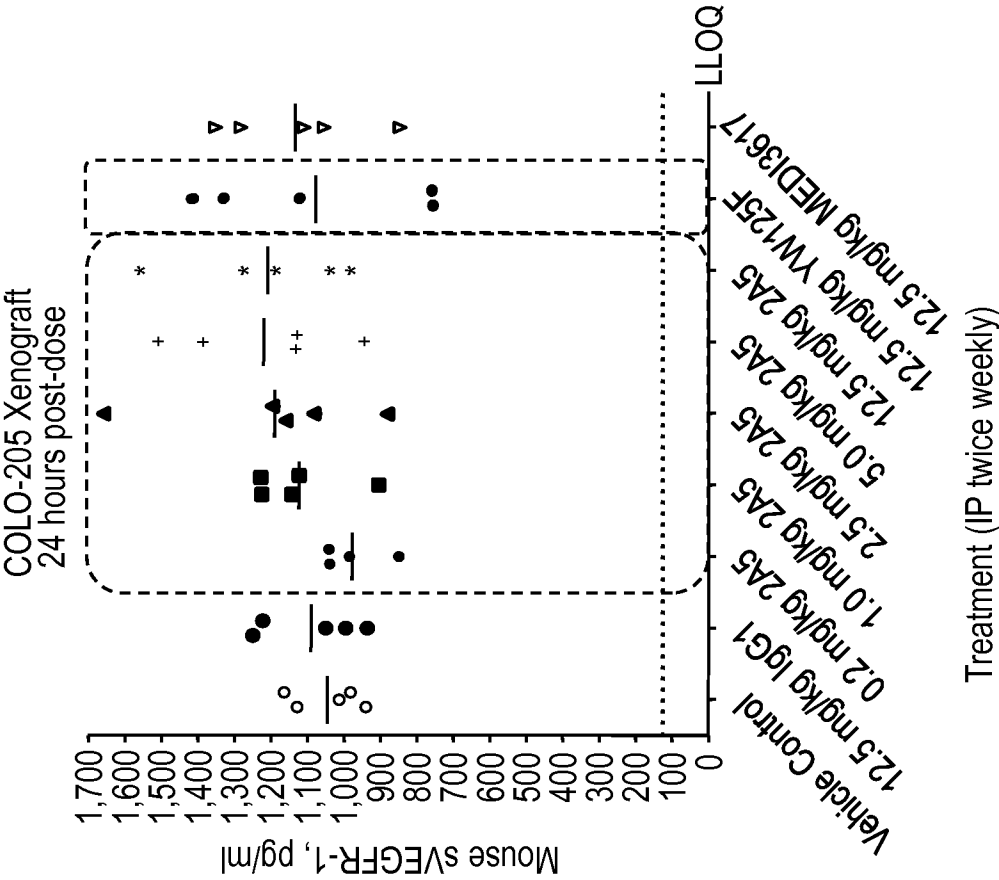


FIG. 12B

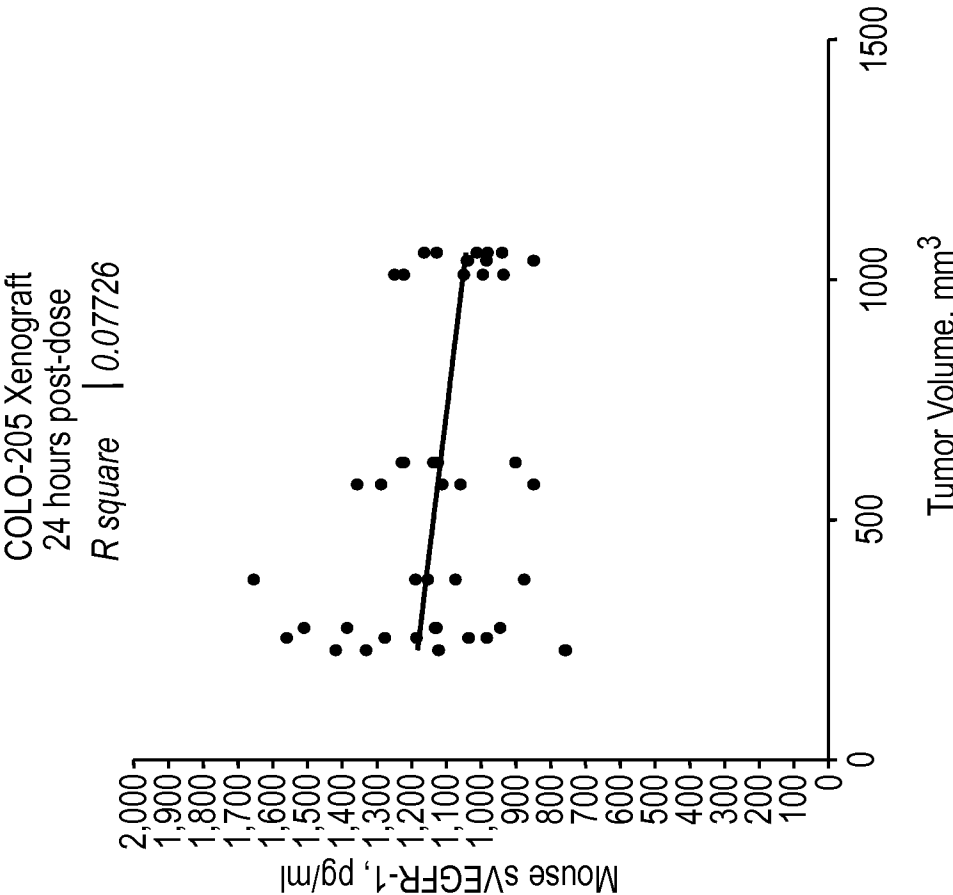


FIG. 13A

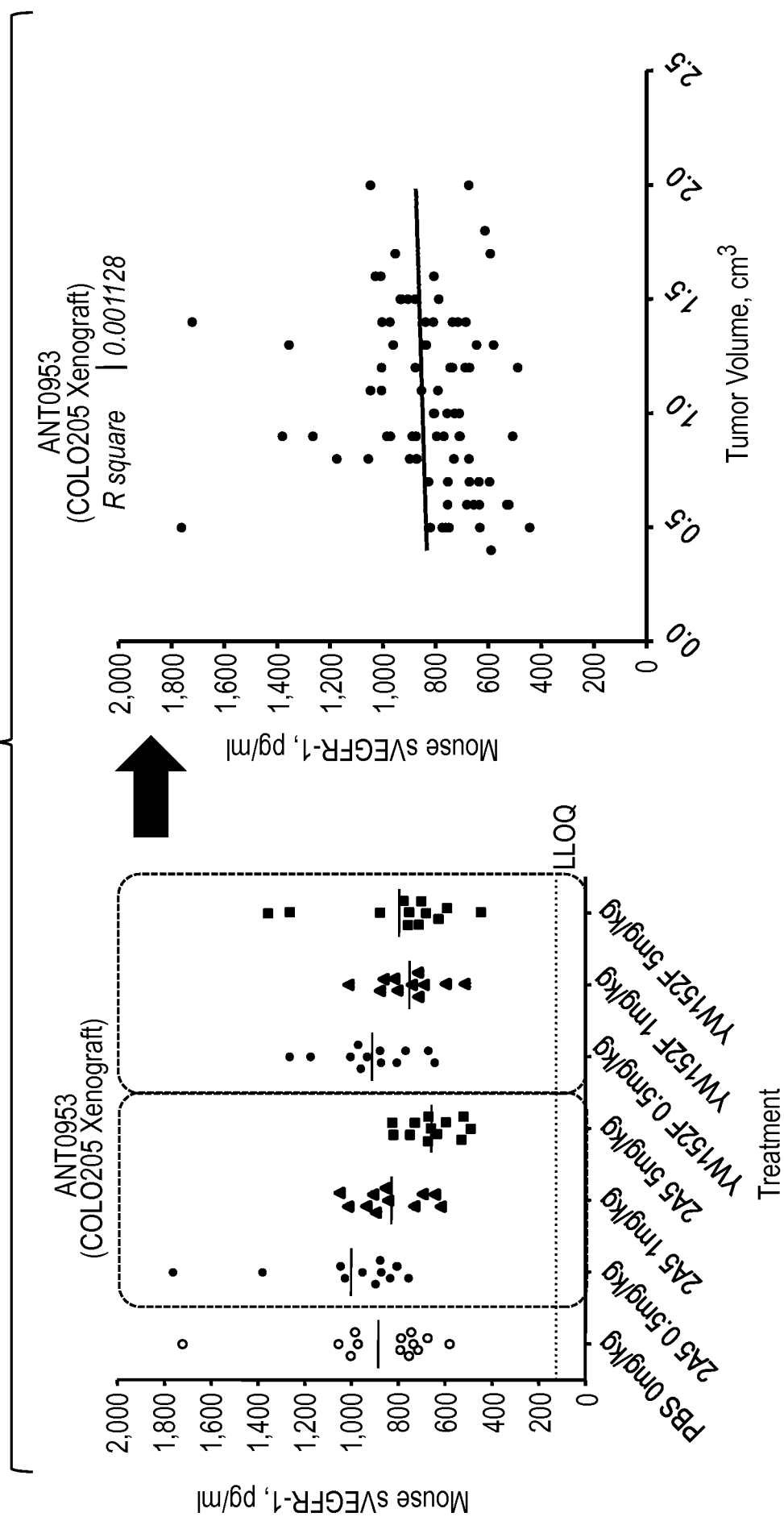
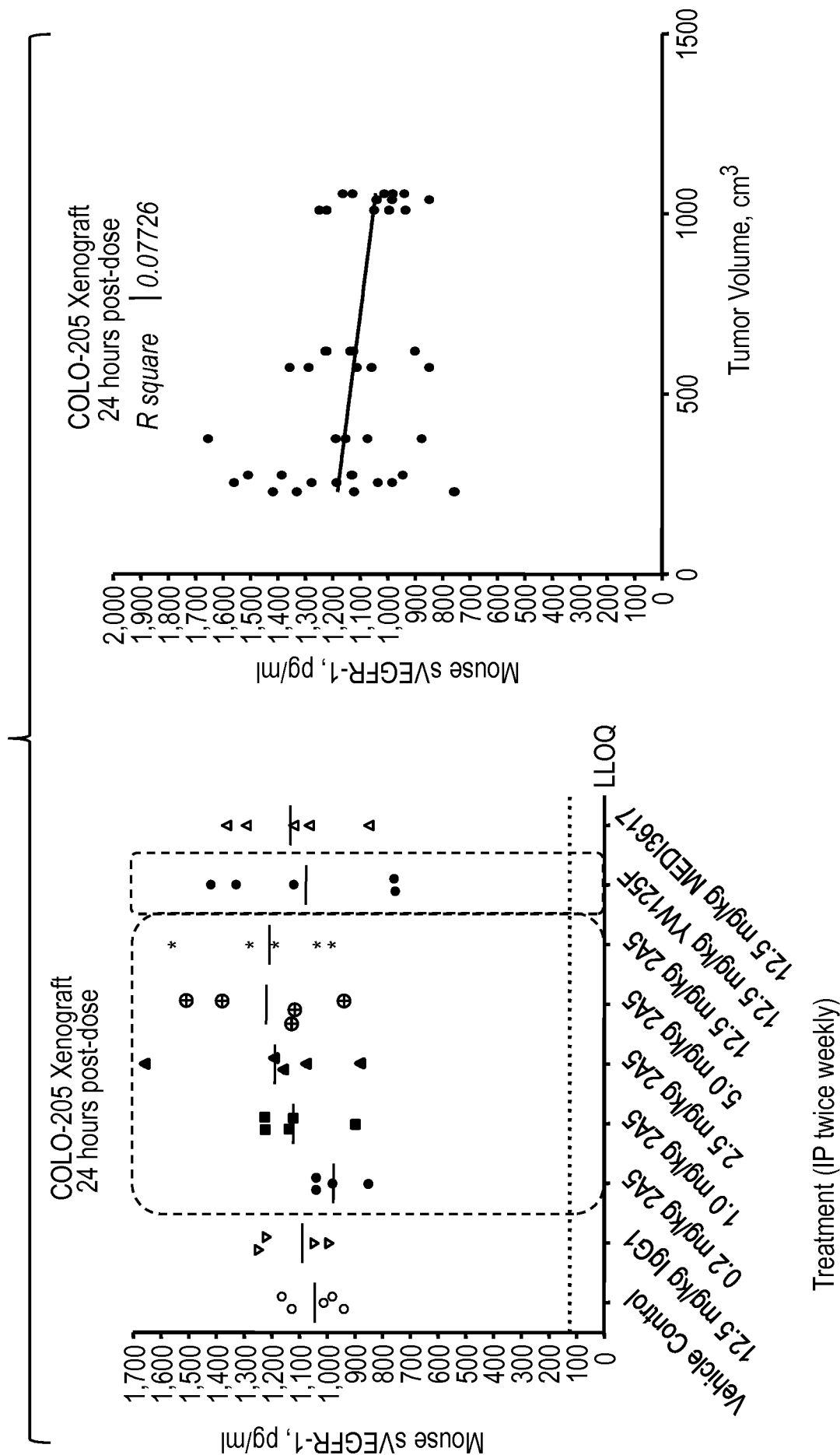


FIG. 13B



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FIG. 14

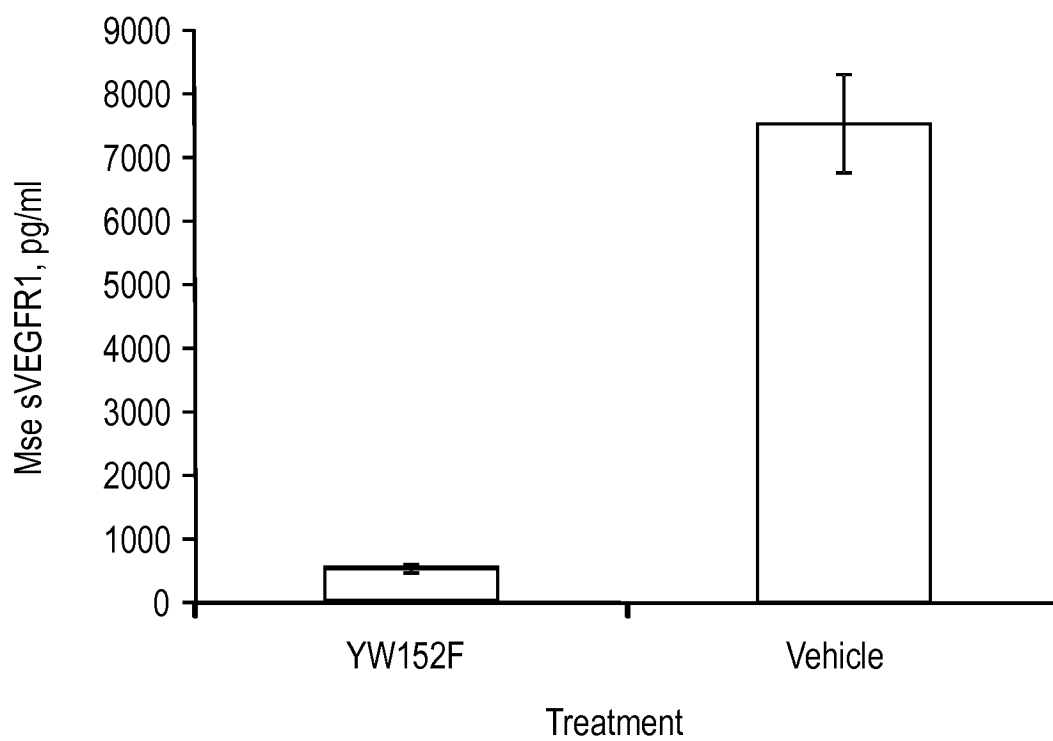


FIG. 15B

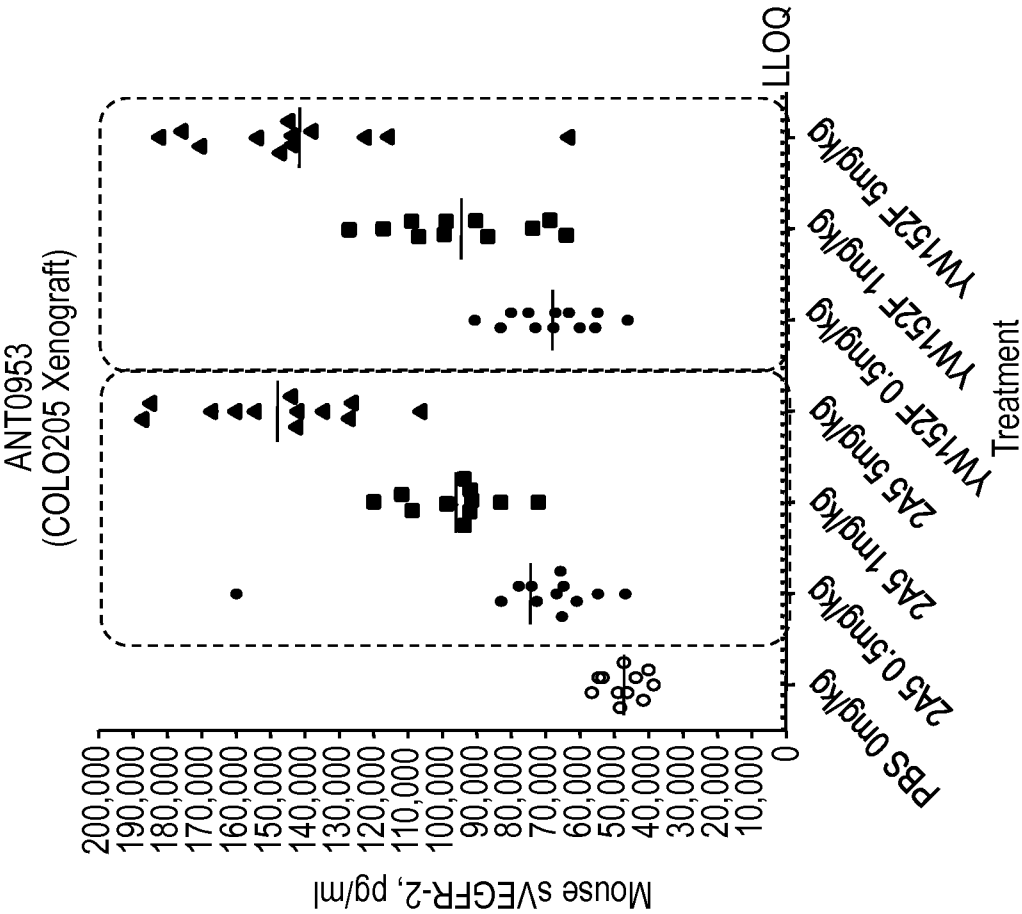


FIG. 15A

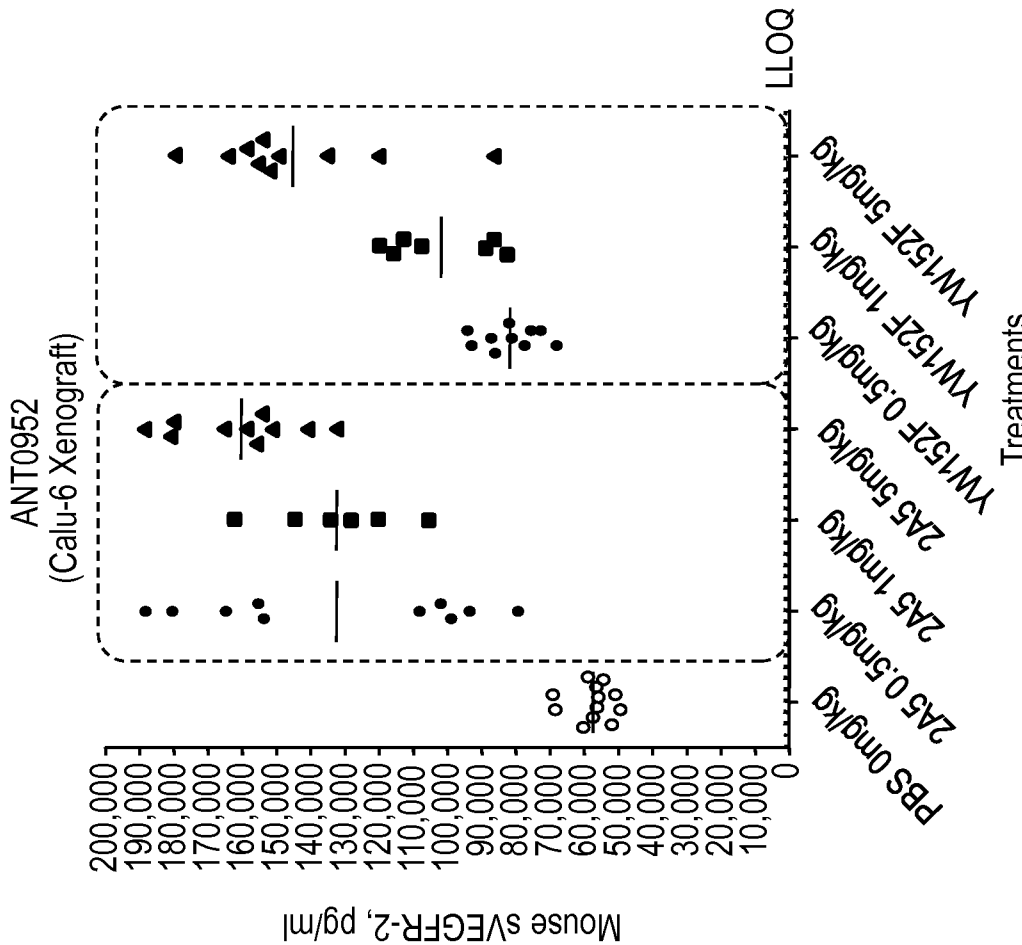


FIG. 16B

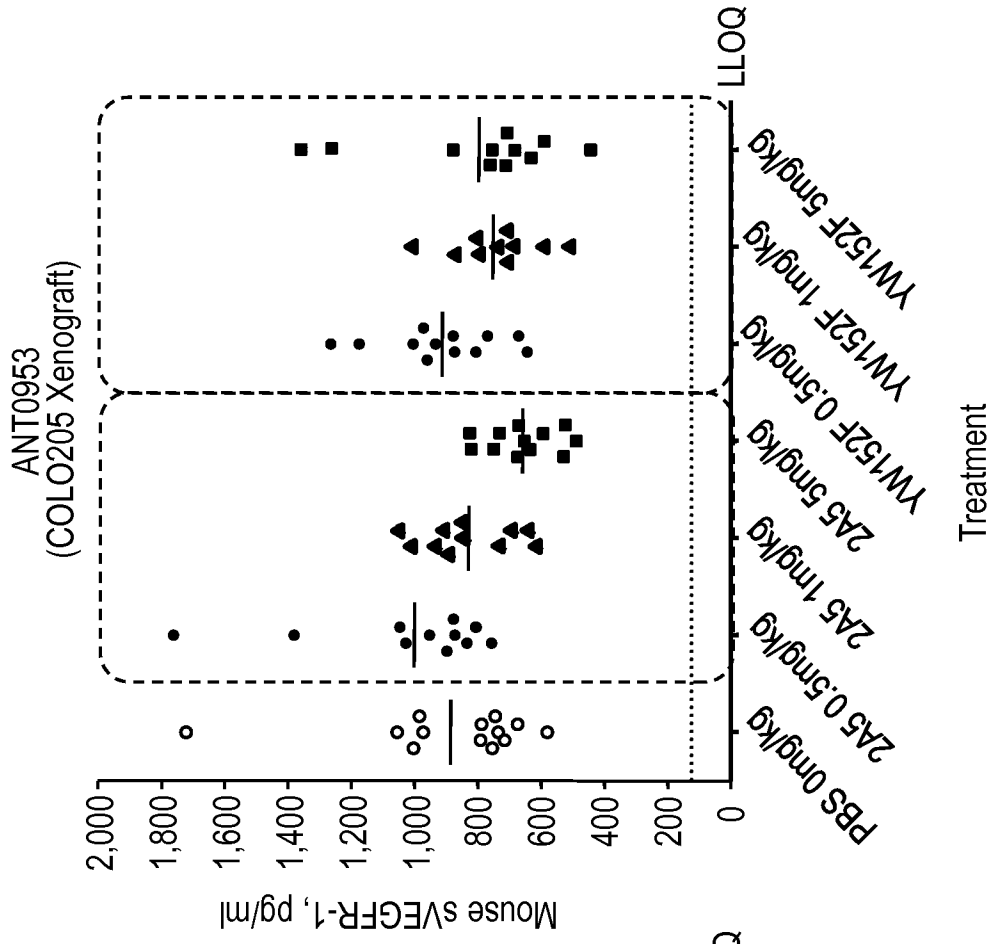


FIG. 16A

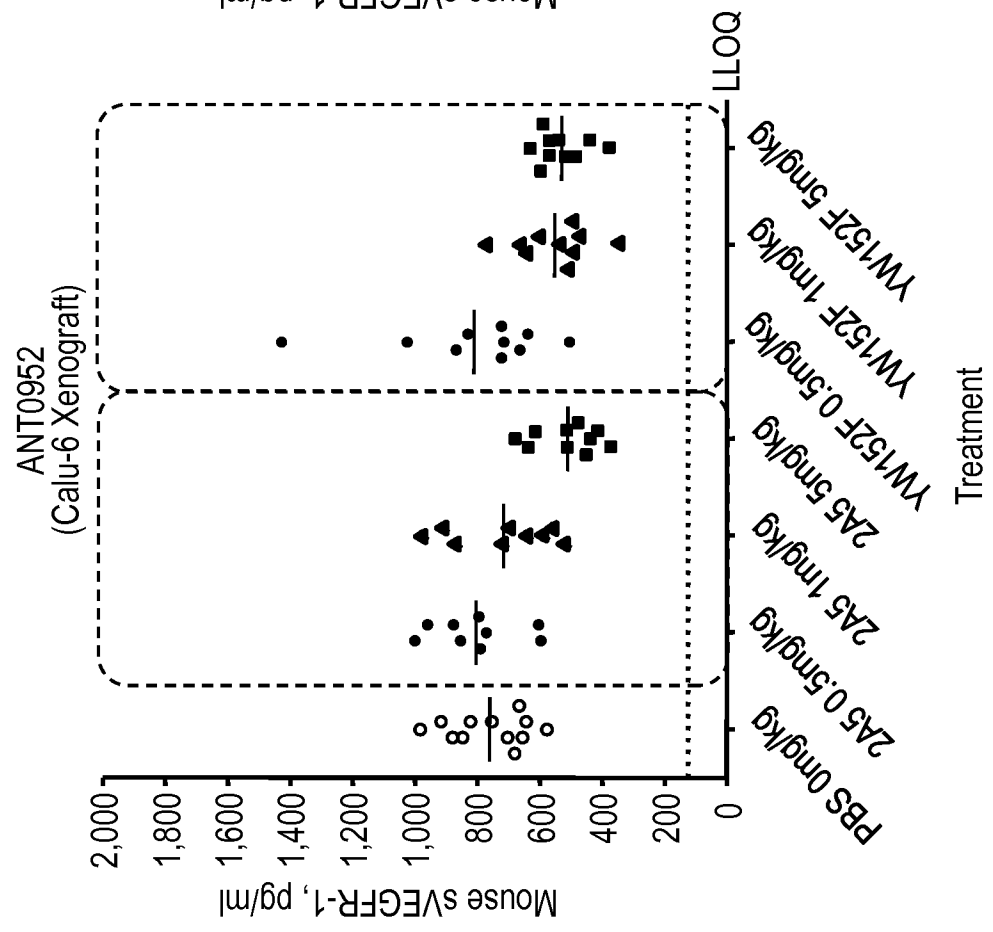


FIG. 17B

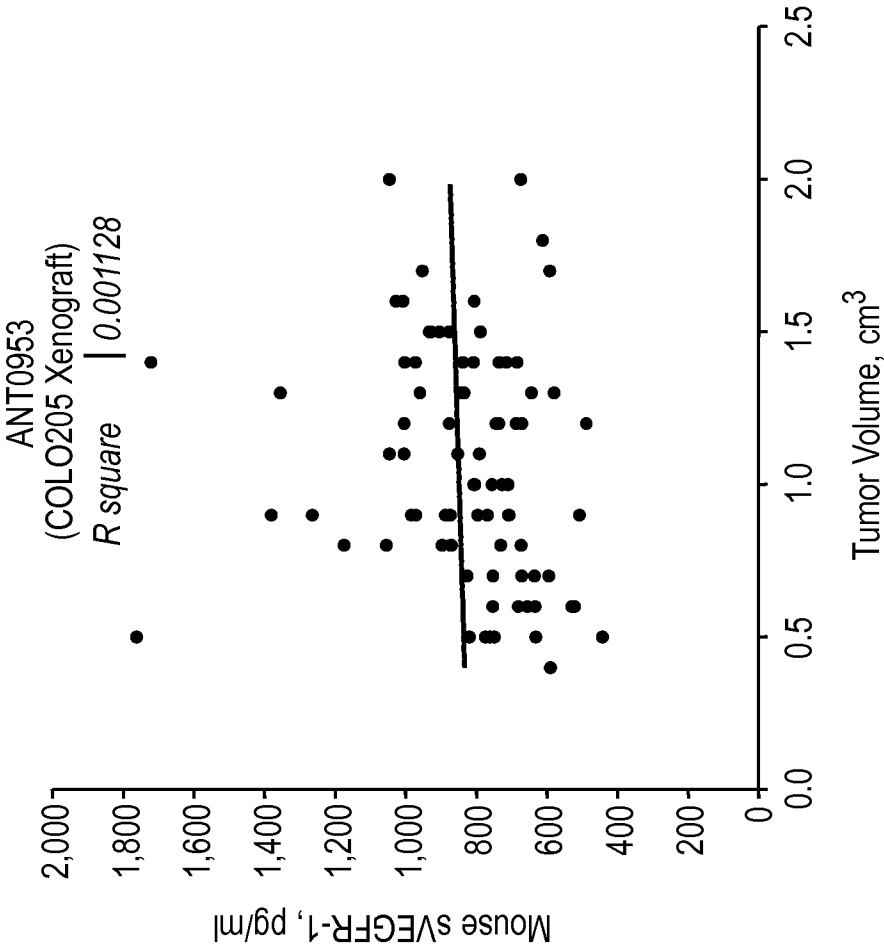


FIG. 17A

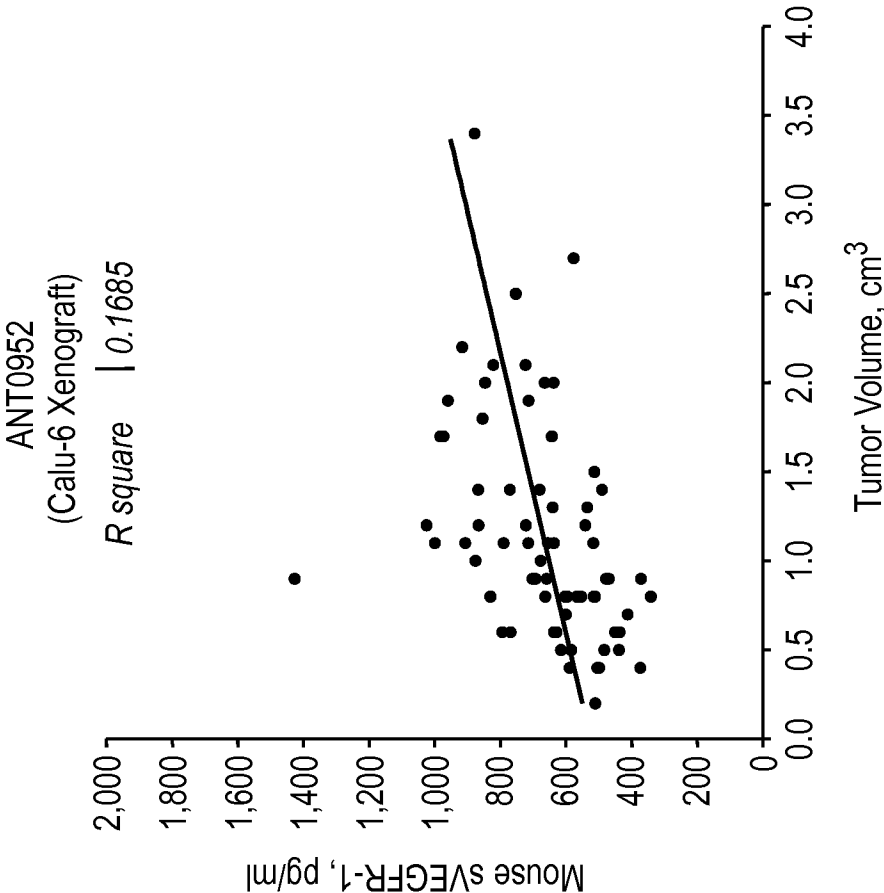


FIG. 18

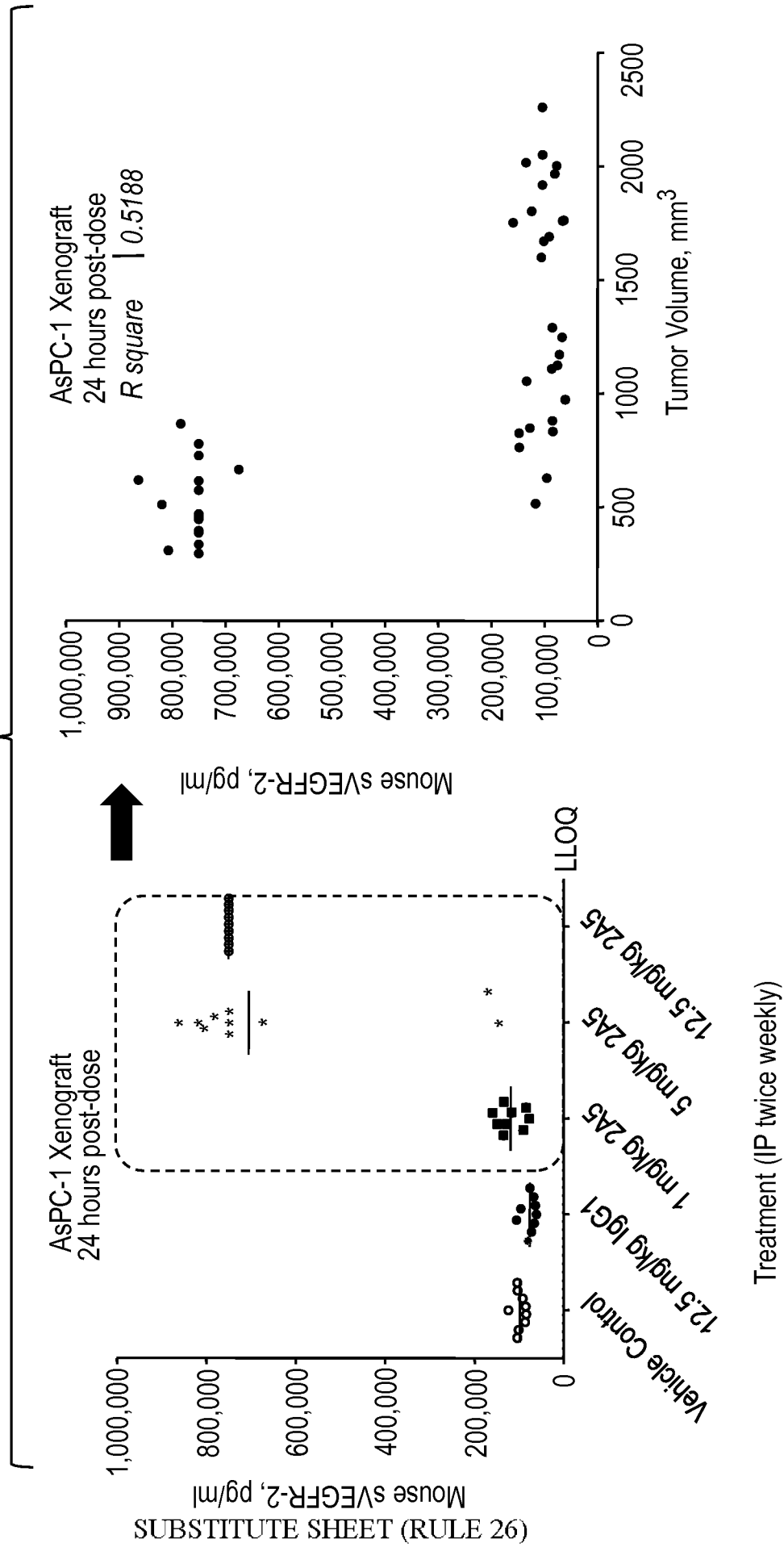


FIG. 19B

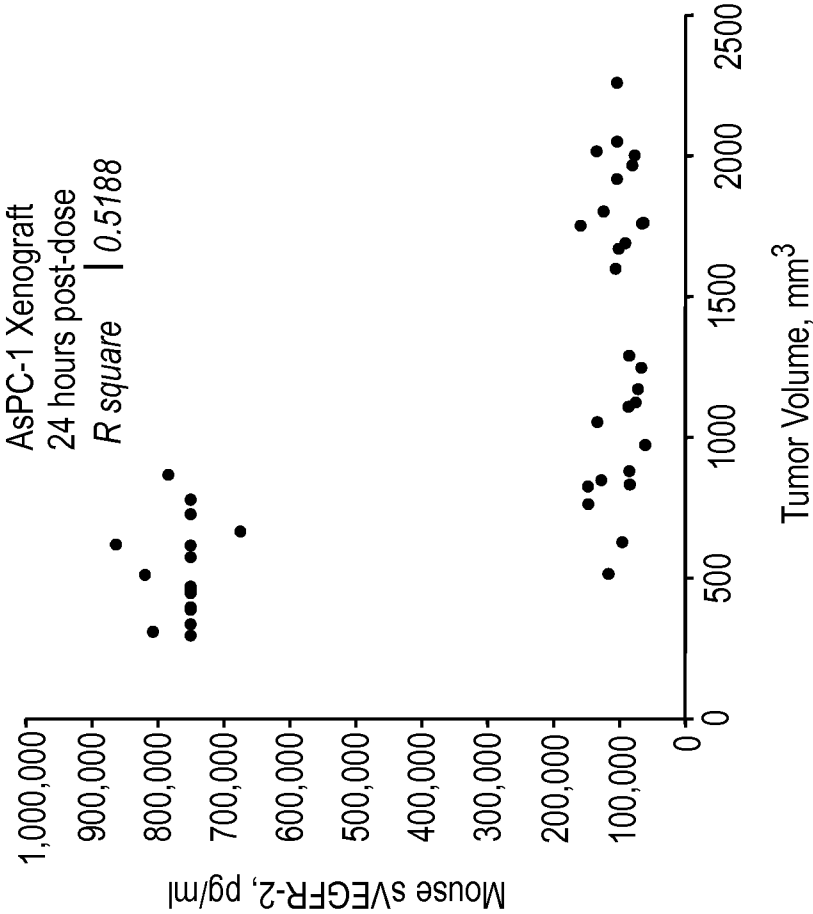


FIG. 19A

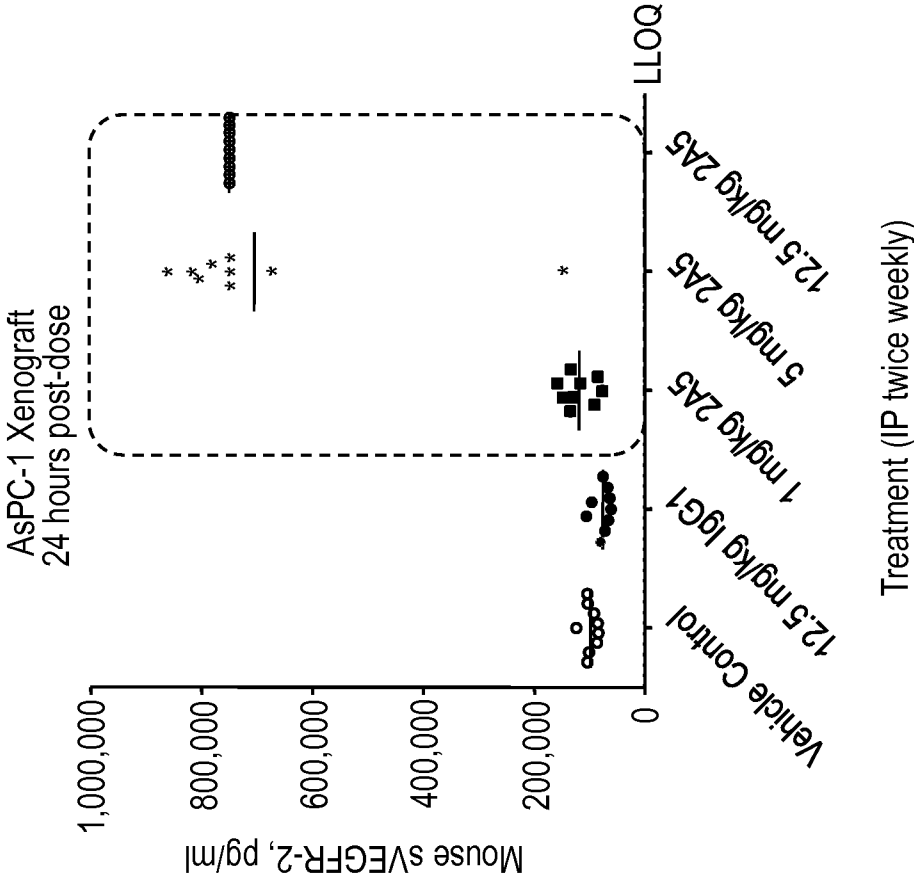
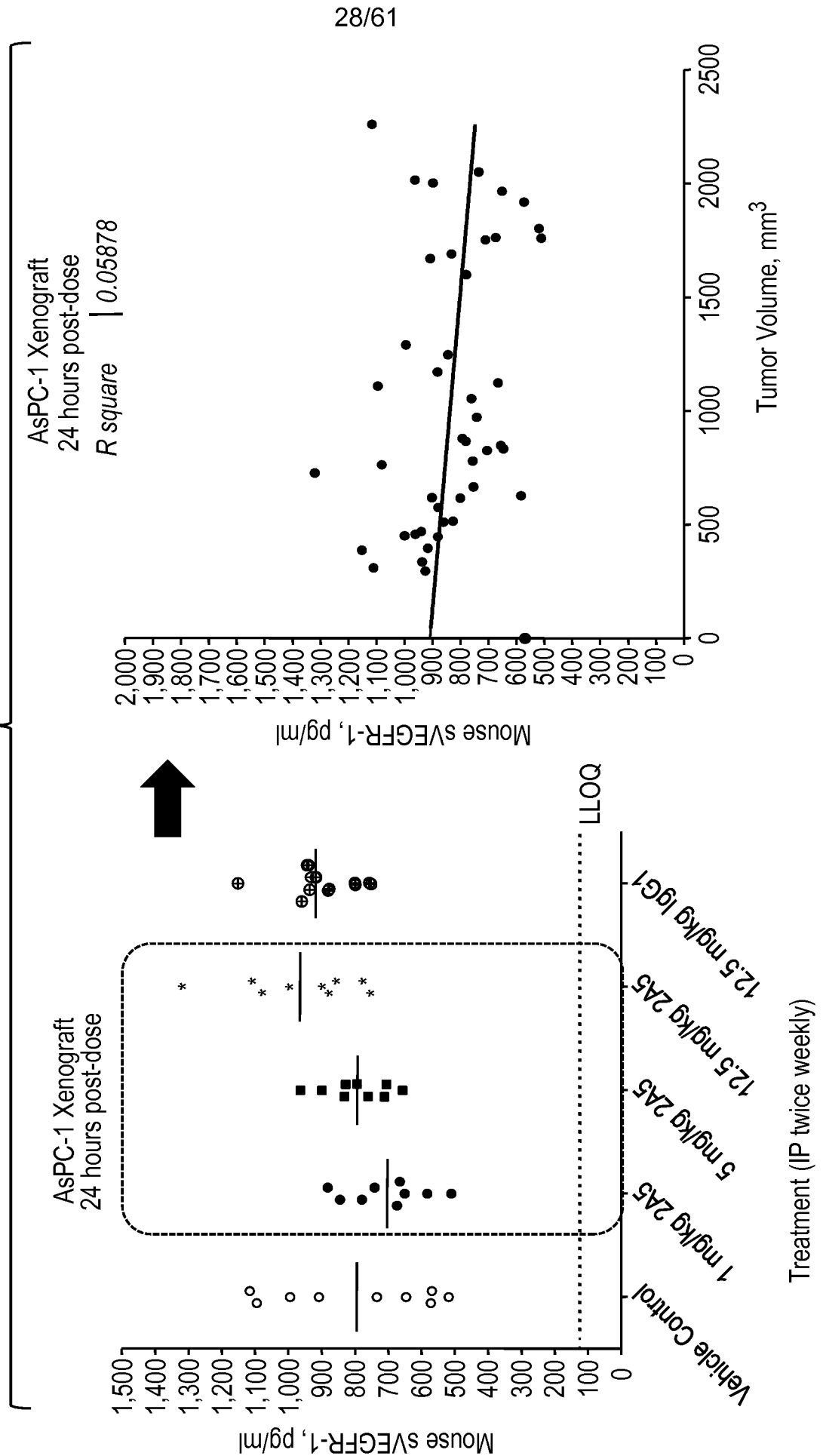


FIG. 20



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FIG. 21

Normal ICR Mouse

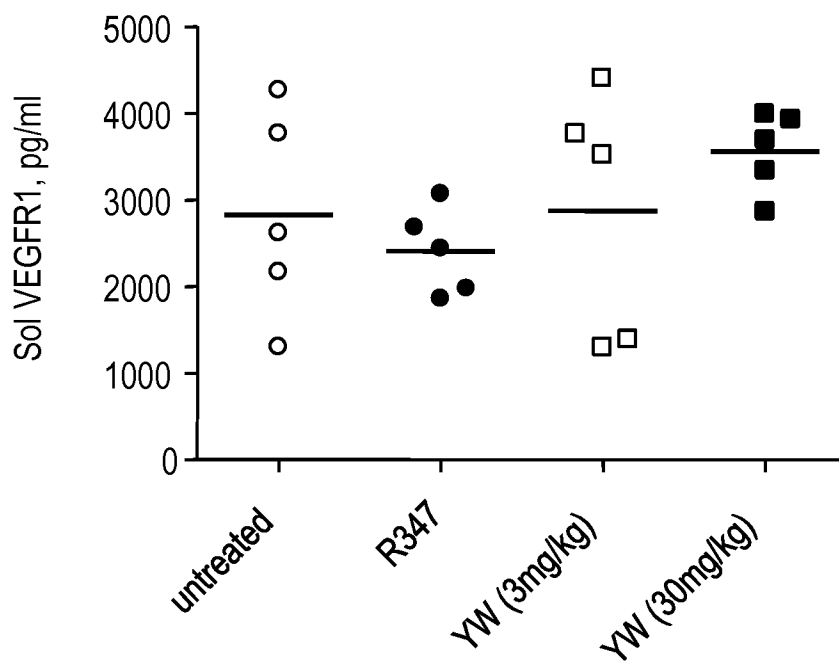
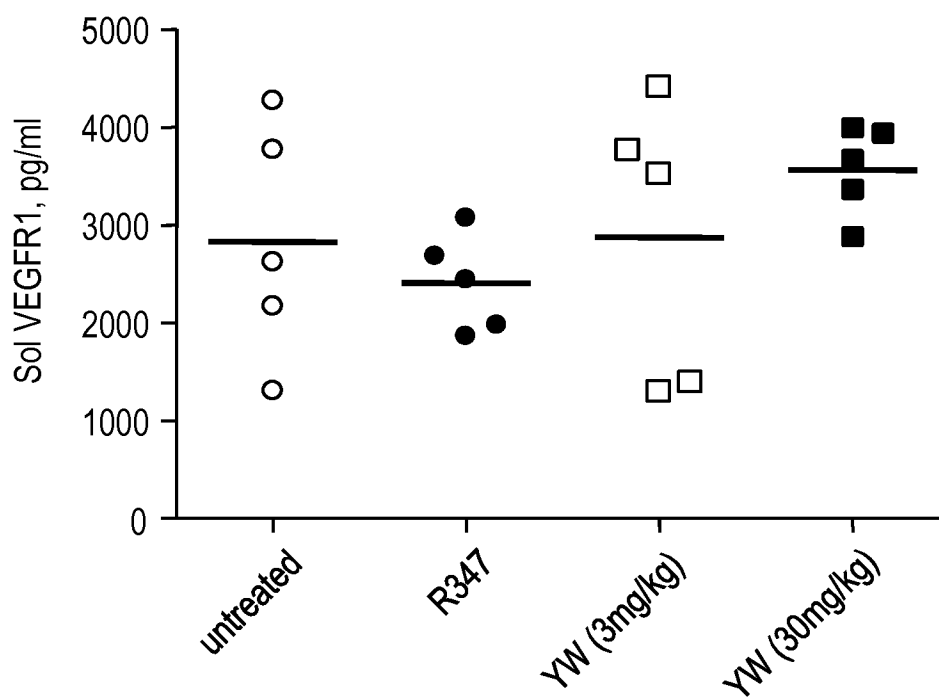


FIG. 22

Normal ICR Mouse



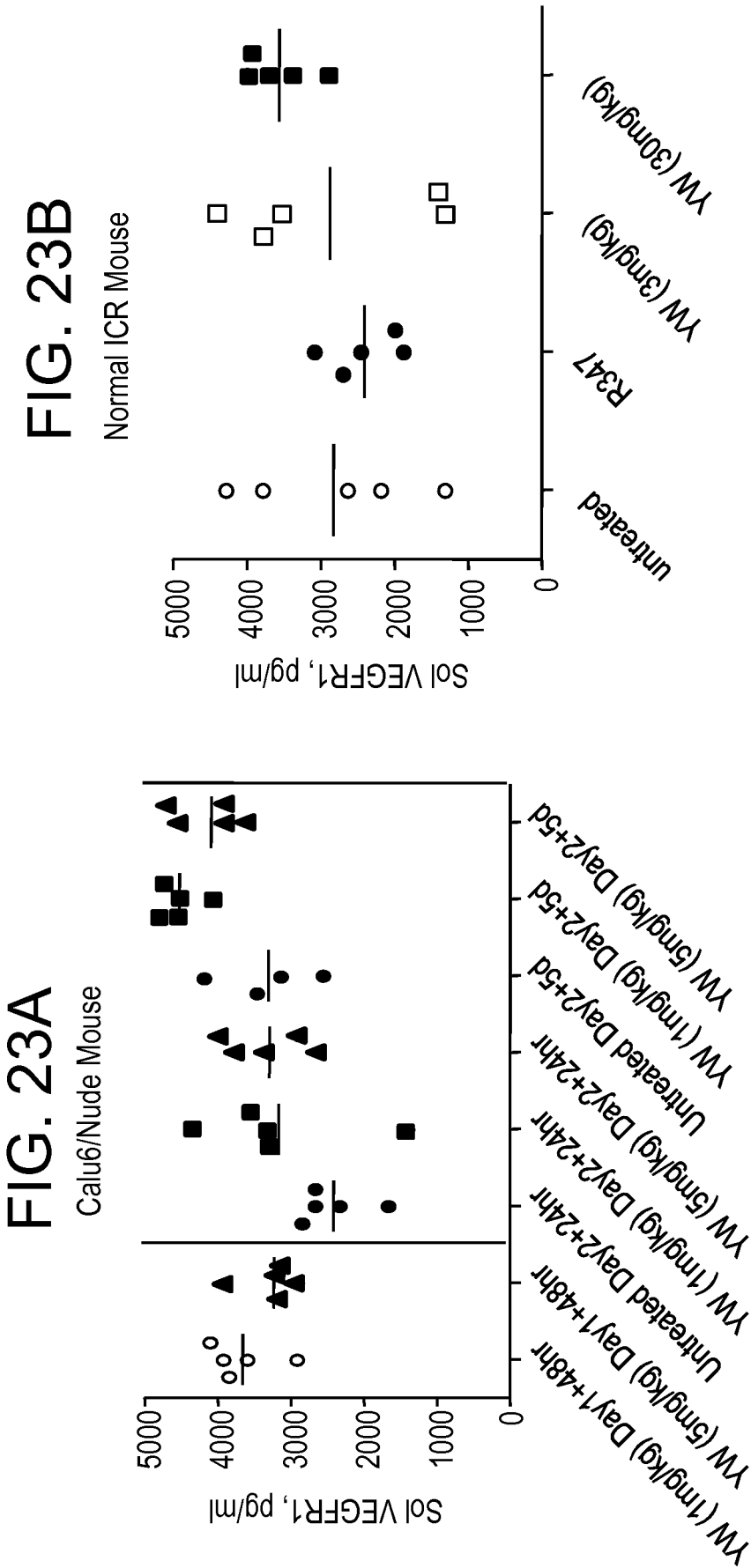


FIG. 23D

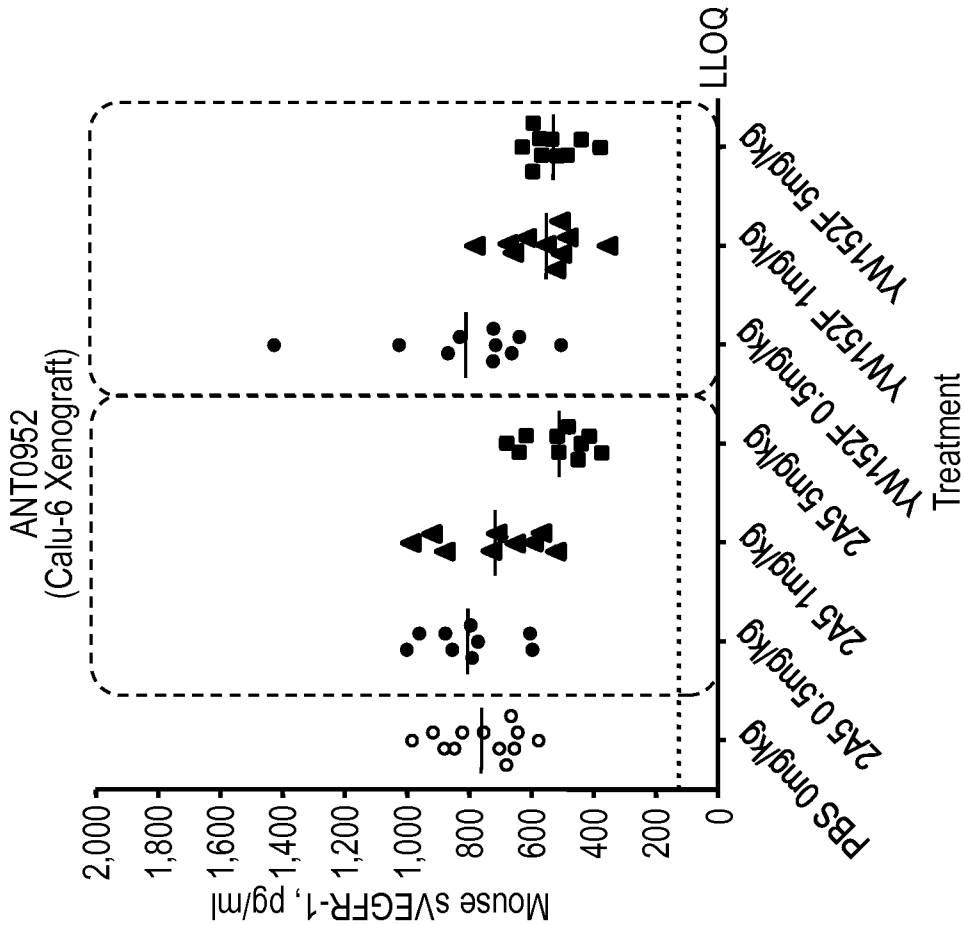


FIG. 23C

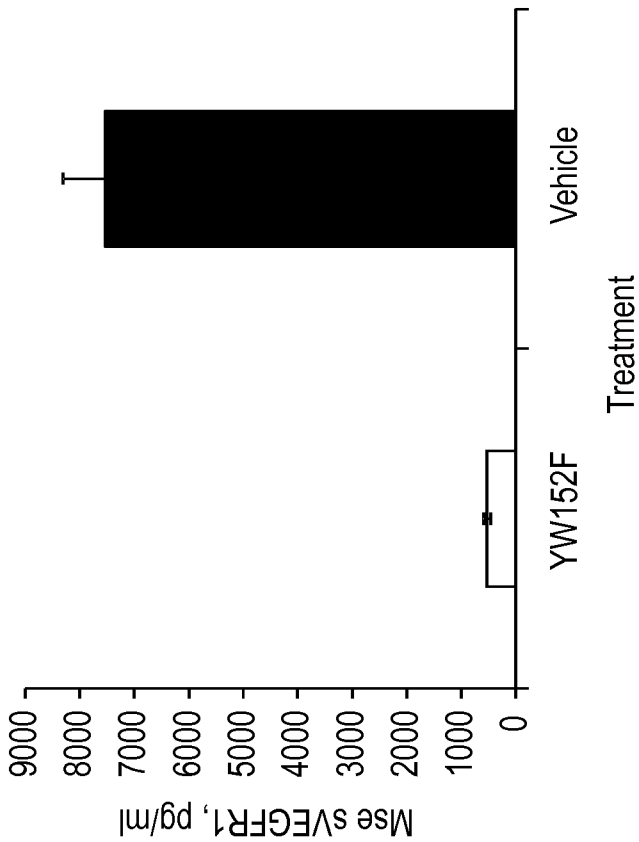


FIG. 23F

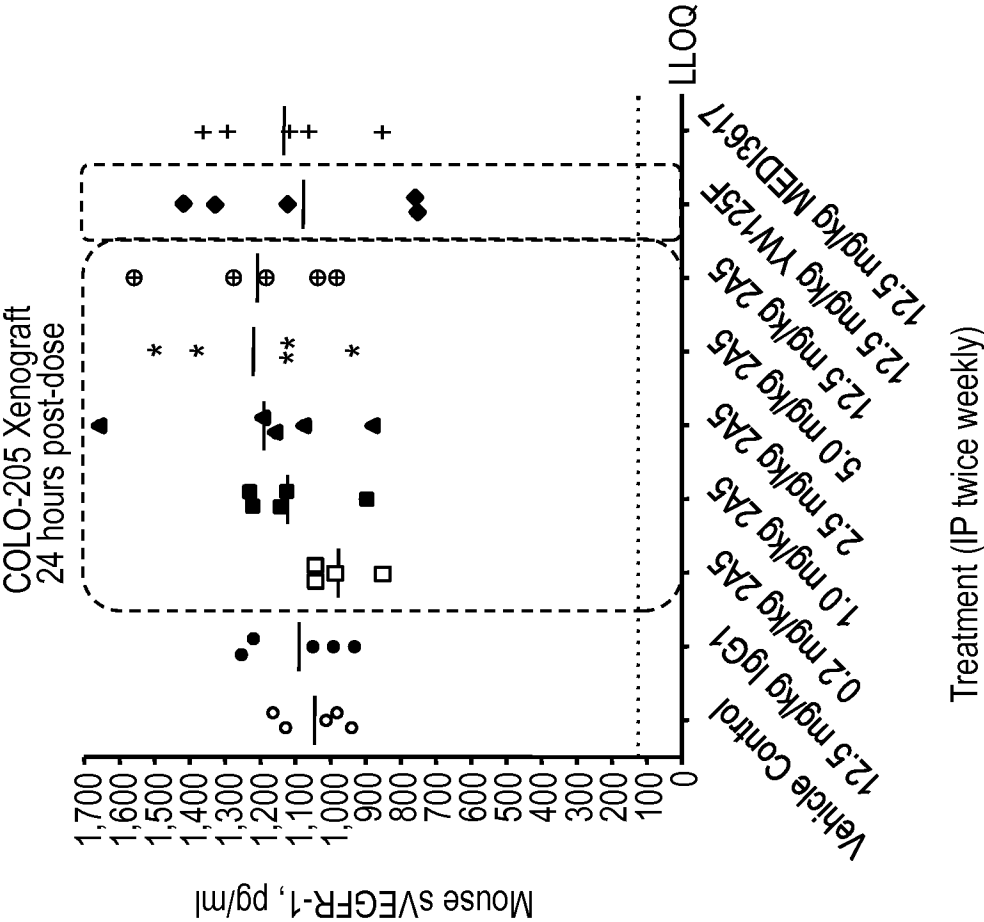


FIG. 23E

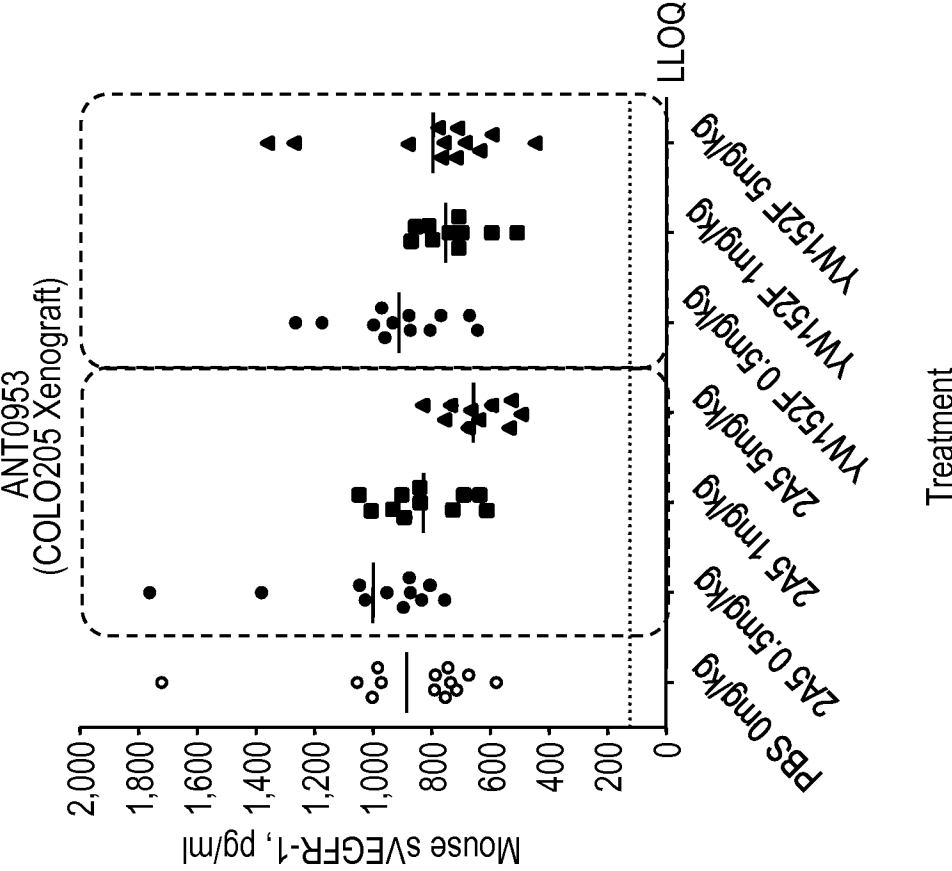


FIG. 24A

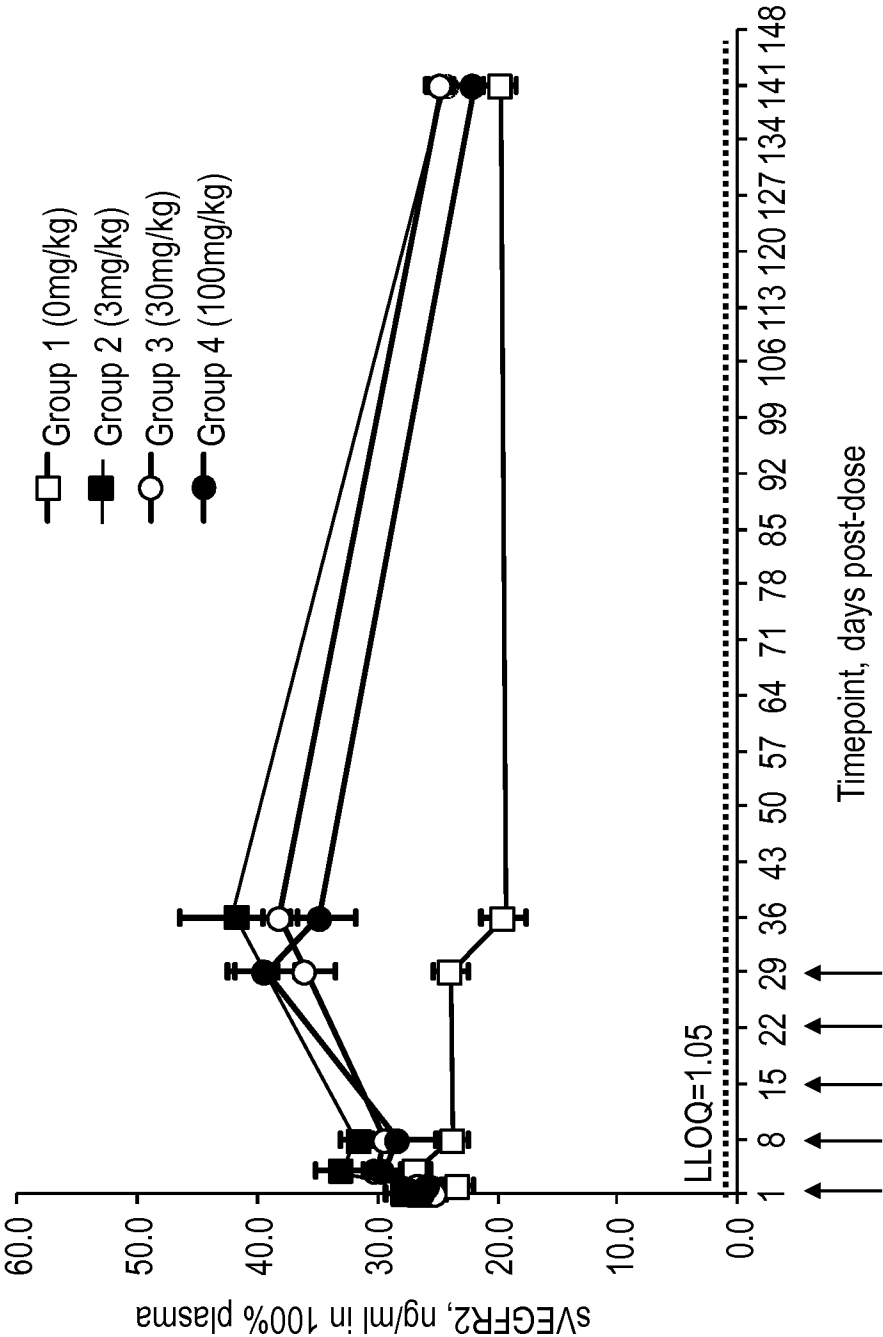


FIG. 24B

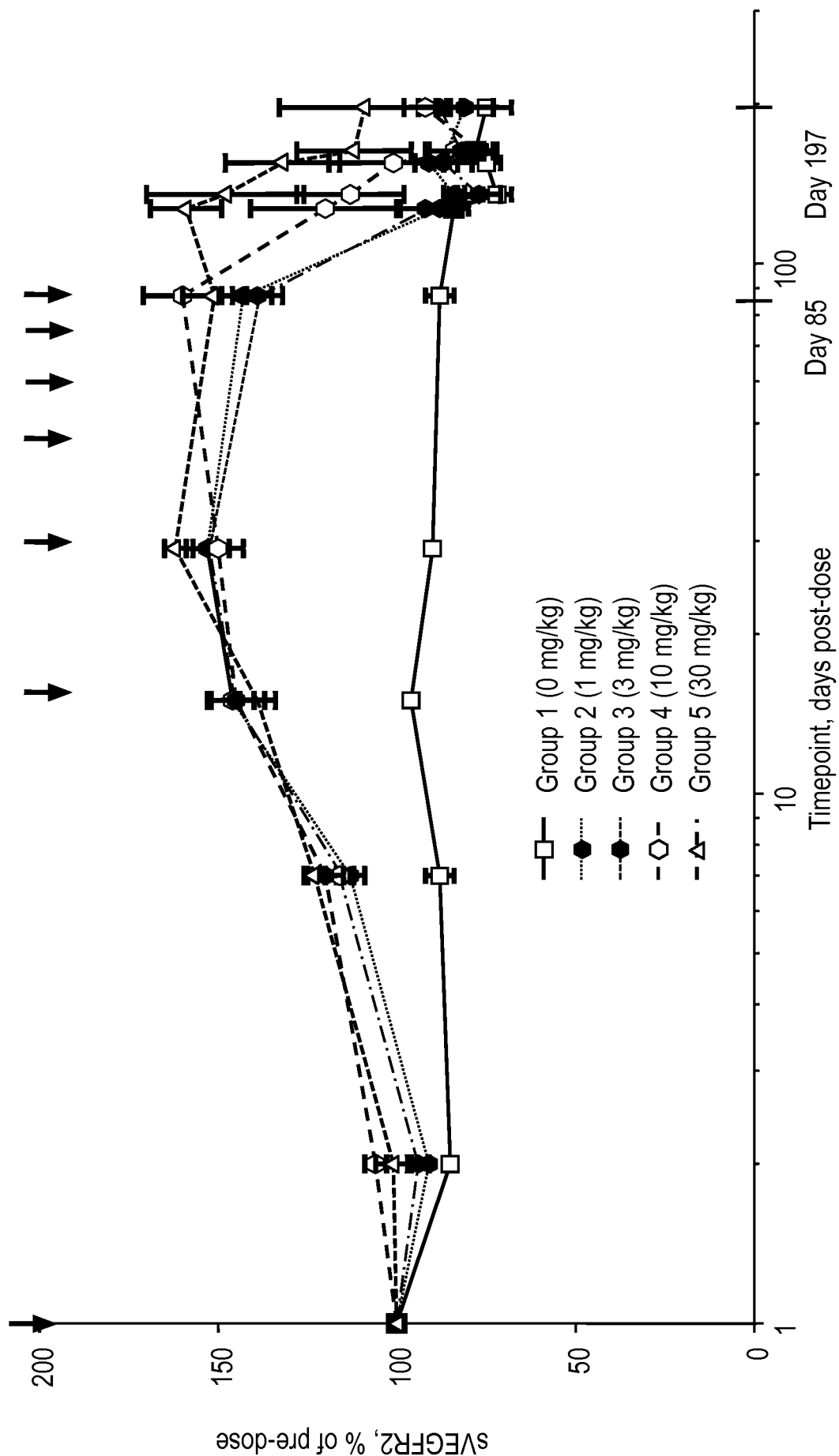
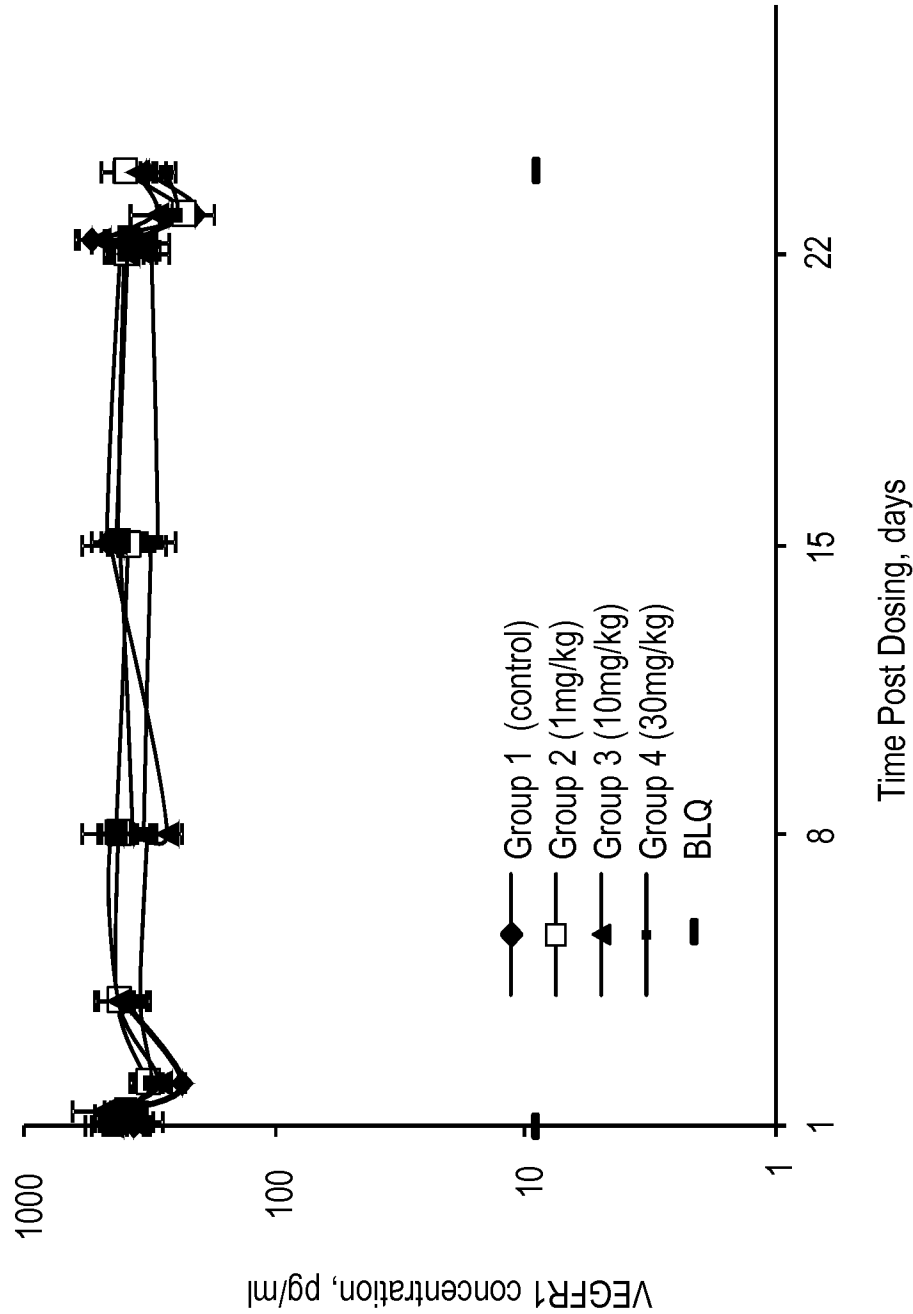
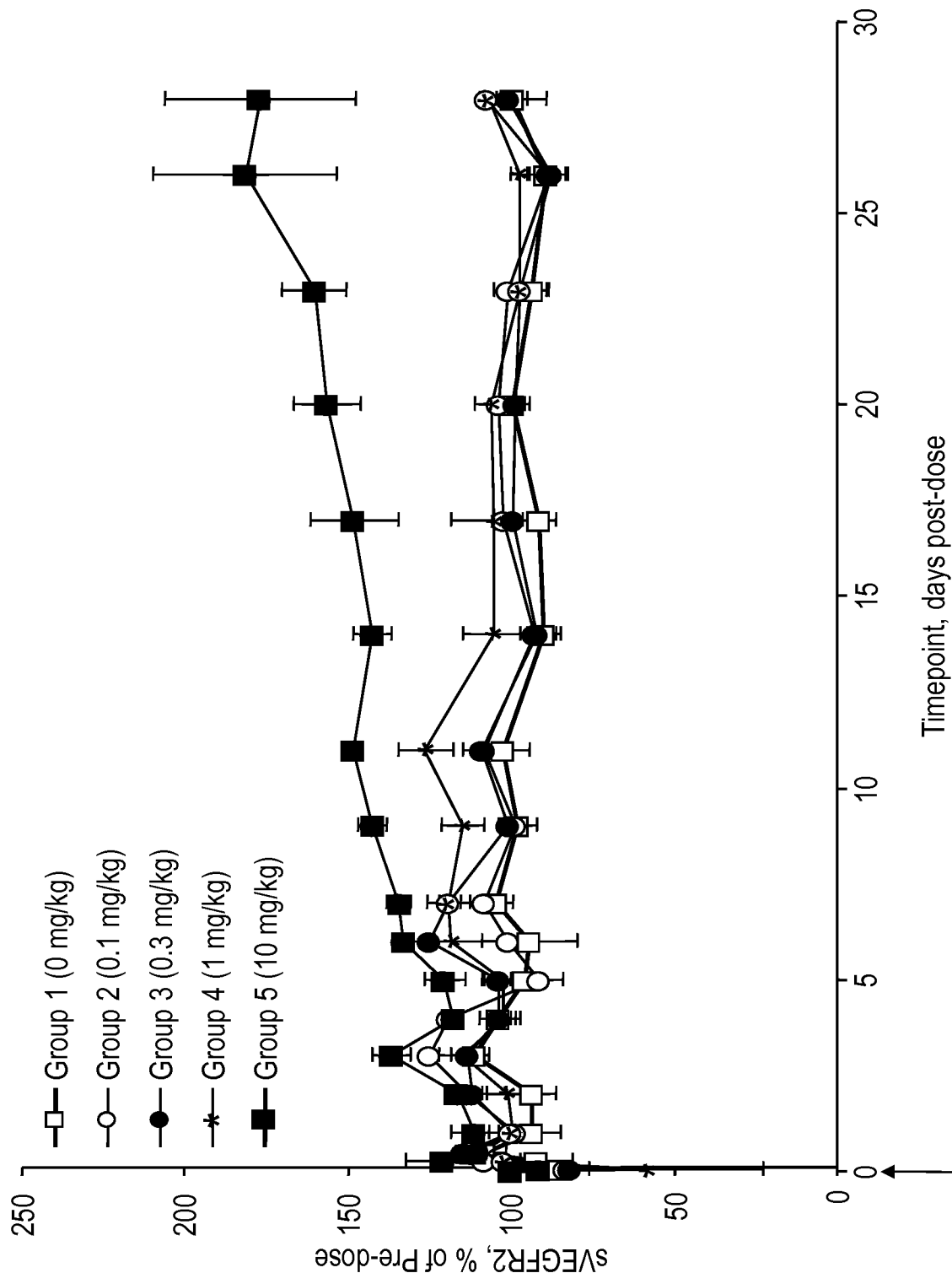


FIG. 24C
VEGFR1 in Cyno Plasma



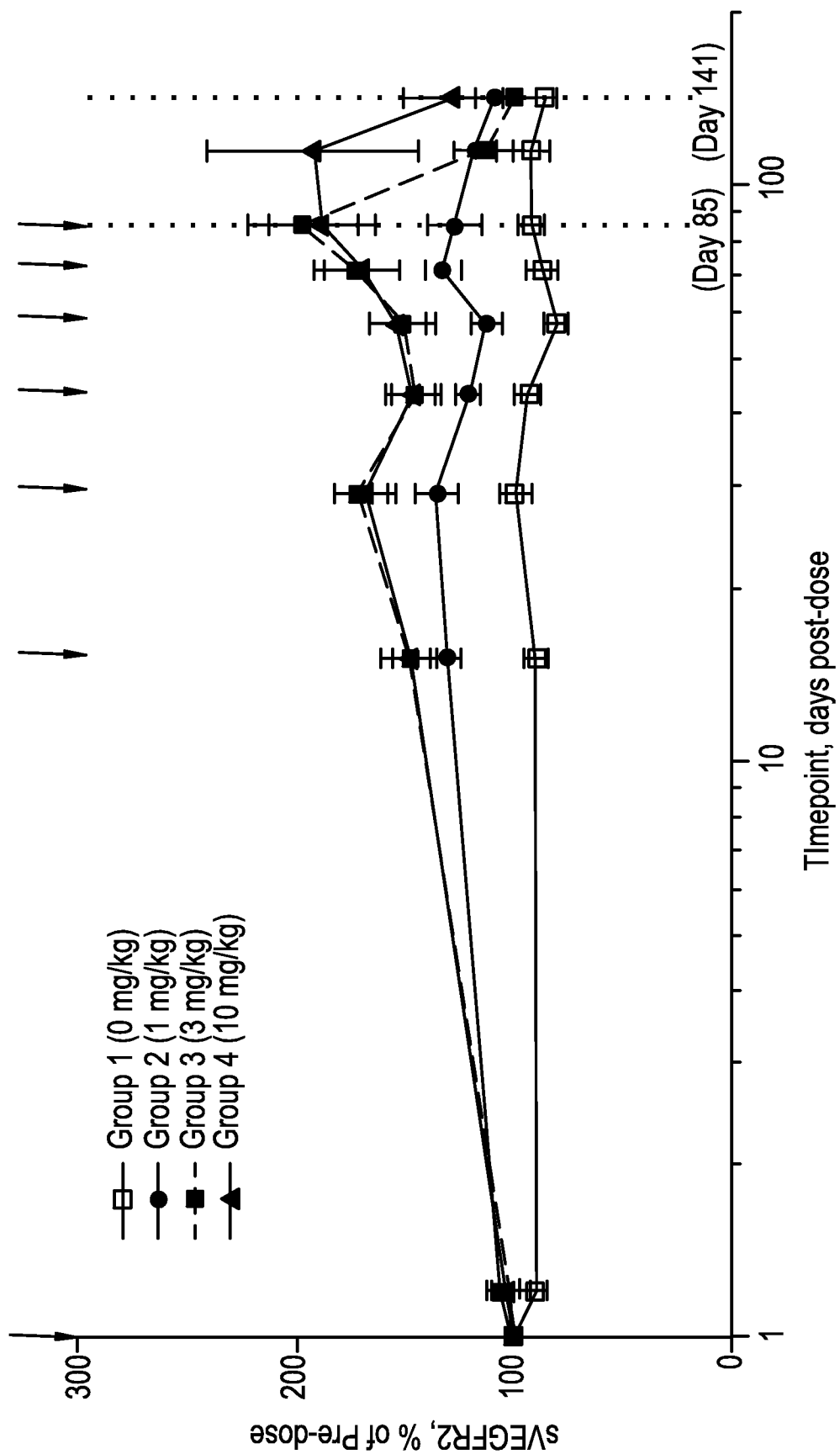
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FIG. 24D



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FIG. 24E



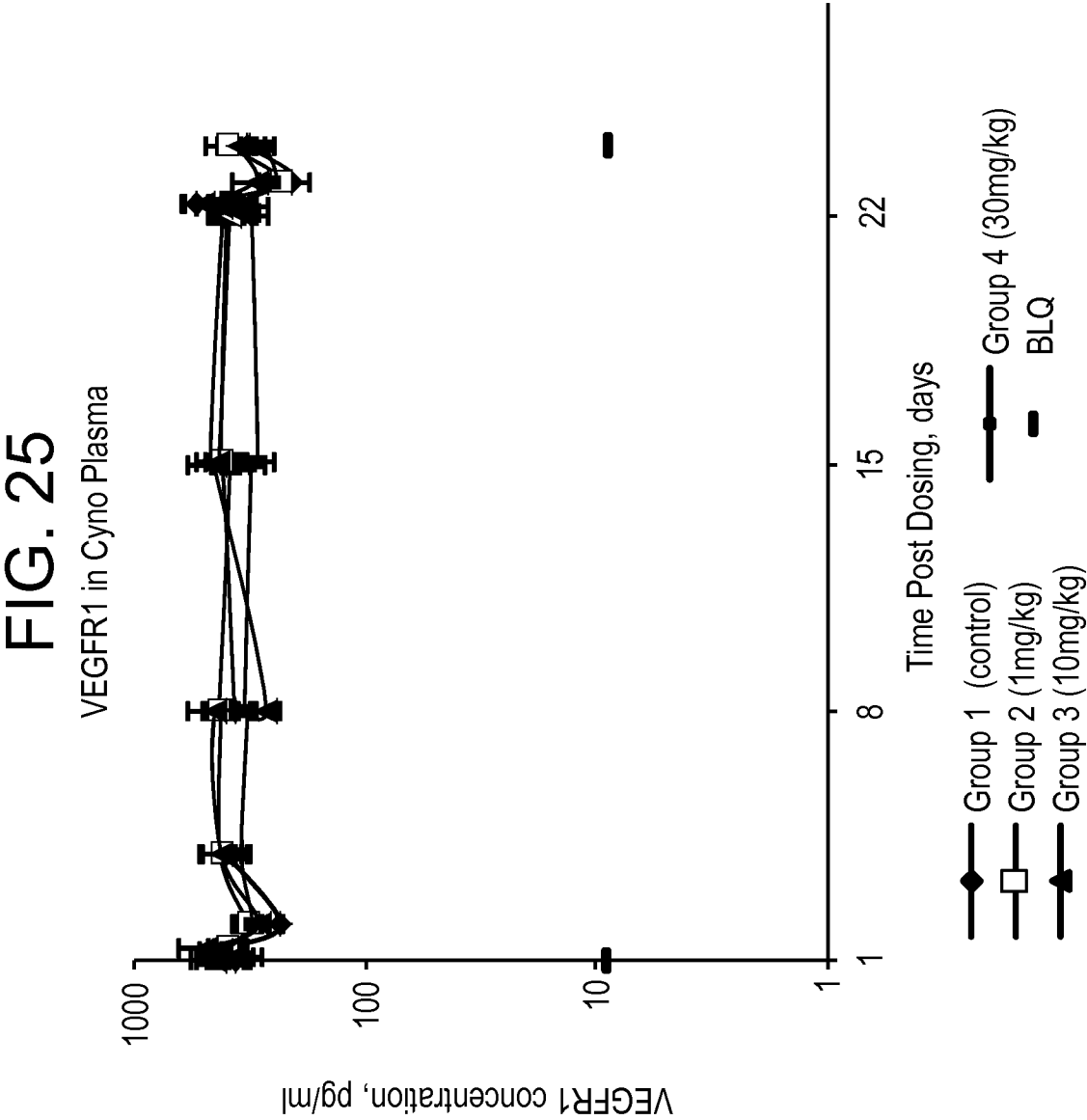


FIG. 26

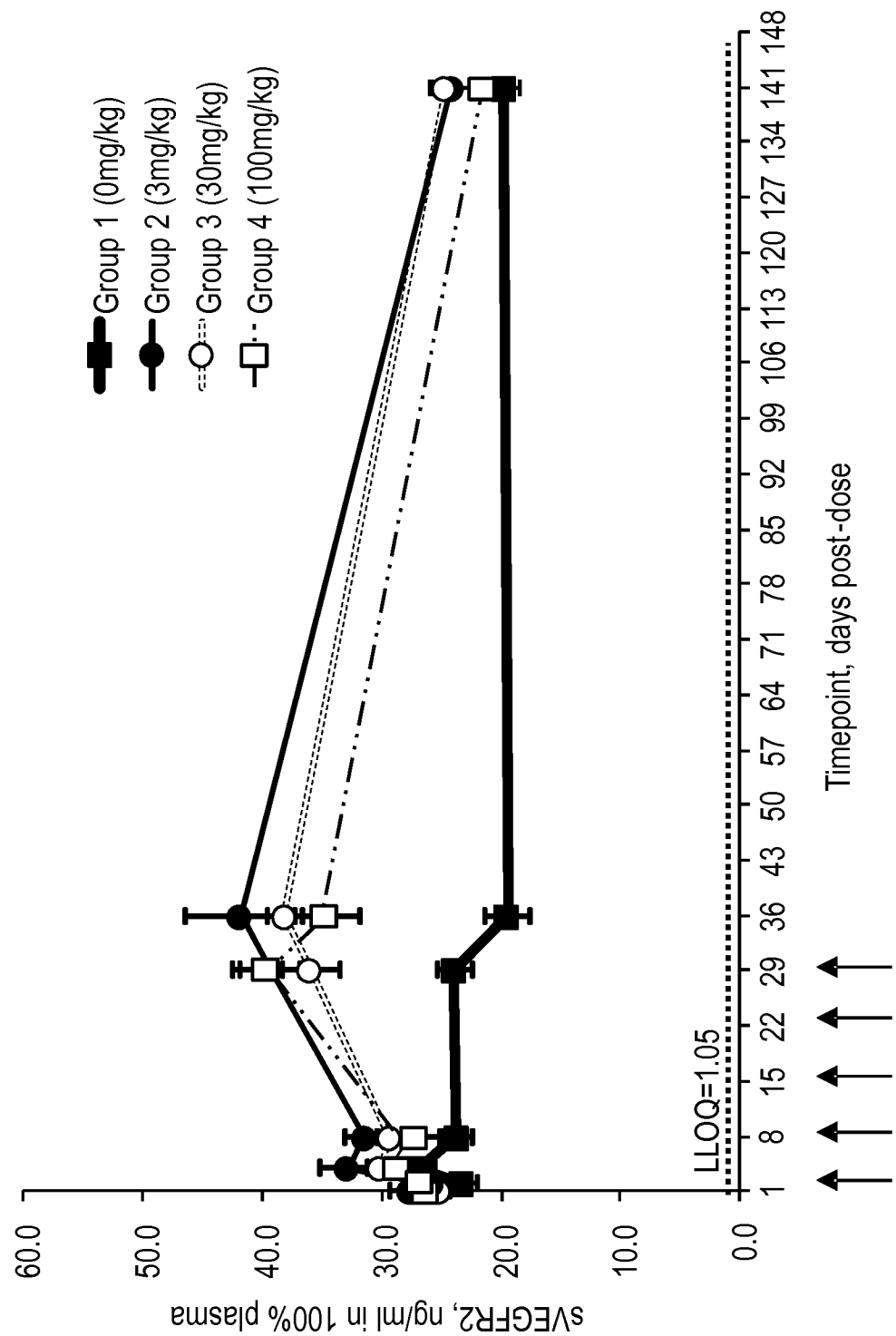


FIG. 27A
PK: MEDI0639 Time-concentration profile

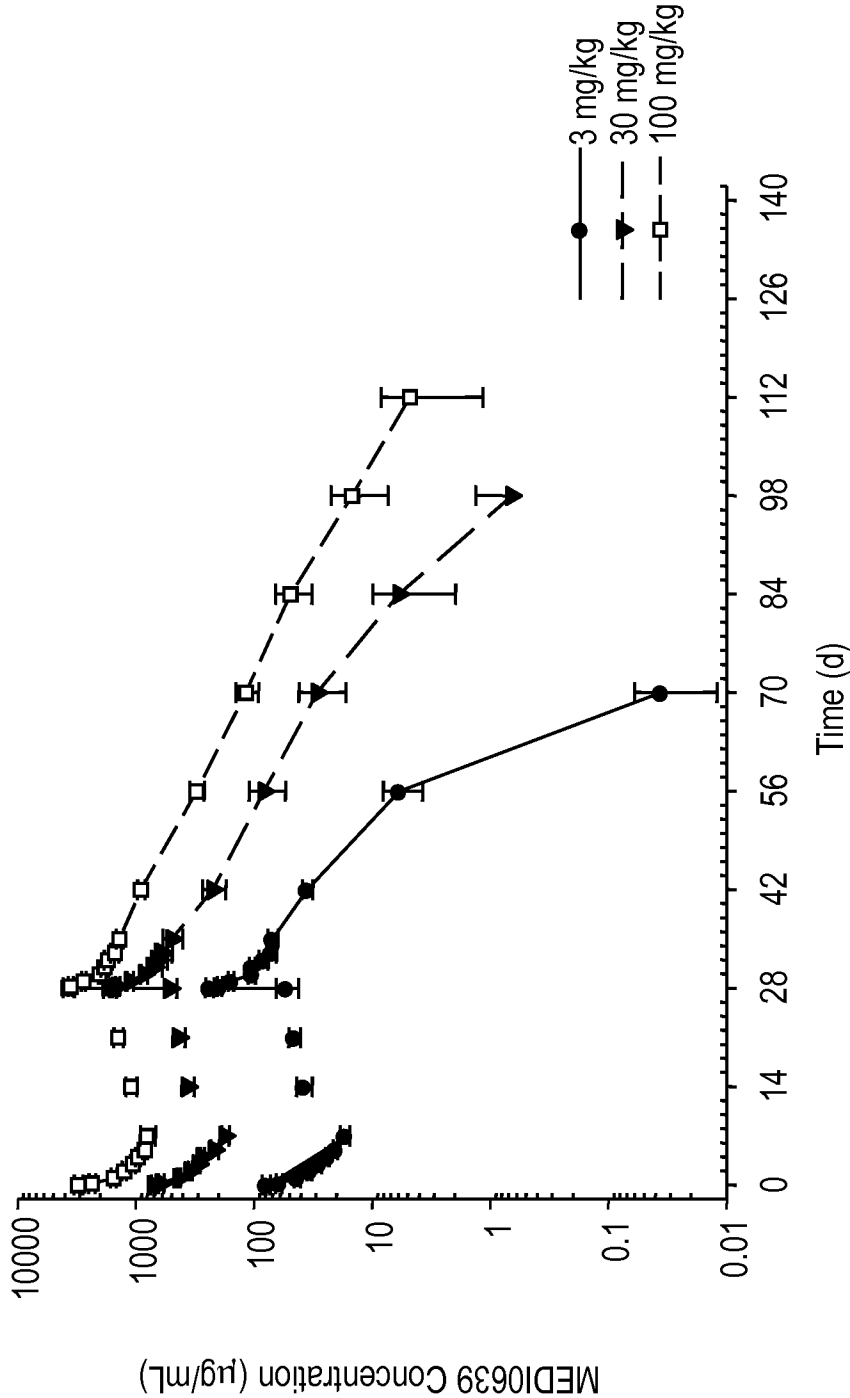
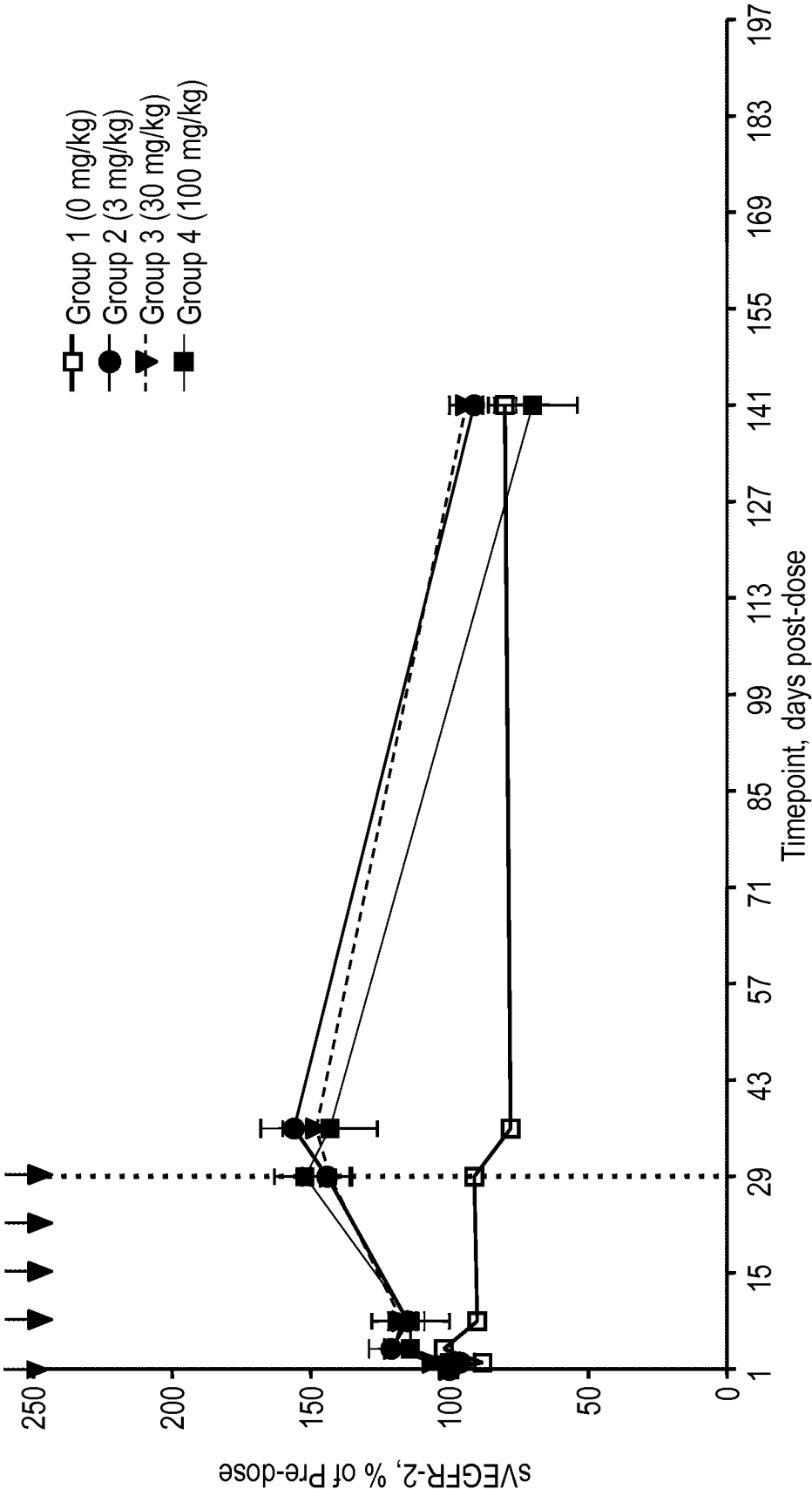


FIG. 27B

PD: sVEGFR2 Levels in Plasma



Pre-dose value graphed on Day 1
Arrows indicate times of dosing (Days 1, 8, 15, 22 and 29)

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FIG. 28

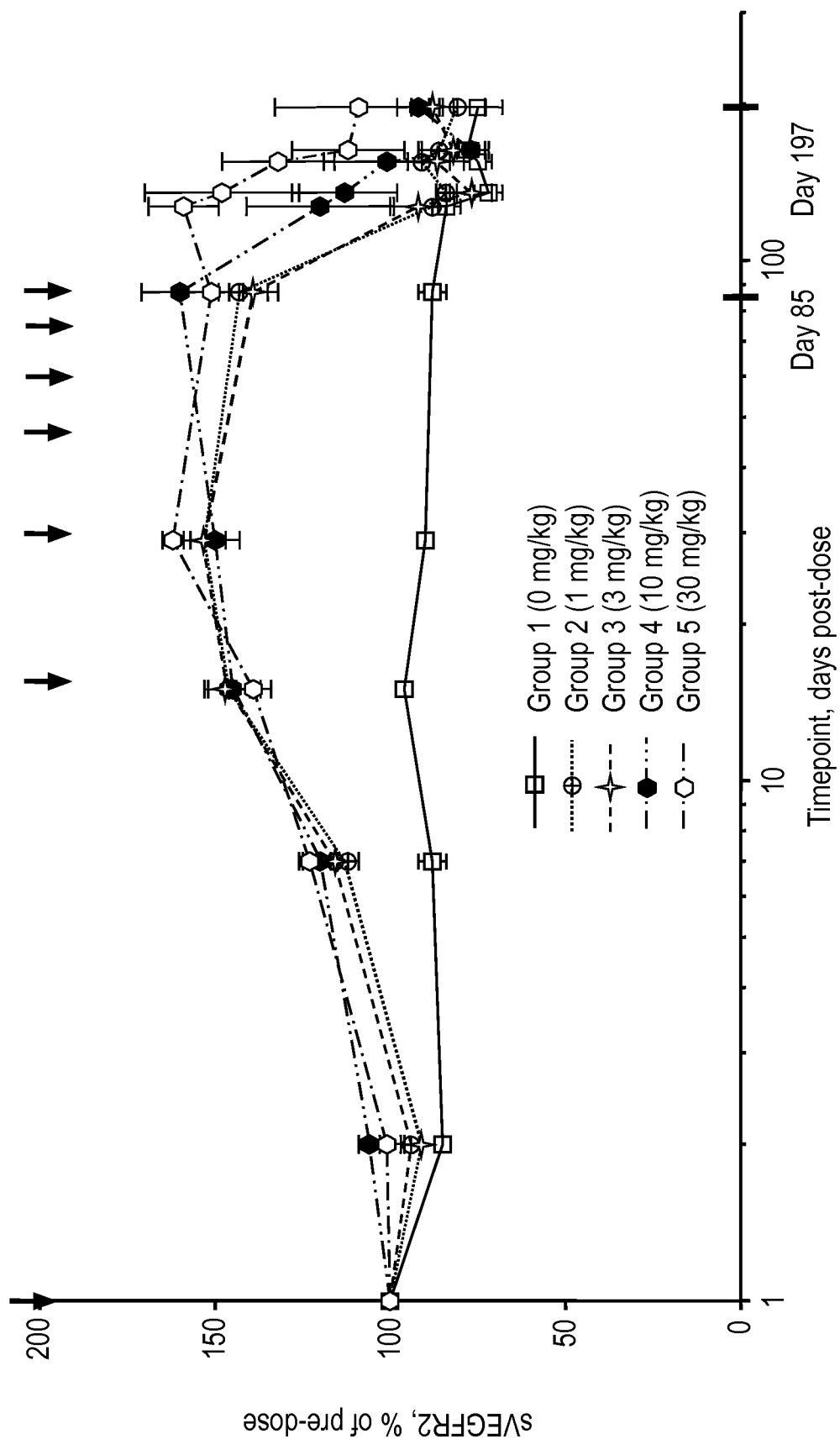


FIG. 29A
PK: MEDI0639 Time-concentration profile

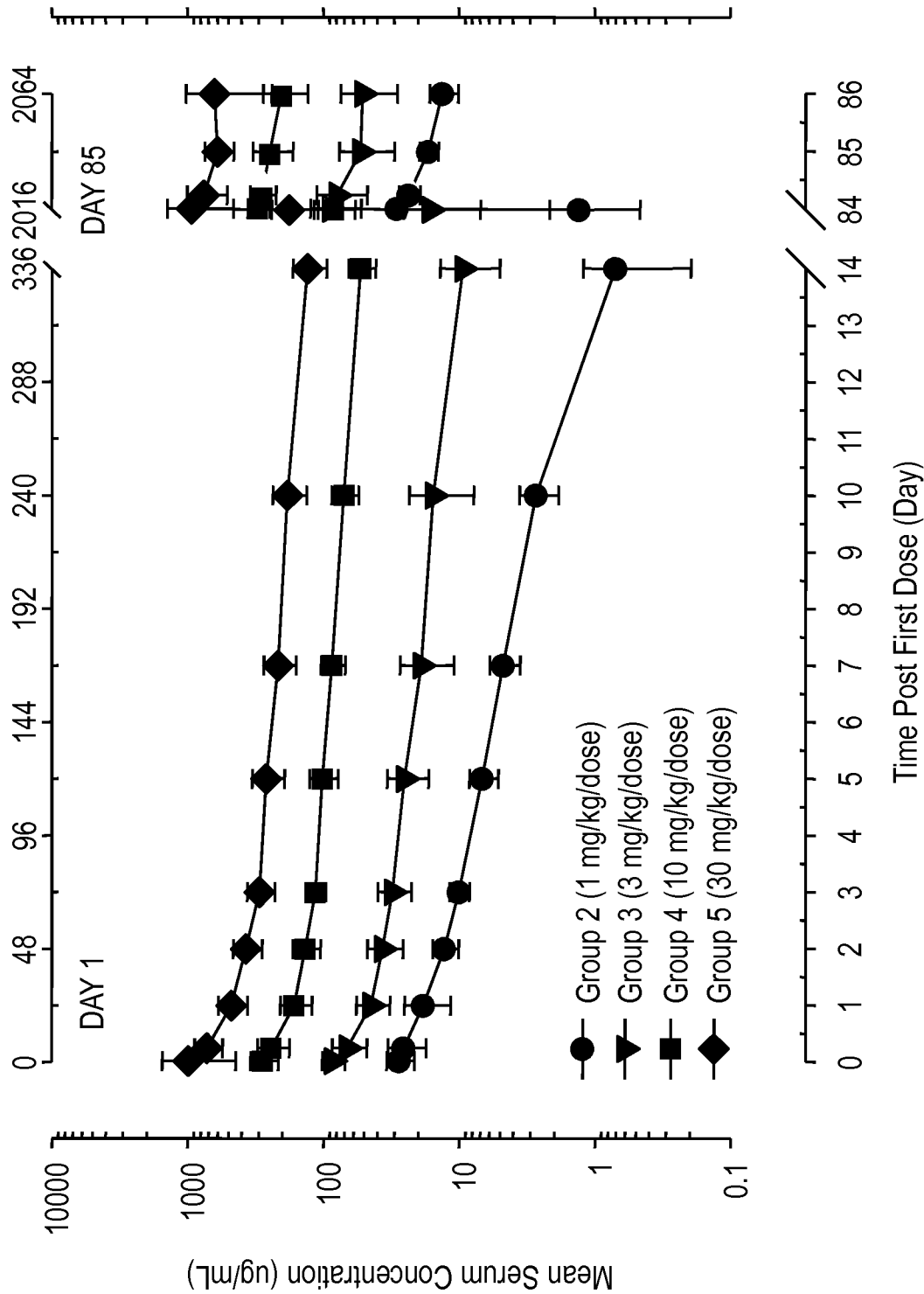


FIG. 29B

PD: sVEGFR2 Levels in Plasma

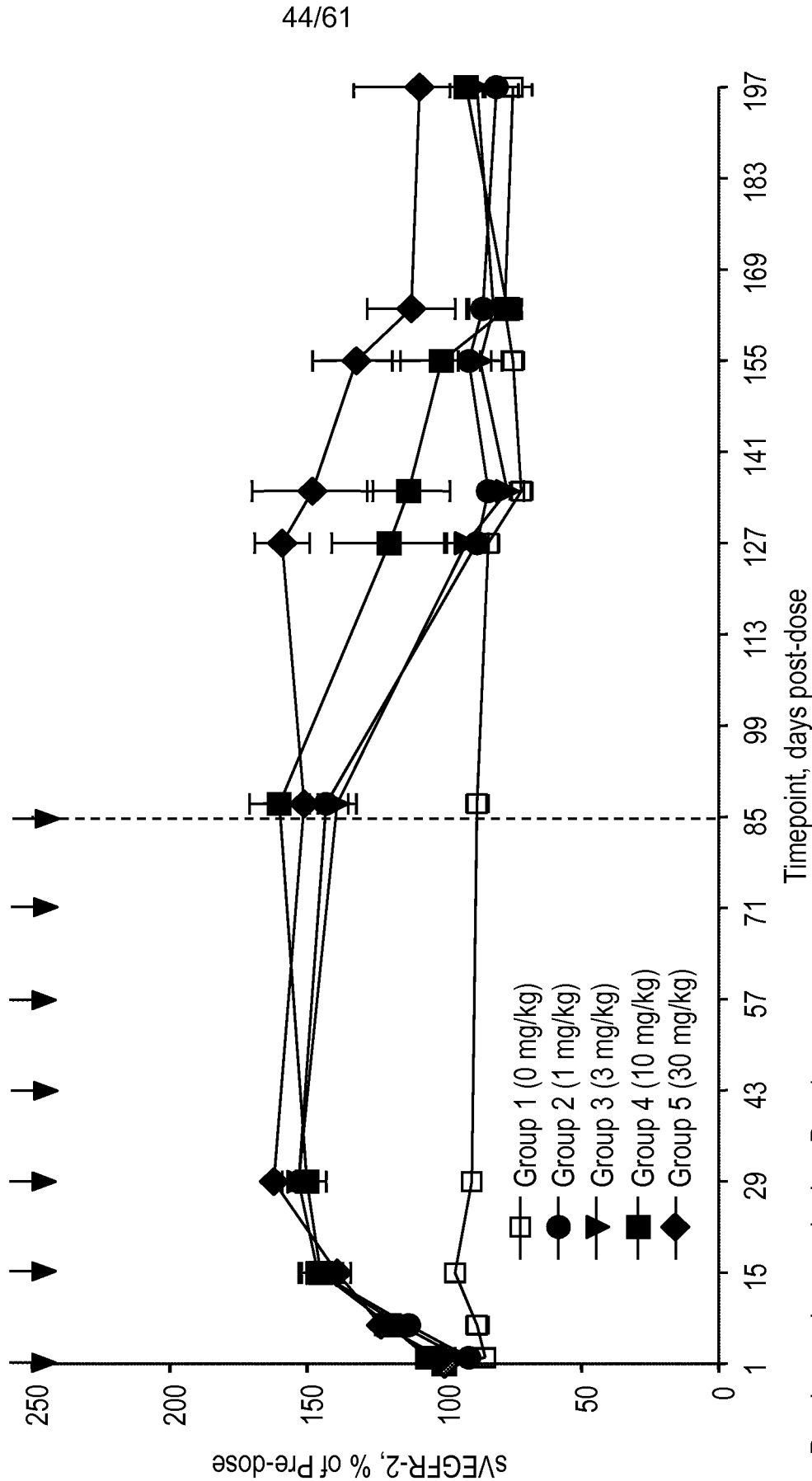


FIG. 30

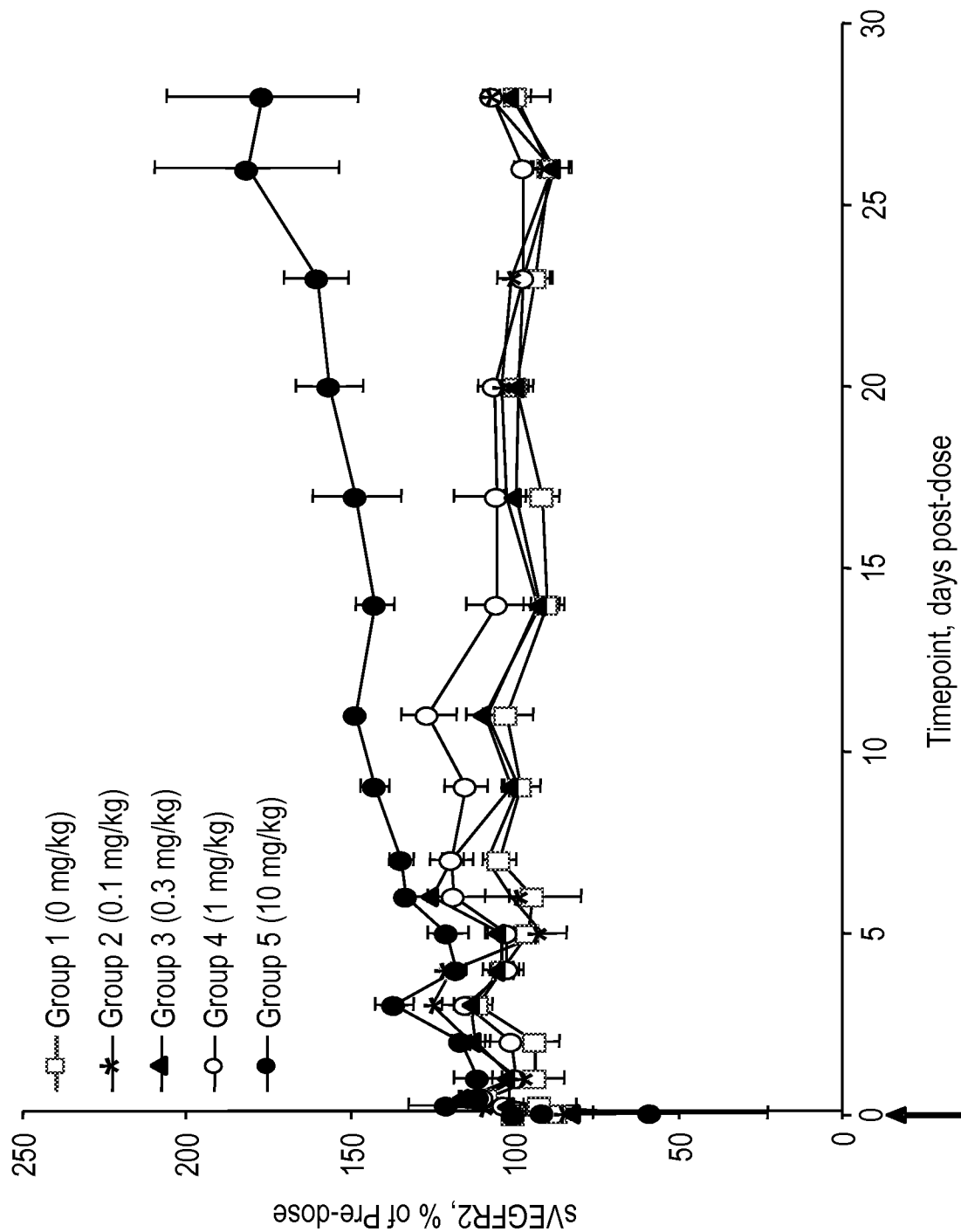
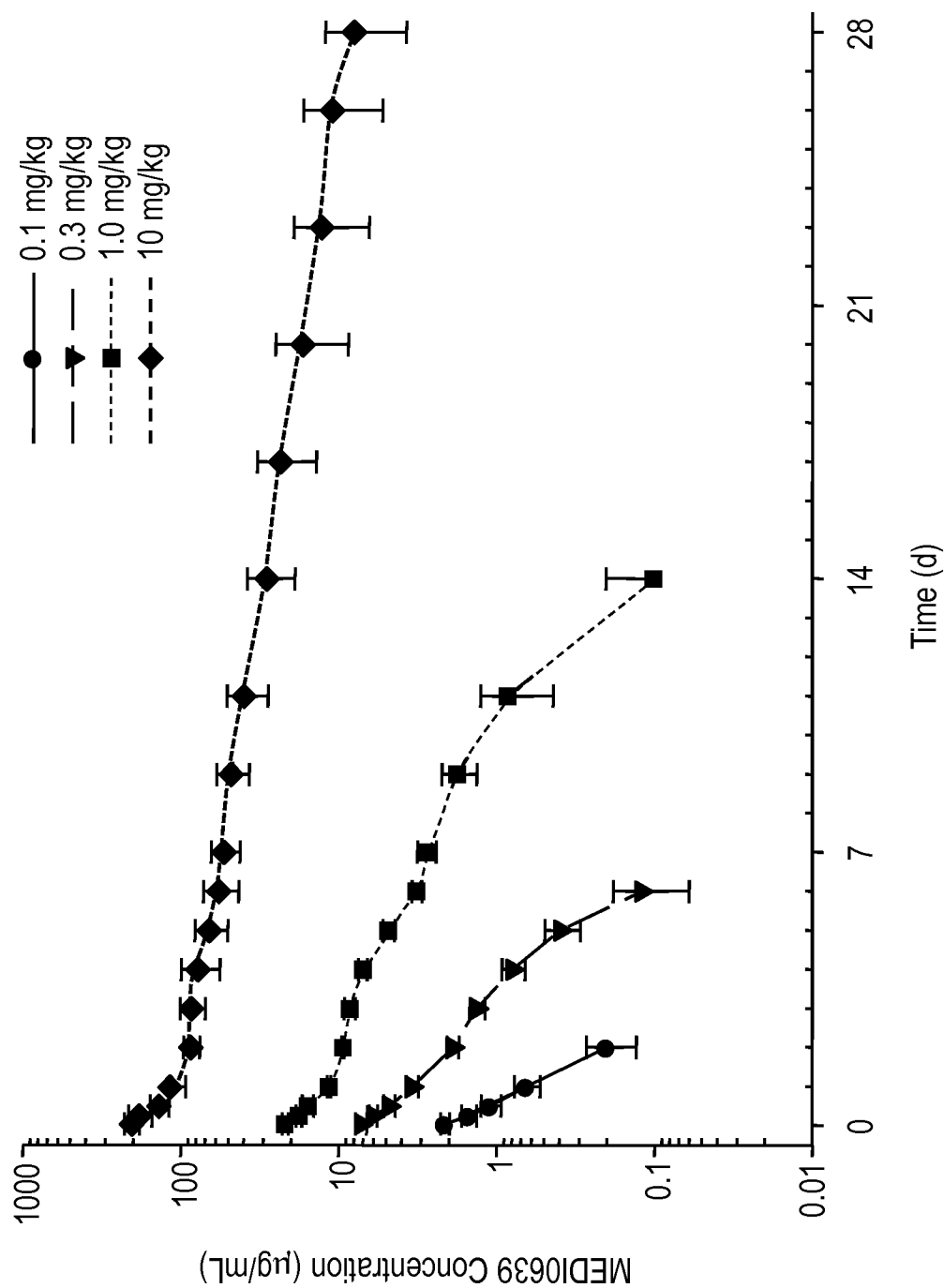


FIG. 31



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FIG. 32

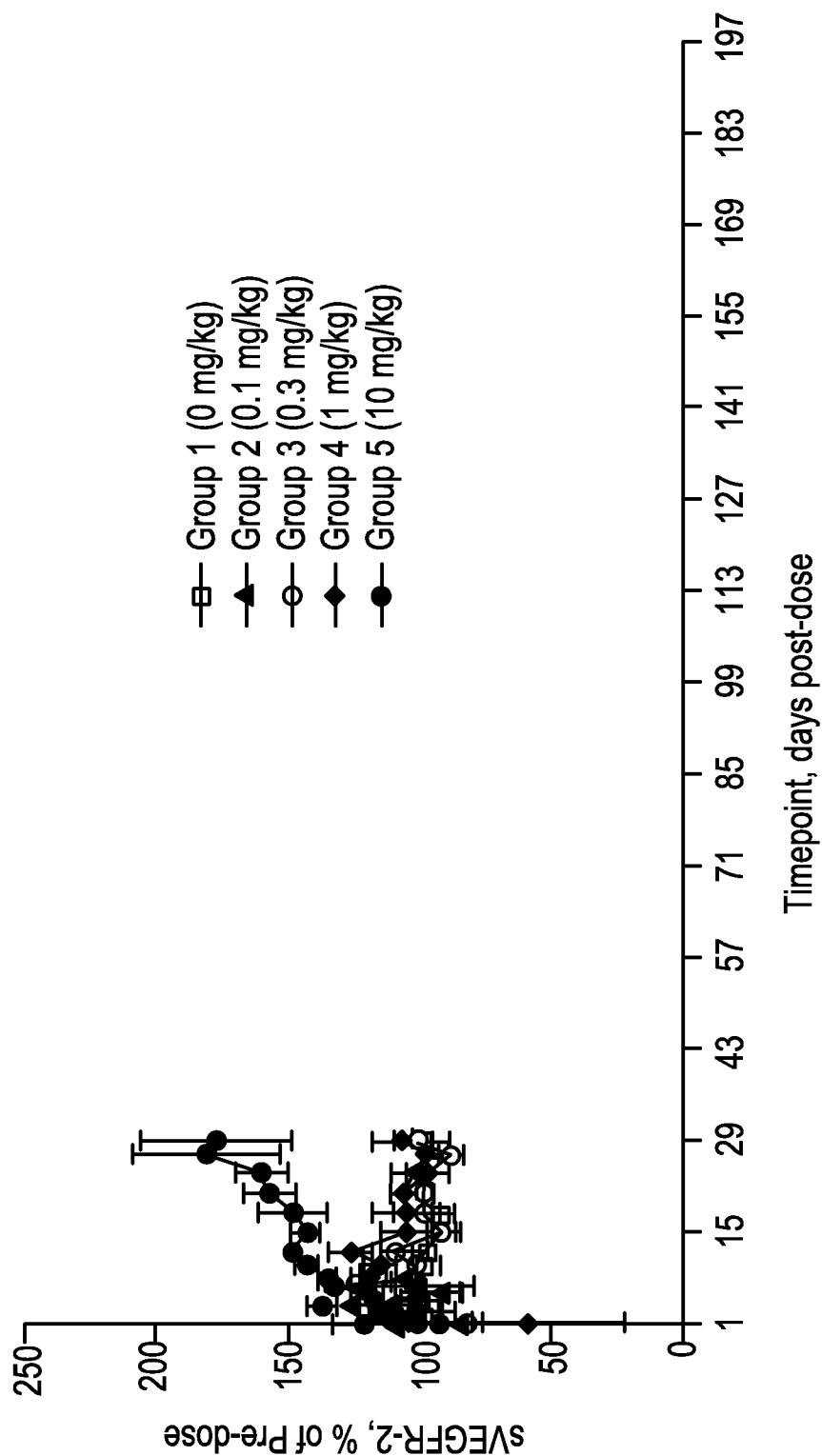


FIG. 33

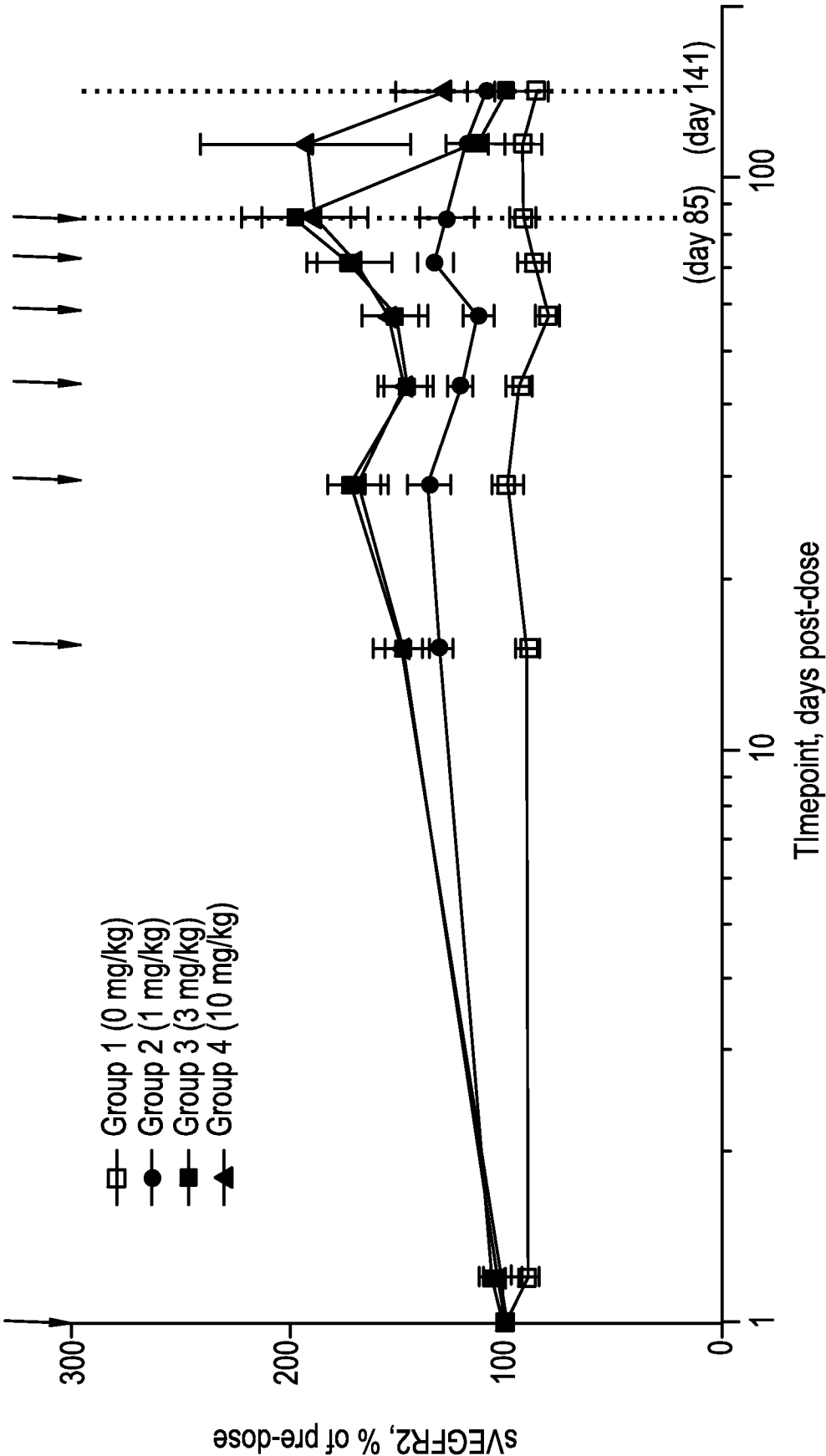


FIG. 34

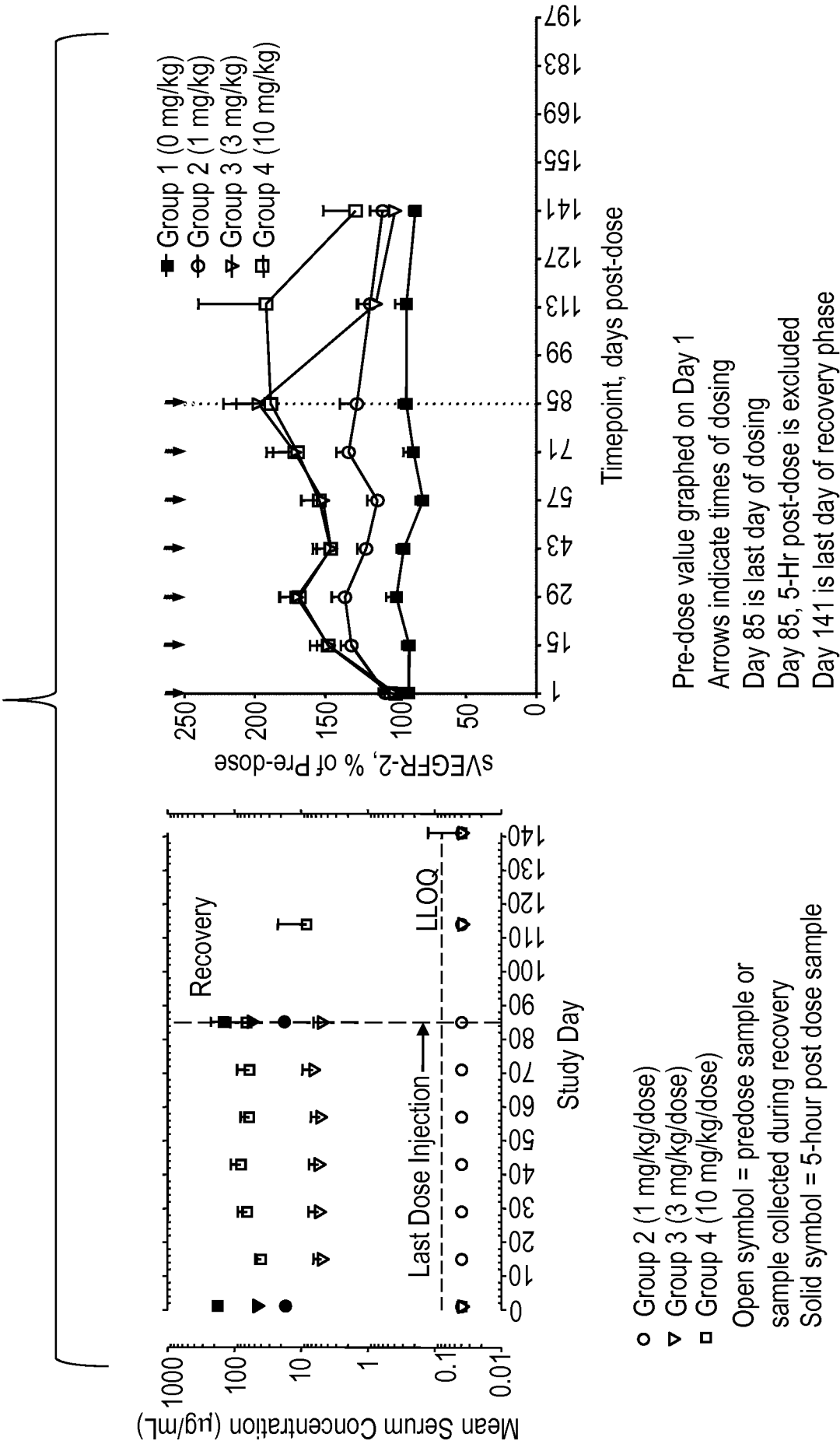


FIG. 35A

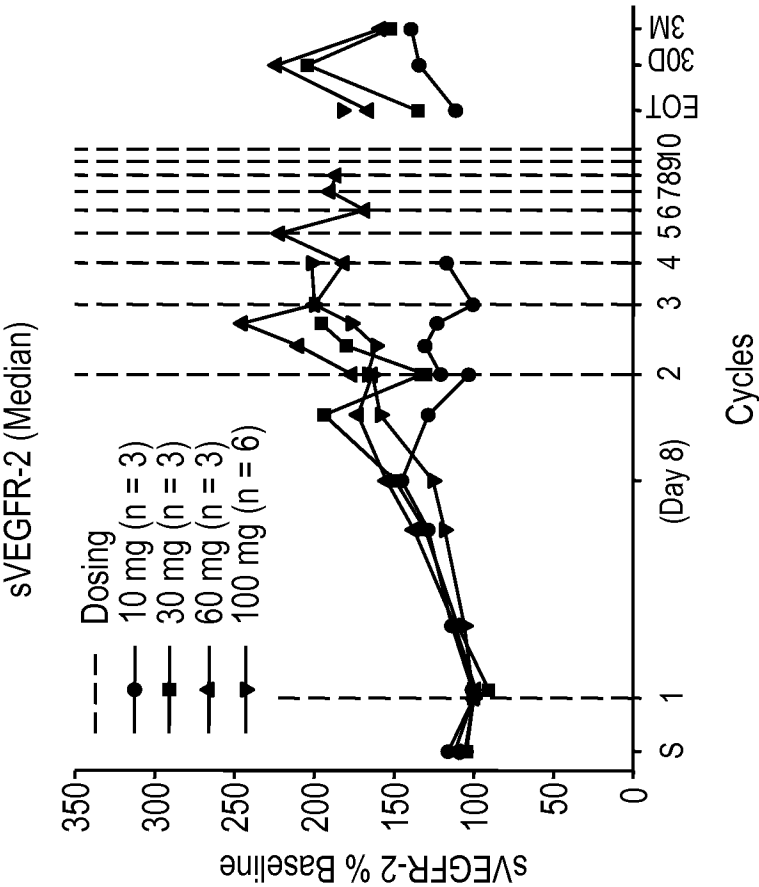
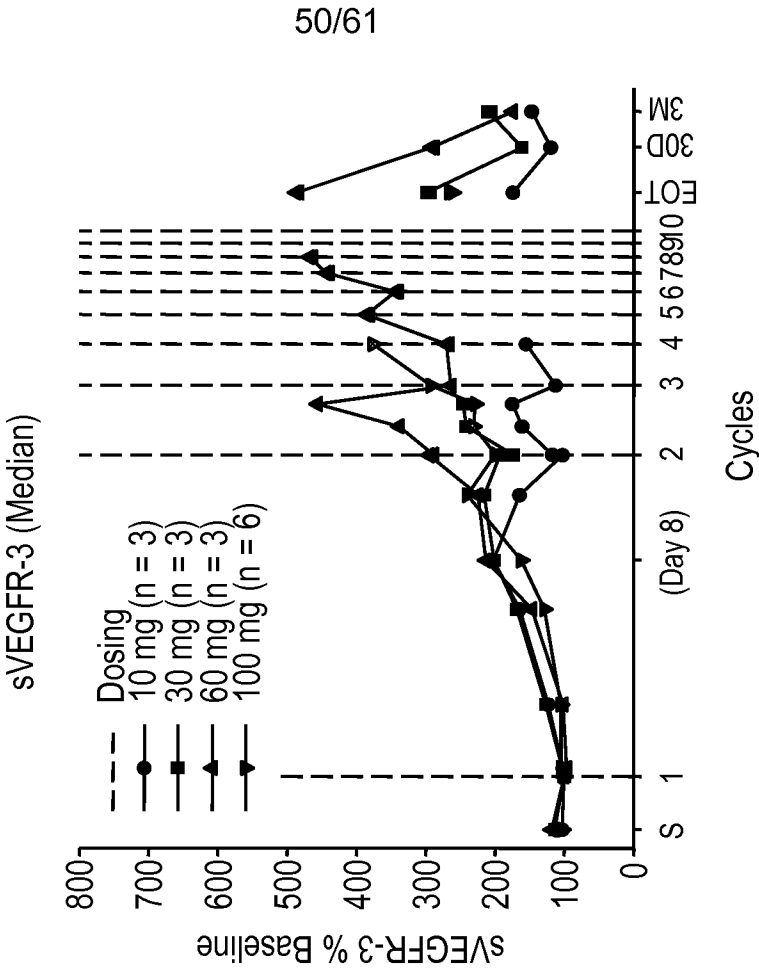


FIG. 35B



•The following time points were not plotted to scale: S = screening; EOT = end of treatment; 30D = 30 day post tx; 3M = 3 months post tx

•Cycle 1 and 2 - full profiles; Cycle 3 and beyond – only predose; Cycles were plotted on log scale.

FIG. 36A

sVEGFR-2 (Median)

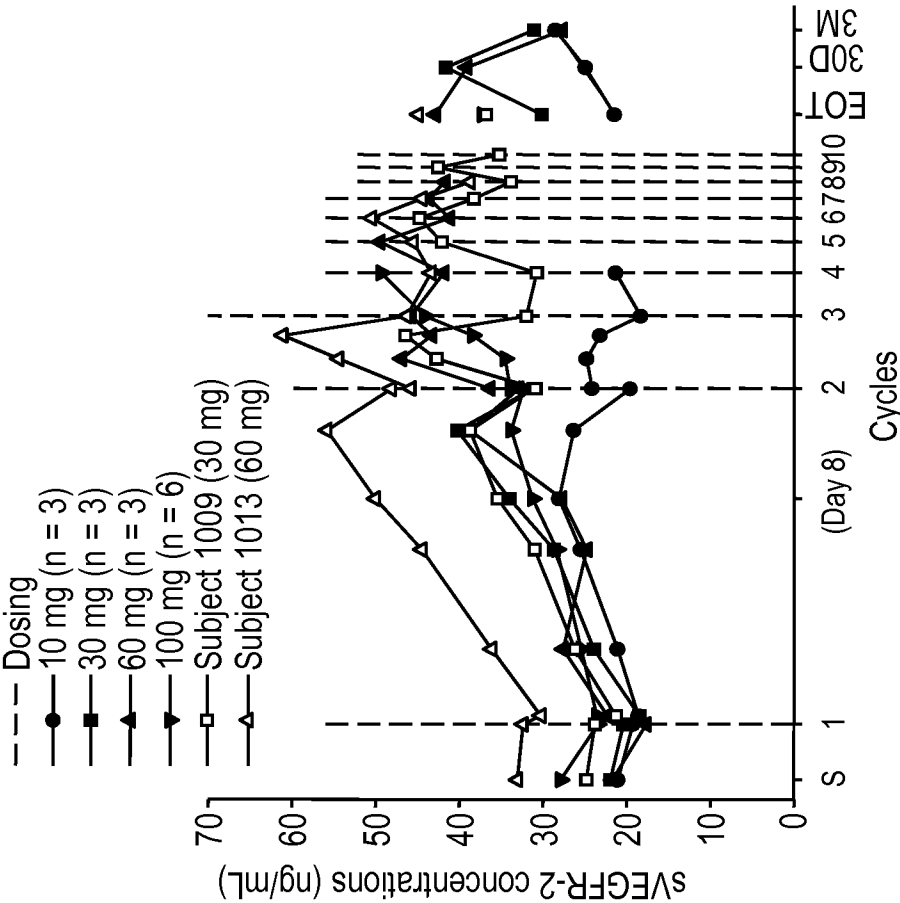


FIG. 36B

sVEGFR-3 (Median)

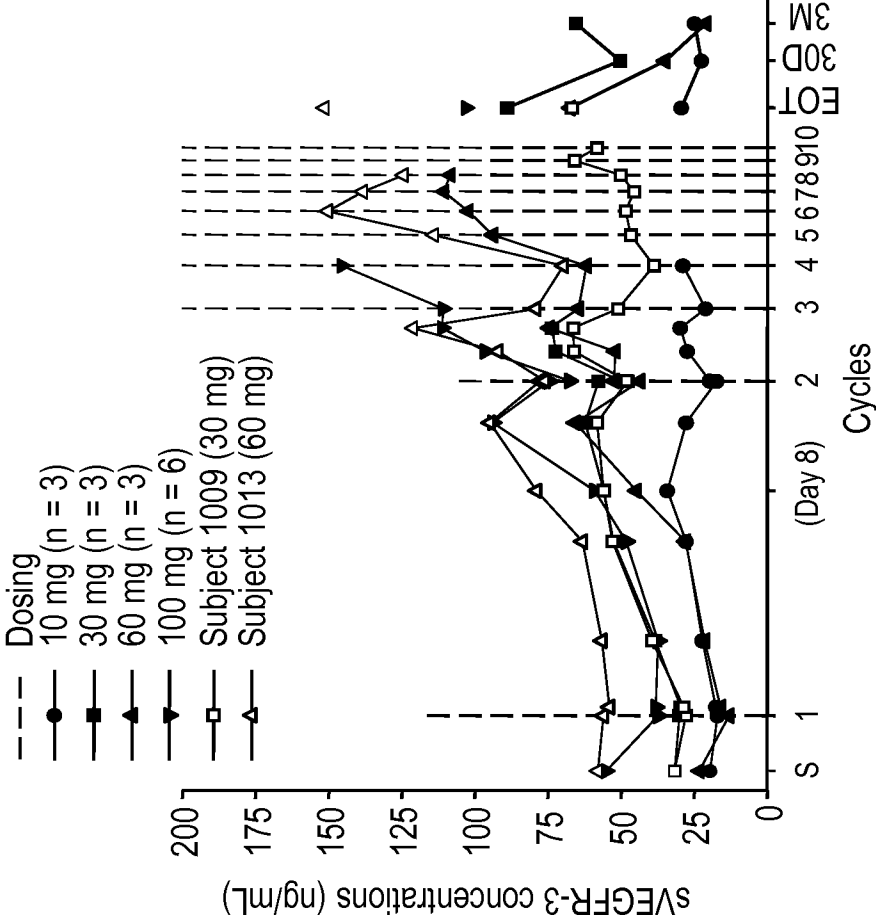


FIG. 37B
60 mg Cohort

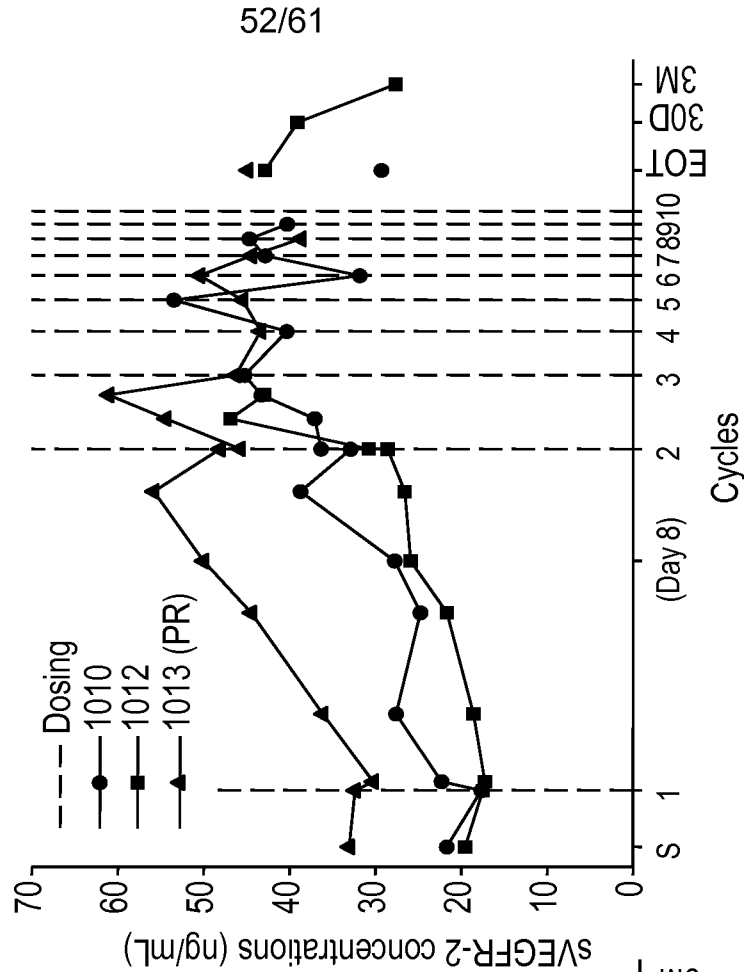


FIG. 37A
30 mg Cohort

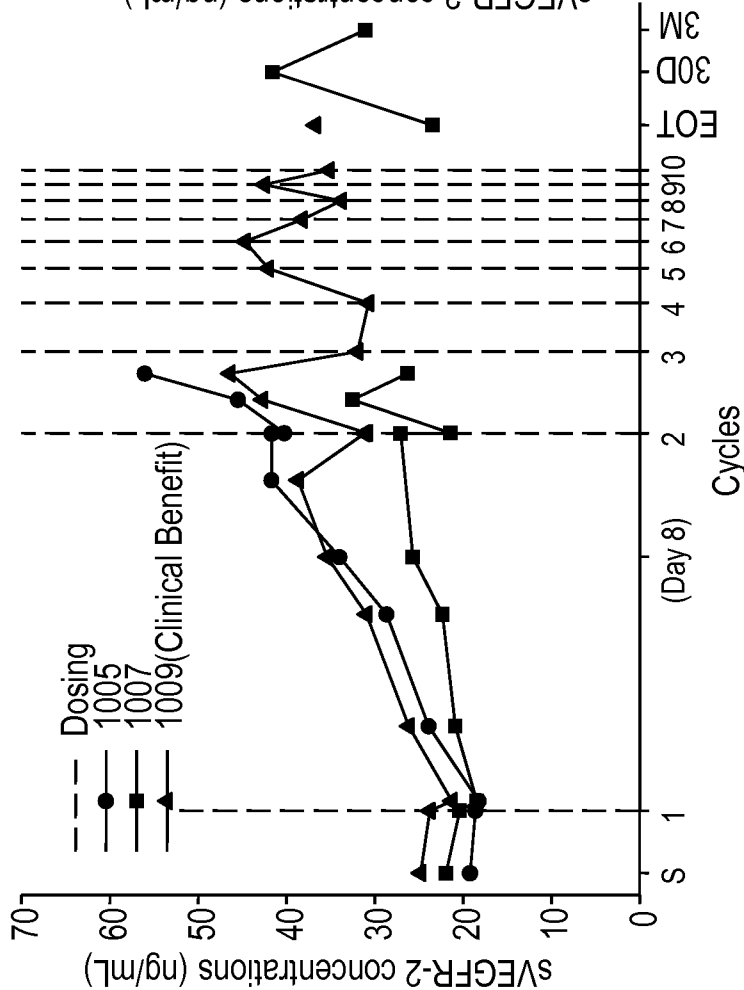


FIG. 37C
100 mg Cohort

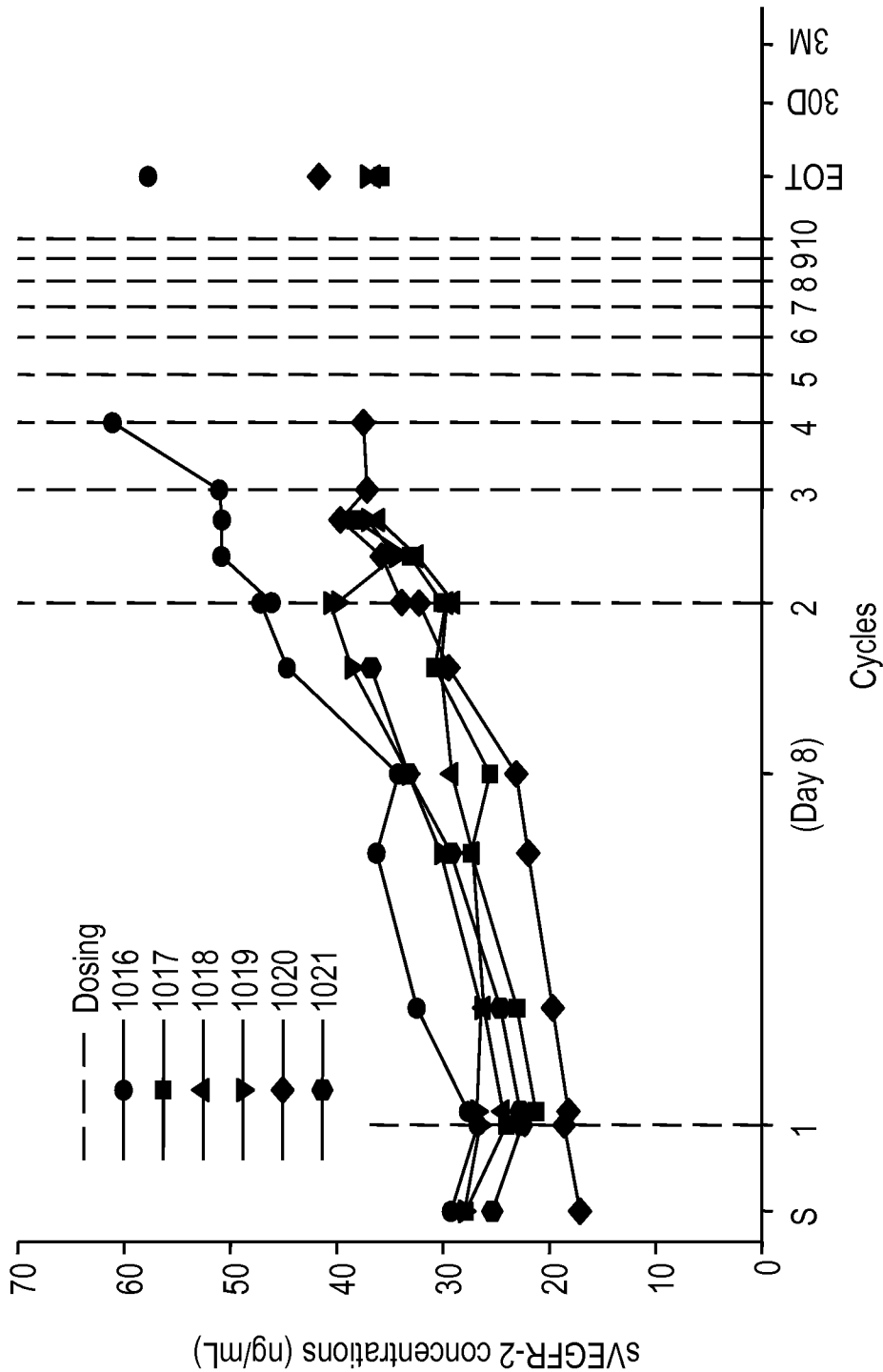


FIG. 38

Visualization of Exposure & Selected Events

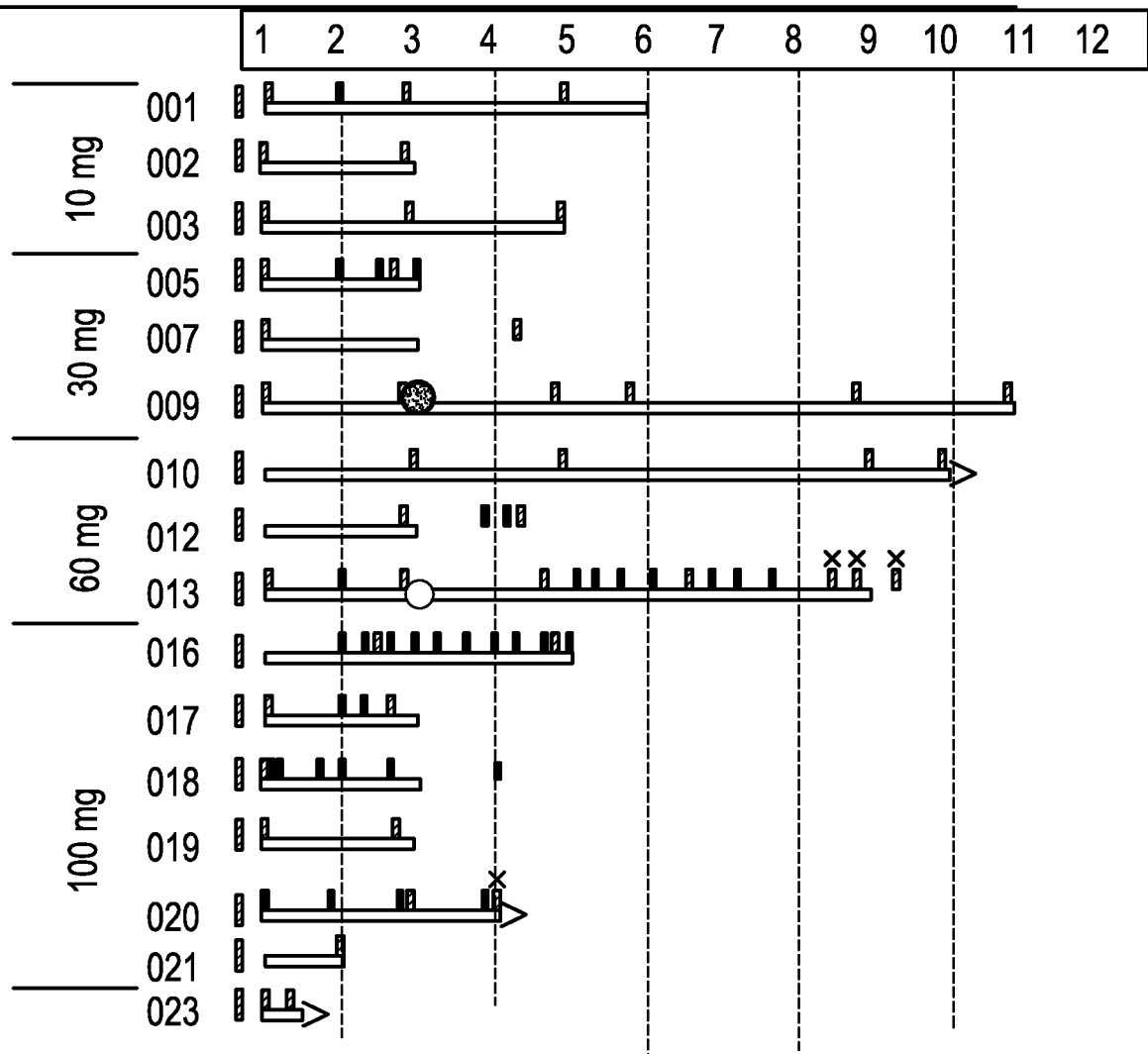


FIG. 39A

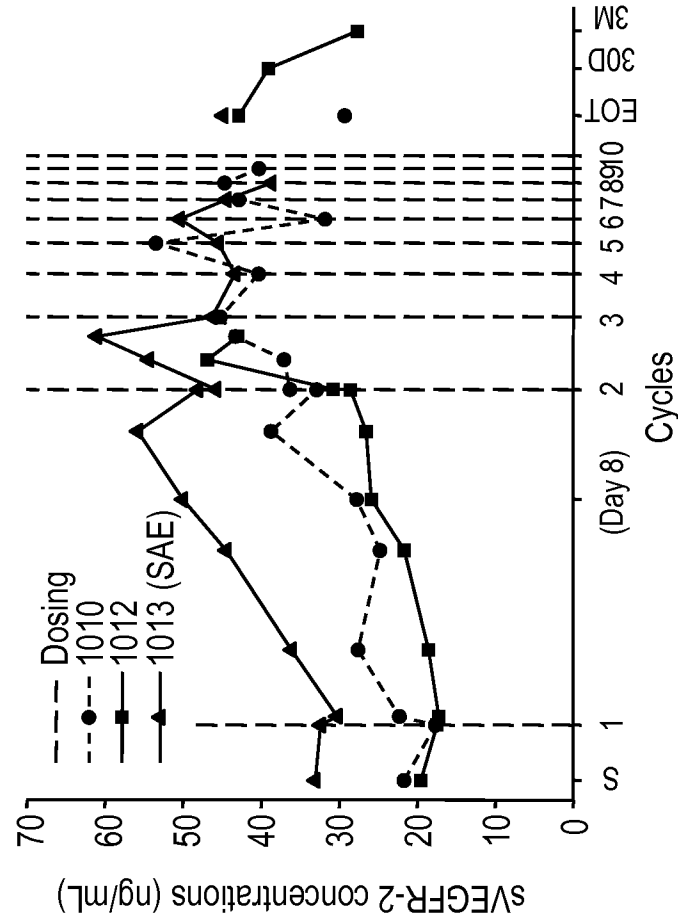
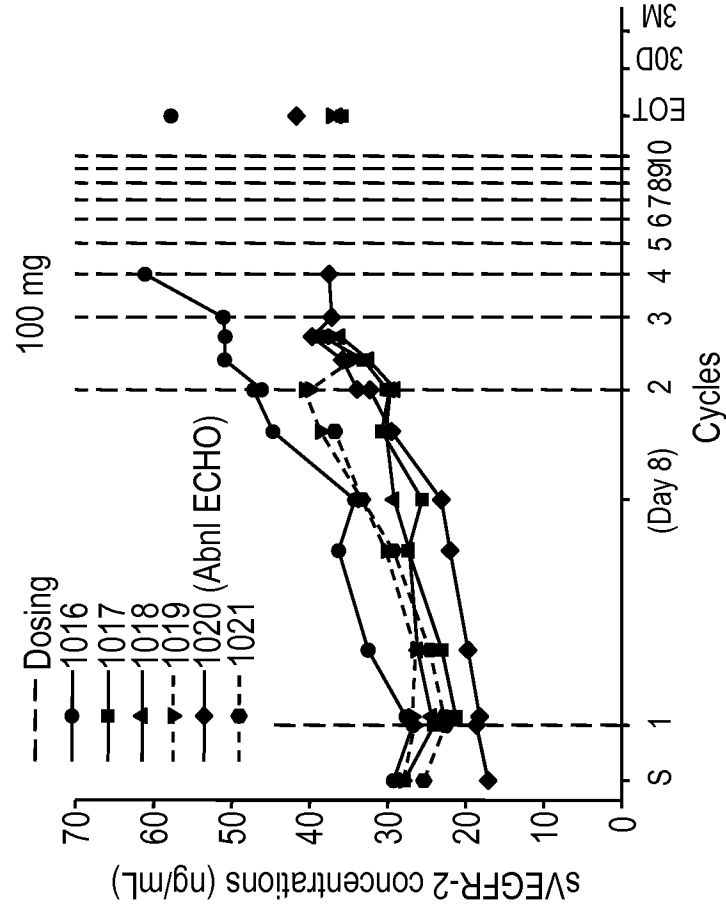
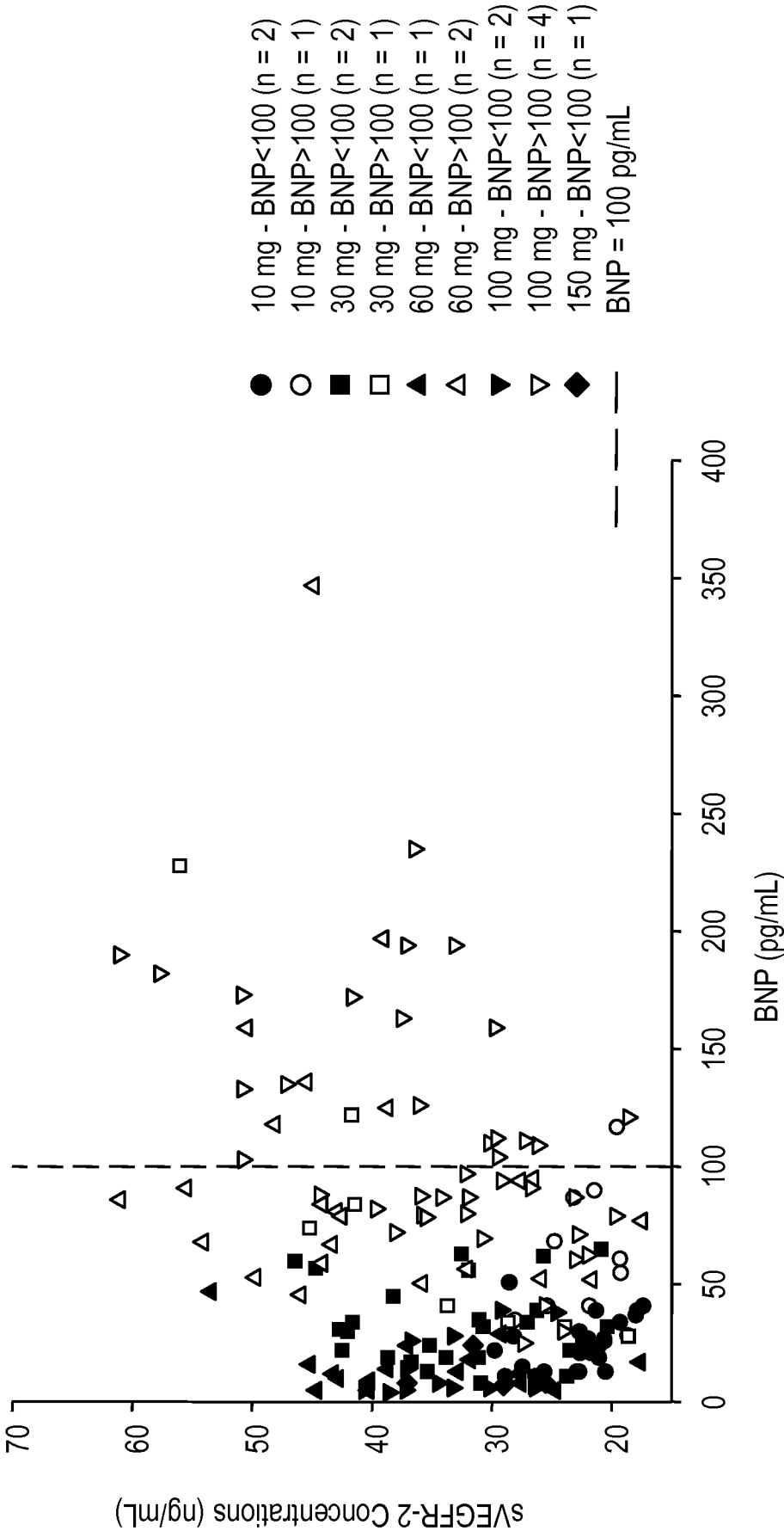


FIG. 39B



- The following time points were not plotted to scale: S = screening; EOT = end of treatment; 30D = 30 day post tx; 3M = 3 months post tx
- Cycle 1 and 2 - full profiles; Cycle 3 and beyond – only predose; Cycles were plotted on log scale.
- Subjects with BNP>100 (solid line)
- Subjects without BNP elevations (dash line)

FIG. 40



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FIG. 41

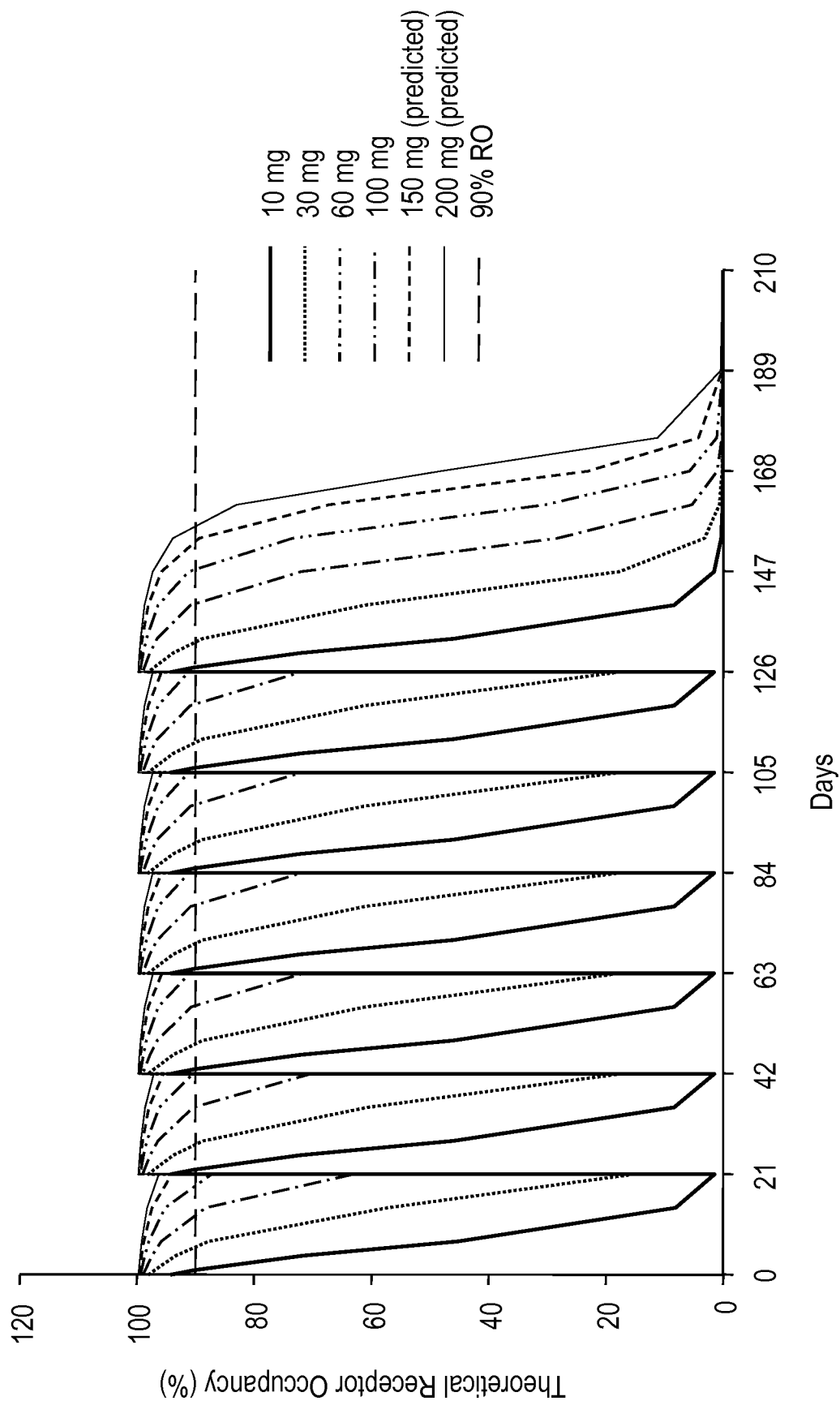


FIG. 42

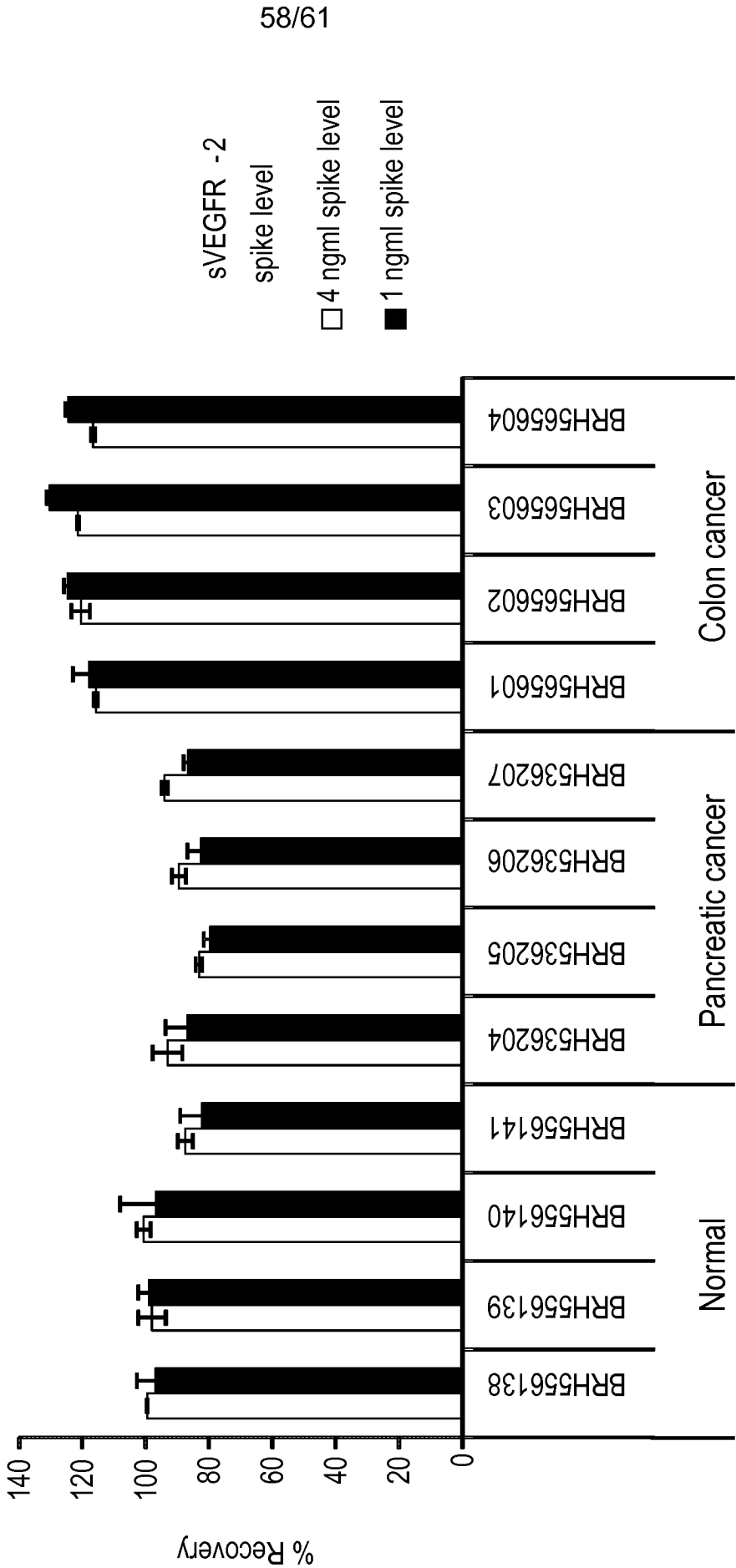
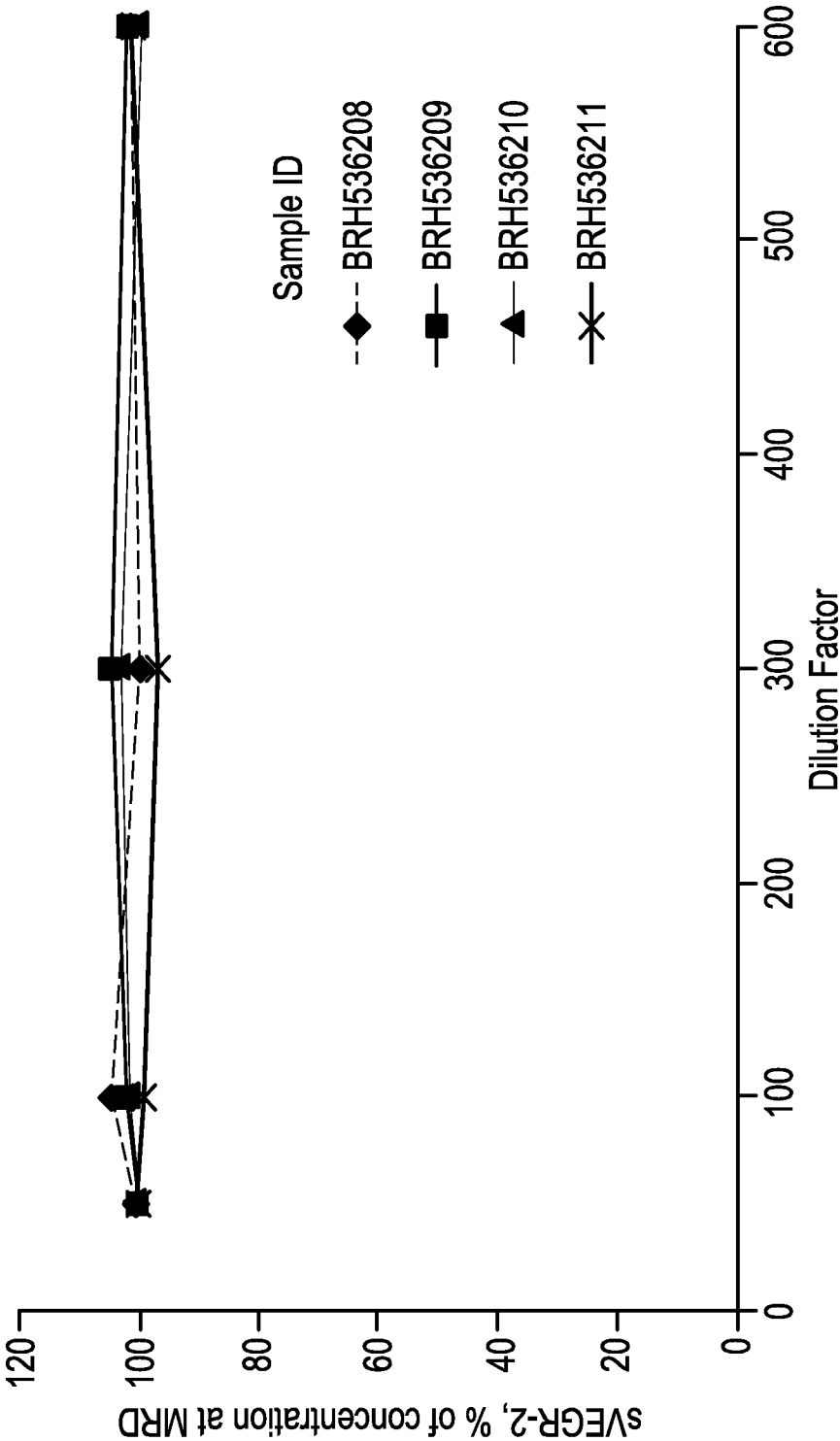


FIG. 43



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FIG. 44

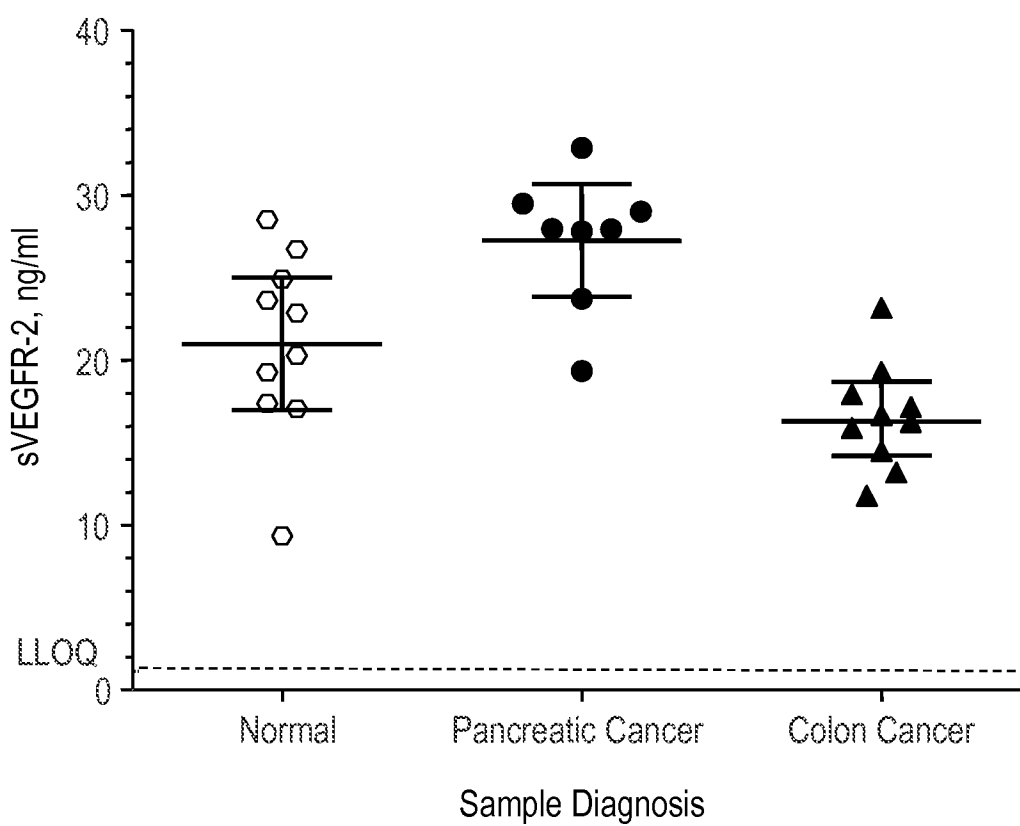
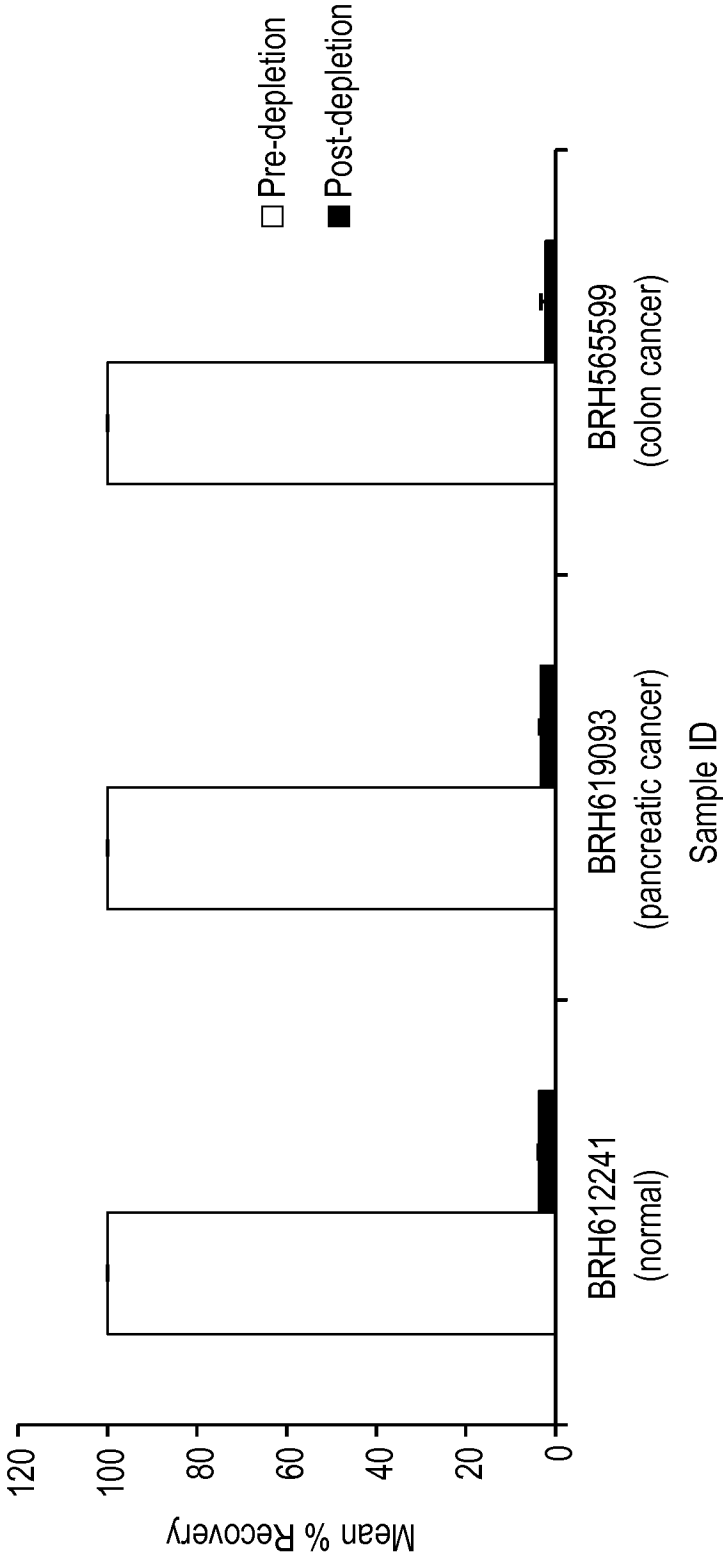


FIG. 45



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 15/17467

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 39/00, C12Q 1/68 (2015.01)

CPC - A61K 38/00

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)

IPC(8)- A61K 39/00, C12Q 1/68 (2015.01)

CPC - A61K 38/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC- 424/139.1, 435/6.13 (keyword search, terms limited)Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase (PGPB, USPT, USOC, EPAB, JPAB, DWPI, TDBD), FreePatentsOnline (US Pat, PubMed, EPO, JPO, WIPO, NPL), GoogleScholar (PL, NPL); search terms: DLL4 sVEGFR sVEGFR2 VEGFR2 side effects biomarker administering DLL4 antagonist patient pharmacodynamic

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2013/0164295 A1 (Gurney et al.) 27 June 2013 (27.06.2013) Abstract, para [0044], [0106]-[0110], [0290]-[0297]	1-6
Y	Dubois et al., Phase I and Pharmacokinetic Study of Sunitinib in Pediatric Patients with Refractory Solid Tumors: A Children's Oncology Group Study, Clin Cancer Res; 17(15); 5113-22 (2011) abstract, pg 5113, col 1, para 1; pg 5118, col 2, para 2 to pg 5119, col 1, para 1, pg 5121, col 1, para 3, Fig. 1C	1-6

☐ Further documents are listed in the continuation of Box C.

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"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

15 June 2015 (15.06.2015)

Date of mailing of the international search report

07 JUL 2015

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Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 15/17467

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.: 7-29, 32-50, 56-58
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:
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 examined, the appropriate additional examination fees must be paid.

Group I, claims 1-6, directed to a method of determining a justified DLL4 antagonist dose to the patient.

Group II, claims 30-31, and 51-55, directed to a method for detecting efficacy of DLL4 antagonist.

The groups 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:

-----Continued in the supplemental box-----

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.: 1-6.

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 15/17467

continuation of Box NO. III Observations where unity of invention is lacking

Special Technical Features

The special technical feature of Group I is a method of determining a justified DLL4 antagonist dose to the patient, not required in Group II.

The special technical feature of Group II is a method for detecting efficacy of DLL4 antagonist.

Common Technical Features

The inventions of Groups I and II share the common feature of administering a DLL4 antagonist to a patient, detecting the level of at least one biomarker chosen from sVEGFR-2 and sVEGFR-3 in the subject for at least one time point after administration of the first DLL4 antagonist; comparing the level of sVEGFR-2 and/or sVEGFR-3 to a reference standard.

However, these technical features are not unifying technical features as they do not make a contribution over the prior art US 2013/0164295 A1 to Gurney et al. (hereinafter Gurney) in view of an article entitled "Phase I and Pharmacokinetic Study of Sunitinib in Pediatric Patients with Refractory Solid Tumors: A Children's Oncology Group Study" by Dubois et al. (hereinafter Dubois). Dubois teaches administering a DLL4 antagonist to a patient (para [0014], the methods of inhibiting the growth of a tumor by administering the DLL4/VEGF binding agents to a subject with a tumor), but does not specifically teach sVEGFR-2 as a biomarker. Dubois teaches administering Sunitinib, a VEGF receptor kinase inhibitor, to treat refractory solid tumors (abstract, pg 5113, col 1, para 1) wherein the angiogenic factor biomarker, plasma soluble VEGFR2 concentration decreased significantly over the course of the first cycle of therapy (pg 5118, col 2, para 2 to pg 5119, col 1, para 1). It would have been obvious to one of ordinary skill in the art to have applied the angiogenic biomarker, soluble VEGFR2, as taught by Dubois, to the method of using DLL antagonist of Gurney, since soluble VEGFR2 is demonstrated to be an useful angiogenic biomaker for tumor therapy, as taught by Dubois. Accordingly, it would have been obvious to one of ordinary skilled in the art to have provided a method comprising administering a DLL4 antagonist to a patient, detecting the level of at least one biomarker chosen from sVEGFR-2 in the subject for at least one time point after administration of the first DLL4 antagonist; comparing the level of sVEGFR-2 to a reference standard.

Therefore, Groups I-II lack unity under PCT Rule 13.

Note:

Claims 7-29, 32-50, 56-58 have been held unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Claim 54 is improperly dependent from 53. For the purposes of the present search and opinion, claim 54 is treated as dependent from claim 52.

Claim 55 is improperly self dependent. For the purposes of the present search and opinion, claim 55 is treated as dependent from the immediately preceding claim, claim 54.