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Novel modulators, including antibodies and derivatives thereof, and methods of using such modulators to treat proliferative disorders are provided.

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(54) Title: ANTI SEZ6 ANTIBODIES AND METHODS OF USE

(57) Abstract: Novel modulators, including antibodies and derivatives thereof, and methods of using such modulators to treat proliferative disorders are provided.

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LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

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CROSS REFERENCED APPLICATIONS

This application claims priority from U. S. Provisional Patent Application Ser. No. 61/603,203 filed on February 24, 2012 which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

The instant application contains a sequence listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on February 19, 2013, is named 11200.0014-00304 and is 450,013 bytes in size.

FIELD OF THE INVENTION

This application generally relates to novel compounds, compositions and methods of their use in diagnosing, preventing, treating or ameliorating proliferative disorders and any expansion, recurrence, relapse or metastasis thereof. In a broad aspect, the present invention relates to the use of seizure related 6 homolog (SEZ6) modulators, including anti-SEZ6 antibodies and fusion constructs, for the treatment, diagnosis or prophylaxis of neoplastic disorders. Selected embodiments of the present invention provide for the use of such SEZ6 modulators, including antibody drug conjugates, for the immunotherapeutic treatment of malignancies preferably comprising a reduction in tumor initiating cell frequency.

BACKGROUND OF THE INVENTION

Stem and progenitor cell differentiation and cell proliferation are normal ongoing processes that act in concert to support tissue growth during organogenesis and cell replacement and repair of most tissues during the lifetime of all living organisms. In the normal course of events cellular differentiation and proliferation is controlled by numerous factors and signals that are generally balanced to maintain cell fate decisions and tissue architecture. Thus, to a large extent it is this controlled microenvironment that regulates cell division and tissue maturation where signals are properly generated based on the needs of the organism. In this regard cell proliferation and differentiation normally occur only as

necessary for the replacement of damaged or dying cells or for growth. Unfortunately, disruption of cell proliferation and/or differentiation can result from a myriad of factors including, for example, the under- or overabundance of various signaling chemicals, the presence of altered microenvironments, genetic mutations or some combination thereof. When normal cellular proliferation and/or differentiation is disturbed or somehow disrupted it can lead to various diseases or disorders including proliferative disorders such as cancer.

Conventional treatments for cancer include chemotherapy, radiotherapy, surgery, immunotherapy (e.g., biological response modifiers, vaccines or targeted therapeutics) or combinations thereof. Unfortunately, certain cancers are non-responsive or minimally responsive to such treatments. For example, in some patients tumors exhibit gene mutations that render them non-responsive despite the general effectiveness of selected therapies. Moreover, depending on the type of cancer and what form it takes some available treatments, such as surgery, may not be viable alternatives. Limitations inherent in current standard of care therapeutics are particularly evident when attempting to treat patients who have undergone previous treatments and have subsequently relapsed. In such cases the failed therapeutic regimens and resulting patient deterioration may contribute to refractory tumors which often manifest themselves as a relatively aggressive disease that ultimately proves to be incurable. Although there have been great improvements in the diagnosis and treatment of cancer over the years, overall survival rates for many solid tumors have remained largely unchanged due to the failure of existing therapies to prevent relapse, tumor recurrence and metastases. Thus, it remains a challenge to develop more targeted and potent therapies for proliferative disorders.

SUMMARY OF THE INVENTION

These and other objectives are provided for by the present invention which, in a broad sense, is directed to methods, compounds, compositions and articles of manufacture that may be used in the treatment of SEZ6 associated disorders (e.g., proliferative disorders or neoplastic disorders). To that end, the present invention provides novel seizure related 6 homolog (or SEZ6) modulators that effectively target tumor cells and/or cancer stem cells and may be used to treat patients suffering from a wide variety of malignancies. As will be discussed in more detail herein, there are at least two naturally occurring SEZ6 isoforms or

variants and the disclosed modulators may comprise or associate selectively with one isoform or the other or with both. Moreover, in certain embodiments the disclosed SEZ6 modulators may further react with one or more SEZ family members (e.g., SEZ6L or SEZ6L2) or, in other embodiments, may be generated and selected for so as to exclusively associate or react with SEZ6 isoform(s). In any event the modulators may comprise any compound that recognizes, competes, agonizes, antagonizes, interacts, binds or associates with a SEZ6 polypeptide or gene (or fragment thereof) and modulates, adjusts, alters, changes or modifies the impact of the SEZ6 protein on one or more physiological pathways. Thus, in a broad sense the present invention is generally directed to isolated SEZ6 modulators and use thereof. In preferred embodiments the invention is more particularly directed to isolated SEZ6 modulators comprising antibodies (i.e., antibodies that immunopreferentially bind, react with or associate with at least one isoform of SEZ6) that, in particularly preferred embodiments, are associated or conjugated to one or more cytotoxic agents. Moreover, as discussed extensively below, such modulators may be used to provide pharmaceutical compositions useful for the prophylaxis, diagnosis or treatment of proliferative disorders.

In selected embodiments of the invention, SEZ6 modulators may comprise a SEZ6 polypeptide or fragments thereof, either in an isolated form or fused or associated with other moieties (e.g., Fc-SEZ6, PEG-SEZ6 or SEZ6 associated with a targeting moiety). In other selected embodiments SEZ6 modulators may comprise SEZ6 antagonists which, for the purposes of the instant application, shall be held to mean any construct or compound that recognizes, competes, interacts, binds or associates with SEZ6 and neutralizes, eliminates, reduces, sensitizes, reprograms, inhibits or controls the growth of neoplastic cells including tumor initiating cells. In preferred embodiments the SEZ6 modulators of the instant invention comprise anti-SEZ6 antibodies, or fragments or derivatives thereof, that have unexpectedly been found to silence, neutralize, reduce, decrease, deplete, moderate, diminish, reprogram, eliminate, or otherwise inhibit the ability of tumor initiating cells to propagate, maintain, expand, proliferate or otherwise facilitate the survival, recurrence, regeneration and/or metastasis of neoplastic cells. In particularly preferred embodiments the antibodies or immunoreactive fragments may be associated with or conjugated to one or more anti-cancer agents (e.g., a cytotoxic agent).

With regard to such modulators it will be appreciated that compatible antibodies may take on any one of a number of forms including, for example, polyclonal and monoclonal antibodies, chimeric, CDR grafted, humanized and human antibodies and immunoreactive fragments and/or variants of each of the foregoing. Preferred embodiments will comprise antibodies that are relatively non-immunogenic such as humanized or fully human constructs. Of course, in view of the instant disclosure those skilled in the art could readily identify one or more complementarity determining regions (CDRs) associated with heavy and light chain variable regions of SEZ6 antibody modulators and use those CDRs to engineer or fabricate chimeric, humanized or CDR grafted antibodies without undue experimentation. Accordingly, in certain preferred embodiments the SEZ6 modulator comprises an antibody that incorporates one or more CDRs as defined in FIGS. 10A and 10B and derived from the light (FIG. 10A) or heavy (FIG. 10B) contiguous chain murine variable regions (SEQ ID NOS: 20-169) set forth therein. Such CDR grafted variable regions having a human framework and variants thereof are also shown in FIG. 10 comprising SEQ ID NOS: 170-199. In preferred embodiments such antibodies will comprise monoclonal antibodies and, in even more preferred embodiments, will comprise chimeric, CDR grafted or humanized antibodies.

Exemplary nucleic acid sequences encoding each of the amino acid sequences set forth in FIGS. 10A and 10B are appended hereto in the sequence listing and comprise SEQ ID NOS: 220 to 399. In this respect it will be appreciated that the invention further comprises nucleic acid molecules (and associated constructs, vectors and host cells) encoding disclosed antibody variable region amino acid sequences including those set forth in the attached sequence listing.

More particularly, in selected embodiments compatible SEZ6 modulators may comprise an antibody having a light chain variable region and a heavy chain variable region wherein said light chain variable region comprises an amino acid sequence having at least 60% identity to an amino acid sequence selected from the group consisting of amino acid sequences as set forth in SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID

NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78 SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 102, SEQ ID NO: 104, SEQ ID NO: 106, SEQ ID NO: 108, SEQ ID NO: 110, SEQ ID NO: 112, SEQ ID NO: 114, SEQ ID NO: 116, SEQ ID NO: 118, SEQ ID NO: 120, SEQ ID NO: 122, SEQ ID NO: 124, SEQ ID NO: 126, SEQ ID NO: 128, SEQ ID NO: 130, SEQ ID NO: 132, SEQ ID NO: 134, SEQ ID NO: 136, SEQ ID NO: 138, SEQ ID NO: 140, SEQ ID NO: 142, SEQ ID NO: 144, SEQ ID NO: 146, SEQ ID NO: 148, SEQ ID NO: 150, SEQ ID NO: 152, SEQ ID NO: 154, SEQ ID NO: 156, SEQ ID NO: 158, SEQ ID NO: 160, SEQ ID NO: 162, SEQ ID NO: 164, SEQ ID NO: 166 and SEQ ID NO: 168 and wherein said heavy chain variable region comprises an amino acid sequence having at least 60% identity to an amino acid sequence selected from the group consisting of amino acid sequences as set forth in SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51 , SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 115, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 121, SEQ ID NO: 123, SEQ ID NO: 125, SEQ ID NO: 127, SEQ ID NO: 129, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137, SEQ ID NO: 139, SEQ ID NO: 141, SEQ ID NO: 143, SEQ ID NO: 145, SEQ ID NO: 147, SEQ ID NO: 149, SEQ ID NO: 151, SEQ ID NO: 153, SEQ ID NO: 155, SEQ ID NO: 157, SEQ ID NO: 159, SEQ ID NO: 161, SEQ ID NO: 163, SEQ ID NO: 165, SEQ ID NO: 167 and SEQ ID NO: 169. In other preferred embodiments the selected modulators will comprise heavy and light chain variable regions that comprise 65, 70, 75 or 80% identity to the aforementioned murine sequences. In still other embodiments the modulators will comprise heavy and light chain variable regions that comprise 85, 90 or even 95% identity to the disclosed murine sequences.

Of course, in view of the instant disclosure those skilled in the art could readily identify CDRs associated with each of the aforementioned heavy and light chain variable regions and use those CDRs to engineer or fabricate chimeric, humanized or CDR grafted antibodies without undue experimentation. As such, in selected embodiments the present invention is directed to anti-SEZ6 antibodies comprising one or more CDRs from a variable region sequence set forth in FIG. 10A or FIG. 10B. In preferred embodiments such antibodies will comprise monoclonal antibodies and, in even more preferred embodiments will comprise chimeric, CDR grafted or humanized antibodies. As discussed in more detail below still other embodiments will comprise such antibodies conjugated or associated with one or more cytotoxic agents.

Another aspect of the invention comprises modulators obtained or derived from SC17.1, SC17.2, SC17.3, SC17.4, SC17.8, SC17.9, SC17.10, SC17.11, SC17.14, SC17.15, SC17.16, SC17.17, SC17.18, SC17.19, SC17.22, SC17.24, SC17.27, SC17.28, SC17.29, SC17.30, SC17.32, SC17.34, SC17.35, SC17.36, SC17.38, SC17.39, SC17.40, SC17.41, SC17.42, SC17.45, SC17.46, SC17.47, SC17.49, SC17.50, SC17.53, SC17.54, SC17.56, SC17.57, SC17.59, SC17.61, SC17.63, SC17.71, SC17.72, SC17.74, SC17.76, SC17.77, SC17.79, SC17.81, SC17.82, SC17.84, SC17.85, SC17.87, SC17.89, SC17.90, SC17.91, SC17.93, SC17.95, SC17.97, SC17.99, SC17.102, SC17.114, SC17.115, SC17.120, SC17121, SC17.122, SC17.140, SC17.151, SC17.156, SC17.161, SC17.166, SC17.187, SC17.191, SC17.193, SC17.199 and SC17.200.

In yet other compatible embodiments the instant invention will comprise the CDR grafted or humanized SEZ6 modulators hSC17.16, hSC17.17, hSC17.24, hSC17.28, SC17.34, hSC17.46, SC17.151, SC17.155, SC17.156, SC17.161 and SC17.200. Still other embodiments are directed to a SEZ6 modulator comprising a humanized antibody wherein said humanized antibody comprises a light chain variable region and a heavy chain variable region wherein said light chain variable region comprises an amino acid sequence having at least 60% identity to an amino acid sequence selected from the group consisting of amino acid sequences as set forth in SEQ ID NO: 170, SEQ ID NO: 172, SEQ ID NO: 174, SEQ ID NO: 176, SEQ ID NO: 178, SEQ ID NO: 180, SEQ ID NO: 182, SEQ ID NO: 184, SEQ ID NO: 186, SEQ ID NO: 188 and SEQ ID NO: 190 and wherein said heavy chain variable region comprises an amino acid sequence having at least 60% identity to an amino acid sequence

selected from the group consisting of amino acid sequences as set forth in SEQ ID NO: 171, SEQ ID NO: 173, SEQ ID NO: 175, SEQ ID NO: 177, SEQ ID NO: 179 and SEQ ID NO: 181, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 187, SEQ ID NO: 189 and SEQ ID NO: 191. Additionally, certain humanized variants of light (SEQ ID NO: 192) and heavy (SEQ ID NO: 193, SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197, SEQ ID NO: 198 and SEQ ID NO: 199) chain variable regions are provided in accordance with the teachings herein. Moreover, as described immediately above nucleic acid sequences encoding the exemplified humanized heavy and light chain variable regions are set forth in the sequence listing appended hereto as SEQ ID NOS: 370 – 399.

Besides the aforementioned aspects, other preferred embodiments of the instant invention will comprise SEZ6 modulators associated or conjugated to one or more drugs to provide modulator conjugates that may be particularly effective in treating proliferative disorders (alone or in combination with other pharmaceutically active agents). More generally, once the modulators of the invention have been fabricated and selected they may be linked with, fused to, conjugated to (e.g., covalently or non-covalently) or otherwise associated with pharmaceutically active or diagnostic moieties or biocompatible modifiers. As used herein the term "conjugate" or "modulator conjugate" or "antibody conjugate" will be used broadly and held to mean any biologically active or detectable molecule or drug associated with the disclosed modulators regardless of the method of association. In this respect it will be understood that such conjugates may, in addition to the disclosed modulators, comprise peptides, polypeptides, proteins, prodrugs which are metabolized to an active agent in vivo, polymers, nucleic acid molecules, small molecules, binding agents, mimetic agents, synthetic drugs, inorganic molecules, organic molecules and radioisotopes. Moreover, as indicated above the selected conjugate may be covalently or non-covalently associated with, or linked to, the modulator and exhibit various stoichiometric molar ratios depending, at least in part, on the method used to effect the conjugation.

Particularly preferred aspects of the instant invention will comprise antibody modulator conjugates or antibody-drug conjugates that may be used for the diagnosis and/or treatment of proliferative disorders. Such conjugates may be represented by the formula M-[L-D]n where M stands for a disclosed modulator or target binding moiety, L is an optional linker or linker unit, D is a compatible drug or prodrug and n is an integer from about 1 to about 20. It will be

appreciated that, unless otherwise dictated by context, the terms "antibody-drug conjugate" or "ADC" or the formula M-[L-D]n shall be held to encompass conjugates comprising both therapeutic and diagnostic moieties. In such embodiments antibody-drug conjugate compounds will typically comprise anti-SEZ6 as the modulator unit (M), a therapeutic or diagnostic moiety (D), and optionally a linker (L) that joins the drug and the antigen binding agent. In a preferred embodiment, the antibody is a SEZ6 mAb comprising at least one CDR from the heavy and light chain variable regions as described above.

As previously indicated one aspect of the invention may comprise the unexpected association of SEZ6 polypeptides with cancer stem cells. Thus, in certain other embodiments the invention will comprise a SEZ6 modulator that reduces the frequency of tumor initiating cells upon administration to a subject. Preferably the reduction in frequency will be determined using *in vitro* or *in vivo* limiting dilution analysis. In particularly preferred embodiments such analysis may be conducted using *in vivo* limiting dilution analysis comprising transplant of live human tumor cells into immunocompromised mice. Alternatively, the limiting dilution analysis may be conducted using *in vitro* limiting dilution analysis comprising limiting dilution deposition of live human tumor cells into *in vitro* colony supporting conditions. In either case, the analysis, calculation or quantification of the reduction in frequency will preferably comprise the use of Poisson distribution statistics to provide an accurate accounting. It will be appreciated that, while such quantification methods are preferred, other, less labor intensive methodology such as flow cytometry or immunohistochemistry may also be used to provide the desired values and, accordingly, are expressly contemplated as being within the scope of the instant invention. In such cases the reduction in frequency may be determined using flow cytometric analysis or immunohistochemical detection of tumor cell surface markers known to enrich for tumor initiating cells.

As such, in another preferred embodiment of the instant invention comprises a method of treating a SEZ6 associated disorder comprising administering a therapeutically effective amount of a SEZ6 modulator to a subject in need thereof whereby the frequency of tumor initiating cells is reduced. Preferably the SEZ6 associated disorder comprises a neoplastic disorder. Again, the reduction in the tumor initiating cell frequency will preferably be determined using *in vitro* or *in vivo* limiting dilution analysis.

In this regard it will be appreciated that the present invention is based, at least in part, upon the discovery that SEZ6 immunogens are associated with tumor perpetuating cells (i.e., cancer stem cells) that are involved in the etiology of various neoplasia. More specifically, the instant application unexpectedly demonstrates that the administration of various exemplary SEZ6 modulators can mediate, reduce, deplete, inhibit or eliminate tumorigenic signaling by tumor initiating cells (i.e., reduce the frequency of tumor initiating cells). This reduced signaling, whether by depletion, neutralization, reduction, elimination, reprogramming or silencing of the tumor initiating cells or by modifying tumor cell morphology (e.g., induced differentiation, niche disruption), in turn allows for the more effective treatment of SEZ6 associated disorders by inhibiting tumorigenesis, tumor maintenance, expansion and/or metastasis and recurrence.

Besides the aforementioned association with cancer stem cells, there is evidence that SEZ6 isoforms may be implicated in the growth, recurrence or metastatic potential of tumors comprising neuroendocrine features. For the purposes of the instant invention such tumors will comprise neuroendocrine tumors and pseudo neuroendocrine tumors. Intervention in the proliferation of such tumorigenic cells using the novel SEZ6 modulators described herein, may thereby ameliorate or treat a disorder by more than one mechanism (i.e., tumor initiating cell reduction and disruption of oncogenic pathway signaling) to provide additive or synergistic effects. Still other preferred embodiments may take advantage of the cellular internalization of cell surface SEZ6 to deliver a modulator mediated anti-cancer agent. In this regard it will be appreciated that the present invention is not limited by any particular mechanism of action but rather encompasses the broad use of the disclosed modulators to treat SEZ6 associated disorders (including various neoplasia).

Thus, in other embodiments the present invention will comprise the use of the disclosed modulators to treat tumors comprising neuroendocrine features in a subject in need thereof. Of course the same modulators may be used for the prophylaxis, prognosis, diagnosis, theragnosis, inhibition or maintenance therapy of these same tumors.

Other facets of the instant invention exploit the ability of the disclosed modulators to potentially disrupt oncogenic pathways while simultaneously silencing tumor initiating cells. Such multi-active SEZ6 modulators (e.g., SEZ6 antagonists) may prove to be particularly effective when used in combination with standard of care anti-cancer agents or debulking

agents. Accordingly preferred embodiments of the instant invention comprise using the disclosed modulators as anti-metastatic agents for maintenance therapy following initial treatments. In addition, two or more SEZ6 antagonists (e.g. antibodies that specifically bind to two discrete epitopes on SEZ6) may be used in combination in accordance with the present teachings. Moreover, as discussed in some detail below, the SEZ6 modulators of the present invention may be used in a conjugated or unconjugated state and, optionally, as a sensitizing agent in combination with a variety of chemical or biological anti-cancer agents.

Accordingly another preferred embodiment of the instant invention comprises a method of sensitizing a tumor in a subject for treatment with an anti-cancer agent comprising the step of administering a SEZ6 modulator to said subject. Other embodiments comprise a method of reducing metastasis or tumor recurrence following treatment comprising administering a SEZ6 modulator to a subject in need thereof. In a particularly preferred aspect of the invention the SEZ6 modulator will specifically result in a reduction of tumor initiating cell frequency as determined using *in vitro* or *in vivo* limiting dilution analysis.

More generally preferred embodiments of the invention comprise a method of treating a SEZ6 associated disorder in a subject in need thereof comprising the step of administering a SEZ6 modulator to the subject. In particularly preferred embodiments the SEZ6 modulator will be associated (e.g., conjugated) with an anti-cancer agent. In yet other embodiments the SEZ6 modulator will internalize following association or binding with SEZ6 on or near the surface of the cell. Moreover the beneficial aspects of the instant invention, including any disruption of signaling pathways and collateral benefits, may be achieved whether the subject tumor tissue exhibits elevated levels of SEZ6 or reduced or depressed levels of SEZ6 as compared with normal adjacent tissue. Particularly preferred embodiments will comprise the treatment of disorders exhibiting elevated levels of SEZ6 on tumorigenic cells as compared to normal tissue or non-tumorigenic cells.

In yet another aspect the present invention will comprise a method of treating a subject suffering from a neoplastic disorder comprising the step of administering a therapeutically effective amount of at least one internalizing SEZ6 modulator. Preferred embodiments will comprise the administration of internalizing antibody modulators wherein, in other selected embodiments, the internalizing antibody modulators are conjugated or associated with a cytotoxic agent.

Other embodiments are directed to a method of treating a subject suffering from a SEZ6 associated disorder comprising the step of administering a therapeutically effective amount of at least one depleting SEZ6 modulator.

In yet another embodiment the present invention provides methods of maintenance therapy wherein the disclosed effectors or modulators are administered over a period of time following an initial procedure (e.g., chemotherapeutic, radiation or surgery) designed to remove at least a portion of the tumor mass. Such therapeutic regimens may be administered over a period of weeks, a period of months or even a period of years wherein the SEZ6 modulators may act prophylactically to inhibit metastasis and/or tumor recurrence. In yet other embodiments the disclosed modulators may be administrated in concert with known debulking regimens to prevent or retard metastasis, tumor maintenance or recurrence.

It will further be appreciated that the SEZ6 modulators of the instant invention may be generated and selected to react with known isoform(s) of SEZ6 or a single isoform of the protein or, conversely, may comprise a pan-SEZ6 modulator that reacts or associates with at least one additional SEZ6 family member (e.g., SEZ6L or SEZ6L2 and isoforms thereof) in addition to SEZ6. More specifically, as disclosed herein preferred modulators such as antibodies may be generated and selected so that they react with domains (or epitopes therein) that are exhibited by SEZ6 only or with domains that are at least somewhat conserved across two or more of the SEZ6 family members.

In yet other preferred embodiments the modulators will associate or bind to a specific epitope, portion, motif or domain of SEZ6. As will be discussed in some detail below both SEZ6 isoforms incorporate an identical extracellular region (see FIG. 1E) comprising at least an N-terminal domain, two alternating Sushi and CUB domains, and three additional tandem Sushi domain repeats. In addition the SEZ6 protein comprises a transmembrane domain and a cytoplasmic domain. Accordingly, in certain embodiments the modulators will bind or associate with the N-terminal domain of SEZ6 (i.e. amino acids 1-335 in the mature protein) or to an epitope therein. Other aspects of the instant invention comprise modulators that associate or bind to a specific epitope located in a particular Sushi domain of SEZ6. In this regard the particular modulator may associate or bind to an epitope located in Sushi Domain 1 (amino acids 336-395), Sushi Domain 2 (amino acids 511-572), Sushi Domain 3 (amino acids 690-748), Sushi Domain 4 (amino acids 750-813) or Sushi Domain 5 (amino acids 817-878).

Other aspects of the instant invention comprise modulators that associate or bind to a specific epitope located in a particular CUB-like domain of SEZ6. In this regard the particular modulator may associate or bind to an epitope located in CUB Domain 1 (amino acids 397-508) or CUB Domain 2 (amino acids 574-685). Of course it will be appreciated that each of the aforementioned domains may comprise more than one epitope and may be associated with more than one bin.

With regard to modulator or antibody "bins" it will be appreciated that the SEZ6 antigen may be analyzed or mapped through competitive antibody binding using art recognized techniques to define specific bins located along the protein. While discussed in more detail herein and shown in Examples 9 and 10 below, two antibodies (one of which may be termed a "reference antibody," "bin delineating antibody" or "delineating antibody") may be considered to be in the same bin if they substantially compete with each other for binding to the target antigen. In such cases the subject antibody epitopes may be identical, substantially identical or close enough (either in a linear sense where they are separated by a few amino acids or conformationally) so that both antibodies are sterically or electrostatically inhibited or precluded from binding to the antigen. Such defined bins may be generally associated with certain SEZ6 domains (e.g. the reference antibody will bind with an epitope contained in a specific domain) though the correlation is not always precise (e.g., there may be more than one bin in a domain or the bin may be defined conformationally and comprise more than one domain). It will be appreciated that those skilled in the art can readily determine the relationship between the SEZ6 domains and empirically determined bins.

With regard to the present invention competitive binding analysis using art-recognized techniques (e.g., ELISA, surface plasmon resonance or bio-layer interferometry) defined at least seven distinct bins, each of which was found to contain a number of antibody modulators. For the purposes of the instant disclosure the seven bins were termed bins A-F and bin U. Bins A-F are unique bins and the antibodies contained in each of these bins compete with each other for binding to the SEZ6 protein. Bin U contains antibodies that do not compete with antibodies in Bins A-F, but may compete for binding with each other. Thus, in selected embodiments the present invention will comprise a modulator residing in a bin selected from the group consisting of bin A, bin B, bin C, bin D, bin E, bin F, and bin U. In other embodiments the present invention comprises a modulator residing in a bin defined by a

reference antibody selected from the group consisting of SC17.1, SC17.2, SC17.3, SC17.4, SC17.8, SC17.9, SC17.10, SC17.11, SC17.14, SC17.15, SC17.16, SC17.17, SC17.18, SC17.19, SC17.22, SC17.24, SC17.27, SC17.28, SC17.29, SC17.30, SC17.32, SC17.34, SC17.35, SC17.36, SC17.38, SC17.39, SC17.40, SC17.41, SC17.42, SC17.45, SC17.46, SC17.47, SC17.49, SC17.50, SC17.53, SC17.54, SC17.56, SC17.57, SC17.59, SC17.61, SC17.63, SC17.71, SC17.72, SC17.74, SC17.76, SC17.77, SC17.79, SC17.81, SC17.82, SC17.84, SC17.85, SC17.87, SC17.89, SC17.90, SC17.91, SC17.93, SC17.95, SC17.97, SC17.99, SC17.102, SC17.114, SC17.115, SC17.120, SC17121, SC17.122, SC17.140, SC17.151, SC17.156, SC17.161, SC17.166, SC17.187, SC17.191, SC17.193, SC17.199 and SC17.200. In still other embodiments the invention will comprise modulators from bin A, modulators from bin B, modulators from bin C, modulators from bin D, modulators from bin E, modulators from bin F or modulators from bin U. Yet other preferred embodiments will comprise a reference antibody modulator and any antibody that competes with the reference antibody.

The term "compete" or "competing antibody" when used in the context of the disclosed modulators means binding competition between antibodies as determined by an assay in which a reference antibody or immunologically functional fragment substantially prevents or inhibits (e.g., greater than 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% or 90%.) specific binding of a test antibody to a common antigen. Compatible methods for determining such competition comprise art known techniques such as, for example, bio-layer interferometry, surface plasmon resonance, flow cytometry, competitive ELISA, etc.

In a selected embodiment the invention comprises a pan-SEZ6 modulator that associates with SEZ6 and at least one other SEZ6 family member (e.g., SEZ6L or SEZ6L2). In other selected embodiments the invention comprises a SEZ6 modulator that immunospecifically associates with one or more isoform of SEZ6 but does not immunospecifically associate with any other SEZ6 family member. In yet other embodiments the present invention comprises a method of treating a subject in need thereof comprising administering a therapeutically effective amount of a pan-SEZ6 modulator. Still other embodiments comprise a method of treating a subject in need thereof comprising administering a therapeutically effective amount of a SEZ6 modulator that

immunospecifically associates with one or more isoforms of SEZ6 but does not immunospecifically associate with any other SEZ6 family member.

Beyond the therapeutic uses discussed above it will also be appreciated that the modulators of the instant invention may be used to detect, diagnose or classify SEZ6 related disorders and, in particular, proliferative disorders. In some embodiments the modulator may be administered to the subject and detected or monitored *in vivo*. Those of skill in the art will appreciate that such modulators may be labeled or associated with markers or reporters as disclosed below and detected using any one of a number of standard techniques (e.g., MRI, CAT scan PET scan, etc.).

Thus, in some embodiments the invention will comprise a method of diagnosing, detecting or monitoring a SEZ6 associated disorder *in vivo* in a subject in need thereof comprising the step of administering a SEZ6 modulator.

In other instances the modulators may be used in an *in vitro* diagnostic setting using art-recognized procedures. As such, a preferred embodiment comprises a method of diagnosing a proliferative disorder in a subject in need thereof comprising the steps of:

a. obtaining a tissue sample from said subject;
b. contacting the tissue sample with at least one SEZ6 modulator; and
c. detecting or quantifying the SEZ6 modulator associated with the sample.

Such methods may be easily discerned in conjunction with the instant application and may be readily performed using generally available commercial technology such as automatic plate readers, dedicated reporter systems, etc. In selected embodiments the SEZ6 modulator will be associated with tumor perpetuating cells present in the sample. In other preferred embodiments the detecting or quantifying step will comprise a reduction of tumor initiating cell frequency and detection thereof. Moreover, limiting dilution analysis may be conducted as previously alluded to above and will preferably employ the use of Poisson distribution statistics to provide an accurate accounting as to the reduction of frequency.

In a similar vein the present invention also provides kits or devices and associated methods that are useful in the diagnosis and monitoring of SEZ6 associated disorders such as cancer. To this end the present invention preferably provides an article of manufacture useful

for diagnosing or treating SEZ6 associated disorders comprising a receptacle comprising a SEZ6 modulator and instructional materials for using said SEZ6 modulator to treat or diagnose the SEZ6 associated disorder. In selected embodiments the devices and associated methods will comprise the step of contacting at least one circulating tumor cell.

Other preferred embodiments of the invention also exploit the properties of the disclosed modulators as an instrument useful for identifying, characterizing, isolating, sectioning or enriching populations or subpopulations of tumor initiating cells through methods such as flow cytometric analysis including fluorescence activated cell sorting (FACS) or laser mediated sectioning.

As such, another preferred embodiment of the instant invention is directed to a method of identifying, isolating, sectioning or enriching a population of tumor initiating cells comprising the step of contacting said tumor initiating cells with a SEZ6 modulator.

The foregoing is a summary and thus contains, by necessity, simplifications, generalizations, and omissions of detail; consequently, those skilled in the art will appreciate that the summary is illustrative only and is not intended to be in any way limiting. Other aspects, features, and advantages of the methods, compositions and/or devices and/or other subject matter described herein will become apparent in the teachings set forth herein. The summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This summary is not intended to identify key features or essential features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject matter.

Brief Description of the Figures

FIGS. 1A - 1E are various representations of SEZ6 including nucleic acid or amino acid sequences pertaining to the SEZ6 modulators described herein. FIGS. 1A and 1B (SEQ ID NOS: 1 and 2) depict the full length mRNA sequence containing the open reading frames (ORFs) (underlined) encoding the SEZ6 variants 1 and 2, respectively. FIGS. 1C and 1D (SEQ ID NOS: 3 and 4) provide the corresponding amino acid sequences of the ORFs denoted in FIGS. 1A and 1B, respectively, with the single underlined amino acid residues indicating the predicted transmembrane spanning domain for each protein isoform and the double underlined amino acid residues indicating the signal peptide; FIG. 1E depicts the

alignment of the two protein isoforms (SEQ ID NOS: 3 and 4) to illustrate the sequence differences in the cytoplasmic termini of each isoform, with the underlined residues indicating the differences between the two sequences; and FIG. 1F provides a schematic representation of the extracellular region of the SEZ6 protein illustrating the positions of the various domains.

FIGS. 2A – 2C provide a tabular representation of the percent identity at the protein level between the closest human isoform of SEZ6 and rhesus, cynomolgus, mouse or rat SEZ6 proteins (FIG. 2A); a tabular listing of various cDNA or protein sequence accessions for each of the reported isoforms of the SEZ6 family of genes (FIG. 2B); and the percent identity at the protein level between the longest isoforms of human SEZ6, SEZ6L, and SEZ6L2 proteins (FIG. 2C).

FIGS. 3A – 3C provide various representations of nucleic acid or amino acid sequences related to the production of the immunogens or cell lines used to generate or characterize the SEZ6 modulators described herein. For human SEZ6 a specific cDNA clone (FIG. 3A; SEQ ID NO: 5) encoding the complete mature human SEZ6 protein (FIG. 3B; SEQ ID NO: 6) was constructed from a commercial cDNA clone (BC146292; SEQ ID NO: 7) with known differences (FIG. 3C) from a database reference sequence, NP_849191 (SEQ ID NO: 3), for the SEZ6 protein.

FIGS. 4A and 4B provide a cDNA (FIG. 4A; SEQ ID NO: 8) used to express an Fc-SEZ6 construct in CHO-S cells and yield a protein immunogen (FIG. 4B; SEQ ID NO: 9), comprising the ECD of human SEZ6 fused to a human IgG2 Fc domain, in which the underlined sequences correspond to the human IgG2 Fc domain, the double underlined sequences correspond to the IgK signal peptide, and the amino acids in bold font correspond to residues contributed by the restriction sites used to clone the hSCRx17 fragment.

FIGS. 5A – 5J provide various representations of nucleic acid or amino acid sequences related to the production of the immunogens or cell lines used to generate or characterize the SEZ6 modulators described herein, wherein the underlined sequences denote the ECD of protein for the specific SEZ6 or SEZ6 family member being illustrated, and the figures comprise the cDNA sequences for the constructs encoding mature murine SEZ6 (FIG. 5A, SEQ ID NO: 10), mature rat SEZ6 (FIG. 5C, SEQ ID NO: 12), mature cynomolgus SEZ6 (FIG. 5E, SEQ ID NO: 14), mature ECD of the human SEZ6L protein (FIG. 5G, SEQ ID NO:

16), or the mature ECD of the human SEZ6L2 protein (FIG. 5I, SEQ ID NO: 18), or the corresponding proteins encoded by these cDNA constructs, namely mature murine SEZ6 (FIG. 5B, SEQ ID NO: 11), mature rat SEZ6 (FIG. 5D, SEQ ID NO: 13), mature cynomolgus SEZ6 (FIG. 5F, SEQ ID NO: 15), the mature ECD of the human SEZ6L protein (FIG. 5H, SEQ ID NO: 17), or the mature ECD of the human SEZ6L2 protein (FIG. 5J, SEQ ID NO: 19).

FIGS. 6A and 6B are depictions of mRNA expression levels of various genes as measured using whole transcriptome (SOLiD) sequencing of mRNA derived from tumor cell subpopulations or normal tissues. FIG. 6A is a tabular representation of genes associated with tumors having neuroendocrine features; and FIG. 6B is a graphical representation of SEZ6 mRNA expression in normal tissues and several non-traditional xenograft (NTX) tumors derived from lung cancers.

FIG. 7A – 7F depict mRNA expression levels analyzed using microarray. FIG. 7A is a graphical representation of unsupervised clustering of microarray profiles for 46 tumor lines and two normal tissues; FIGS. 7B and 7C are tabular representations of normalized intensity values corresponding to relative expression levels of selected genes related to neuroendocrine phenotypes (FIG. 7B) or the Notch signaling pathway (FIG. 7C) wherein unshaded cells and relatively low numbers indicate little to no expression and darker cells and relatively higher numbers indicate higher expression levels; FIG. 7D is a graphical representation showing relative expression levels of HES6 mRNA in various tumors and control tissues as measured using qRT-PCR; FIG. 7E is a tabular representation of normalized intensity values corresponding to relative expression levels of selected genes indicative of neurogenesis, neural commitment, or differentiation towards neural fates, with un-shaded cells indicating little to no expression and darker cells indicating higher expression levels; and FIG. 7F is a graphical representation of normalized intensity values corresponding to relative expression of SEZ6 in various NTX tumor lines.

FIGS. 8A and 8B are graphical representations showing relative expression levels of SEZ6 mRNA transcripts as measured by RT-PCR in a variety of RNA samples isolated from normal tissues or bulk neuroendocrine NTX tumors (FIG. 8A) and a variety of other NTX tumors (FIG. 8B).

FIGS. 9A and 9B are graphical representations showing the absolute (FIG. 9A) or

normalized (FIG. 9B) mRNA expression levels of human SEZ6 as measured by RT-PCR in whole tumor specimens (grey dot) or matched normal adjacent tissue (NAT; white dot) from patients with one of eighteen different solid tumor types.

FIGS. 10A and 10B provide, in a tabular form, the continuous amino acid sequences of heavy and light chain variable regions of a number of murine and humanized exemplary SEZ6 modulators isolated, cloned and engineered as described in the Examples herein.

FIG. 11 sets forth various characteristics of exemplary modulators of the invention. FIG. 11A shows the biochemical and immunological properties of exemplary SEZ6 modulators as represented in a tabular format; and Figure 11B provides a correlation between the domain to which an antibody binds and the antibody's efficacy in an in vitro killing assay.

FIGS. 12A and 12B show detection of expression of SEZ6. FIG. 12A shows SEZ6 expression in HEK-293T cells engineered to over-express human SEZ6 protein (h293T-HuSEZ6) using the anti-SEZ6 antibody SC17.33; FIG. 12B shows the relative protein expression of human SEZ6 in various NTX tumor and normal tissue lysates as measured using an electrochemiluminescent assay.

FIGS. 13A and 13B show detection by flow cytometry of SEZ6 protein expression on NTX tumor cells using various anti-SEZ6 antibodies (FIG. 13A); whereas FIG. 13B shows enhanced expression of SEZ6 protein in CSCs compared to NTG subpopulations using various anti-SEZ6 antibodies (FIG. 13B).

FIGS. 14A and 14B show that CSCs expressing SEZ6 exhibit enhanced tumorigenicity compared to CSCs that do not express SEZ6. FIG. 14A is a contour plot showing cell sorting by FACS of the cells in a lung tumor (LU37) on the basis of expression of CD324 (a marker of CSCs) and SEZ6; FIG. 14B is a graphical representation of the growth of tumor cells that are either $CD324^{+}SEZ6^{+}$ (black circles) or $CD324^{+}SEZ6^{-}$ (white circles) after implantation into immunocompromised mice. Tumor cells expressing both CD324 and SEZ6 exhibit enhanced tumorigenicity.

FIGS. 15A and 15B provide, respectively, a tabular and graphical representation illustrating that the disclosed modulators may effectively be used as targeting moieties to direct cytotoxic payloads to cells engineered to express SEZ6 (FIG. 15A) and NTX lung tumors (LU80, LU37 and LU100) grown *in vitro* (FIG. 15B) where the decrease in

normalized relative luminescence units (RLU) is indicative of cell killing through internalization of the saporin toxin.

FIG. 16 is a tabular representation of immunohistochemistry results showing expression of SEZ6 on various NTX tumors.

FIGS. 17A and 17B depict the ability of conjugated anti-SEZ6 mouse antibodies to retard *in vitro and in vivo* growth of NTX tumor cells. FIG. 17A shows the results of an *in vitro* killing assay using anti-SEZ6 ADCs on SEZ6-overexpressing HEK293 cells; whereas FIG. 17B shows the effect of anti-SEZ6 ADCs on *in vivo* growth of SCLC (LU86) and LCNEC (LU50) tumors.

FIGS. 18A and 18B depict the ability of conjugated humanized anti-SEZ6 antibodies to retard *in vivo* growth of four SCLC tumors (LU80, LU64, LU111 and LU117) and achieve durable remission in immunodeficient mice.

DETAILED DESCRIPTION OF THE INVENTION

I. Introduction

While the present invention may be embodied in many different forms, disclosed herein are specific illustrative embodiments thereof that exemplify the principles of the invention. It should be emphasized that the present invention is not limited to the specific embodiments illustrated. Moreover, any section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. Finally, for the purposes of the instant disclosure all identifying sequence Accession numbers may be found in the NCBI Reference Sequence (RefSeq) database and/or the NCBI GenBank® archival sequence database unless otherwise noted.

As previously alluded to, it has surprisingly been found that the expression of SEZ6 is associated with neoplastic growth and proliferative disorders, particularly in the instance of tumors with neuroendocrine features, and that SEZ6 and variants or isoforms thereof provide useful tumor markers which may be exploited in the treatment of related diseases. Moreover, as shown in the instant application it has unexpectedly been found that SEZ6 markers or determinants such as cell surface SEZ6 protein are associated with cancer stem cells (also known as tumor perpetuating cells) and may be effectively exploited to eliminate or silence the same. The ability to selectively reduce or eliminate cancer stem cells (e.g., through the

use of conjugated SEZ6 modulators) is particularly surprising in that such cells are known to generally be resistant to many conventional treatments. That is, the effectiveness of traditional, as well as more recent targeted treatment methods, is often limited by the existence and/or emergence of resistant cancer stem cells that are capable of perpetuating the cancer even in the face of these diverse treatment methods. Further, determinants associated with cancer stem cells often make poor therapeutic targets due to low or inconsistent expression, failure to remain associated with the tumorigenic cell or failure to present at the cell surface. In sharp contrast to the teachings of the prior art, the instantly disclosed compounds and methods effectively overcome this inherent resistance to specifically eliminate, deplete, silence or promote the differentiation of such cancer stem cells thereby negating their ability to sustain or re-induce the underlying tumor growth.

More specifically, it has been discovered that SEZ6 modulators such as those disclosed herein may advantageously be used in the prognosis, diagnosis, theragnosis, treatment or prevention of proliferative disorders (e.g. neoplastic disorders) in subjects in need thereof. Accordingly, while preferred embodiments of the invention will be discussed extensively below, particularly in terms of particular domains, regions or epitopes or in the context of cancer stem cells or tumors comprising neuroendocrine features and their interactions with the disclosed modulators, those skilled in the art will appreciate that the scope of the instant invention is not limited by such exemplary embodiments. Rather, the most expansive embodiments of the present invention and the appended claims are broadly and expressly directed to SEZ6 modulators (including conjugated modulators) and their use in the prognosis, diagnosis, theragnosis, treatment or prevention of a variety of SEZ6 associated or mediated disorders, including neoplastic or proliferative disorders, regardless of any particular mechanism of action or specifically targeted tumor, cellular or molecular component.

To that end, and as demonstrated in the instant application, it has unexpectedly been found that the disclosed SEZ6 modulators can effectively be used to target and eliminate or otherwise incapacitate proliferative or tumorigenic cells and treat SEZ6 associated disorders (e.g., neoplasia). As used herein a "SEZ6 associated disorder" shall be held to mean any disorder or disease (including proliferative disorders) that is marked, diagnosed, detected or identified by a phenotypic or genotypic aberration of SEZ6 genetic components or expression

during the course or etiology of the disease or disorder. In this regard a SEZ6 phenotypic aberration or determinant may, for example, comprise elevated or depressed levels of SEZ6 protein expression, abnormal SEZ6 protein expression on certain definable cell populations or abnormal SEZ6 protein expression at an inappropriate phase or stage of a cell lifecycle. Of course, it will be appreciated that similar expression patterns of genotypic determinants (e.g., mRNA transcription levels) of SEZ6 may also be used to classify or detect SEZ6 associated disorders.

As used herein the term "determinant" or "SEZ6 determinant" shall mean any detectable trait, property, marker or factor that is identifiably associated with, or specifically found in or on a particular cell, cell population or tissue including those identified in or on a tissue, cell or cell population affected by a SEZ6 associated disease or disorder. In selected preferred embodiments the SEZ6 modulators may associate, bind or react directly with the SEZ6 determinant (e.g., cell surface SEZ6 protein or SEZ6 mRNA) and thereby ameliorate the disorder. More generally determinants may be morphological, functional or biochemical in nature and may be genotypic or phenotypic. In other preferred embodiments the determinant is a cell surface antigen or genetic component that is differentially or preferentially expressed (or is not) by specific cell types (e.g., cancer stem cells) or by cells under certain conditions (e.g., during specific points of the cell cycle or cells in a particular niche). In still other preferred embodiments the determinant may comprise a gene or genetic entity that is differently regulated (up or down) in a specific cell or discrete cell population, a gene that is differentially modified with regard to its physical structure and chemical composition or a protein or collection of proteins physically associated with a gene that show differential chemical modifications. Determinants contemplated herein are specifically held to be positive or negative and may denote a cell, cell subpopulation or tissue (e.g., tumors) by its presence (positive) or absence (negative).

In a similar vein "SEZ6 modulators" of the invention broadly comprise any compound that recognizes, reacts, competes, antagonizes, interacts, binds, agonizes, or associates with a SEZ6 variant or isoform (or specific domains, regions or epitopes thereof) or its genetic component. By these interactions, the SEZ6 modulators may advantageously eliminate, reduce or moderate the frequency, activity, recurrence, metastasis or mobility of tumorigenic cells (e.g., tumor perpetuating cells or cancer stem cells). Exemplary modulators disclosed

herein comprise nucleotides, oligonucleotides, polynucleotides, peptides or polypeptides. In certain preferred embodiments the selected modulators will comprise antibodies to a SEZ6 protein isoform or immunoreactive fragments or derivatives thereof. Such antibodies may be antagonistic or agonistic in nature and may optionally be conjugated or associated with a therapeutic or diagnostic agent. Moreover, such antibodies or antibody fragments may comprise depleting, neutralizing or internalizing antibodies. In other embodiments, modulators within the instant invention will constitute a SEZ6 construct comprising a SEZ6 isoform or a reactive fragment thereof. It will be appreciated that such constructs may comprise fusion proteins and can include reactive domains from other polypeptides such as immunoglobulins or biological response modifiers. In still other aspects, the SEZ6 modulator will comprise a nucleic acid moiety (e.g. miRNA, siRNA, shRNA, antisense constructs, etc.) that exerts the desired effects at a genomic level. Still other modulators compatible with the instant teachings will be discussed in detail below.

More generally SEZ6 modulators of the present invention broadly comprise any compound that recognizes, reacts, competes, antagonizes, interacts, binds, agonizes, or associates with a SEZ6 determinant (genotypic or phenotypic) including cell surface SEZ6 protein. Whichever form of modulator is ultimately selected it will preferably be in an isolated and purified state prior to introduction into a subject. In this regard the term "isolated SEZ6 modulator" or "isolated SEZ6 antibody" shall be construed in a broad sense and in accordance with standard pharmaceutical practice to mean any preparation or composition comprising the modulator in a state substantially free of unwanted contaminants (biological or otherwise). Moreover these preparations may be purified and formulated as desired using various art recognized techniques. Of course, it will be appreciated that such "isolated" preparations may be intentionally formulated or combined with inert or active ingredients as desired to improve the commercial, manufacturing or therapeutic aspects of the finished product and provide pharmaceutical compositions. In a broader sense the same general considerations may be applied to an "isolated" SEZ6 isoform or variant or an "isolated" nucleic acid encoding the same.

Further, it has surprisingly been found that modulators interacting, associating or binding to particular SEZ6 domains, motifs or epitopes are especially effective in eliminating tumorigenic cells and/or silencing or attenuating cancer stem cell effects on tumor growth or

propagation. That is, while modulators that react or associate with domains that are proximal to the cell surface (e.g. one of the Sushi or CUB-like domains) are effective in depleting or neutralizing tumorigenic cells it has unexpectedly been discovered that modulators associating or binding to domains, motifs or regions that are relatively more distal to the cell surface are also effective in eliminating, neutralizing, depleting or silencing tumorigenic cells. This is especially true of conjugated modulators such as, for example, anti-SEZ6 antibody drug conjugates comprising a cytotoxic agent.

While the present invention expressly contemplates the use of any SEZ6 modulator in the treatment of any SEZ6 disorder, including any type of neoplasia, in particularly preferred embodiments the disclosed modulators may be used to prevent, treat or diagnose tumors comprising neuroendocrine features (genotypic or phenotypic) including neuroendocrine tumors. True or "canonical neuroendocrine tumors" (NETs) arise from the dispersed endocrine system and are typically highly aggressive. Neuroendocrine tumors occur in the kidney, genitourinary tract (bladder, prostate, ovary, cervix, and endometrium), gastrointestinal tract (stomach, colon), thyroid (medullary thyroid cancer), and lung (small cell lung carcinoma and large cell neuroendocrine carcinoma). Moreover, the disclosed modulators may advantageously be used to treat, prevent or diagnose pseudo neuroendocrine tumors (pNETs) that genotypically or phenotypically mimic, comprise, resemble or exhibit common traits with canonical neuroendocrine tumors. "Pseudo neuroendocrine tumors" are tumors that arise from cells of the diffuse neuroendocrine system or from cells in which a neuroendocrine differentiation cascade has been aberrantly reactivated during the oncogenic process. Such pNETs commonly share certain genotypic, phenotypic or biochemical characteristics with traditionally defined neuroendocrine tumors, including the ability to produce subsets of biologically active amines, neurotransmitters, and peptide hormones. Accordingly, for the purposes of the instant invention the phrases "tumors comprising neuroendocrine features" or "tumors exhibiting neuroendocrine features" shall be held to comprise both neuroendocrine tumors and pseudo neuroendocrine tumors unless otherwise dictated by context.

Besides the association with tumors generally discussed above, there are also indications of phenotypic or genotypic association between selected tumor initiating cells (TIC) and SEZ6 determinants. In this regard selected TICs (e.g., cancer stem cells) may

express elevated levels of SEZ6 proteins when compared to normal tissue and non-tumorigenic cells (NTG), which together typically comprise much of a solid tumor. Thus, SEZ6 determinants may comprise a tumor associated marker (or antigen or immunogen) and the disclosed modulators may provide effective agents for the detection and suppression of TIC and associated neoplasia due to altered levels of the proteins on cell surfaces or in the tumor microenvironment. Accordingly, SEZ6 modulators, including immunoreactive antagonists and antibodies that associate, bind or react with the proteins, may effectively reduce the frequency of tumor initiating cells and could be useful in eliminating, depleting, incapacitating, reducing, promoting the differentiation of, or otherwise precluding or limiting the ability of these tumor-initiating cells to lie dormant and/or continue to fuel tumor growth, metastasis or recurrence in a patient. In this regard those skilled in the art will appreciate that the present invention further provides SEZ6 modulators and their use in reducing the frequency of tumor initiating cells.

II. SEZ6 Physiology

SEZ6 (also known as seizure related 6 homolog) is a type I transmembrane protein originally cloned from mouse cerebrum cortex-derived cells treated with the convulsant pentylentetrazole (Shimizu-Nishikawa, 1995; PMID: 7723619). Representative SEZ6 protein orthologs include, but are not limited to, human (NP_849191; NP_001092105), chimpanzee (XP_511368, NP_001139913), mouse (NP_067261), and rat (NP_001099224). In humans, the SEZ6 gene consists of 17 exons spanning 51.1 kBp located on chromosome 17q11.2. Alternate splice acceptor sites only 16 base pairs apart within the last exon gives rise to two processed transcripts, one of approximately 4210 bases (NM_178860; FIG. 1A) and one of approximately 4194 bases (NM_001098635, FIG. 1B). The former transcript encodes a 994 amino acid protein (NP_849191; FIG. 1C), whereas the latter encodes a 993 amino acid protein (NP_001092105; FIG. 1D). These two protein isoforms of SEZ6 share overall 100% identity across their extracellular domains and their transmembrane domains, differing only in the final ten amino acid residues (FIG. 1E). A third splice variant has been reported to generate a secreted from of SEZ6 (Shimizu-Nishikawa, 1995; PMID: 7723619), however it has not been included in the RefSeqs associated within the NCBI database Gene page entry. The modulators of the invention may bind to any of the splice variants.

The biological relevance of the isoforms is unclear, although one study has suggested opposing actions for the membrane versus soluble proteins when their expression is restored in neurons from murine SEZ6 knockout mice (Gunnersen *et al.* 2007, PMID: 18031681). Cross species protein sequence identity for the SEZ6 proteins are listed in FIG. 2A. In the human genome, there are two closely related genes- seizure related 6 homolog-like (SEZ6L) and seizure related 6 homolog like-2 (SEZ6L2), each of which has multiple splice variants encoding numerous isoforms (FIG. 2B). Percent identities for the longest protein of each of the members of this family of SEZ6-like proteins in humans are shown in FIG. 2C. Taken together SEZ6, SEZ6L and SEZ6L2, including their various isoforms, will be termed the SEZ6 family for the purposes of the instant application. SEZ6 modulators of the invention comprise modulators that are specific for each of SEZ6, SEZ6L or SEZ6L2. Alternatively, the modulators of the invention may cross react with SEZ6 and one or both of SEZ6L and/or SEZ6L2.

The mature SEZ6 protein is composed of a series of structural domains: a cytoplasmic domain, a transmembrane domain and an extracellular domain comprising a unique N-terminal domain, followed by two alternating Sushi and CUB-like domains, and three additional tandem Sushi domain repeats. Two isoforms of the SEZ6 antigen exist, and differ only on the extreme carboxy terminal, cytoplasmic domain.

FIG. 1F provides a schematic diagram of the extracellular region of the SEZ6 protein, illustrating the general juxtaposition of the Sushi and CUB domains, and the N-terminal domain. Generally, the domains are recognized as occurring at about amino acid residues 336-395 (Sushi Domain 1), 397-508 (CUB Domain 1), 511-572 (Sushi Domain 2), 574-685 (CUB Domain 2), 690-748 (Sushi Domain 3), 750-813 (Sushi Domain 4), 817-878 (Sushi Domain 5), with the N terminal domain at about amino acid residues 1-335, and a compositional bias of proline-rich residues at about amino acid residues 71-169.

The Sushi repeats are similar to the short consensus repeats found in the other human complement regulatory proteins (i.e., complement C3b/C4b binding sites). The CUB-like domains are similar to CUB domains found in other mammalian complement binding proteins which are associated with a wide range of proteins that participate in numerous biological processes other than complement activation, including but not limited to patterning, axon guidance, inflammation, and tumor suppression (Bork and Beckman, 1993, PMID: 8510165).

Both the Sushi and CUB domains imply a function for SEZ6 involving binding of other proteins extracellularly. Proteins containing CUB domains also have been linked to cell signaling pathways, and consistent with this function, the SEZ6 C-terminal cytoplasmic domains contain the Asn-Pro-Thr-Tyr motif (SEQ ID NO: 403), which is a potential target for phosphorylation by Src tyrosine kinase family members. If true, this would link SEZ6 to a cellular signal transduction pathway leading to the activation of Ras, suggesting that SEZ6 may be a neurotrophic receptor.

Note that, the terms "mature protein" or "mature polypeptide" as used herein refers to the form(s) of the SEZ6 protein produced without the signal peptide of 19 amino acids that may be cleaved prior to cell surface expression. Unless otherwise indicated SEZ6 amino acid numbering (for domains, regions, epitopes, etc.) will be in the context of a mature protein without the leader.

SEZ6 is detectable by RT-PCR at low levels in kidney, liver, heart, lung and thymus of rodents, although strong protein expression was seen only in brain, with a significant level expressed in testis (Herbst and Nicklin, 1997, PMID: 9073173). Using polyclonal sera to SEZ6, protein expression was detected in day 13 of developing mouse forebrain. Strong staining was detected in the post-mitotic, maturing neurons of the developing cortical plate and sub-plate. This staining is diminished in the adult brain where the SEZ6 expression can be detected in other brain regions associated with ongoing morphological plasticity, such as the hippocampus, cerebellum, and olfactory bulb and in neurons of the retina and spinal cord (Gunnersen *et al.*, 2007, PMID: 18031681). The densest signals are found in regions with greatest concentration of neuronal cell bodies. In spite of widespread retinal expression of SEZ6, retinal function in the absence of SEZ6 was not affected (Gunnersen *et al.*, 2009, PMID: 19662096). The SEZ6 staining pattern is closely tied with the emergence of the neocortical layers and hippocampus, and implies a forebrain-specific role for this gene during development. In human and mice SEZ6 was found to be differentially expressed in highly specific regions of the neocortex (Gunnersen *et al.*, 2007, supra).

Mutations in the human SEZ6 gene have been linked to febrile seizures (FS), a convulsion associated with a rise in body temperature and the most common type of seizure in childhood (Yu *et al.*, 2007, PMID:17086543). FS may be classified as simple or complex, depending upon duration, recurrence, and extent of the body affected by the seizure. In a

Chinese cohort, no mutations in SEZ6 were found in 15 healthy controls, but mutations were found in 21 of 60 patients with FS, with the most common type of mutation being a heterozygous, cytosine insertion (frame shift mutation) at position 1435 of the cDNA. The mutation incidence was significantly higher in patients with complex FS and in patients with a positive family history. As there is an 80% chance that children with complex FS will have seizures later in life, the authors suggest that screening for mutations in SEZ6 may be valuable in predicting FS recurrence or the development of epilepsy (Yu *et al.*, 2007, supra). Later studies have questioned the incidence, relevance, and ability of this study to have adequate power to imply causality, but do support that SEZ6 may be one gene among many that may play a role in seizure disorders (Mulley *et al.*, 2011, PMID: 21785725).

The specific molecular functions of SEZ6 remain unclear. As discussed above, analysis of the structural modules of the protein identified by homology and sequence analysis suggest a possible role in signaling, cell-cell communication, and neural development. The neuronal dendritic branching and connectivity that form the signaling networks that constitute the brain's circuitry arise and are specified both by intrinsic molecular programs in the neural cell as well as extrinsic signals. The process of dendritic growth in pyramidal neurons, the principal neuron in the mammalian forebrain, yields neurons with distinctive morphologies - a pyramidal cell body, and two distinct, complex dendritic trees: one emerging from the apex and the other from the base of the cell body. Gunnersen *et al.* (2007, supra) have shown that SEZ6 null mice exhibit an excess of short dendrites in the dendritic trees of these neurons, yet display no increase in the overall dendritic field, the range of neurons with which a given neuron connects. Restoring the expression of the membrane bound SEZ6 isoforms in the knockout neurons results in an anti-branching effect. In behavioral tests the SEZ6 null mice display specific exploratory, motor, and cognitive deficits. These data suggest that SEZ6 is important for the achievement of the necessary balance between dendrite elongation and branching during the elaboration of a complex dendritic arbor during development.

Together, the studies above strongly suggest that the SEZ6 protein is important in the context of neural development, and is likely to have some role in cell-cell communication and signaling. Inappropriate reactivation of developmental signaling pathways or disregulation of normal signaling pathways are commonly observed in tumors (Harris *et al.*, 2012). One collection of tumors sharing features indicative of partial reactivation of developmental

programs are tumors with neuroendocrine phenotypes (Yao 2008; PMID: 18565894), in which various hormone and endocrine markers are expressed and/or secreted, and various neural markers indicative of neurogenesis, neural commitment, or differentiation towards neural fates are expressed. Tumors with neuroendocrine features arise infrequently in a wide range of primary sites, and while their exhaustive classification remains problematic (Yao; PMID: 18565894; Klimstra 2010; PMID: 20664470; Klöppel, 2011; PMID: 22005112), they may be classified into four major types: low grade benign carcinoids, low-grade well-differentiated neuroendocrine tumors with malignant behavior, tumors with mixed neuroendocrine and epithelial features, and high-grade poorly differentiated neuroendocrine carcinomas. Of these classifications, the poorly differentiated neuroendocrine carcinomas, which include small cell lung cancer (SCLC) and subsets of non-small cell lung cancer (NSCLC), are cancer types with dismal prognoses. It has been postulated that SCLC is bronchogenic in origin, arising in part from pulmonary neuroendocrine cells (Galluzzo and Bocchetta, 2011; PMID: 21504320). Whatever the cellular source of origin for these tumors, it is clear that they show a poorly differentiated endocrine phenotype, often are highly proliferative and aggressive, and frequently over-express neural proteins. The resultant elevation of neural expression markers in these tumors that otherwise may be primarily restricted to the nervous system or show limited expression during development, of which SEZ6 may be an exemplar, may therefore offer a unique therapeutic target for tumors with the neuroendocrine phenotype.

III. Cancer Stem Cells

As alluded to above it has surprisingly been discovered that aberrant SEZ6 expression (genotypic and/or phenotypic) is associated with various tumorigenic cell subpopulations. In this respect the present invention provides SEZ6 modulators that may be particularly useful for targeting such cells, and especially tumor perpetuating cells, thereby facilitating the treatment, management or prevention of neoplastic disorders. Thus, in preferred embodiments modulators of SEZ6 determinants (phenotypic or genotypic) may be advantageously be used to reduce tumor initiating cell frequency in accordance with the present teachings and thereby facilitate the treatment or management of proliferative disorders.

For the purposes of the instant application the term "tumor initiating cell" (TIC) encompasses both "tumor perpetuating cells" (TPC; i.e., cancer stem cells or CSC) and highly proliferative "tumor progenitor cells" (termed TProg), which together generally comprise a unique subpopulation (i.e. 0.1-40%) of a bulk tumor or mass. For the purposes of the instant disclosure the terms "tumor perpetuating cells" and "cancer stem cells" or "neoplastic stem cells" are equivalent and may be used interchangeably herein. TPC differ from TProg in that TPC can completely recapitulate the composition of tumor cells existing within a tumor and have unlimited self-renewal capacity as demonstrated by serial transplantation (two or more passages through mice) of low numbers of isolated cells, whereas TProg will not display unlimited self-renewal capacity.

Those skilled in the art will appreciate that fluorescence-activated cell sorting (FACS) using appropriate cell surface markers is a reliable method to isolate highly enriched cancer stem cell subpopulations (e.g., > 99.5% purity) due, at least in part, to its ability to discriminate between single cells and clumps of cells (i.e. doublets, etc.). Using such techniques it has been shown that when low cell numbers of highly purified TProg cells are transplanted into immunocompromised mice they can fuel tumor growth in a primary transplant. However, unlike purified TPC subpopulations the TProg generated tumors do not completely reflect the parental tumor in phenotypic cell heterogeneity and are demonstrably inefficient at reinitiating serial tumorigenesis in subsequent transplants. In contrast, TPC subpopulations completely reconstitute the cellular heterogeneity of parental tumors and can efficiently initiate tumors when serially isolated and transplanted. Thus, those skilled in the art will recognize that a definitive difference between TPC and TProg, though both may be tumor generating in primary transplants, is the unique ability of TPC to perpetually fuel heterogeneous tumor growth upon serial transplantation at low cell numbers. Other common approaches to characterize TPC involve morphology and examination of cell surface markers, transcriptional profile, and drug response although marker expression may change with culture conditions and with cell line passage *in vitro*.

Accordingly, for the purposes of the instant invention tumor perpetuating cells, like normal stem cells that support cellular hierarchies in normal tissue, are preferably defined by their ability to self-renew indefinitely while maintaining the capacity for multilineage differentiation. Tumor perpetuating cells are thus capable of generating both tumorigenic

progeny (i.e., tumor initiating cells: TPC and TProg) and non-tumorigenic (NTG) progeny. As used herein a "non-tumorigenic cell" (NTG) refers to a tumor cell that arises from tumor initiating cells, but does not itself have the capacity to self-renew or generate the heterogeneous lineages of tumor cells that comprise a tumor. Experimentally, NTG cells are incapable of reproducibly forming tumors in mice, even when transplanted in excess cell numbers.

As indicated, TProg are also categorized as tumor initiating cells (or TIC) due to their limited ability to generate tumors in mice. TProg are progeny of TPC and are typically capable of a finite number of non-self-renewing cell divisions. Moreover, TProg cells may further be divided into early tumor progenitor cells (ETP) and late tumor progenitor cells (LTP), each of which may be distinguished by phenotype (e.g., cell surface markers) and different capacities to recapitulate tumor cell architecture. In spite of such technical differences, both ETP and LTP differ functionally from TPC in that they are generally less capable of serially reconstituting tumors when transplanted at low cell numbers and typically do not reflect the heterogeneity of the parental tumor. Notwithstanding the foregoing distinctions, it has also been shown that various TProg populations can, on rare occasion, gain self-renewal capabilities normally attributed to stem cells and themselves become TPC (or CSC). In any event both types of tumor-initiating cells are likely represented in the typical tumor mass of a single patient and are subject to treatment with the modulators as disclosed herein. That is, the disclosed compositions are generally effective in reducing the frequency or altering the chemosensitivity of such SEZ6 positive tumor initiating cells regardless of the particular embodiment or mix represented in a tumor.

In the context of the instant invention, TPC are more tumorigenic, relatively more quiescent and often more chemoresistant than the TProg (both ETP and LTP), NTG cells and the tumor-infiltrating non-TPC derived cells (e.g., fibroblasts/stroma, endothelial & hematopoietic cells) that comprise the bulk of a tumor. Given that conventional therapies and regimens have, in large part, been designed to both debulk tumors and attack rapidly proliferating cells, TPC are likely to be more resistant to conventional therapies and regimens than the faster proliferating TProg and other bulk tumor cell populations. Further, TPC often express other characteristics that make them relatively chemoresistant to conventional therapies, such as increased expression of multi-drug resistance transporters, enhanced DNA

repair mechanisms and anti-apoptotic proteins. These properties, each of which contribute to drug tolerance by TPC, constitute a key reason for the failure of standard oncology treatment regimens to ensure long-term benefit for most patients with advanced stage neoplasia; i.e. the failure to adequately target and eradicate those cells that fuel continued tumor growth and recurrence (i.e. TPC or CSC).

Unlike many prior art treatments, the novel compositions of the present invention preferably reduce the frequency of tumor initiating cells upon administration to a subject regardless of the form or specific target (e.g., genetic material, SEZ6antibody or ligand fusion construct) of the selected modulator. As noted above, the reduction in tumor initiating cell frequency may occur as a result of a) elimination, depletion, sensitization, silencing or inhibition of tumor initiating cells; b) controlling the growth, expansion or recurrence of tumor initiating cells; c) interrupting the initiation, propagation, maintenance, or proliferation of tumor initiating cells; or d) by otherwise hindering the survival, regeneration and/or metastasis of the tumorigenic cells. In some embodiments, the reduction in the frequency of tumor initiating cells occurs as a result of a change in one or more physiological pathways. The change in the pathway, whether by reduction or elimination of the tumor initiating cells or by modifying their potential (e.g., induced differentiation, niche disruption) or otherwise interfering with their ability to influence the tumor environment or other cells, in turn allows for the more effective treatment of SEZ6 associated disorders by inhibiting tumorigenesis, tumor maintenance and/or metastasis and recurrence.

Among art-recognized methods that can be used to assess such a reduction in the frequency of tumor initiating cells is limiting dilution analysis either *in vitro* or *in vivo*, preferably followed by enumeration using Poisson distribution statistics or assessing the frequency of predefined definitive events such as the ability to generate tumors *in vivo* or not. While such limiting dilution analysis comprise preferred methods of calculating reduction of tumor initiating cell frequency other, less demanding methods, may also be used to effectively determine the desired values, albeit slightly less accurately, and are entirely compatible with the teachings herein. Thus, as will be appreciated by those skilled in the art, it is also possible to determine reduction of frequency values through well-known flow cytometric or immunohistochemical means. As to all the aforementioned methods see, for example, Dylla

et al. 2008, PMID: 18560594 & Hoey et al. 2009, PMID: 19664991; each of which is incorporated herein by reference in its entirety and, in particular, for the disclosed methods.

With respect to limiting dilution analysis, *in vitro* enumeration of tumor initiating cell frequency may be accomplished by depositing either fractionated or unfractionated human tumor cells (e.g. from treated and untreated tumors, respectively) into *in vitro* growth conditions that foster colony formation. In this manner, colony forming cells might be enumerated by simple counting and characterization of colonies, or by analysis consisting of, for example, the deposition of human tumor cells into plates in serial dilutions and scoring each well as either positive or negative for colony formation at least 10 days after plating. *In vivo* limiting dilution experiments or analyses, which are generally more accurate in their ability to determine tumor initiating cell frequency encompass the transplantation of human tumor cells, from either untreated control or treated populations, for example, into immunocompromised mice in serial dilutions and subsequently scoring each mouse as either positive or negative for tumor formation at least 60 days after transplant. The derivation of cell frequency values by limiting dilution analysis *in vitro* or *in vivo* is preferably done by applying Poisson distribution statistics to the known frequency of positive and negative events, thereby providing a frequency for events fulfilling the definition of a positive event; in this case, colony or tumor formation, respectively.

As to other methods compatible with the instant invention that may be used to calculate tumor initiating cell frequency, the most common comprise quantifiable flow cytometric techniques and immunohistochemical staining procedures. Though not as precise as the limiting dilution analysis techniques described immediately above, these procedures are much less labor intensive and provide reasonable values in a relatively short time frame. Thus, it will be appreciated that a skilled artisan may use flow cytometric cell surface marker profile determination employing one or more antibodies or reagents that bind art-recognized cell surface proteins known to enrich for tumor initiating cells (e.g., potentially compatible markers as are set forth in PCT application 2012/031280 which is incorporated herein in its entirety) and thereby measure TIC levels from various samples. In still another compatible method one skilled in the art might enumerate TIC frequency *in situ* (e.g., in a tissue section) by immunohistochemistry using one or more antibodies or reagents that are able to bind cell surface proteins thought to demarcate these cells.

Those skilled in the art will recognize that numerous markers (or their absence) have been associated with various populations of cancer stem cells and used to isolate or characterize tumor cell subpopulations. In this respect exemplary cancer stem cell markers comprise OCT4, Nanog, STAT3, EPCAM, CD24, CD34, NB84, TrkA, GD2, CD133, CD20, CD56, CD29, B7H3, CD46, transferrin receptor, JAM3, carboxypeptidase M, ADAM9, oncostatin M, Lgr5, Lgr6, CD324, CD325, nestin, Sox1, Bmi-1, eed, easyh1, easyh2, mf2, yy1, smarcA3, smarckA5, smarcD3, smarcE1, mllt3, FZD1, FZD2, FZD3, FZD4, FZD6, FZD7, FZD8, FZD9, FZD10, WNT2, WNT2B, WNT3, WNT5A, WNT10B, WNT16, AXIN1, BCL9, MYC, (TCF4) SLC7A8, IL1RAP, TEM8, TMPRSS4, MUC16, GPRC5B, SLC6A14, SLC4A11, PPAP2C, CAV1, CAV2, PTPN3, EPHA1, EPHA2, SLC1A1, CX3CL1, ADORA2A, MPZL1, FLJ10052, C4.4A, EDG3, RARRES1, TMEPAI, PTS, CEACAM6, NID2, STEAP, ABCA3, CRIM1, IL1R1, OPN3, DAF, MUC1, MCP, CPD, NMA, ADAM9, GJA1, SLC19A2, ABCA1, PCDH7, ADCY9, SLC39A1, NPC1, ENPP1, N33, GPNMB, LY6E, CELSR1, LRP3, C20orf52, TMEPAI, FLVCR, PCDHA10, GPR54, TGFBR3, SEMA4B, PCDHB2, ABCG2, CD166, AFP, BMP-4, β-catenin, CD2, CD3, CD9, CD14, CD31, CD38, CD44, CD45, CD74, CD90, CXCR4, decorin, EGFR, CD105, CD64, CD16, CD16a, CD16b, GLI1, GLI2, CD49b, and CD49f. See, for example, Schulenburg et al., 2010, PMID: 20185329, U.S.P.N. 7,632,678 and U.S.P.Ns. 2007/0292414, 2008/0175870, 2010/0275280, 2010/0162416 and 2011/0020221 each of which is incorporated herein by reference. It will further be appreciated that each of the aforementioned markers may also be used as a secondary target antigen in the context of the bispecific or multispecific antibodies of the instant invention.

Similarly, non-limiting examples of cell surface phenotypes associated with cancer stem cells of certain tumor types include $CD44^{hi}CD24^{low}$, $ALDH^{+}$, $CD133^{+}$, $CD123^{+}$, $CD34^{+}CD38^{-}$, $CD44^{+}CD24^{-}$, $CD46^{hi}CD324^{+}CD66c^{-}$, $CD133^{+}CD34^{+}CD10^{-}CD19^{-}$, $CD138^{-}CD34^{-}CD19^{+}$, $CD133^{+}RC2^{+}$, $CD44^{+}\alpha_2\ \beta_1{}^{hi}CD133^{+}$, $CD44^{+}CD24^{+}ESA^{+}$, $CD271^{+}$, $ABCB5^{+}$ as well as other cancer stem cell surface phenotypes that are known in the art. See, for example, Schulenburg et al., 2010, supra, Visvader et al., 2008, PMID: 18784658 and U.S.P.N. 2008/0138313, each of which is incorporated herein in its entirety by reference. Those skilled in the art will appreciate that marker phenotypes such as those exemplified immediately above may be used in conjunction with standard flow cytometric analysis and

cell sorting techniques to characterize, isolate, purify or enrich TIC and/or TPC cells or cell populations for further analysis. Of interest with regard to the instant invention CD46, CD324 and, optionally, CD66c are either highly or heterogeneously expressed on the surface of many human colorectal ("CR"), breast ("BR"), non-small cell lung (NSCLC), small cell lung (SCLC), pancreatic ("PA"), melanoma ("Mel"), ovarian ("OV"), and head and neck cancer ("HN") tumor cells, regardless of whether the tumor specimens being analyzed were primary patient tumor specimens or patient-derived NTX tumors.

Using any of the above-referenced methods and selected markers as known in the art it is then possible to quantify the reduction in frequency of TIC (or the TPC therein) provided by the disclosed SEZ6 modulators (including those conjugated to cytotoxic agents) in accordance with the teachings herein. In some instances, the compounds of the instant invention may reduce the frequency of TIC or TPC (by a variety of mechanisms noted above, including elimination, induced differentiation, niche disruption, silencing, etc.) by 10%, 15%, 20%, 25%, 30% or even by 35%. In other embodiments, the reduction in frequency of TIC or TPC may be on the order of 40%, 45%, 50%, 55%, 60% or 65%. In certain embodiments, the disclosed compounds my reduce the frequency of TIC or TPC by 70%, 75%, 80%, 85%, 90% or even 95%. Of course it will be appreciated that any reduction of the frequency of the TIC or TPC likely results in a corresponding reduction in the tumorigenicity, persistence, recurrence and aggressiveness of the neoplasia.

IV. SEZ6 Modulators

In any event, the present invention is directed to the use of SEZ6 modulators, including SEZ6 antagonists, for the diagnosis, theragnosis, treatment and/or prophylaxis of various disorders including any one of a number of SEZ6 associated malignancies. The disclosed modulators may be used alone or in conjunction with a wide variety of anti-cancer compounds such as chemotherapeutic or immunotherapeutic agents (e.g., therapeutic antibodies) or biological response modifiers. In other selected embodiments, two or more discrete SEZ6 modulators may be used in combination to provide enhanced anti-neoplastic effects or may be used to fabricate multispecific constructs.

In certain embodiments, the SEZ6 modulators of the present invention will comprise nucleotides, oligonucleotides, polynucleotides, peptides or polypeptides. More particularly,

exemplary modulators of the invention may comprise antibodies and antigen-binding fragments or derivatives thereof, proteins, peptides, glycoproteins, glycopeptides, glycolipids, polysaccharides, oligosaccharides, nucleic acids, antisense constructs, siRNA, miRNA, bioorganic molecules, peptidomimetics, pharmacological agents and their metabolites, transcriptional and translation control sequences, and the like. In certain embodiments the modulators will comprise soluble SEZ6 (sSEZ6) or a form, variant, derivative or fragment thereof including, for example, SEZ6 fusion constructs (e.g., SEZ6-Fc, SEZ6-targeting moiety, etc.) or SEZ6-conjugates (e.g., SEZ6-PEG, SEZ6-cytotoxic agent, SEZ6-brm, etc.). It will also be appreciated that, in other embodiments, the SEZ6 modulators comprise antibodies or immunoreactive fragments or derivatives thereof. In particularly preferred embodiments the modulators of the instant invention will comprise neutralizing antibodies or derivatives or fragments thereof. In other embodiments the SEZ6 modulators may comprise internalizing antibodies or fragments thereof. In still other embodiments the SEZ6 modulators may comprise depleting antibodies or fragments thereof. Moreover, as with the aforementioned fusion constructs, these antibody modulators may be conjugated, linked or otherwise associated with selected cytotoxic agents, polymers, biological response modifiers (BRMs) or the like to provide directed immunotherapies with various (and optionally multiple) mechanisms of action. As alluded to above such antibodies may be pan-SEZ6 antibodies and associate with two or more SEZ6 family members (e.g., SEZ6 and SEZ6L as shown in FIG. 11A) or immunospecific antibodies that selectively react with one or both isoforms of SEZ6. In yet other embodiments the modulators may operate on the genetic level and may comprise compounds as antisense constructs, siRNA, miRNA and the like that interact or associate with the genotypic component of a SEZ6 determinant.

It will further be appreciated that the disclosed SEZ6 modulators may deplete, silence, neutralize, eliminate or inhibit growth, propagation or survival of tumor cells, including TPC, and/or associated neoplasia through a variety of mechanisms, including agonizing or antagonizing selected pathways or eliminating specific cells depending, for example, on the form of SEZ6 modulator, any associated payload or dosing and method of delivery. Thus, while preferred embodiments disclosed herein are directed to the depletion, inhibition or silencing of specific tumor cell subpopulations such as tumor perpetuating cells or to modulators that interact with a specific epitope or domain, it must be emphasized that such

embodiments are merely illustrative and not limiting in any sense. Rather, as set forth in the appended claims, the present invention is broadly directed to SEZ6 modulators and their use in the treatment, management or prophylaxis of various SEZ6 associated disorders irrespective of any particular mechanism, binding region or target tumor cell population.

Regardless of the form of the modulator selected it will be appreciated that the chosen compound may be antagonistic in nature. As used herein an "antagonist" refers to a molecule capable of neutralizing, blocking, inhibiting, abrogating, reducing or interfering with the activities of a particular or specified target (e.g., SEZ6), including the binding of receptors to ligands or the interactions of enzymes with substrates. In this respect it will be appreciated that SEZ6 antagonists of the instant invention may comprise any ligand, polypeptide, peptide, fusion protein, antibody or immunologically active fragment or derivative thereof that recognizes, reacts, binds, combines, competes, associates or otherwise interacts with the SEZ6 protein or fragment thereof and eliminates, silences, reduces, inhibits, hinders, restrains or controls the growth of tumor initiating cells or other neoplastic cells including bulk tumor or NTG cell. Compatible antagonists may further include small molecule inhibitors, aptamers, antisense constructs, siRNA, miRNA and the like, receptor or ligand molecules and derivatives thereof which recognize or associate with a SEZ6 genotypic or phenotypic determinant thereby altering expression patterns or sequestering its binding or interaction with a substrate, receptor or ligand.

As used herein an antagonist refers to a molecule capable of neutralizing, blocking, inhibiting, abrogating, reducing or interfering with the activities of a particular or specified protein, including the binding of receptors to ligands or the interactions of enzymes with substrates. More generally antagonists of the invention may comprise antibodies and antigen-binding fragments or derivatives thereof, proteins, peptides, glycoproteins, glycopeptides, glycolipids, polysaccharides, oligosaccharides, nucleic acids, antisense constructs, siRNA, miRNA, bioorganic molecules, peptidomimetics, pharmacological agents and their metabolites, transcriptional and translation control sequences, and the like. Antagonists may also include small molecule inhibitors, fusion proteins, receptor molecules and derivatives which bind specifically to the protein thereby sequestering its binding to its substrate target, antagonist variants of the protein, antisense molecules directed to the protein, RNA aptamers, and ribozymes against the protein.

As used herein and applied to two or more molecules or compounds, the terms "recognizes" or "associates" shall be held to mean the reaction, binding, specific binding, combination, interaction, connection, linkage, uniting, coalescence, merger or joining, covalently or non-covalently, of the molecules whereby one molecule exerts an effect on the other molecule.

Moreover, as demonstrated in the examples herein (e.g., see FIG. 11), some modulators of human SEZ6 may, in certain cases, cross-react with SEZ6 from a species other than human (e.g., rat or cynomolgus monkey). In other cases exemplary modulators may be specific for one or more isoforms of human SEZ6 and will not exhibit cross-reactivity with SEZ6 orthologs from other species. Of course, in conjunction with the teachings herein such embodiments may comprise pan-SEZ6 antibodies that associate with two or more SEZ6 family members from a single species or antibodies that exclusively associate with SEZ6.

In any event, and as will be discussed in more detail below, those skilled in the art will appreciate that the disclosed modulators may be used in a conjugated or unconjugated form. That is, the modulator may be associated with or conjugated to (e.g. covalently or non-covalently) pharmaceutically active compounds, biological response modifiers, anti-cancer agents, cytotoxic or cytostatic agents, diagnostic moieties or biocompatible modifiers. In this respect it will be understood that such conjugates may comprise peptides, polypeptides, proteins, fusion proteins, nucleic acid molecules, small molecules, mimetic agents, synthetic drugs, inorganic molecules, organic molecules and radioisotopes. Moreover, as indicated herein the selected conjugate may be covalently or non-covalently linked to the SEZ6 modulator in various molar ratios depending, at least in part, on the method used to effect the conjugation.

V. Modulator Fabrication and Supply

A. Antibody Modulators

1. Overview

As previously alluded to particularly preferred embodiments of the instant invention comprise SEZ6 modulators in the form of antibodies that preferentially associate with one or more isoforms of SEZ6 (and, optionally, may cross-react with other SEZ6 family members). Those of ordinary skill in the art will appreciate the well developed knowledge base on

antibodies such as set forth, for example, in Abbas *et al.*, Cellular and Molecular Immunology, 6th ed., W.B. Saunders Company (2010) or Murphey *et al.*, Janeway's Immunobiology, 8th ed., Garland Science (2011), each of which is incorporated herein by reference in its entirety.

The term "antibody" is intended to cover polyclonal antibodies, multiclonal antibodies, monoclonal antibodies, chimeric antibodies, humanized and primatized antibodies, human antibodies, recombinantly produced antibodies, intrabodies, multispecific antibodies, bispecific antibodies, monovalent antibodies, multivalent antibodies, anti-idiotypic antibodies, synthetic antibodies, including muteins and variants thereof; antibody fragments such as Fab fragments, F(ab') fragments, single-chain FvFcs, single-chain Fvs; and derivatives thereof including Fc fusions and other modifictaions, and any other immunologically active molecule so long as they exhibit the desired biological activity (i.e., antigen association or binding). Moreover, the term further comprises all classes of antibodies (i.e. IgA, IgD, IgE, IgG, and IgM) and all isotypes (i.e., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2), as well as variations thereof unless otherwise dictated by context. Heavy-chain constant domains that correspond to the different classes of antibodies are denoted by the corresponding lower case Greek letter α, δ, ε, γ, and μ, respectively. Light chains of the antibodies from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

While all such antibodies are within the scope of the present invention, preferred embodiments comprising the IgG class of immunoglobulin will be discussed in some detail herein solely for the purposes of illustration. It will be understood that such disclosure is, however, merely demonstrative of exemplary compositions and methods of practicing the present invention and not in any way limiting of the scope of the invention or the claims appended hereto.

As is well known, the variable domains of both the light (V_L) and heavy (V_H) chain portions determine antigen recognition and specificity and the constant domains of the light chain (C_L) and the heavy chain (C_H1, C_H2 or C_H3) confer and regulate important biological properties such as secretion, transplacental mobility, circulation half-life, complement binding, and the like.

The "variable" region includes hypervariable sites that manifest themselves in three segments commonly termed complementarity determining regions (CDRs), in both the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains flanking the CDRs are termed framework regions (FRs). For example, in naturally occurring monomeric immunoglobulin G (IgG) antibodies, the six CDRs present on each arm of the "Y" are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen binding site as the antibody assumes its three dimensional configuration in an aqueous environment. Thus, each naturally occurring IgG antibody comprises two identical binding sites proximal to the amino-terminus of each arm of the Y.

It will be appreciated that the position of CDRs can be readily identified by one of ordinary skill in the art using standard techniques. Also familiar to those in the art is the numbering system described in Kabat *et al.* (1991, NIH Publication 91-3242, National Technical Information Service, Springfield, Va.). In this regard Kabat et al. defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable domain sequence, without reliance on any experimental data beyond the sequence itself. Unless otherwise specified, references to the numbering of specific amino acid residue positions in an antibody are according to the Kabat numbering system.

Thus, according to Kabat, in the V_H, residues 31-35 comprise CDR1, residues 50-65 make up CDR2, and 95-102 comprise CDR3, while in the V_L, residues 24-34 are CDR1, 50-56 comprise CDR2, and 89-97 make up CDR3. For context, in a V_H, FR1 corresponds to the domain of the variable region encompassing amino acids 1-30; FR2 corresponds to the domain of the variable region encompassing amino acids 36-49; FR3 corresponds to the domain of the variable region encompassing amino acids 66-94, and FR4 corresponds to the domain of the variable region from amino acids 103 to the end of the variable region. The FRs for the light chain are similarly separated by each of the light chain variable region CDRs.

Note that CDRs vary considerably from antibody to antibody (and by definition will not exhibit homology with the Kabat consensus sequences). In addition, the identity of certain individual residues at any given Kabat site number may vary from antibody chain to antibody chain due to interspecies or allelic divergence. Alternative numbering is set forth in Chothia

et al., *J. Mol. Biol.* 196:901-917 (1987) and MacCallum *et al.*, *J. Mol. Biol.* 262:732-745 (1996), although as in Kabat, the FR boundaries are separated by the respective CDR termini as described above. See also Chothia et al., Nature 342, pp. 877-883 (1989) and S. Dubel, ed., *Handbook of Therapeutic Antibodies,* 3rd ed., WILEY-VCH Verlag GmbH and Co. (2007), where the definitions include overlapping or subsets of amino acid residues when compared against each other. Each of the aforementioned references is incorporated herein by reference in its entirety and the amino acid residues which comprise binding regions or CDRs as defined by each of the above cited references and are set forth for comparison below.

CDR Definitions

	Kabat[1]	Chothia[2]	MacCallum[3]
V_H CDR1	31-35	26-32	30-35
V_H CDR2	50-65	50-58	47-58
V_H CDR3	95-102	95-102	93-101
V_L CDR1	24-34	23-34	30-36
V_L CDR2	50-56	50-56	46-55
V_L CDR3	89-97	89-97	89-96

[1]Residue numbering follows the nomenclature of Kabat *et al.*, supra
[2]Residue numbering follows the nomenclature of Chothia *et al.*, supra
[3]Residue numbering follows the nomenclature of MacCallum *et al.*, supra

In the context of the instant invention it will be appreciated that any of the disclosed light and heavy chain CDRs derived from the murine variable region amino acid sequences set forth in FIG. 10A or FIG. 10B may be combined or rearranged to provide optimized anti-SEZ6 (e.g. humanized, CDR grafted or chimeric anti-hSEZ6) antibodies in accordance with the instant teachings. That is, one or more of the CDRs derived from the light chain variable region amino acid sequences set forth in FIG. 10A (SEQ ID NOS: 20 – 168, even numbers) or the heavy chain variable region amino acid sequences set forth in FIG. 10B (SEQ ID NOS: 21 – 169, odd numbers) may be incorporated in a SEZ6 modulator and, in particularly preferred embodiments, in a CDR grafted or humanized antibody that immunospecifically associates with one or more SEZ6 isoforms. Examples of light (SEQ ID NOS: 170 – 192, even

numbers) and heavy (SEQ ID NOS: 171 – 193, odd numbers and 194 - 199) chain variable region amino acid sequences of such humanized modulators are also set forth in FIGS. 10A and 10B.

Note that hSC17.200vL1 (SEQ ID NO: 192) is a variant of the humanized light chain construct hSC17.200 (SEQ ID NO: 190), hSC17.155vH1 – vH6 (SEQ ID NOS: 193-198) are variants of the heavy chain construct hSC.155 (SEQ ID NO: 184) which is derived from SC17.90 (SEQ ID NO: 127) and that hSC161vH1 (SEQ ID NO: 199) is a variant of the heavy chain construct hSC17.161 (SEQ ID NO: 189). As will be discussed in more detail below these variants were constructed and tested to optimize one or more biochemical properties of the parent antibody.

Taken together these novel amino acid sequences depict seventy-five murine and eleven humanized exemplary modulators (along with reported variants) in accordance with the instant invention. Moreover, corresponding nucleic acid sequences of each of the seventy-five exemplary murine modulators and eleven humanized modulators and variants set forth in FIGS. 10A and 10B are included in sequence listing of the instant application (SEQ ID NOS: 220 – 399).

In FIGS. 10A and 10B the annotated CDRs are defined using Chothia numbering. However, as discussed herein and demonstrated in Example 8 below, one skilled in the art could readily define, identify, derive and/or enumerate the CDRs as defined by Kabat et al., Chothia et al. or MacCallum et al. for each respective heavy and light chain sequence set forth in FIG. 10A or FIG. 10B. Accordingly, each of the subject CDRs and antibodies comprising CDRs defined by all such nomenclature are expressly included within the scope of the instant invention. More broadly, the terms "variable region CDR amino acid residue" or more simply "CDR" includes amino acids in a CDR as identified using any sequence or structure based method as set forth above.

2. Antibody Modulator Generation

a. Polyclonal antibodies

The production of polyclonal antibodies in various host animals, including rabbits, mice, rats, etc. is well known in the art. In some embodiments, polyclonal anti-SEZ6 antibody-containing serum is obtained by bleeding or sacrificing the animal. The serum may

be used for research purposes in the form obtained from the animal or, in the alternative, the anti-SEZ6 antibodies may be partially or fully purified to provide immunoglobulin fractions or homogeneous antibody preparations.

Briefly the selected animal is immunized with a SEZ6 immunogen (e.g., soluble SEZ6 or sSEZ6) which may, for example, comprise selected isoforms, domains and/or peptides, or live cells or cell preparations expressing SEZ6 or immunoreactive fragments thereof. Art known adjuvants that may be used to increase the immunological response, depending on the inoculated species include, but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants may protect the antigen from rapid dispersal by sequestering it in a local deposit, or they may contain substances that stimulate the host to secrete factors that are chemotactic for macrophages and other components of the immune system. Preferably the immunization schedule will involve two or more administrations of the selected immunogen spread out over a predetermined period of time.

The amino acid sequence of a SEZ6 protein as shown in FIGS. 1C or 1D can be analyzed to select specific regions of the SEZ6 protein for generating antibodies. For example, hydrophobicity and hydrophilicity analyses of a SEZ6 amino acid sequence are used to identify hydrophilic regions in the SEZ6 structure. Regions of a SEZ6 protein that show immunogenic structure, as well as other regions and domains, can readily be identified using various other methods known in the art, such as Chou-Fasman, Garnier-Robson, Kyte-Doolittle, Eisenberg, Karplus-Schultz or Jameson-Wolf analysis. Average Flexibility profiles can be generated using the method of Bhaskaran R., Ponnuswamy P. K., 1988, Int. J. Pept. Protein Res. 32:242-255. Beta-turn profiles can be generated using the method of Deleage, G., Roux B., 1987, Protein Engineering 1:289-294. Thus, each SEZ6 region, domain or motif identified by any of these programs or methods is within the scope of the present invention and may be isolated or engineered to provide immunogens giving rise to modulators comprising desired properties. Preferred methods for the generation of SEZ6 antibodies are further illustrated by way of the Examples provided herein. Methods for preparing a protein or polypeptide for use as an immunogen are well known in the art. Also well known in the art

are methods for preparing immunogenic conjugates of a protein with a carrier, such as BSA, KLH or other carrier protein. In some circumstances, direct conjugation using, for example, carbodiimide reagents are used; in other instances linking reagents are effective. Administration of a SEZ6 immunogen is often conducted by injection over a suitable time period and with use of a suitable adjuvant, as is understood in the art. During the immunization schedule, titers of antibodies can be taken as described in the Examples below to determine adequacy of antibody formation.

b. Monoclonal antibodies

In addition, the invention contemplates use of monoclonal antibodies. As known in the art, the term "monoclonal antibody" (or mAb) refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible mutations (e.g., naturally occurring mutations), that may be present in minor amounts. In certain embodiments, such a monoclonal antibody includes an antibody comprising a polypeptide sequence that binds or associates with an antigen wherein the antigen-binding polypeptide sequence was obtained by a process that includes the selection of a single target binding polypeptide sequence from a plurality of polypeptide sequences.

More generally, and as exemplified in Example 6 herein, monoclonal antibodies can be prepared using a wide variety of techniques known in the art including hybridoma, recombinant techniques, phage display technologies, transgenic animals (e.g., a XenoMouse®) or some combination thereof. For example, monoclonal antibodies can be produced using hybridoma and art-recognized biochemical and genetic engineering techniques such as described in more detail in An, Zhigiang (ed.) *Therapeutic Monoclonal Antibodies: From Bench to Clinic*, John Wiley and Sons, 1st ed. 2009; Shire et. al. (eds.) *Current Trends in Monoclonal Antibody Development and Manufacturing*, Springer Science + Business Media LLC, 1st ed. 2010; Harlow *et al.*, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 2nd ed. 1988; Hammerling, *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) each of which is incorporated herein in its entirety by reference. It should be understood that a selected binding sequence can be further altered, for example, to improve affinity for the target, to humanize the target binding sequence, to improve its production in cell culture, to reduce its immunogenicity in vivo, to

create a multispecific antibody, etc., and that an antibody comprising the altered target binding sequence is also an antibody of this invention.

c. Chimeric antibodies

In another embodiment, the antibody of the invention may comprise chimeric antibodies derived from covalently joined protein segments from at least two different species or types of antibodies. As known in the art, the term "chimeric" antibodies is directed to constructs in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. P.N. 4,816,567; Morrison *et al.*, Proc. Natl. Acad. Sci. USA, 81:6851-6855 (1984)).

In one embodiment, a chimeric antibody in accordance with the teachings herein may comprise murine V_H and V_L amino acid sequences and constant regions derived from human sources. In other compatible embodiments a chimeric antibody of the present invention may comprise a humanized antibody as described below. In another embodiment, the so-called "CDR-grafted" antibody, the antibody comprises one or more CDRs from a particular species or belonging to a particular antibody class or subclass, while the remainder of the antibody chain(s) is/are identical with or homologous to a corresponding sequence in antibodies derived from another species or belonging to another antibody class or subclass. For use in humans, selected rodent CDRs may be grafted into a human antibody, replacing one or more of the naturally occurring variable regions or CDRs of the human antibody. These constructs generally have the advantages of providing full strength modulator functions (e.g., CDC (complement dependent cytotoxicity), ADCC (antibody-dependent cell-mediated cytotoxicity), etc.) while reducing unwanted immune responses to the antibody by the subject.

d. Humanized antibodies

Similar to the CDR-grafted antibody is a "humanized" antibody. As used herein, "humanized" forms of non-human (e.g., murine) antibodies are chimeric antibodies that contain a minimal sequence derived from one or more non-human immunoglobulins. In one

embodiment, a humanized antibody is a human immunoglobulin (recipient or acceptor antibody) in which residues from a CDR of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, rabbit, or nonhuman primate having the desired specificity, affinity, and/or capacity. In certain preferred embodiments, residues in one or more FRs in the variable domain of the human immunoglobulin are replaced by corresponding non-human residues from the donor antibody to help maintain the appropriate three-dimensional configuration of the grafted CDR(s) and thereby improve affinity. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody to, for example, further refine antibody performance.

CDR grafting and humanized antibodies are described, for example, in U.S.P.Ns. 6,180,370 and 5,693,762. The humanized antibody optionally may also comprise at least a portion of an immunoglobulin Fc, typically that of a human immunoglobulin. For further details, see, e.g., Jones *et al.*, *Nature* 321:522-525 (1986); and U.S.P.Ns. 6,982,321 and 7,087,409. Still another method is termed "humaneering" which is described, for example, in U.S.P.N. 2005/0008625. Additionally, a non-human antibody may also be modified by specific deletion of human T-cell epitopes or "deimmunization" by the methods disclosed in WO 98/52976 and WO 00/34317. Each of the aforementioned references are incorporated herein in their entirety.

Humanized antibodies may also be bioengineered using common molecular biology techniques, such as isolating, manipulating, and expressing nucleic acid sequences that encode all or part of immunoglobulin variable regions from at least one of a heavy or light chain. In addition to the sources of such nucleic acid noted above, human germline sequences are available as disclosed, for example, in Tomlinson, I. A. *et al.* (1992) *J. Mol. Biol.* 227:776-798; Cook, G. P. *et al.* (1995) *Immunol. Today* 16: 237-242; Chothia, D. *et al.* (1992) *J. Mol. Biol.* 227:799-817; and Tomlinson *et al.* (1995) *EMBO J* 14:4628-4638. The V-BASE directory (VBASE2 - Retter *et al.*, Nucleic Acid Res. 33; 671-674, 2005) provides a comprehensive directory of human immunoglobulin variable region sequences (compiled by Tomlinson, I. A. *et al.* MRC Centre for Protein Engineering, Cambridge, UK). Consensus human FRs can also be used, e.g., as described in U.S.P.N. 6,300,064.

In selected embodiments, and as detailed in Example 8 below, at least 60%, 65%, 70%, 75%, or 80% of the humanized antibody heavy or light chain variable region amino acid residues will correspond to those of the recipient FR and CDR sequences. In other embodiments at least 85% or 90% of the humanized antibody variable region residues will correspond to those of the recipient FR and CDR sequences. In a further preferred embodiment, greater than 95% of the humanized antibody variable region residues will correspond to those of the recipient FR and CDR sequences.

e. Human antibodies

In another embodiment, the antibodies may comprise fully human antibodies. The term "human antibody" refers to an antibody which possesses an amino acid sequence that corresponds to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies.

Human antibodies can be produced using various techniques known in the art. One technique is phage display in which a library of (preferably human) antibodies is synthesized on phages, the library is screened with the antigen of interest or an antibody-binding portion thereof, and the phage that binds the antigen is isolated, from which one may obtain the immunoreactive fragments. Methods for preparing and screening such libraries are well known in the art and kits for generating phage display libraries are commercially available (e.g., the Pharmacia Recombinant Phage Antibody System, catalog no. 27-9400-01; and the Stratagene SurfZAP™ phage display kit, catalog no. 240612). There also are other methods and reagents that can be used in generating and screening antibody display libraries (see, e.g., U.S.P.N. 5,223,409; PCT Publication Nos. WO 92/18619, WO 91/17271, WO 92/20791, WO 92/15679, WO 93/01288, WO 92/01047, WO 92/09690; and Barbas *et al.*, *Proc. Natl. Acad. Sci. USA* 88:7978-7982 (1991)).

In one embodiment, recombinant human antibodies may be isolated by screening a recombinant combinatorial antibody library prepared as above. In one embodiment, the library is a scFv phage display library, generated using human V_L and V_H cDNAs prepared from mRNA isolated from B-cells.

The antibodies produced by naive libraries (either natural or synthetic) can be of moderate affinity (K_a of about 10^6 to 10^7 M^{-1}), but affinity maturation can also be mimicked *in vitro* by constructing and reselecting from secondary libraries as described in the art. For

example, mutation can be introduced at random in vitro by using error-prone polymerase (reported in Leung *et al.*, *Technique*, 1: 11-15 (1989)). Additionally, affinity maturation can be performed by randomly mutating one or more CDRs, e.g. using PCR with primers carrying random sequence spanning the CDR of interest, in selected individual Fv clones and screening for higher-affinity clones. WO 9607754 described a method for inducing mutagenesis in a CDR of an immunoglobulin light chain to create a library of light chain genes. Another effective approach is to recombine the V_H or V_L domains selected by phage display with repertoires of naturally occurring V domain variants obtained from unimmunized donors and to screen for higher affinity in several rounds of chain reshuffling as described in Marks *et al.*, *Biotechnol.*, 10: 779-783 (1992). This technique allows the production of antibodies and antibody fragments with a dissociation constant K_D (k_{off}/k_{on}) of about 10^{-9} M or less.

In other embodiments, similar procedures may be employed using libraries comprising eukaryotic cells (e.g., yeast) that express binding pairs on their surface. See, for example, U.S.P.N. 7,700,302 and U.S.S.N. 12/404,059. In one embodiment, the human antibody is selected from a phage library, where that phage library expresses human antibodies (Vaughan *et al.* Nature *Biotechnology* 14:309-314 (1996): Sheets *et al. Proc. Natl. Acad. Sci. USA* 95:6157-6162 (1998). In other embodiments, human binding pairs may be isolated from combinatorial antibody libraries generated in eukaryotic cells such as yeast. See e.g., U.S.P.N. 7,700,302. Such techniques advantageously allow for the screening of large numbers of candidate modulators and provide for relatively easy manipulation of candidate sequences (e.g., by affinity maturation or recombinant shuffling).

Human antibodies can also be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated and human immunoglobulin genes have been introduced. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S.P.Ns. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and U.S.P.Ns. 6,075,181 and 6,150,584 regarding XenoMouse® technology; and Lonberg and Huszar, *Intern. Rev. Immunol.* 13:65-93 (1995). Alternatively, the human antibody may be prepared via immortalization of human B

lymphocytes producing an antibody directed against a target antigen (such B lymphocytes may be recovered from an individual suffering from a neoplastic disorder or may have been immunized *in vitro*). See, e.g., Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner *et al.*, *J. Immunol*, 147 (1):86-95 (1991); and U.S.P.N. 5,750,373.

3. Further Processing

No matter how obtained, modulator-producing cells (e.g., hybridomas, yeast colonies, etc.) may be selected, cloned and further screened for desirable characteristics including, for example, robust growth, high antibody production and, as discussed in more detail below, desirable antibody characteristics. Hybridomas can be expanded *in vivo* in syngeneic animals, in animals that lack an immune system, e.g., nude mice, or in cell culture *in vitro*. Methods of selecting, cloning and expanding hybridomas and/or colonies, each of which produces a discrete antibody species, are well known to those of ordinary skill in the art.

B. Recombinant Modulator Production

1. Overview

Once the source is perfected DNA encoding the desired SEZ6 modulators may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding antibody heavy and light chains). Isolated and subcloned hybridoma cells (or phage or yeast derived colonies) may serve as a preferred source of such DNA if the modulator is an antibody. If desired, the nucleic acid can further be manipulated as described herein to create agents including fusion proteins, or chimeric, humanized or fully human antibodies. More particularly, isolated DNA (which may be modified) can be used to clone constant and variable region sequences for the manufacture antibodies.

Accordingly, in exemplary embodiments antibodies may be produced recombinantly, using conventional procedures (such as those set forth in Al-Rubeai; An, and Shire et. al. all supra, and Sambrook J. & Russell D. Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2000); Ausubel *et al.*, Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in

Molecular Biology, Wiley, John & Sons, Inc. (2002)) in which the isolated and subcloned hybridoma cells (or phage or yeast derived colonies) serve as a preferred source of nucleic acid molecules.

The term "nucleic acid molecule", as used herein, is intended to include DNA molecules and RNA molecules and artificial variants thereof (e.g., peptide nucleic acids), whether single-stranded or double-stranded. The nucleic acids may encode one or both chains of an antibody of the invention, or a fragment or derivative thereof. The nucleic acid molecules of the invention also include polynucleotides sufficient for use as hybridization probes, PCR primers or sequencing primers for identifying, analyzing, mutating or amplifying a polynucleotide encoding a polypeptide; anti-sense nucleic acids for inhibiting expression of a polynucleotide, and as well as complementary sequences. The nucleic acids can be any length. They can be, for example, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 750, 1,000, 1,500, 3,000, 5,000 or more nucleotides in length, and/or can comprise one or more additional sequences, for example, regulatory sequences, and/or be part of a larger nucleic acid, for example, a vector. It will be appreciated that such nucleic acid sequences can further be manipulated to create modulators including chimeric, humanized or fully human antibodies. More particularly, isolated nucleic acid molecules (which may be modified) can be used to clone constant and variable region sequences for the manufacture antibodies as described in U.S.P.N. 7,709,611.

The term "isolated nucleic acid" means a that the nucleic acid was (i) amplified in vitro, for example by polymerase chain reaction (PCR), (ii) recombinantly produced by cloning, (iii) purified, for example by cleavage and gel-electrophoretic fractionation, or (iv) synthesized, for example by chemical synthesis. An isolated nucleic acid is a nucleic acid that is available for manipulation by recombinant DNA techniques.

Whether the source of the nucleic acid encoding the desired immunoreactive portion of the antibody is obtained or derived from phage display technology, yeast libraries, hybridoma-based technology or synthetically, it is to be understood that the present invention encompasses the nucleic acid molecules and sequences encoding the antibodies or antigen-binding fragments or derivatives thereof. Further, the instant invention is directed to vectors and host cells comprising such nucleic acid molecules.

2. Hybridization and Sequence Identity

As indicated, the invention further provides nucleic acids that hybridize to other nucleic acids under particular hybridization conditions. More specifically the invention encompasses nucleic acids molecules that hybridize under moderate or high stringency hybridization conditions (e.g., as defined below), to the nucleic acid molecules of the invention. Methods for hybridizing nucleic acids are well-known in the art. As is well known, a moderately stringent hybridization conditions comprise a prewashing solution containing 5x sodium chloride/sodium citrate (SSC), 0.5% SDS, 1.0 mM EDTA (pH 8.0), hybridization buffer of about 50% formamide, 6xSSC, and a hybridization temperature of 55°C (or other similar hybridization solutions, such as one containing about 50% formamide, with a hybridization temperature of 42°C), and washing conditions of 60°C, in 0.5xSSC, 0.1% SDS. By way of comparison hybridization under highly stringent hybridization conditions comprise washing with 6xSSC at 45°C, followed by one or more washes in 0.1xSSC, 0.2% SDS at 68°C. Furthermore, one of skill in the art can manipulate the hybridization and/or washing conditions to increase or decrease the stringency of hybridization such that nucleic acids comprising nucleotide sequences that are at least 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% identical to each other typically remain hybridized to each other.

The invention also includes nucleic acid molecules that are "substantially identical" to the described nucleic acid molecules. In one embodiment, the term substantially identical with regard to a nucleic acid sequence means may be construed as a sequence of nucleic acid molecules exhibiting at least about 65%, 70%, 75%, 80%, 85%, or 90% sequence identity. In other embodiments, the nucleic acid molecules exhibit 95% or 98% sequence identity to the reference nucleic acid sequence.

The basic parameters affecting the choice of hybridization conditions and guidance for devising suitable conditions are set forth by, for example, Sambrook, Fritsch, and Maniatis (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., chapters 9 and 11; and Current Protocols in Molecular Biology, 1995, Ausubel et al., eds., John Wiley & Sons, Inc., sections 2.10 and 6.3-6.4), and can be readily determined by those having ordinary skill in the art based on, for example, the length and/or base composition of the nucleic acid.

Sequence similarity for polypeptides, which is also referred to as sequence identity, is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, the sequence analysis tool GCG (Accelrys Software Inc.) contains programs such as "GAP" and "BEST-FIT" which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. (See, e.g., GCG Version 6.1 or Durbin et. Al., *Biological Sequence Analysis: Probabilistic models of proteins and nucleic acids*., Cambridge Press (1998)).

Polypeptide sequences can also be compared using FASTA using default or recommended parameters, a program in GCG Version 6.1. FASTA (e.g., FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) supra). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially blastp or tblastn, using default parameters. See, e.g., Altschul et al. (1990) J. Mol. Biol. 215: 403 410 and Altschul et al. (1997) Nucleic Acids Res. 25:3389 402, each of which is herein incorporated by reference.

In this regard the invention also includes nucleic acid molecules that encode polypeptides that are "substantially identical" with respect to an antibody variable region polypeptide sequence (e.g., either the donor light or heavy chain variable region, acceptor light or heavy chain variable region or resulting humanized construct). As applied to such polypeptides, the term "substantial identity" or "substantially identical" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BEST-FIT using default gap weights, share at least 60% or 65% sequence identity, preferably at least 70%, 75%, 80%, 85%, or 90% sequence identity, even more preferably at least 93%, 95%, 98% or 99% sequence identity. Preferably, residue positions which are not identical differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a

conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution.

3. Expression

The varied processes of recombinant expression, i.e., the production of RNA or of RNA and protein/peptide, are well known as set forth, for example, in Berger and Kimmel, Guide to Molecular Cloning Techniques, *Methods in Enzymology* volume 152 Academic Press, Inc., San Diego, Calif.; Sambrook *et al.*, *Molecular Cloning-A Laboratory Manual* (3rd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., (2000); and *Current Protocols in Molecular Biology*, F. M. Ausubel *et al.*, eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (supplemented through 2006).

Certain terms of interest include "expression control sequence" which comprises promoters, ribosome binding sites, enhancers and other control elements which regulate transcription of a gene or translation of mRNA. As is well known, a "promoter" or "promoter region" relates to a nucleic acid sequence which generally is located upstream (5') to the nucleic acid sequence being expressed and controls expression of the sequence by providing a recognition and binding site for RNA-polymerase.

Exemplary promoters which are compatible according to the invention include promoters for SP6, T3 and T7 polymerase, human U6 RNA promoter, CMV promoter, and artificial hybrid promoters thereof (e.g. CMV) where a part or parts are fused to a part or parts of promoters of genes of other cellular proteins such as e.g. human GAPDH (glyceraldehyde-3-phosphate dehydrogenase), and including or not including (an) additional intron(s).

In certain embodiments, the nucleic acid molecule may be present in a vector, where appropriate with a promoter, which controls expression of the nucleic acid. The well known term "vector" comprises any intermediary vehicle for a nucleic acid which enables said nucleic acid, for example, to be introduced into prokaryotic and/or eukaryotic cells and, where appropriate, to be integrated into a genome. Methods of transforming mammalian cells are well known in the art. See, for example, U.S.P.Ns. 4,399,216, 4,912,040, 4,740,461, and

4,959,455. The vectors may include a nucleotide sequence encoding an antibody of the invention (e.g., a whole antibody, a heavy or light chain of an antibody, a V_H or V_L of an antibody, or a portion thereof, or a heavy- or light-chain CDR, a single chain Fv, or fragments or variants thereof), operably linked to a promoter (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S.P.N. 5,122,464).

A variety of host-expression vector systems are commercially available, and many are compatible with the teachings herein and may be used to express the modulators of the invention. Such systems include, but are not limited to, microorganisms such as bacteria (e.g., E. coli, B. subtilis, streptomyces) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing modulator coding sequences; yeast (e.g., Saccharomyces, Pichia) transfected with recombinant yeast expression vectors containing modulator coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing modulator coding sequences; plant cell systems (e.g., Nicotiana, Arabidopsis, duckweed, corn, wheat, potato, etc.) infected with recombinant viral expression vectors (e.g., cauliflower mosaic virus; tobacco mosaic virus) or transfected with recombinant plasmid expression vectors (e.g., Ti plasmid) containing modulator coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells, etc.) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter).

As used herein, the term "host cell" covers any kind of cellular system which can be engineered to generate the polypeptides and antigen-binding molecules of the present invention. In one embodiment, the host cell is engineered to allow the production of an antigen binding molecule with modified glycoforms. In a preferred embodiment, the antigen binding molecule, or variant antigen binding molecule, is an antibody, antibody fragment, or fusion protein. In certain embodiments, the host cells have been further manipulated to express increased levels of one or more polypeptides having N-acetylglucosaminyltransferase III (GnTI11) activity. Compatible host cells include cultured cells, e.g., mammalian cultured cells, such as CHO cells, BHK cells, NSO cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells or hybridoma cells, yeast cells, insect cells,

and plant cells, to name only a few, but also cells comprised within a transgenic animal, transgenic plant or cultured plant or animal tissue.

For long-term, high-yield production of recombinant proteins stable expression is preferred. Accordingly, cell lines that stably express the selected modulator may be engineered using standard art-recognized techniques. Rather than using expression vectors that contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Any of the selection systems well known in the art may be used, including the glutamine synthetase gene expression system (the GS system) which provides an efficient approach for enhancing expression under certain conditions. The GS system is discussed in whole or part in connection with EP patents 0 216 846, 0 256 055, 0 323 997 and 0 338 841 and U.S.P.N.s 5,591,639 and 5,879,936 each of which is incorporated herein by reference. Another preferred expression system, the Freedom™ CHO-S Kit is commercially provided by Life Technologies (Catalog Number A13696-01) also allows for the development of stable cell lines that may be used for modulator production.

Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express a molecule of the invention in situ. The host cell may be co-transfected with two expression vectors of the invention, for example, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide.

Thus, in certain embodiments, the present invention provides recombinant host cells allowing for the expression of antibodies or portions thereof. Antibodies produced by expression in such recombinant host cells are referred to herein as recombinant antibodies. The present invention also provides progeny cells of such host cells, and antibodies produced by the same.

C. Chemical Synthesis

In addition, the modulators may be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W.H.

Freeman & Co., N.Y., and Hunkapiller, M., *et al.*, 1984, Nature 310:105-111). Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs (such as D-isomers of the common amino acids, 2,4-diaminobutyric acid, a-amino isobutyric acid, 4-aminobutyric acid, and the like) can be introduced as a substitution or addition into a polypeptide sequence.

D. Transgenic Systems

In other embodiments modulators may be produced transgenically through the generation of a mammal or plant that is transgenic for recombinant molecules such as the immunoglobulin heavy and light chain sequences and that produces the desired compounds in a recoverable form. This includes, for example, the production of protein modulators (e.g., antibodies) in, and recovery from, the milk of goats, cows, or other mammals. See, e.g., U.S.P.Ns. 5,827,690, 5,756,687, 5,750,172, and 5,741,957. In some embodiments, non-human transgenic animals that comprise human immunoglobulin loci are immunized to produce antibodies.

Other transgenic techniques are set forth in Hogan *et al.*, Manipulating the Mouse Embryo: A Laboratory Manual 2nd ed., Cold Spring Harbor Press (1999); Jackson *et al.*, Mouse Genetics and Transgenics: A Practical Approach, Oxford University Press (2000); and Pinkert, Transgenic Animal Technology: A Laboratory Handbook, Academic Press (1999) and U.S. P.N. 6,417,429. In some embodiments, the non-human animals are mice, rats, sheep, pigs, goats, cattle or horses, and the desired product is produced in blood, milk, urine, saliva, tears, mucus and other bodily fluids from which it is readily obtainable using art-recognized purification techniques.

Other compatible production systems include methods for making antibodies in plants such as described, for example, in U.S.P.Ns. 6,046,037 and 5,959,177 which are incorporated herein with respect to such techniques.

E. Isolation/Purification

Once a modulator of the invention has been produced by recombinant expression or any other of the disclosed techniques, it may be purified by any method known in the art for purification of immunoglobulins or proteins. In this respect the modulator may be "isolated" which means that it has been identified and separated and/or recovered from a component of

its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the polypeptide and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. Isolated modulators include a modulator *in situ* within recombinant cells because at least one component of the polypeptide's natural environment will not be present.

If the desired molecule is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, may be removed, for example, by centrifugation or ultrafiltration. Where the modulator is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Pellicon ultrafiltration unit (Millipore Corp.). Once the insoluble contaminants are removed the modulator preparation may be further purified using standard techniques such as, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography of particular interest. In this regard protein A can be used to purify antibodies that are based on human IgG1, IgG2 or IgG4 heavy chains (Lindmark, *et al.*, J Immunol Meth 62:1 (1983)) while protein G is recommended for all mouse isotypes and for human IgG3 (Guss, *et al.*, EMBO J 5:1567 (1986)). Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, reverse phase HPLC, chromatography on silica, chromatography on heparin, sepharose chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE and ammonium sulfate precipitation are also available depending on the antibody to be recovered. In particularly preferred embodiments the modulators of the instant invention will be purified, at least in part, using Protein A or Protein G affinity chromatography.

VI. SEZ6 Modulator Fragments and Derivatives

Whatever generation and production methodology is selected, modulators of the instant invention will react, bind, combine, complex, connect, attach, join, interact or otherwise associate with a target determinant (e.g., antigen) and thereby provide the desired results. Where the modulator comprises an antibody or fragment, construct or derivative thereof such associations may be through one or more "binding sites" or "binding components" expressed on the antibody, where a binding site comprises a region of a polypeptide that is responsible

for selectively binding to a target molecule or antigen of interest. Binding domains comprise at least one binding site (e.g., an intact IgG antibody will have two binding domains and two binding sites). Exemplary binding domains include an antibody variable domain, a receptor-binding domain of a ligand, a ligand-binding domain of a receptor or an enzymatic domain.

A. Antibodies

As noted above, the term "antibody" is intended to cover, at least, polyclonal antibodies, multiclonal antibodies, chimeric antibodies, CDR grafted antibodies, humanized and primatized antibodies, human antibodies, recombinantly produced antibodies, intrabodies, multispecific antibodies, bispecific antibodies, monovalent antibodies, multivalent antibodies, anti-idiotypic antibodies, as well as synthetic antibodies.

B. Fragments

Regardless of which form of the modulator (e.g. chimeric, humanized, etc.) is selected to practice the invention it will be appreciated that immunoreactive fragments of the same may be used in accordance with the teachings herein. An "antibody fragment" comprises at least a portion of an intact antibody. As used herein, the term "fragment" of an antibody molecule includes antigen-binding fragments of antibodies, and the term "antigen-binding fragment" refers to a polypeptide fragment of an immunoglobulin or antibody that immunospecifically binds or reacts with a selected antigen or immunogenic determinant thereof or competes with the intact antibody from which the fragments were derived for specific antigen binding.

Exemplary fragments include: V_L, V_H, scFv, F(ab')2 fragment, Fab fragment, Fd fragment, Fv fragment, single domain antibody fragments, diabodies, linear antibodies, single-chain antibody molecules and multispecific antibodies formed from antibody fragments. In addition, an active fragment comprises a portion of the antibody that retains its ability to interact with the antigen/substrates or receptors and modify them in a manner similar to that of an intact antibody (though maybe with somewhat less efficiency).

In other embodiments, an antibody fragment is one that comprises the Fc region and that retains at least one of the biological functions normally associated with the Fc region

when present in an intact antibody, such as FcRn binding, antibody half-life modulation, ADCC function and complement binding. In one embodiment, an antibody fragment is a monovalent antibody that has an *in vivo* half-life substantially similar to an intact antibody. For example, such an antibody fragment may comprise an antigen binding arm linked to an Fc sequence capable of conferring *in vivo* stability to the fragment.

As would be well recognized by those skilled in the art, fragments can be obtained via chemical or enzymatic treatment (such as papain or pepsin) of an intact or complete antibody or antibody chain or by recombinant means. See, e.g., Fundamental Immunology, W. E. Paul, ed., Raven Press, N.Y. (1999), for a more detailed description of antibody fragments.

C. Derivatives

The invention further includes immunoreactive modulator derivatives and antigen binding molecules comprising one or more modifications.

1. Multivalent Antibodies

In one embodiment, the modulators of the invention may be monovalent or multivalent (e.g., bivalent, trivalent, etc.). As used herein, the term "valency" refers to the number of potential target binding sites associated with an antibody. Each target binding site specifically binds one target molecule or specific position or locus on a target molecule. When an antibody is monovalent, each binding site of the molecule will specifically bind to a single antigen position or epitope. When an antibody comprises more than one target binding site (multivalent), each target binding site may specifically bind the same or different molecules (e.g., may bind to different ligands or different antigens, or different epitopes or positions on the same antigen). See, for example, U.S.P.N. 2009/0130105. In each case at least one of the binding sites will comprise an epitope, motif or domain associated with a SEZ6 isoform.

In one embodiment, the modulators are bispecific antibodies in which the two chains have different specificities, as described in Millstein *et al.*, 1983, *Nature*, 305:537-539. Other embodiments include antibodies with additional specificities such as trispecific antibodies. Other more sophisticated compatible multispecific constructs and methods of their fabrication are set forth in U.S.P.N. 2009/0155255, as well as WO 94/04690; Suresh *et al.*, 1986, *Methods in Enzymology*, 121:210; and WO96/27011.

As alluded to above, multivalent antibodies may immunospecifically bind to different epitopes of the desired target molecule or may immunospecifically bind to both the target molecule as well as a heterologous epitope, such as a heterologous polypeptide or solid support material. While preferred embodiments of the anti-SEZ6 antibodies only bind two antigens (i.e. bispecific antibodies), antibodies with additional specificities such as trispecific antibodies are also encompassed by the instant invention. Bispecific antibodies also include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S.P.N. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. P.N. 4,676,980, along with a number of cross-linking techniques.

In yet other embodiments, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences, such as an immunoglobulin heavy chain constant domain comprising at least part of the hinge, C_H2, and/or C_H3 regions, using methods well known to those of ordinary skill in the art.

2. Fc Region Modifications

In addition to the various modifications, substitutions, additions or deletions to the variable or binding region of the disclosed modulators (e.g., Fc-SEZ6 or anti-SEZ6 antibodies) set forth above, those skilled in the art will appreciate that selected embodiments of the present invention may also comprise substitutions or modifications of the constant region (i.e. the Fc region). More particularly, it is contemplated that the SEZ6 modulators of the invention may contain *inter alia* one or more additional amino acid residue substitutions, mutations and/or modifications which result in a compound with preferred characteristics including, but not limited to: altered pharmacokinetics, increased serum half life, increase binding affinity, reduced immunogenicity, increased production, altered Fc ligand binding to an Fc receptor (FcR), enhanced or reduced “ADCC” (antibody-dependent cell mediated cytotoxicity) or “CDC” (complement-dependent cytotoxicity) activity, altered glycosylation

and/or disulfide bonds and modified binding specificity. In this regard it will be appreciated that these Fc variants may advantageously be used to enhance the effective anti-neoplastic properties of the disclosed modulators.

To this end certain embodiments of the invention may comprise substitutions or modifications of the Fc region, for example the addition of one or more amino acid residue, substitutions, mutations and/or modifications to produce a compound with enhanced or preferred Fc effector functions. For example, changes in amino acid residues involved in the interaction between the Fc domain and an Fc receptor (e.g., FcγRI, FcγRIIA and B, FcγRIII and FcRn) may lead to increased cytotoxicity and/or altered pharmacokinetics, such as increased serum half-life (see, for example, Ravetch and Kinet, Annu. Rev. Immunol 9:457-92 (1991); Capel *et al.*, Immunomethods 4:25-34 (1994); and de Haas *et al.*, J. Lab. Clin. Med. 126:330-41 (1995) each of which is incorporated herein by reference).

In selected embodiments, antibodies with increased *in vivo* half-lives can be generated by modifying (e.g., substituting, deleting or adding) amino acid residues identified as involved in the interaction between the Fc domain and the FcRn receptor (see, e.g., International Publication Nos. WO 97/34631; WO 04/029207; U.S.P.N. 6,737,056 and U.S.P.N. 2003/0190311. With regard to such embodiments, Fc variants may provide half-lives in a mammal, preferably a human, of greater than 5 days, greater than 10 days, greater than 15 days, preferably greater than 20 days, greater than 25 days, greater than 30 days, greater than 35 days, greater than 40 days, greater than 45 days, greater than 2 months, greater than 3 months, greater than 4 months, or greater than 5 months. The increased half-life results in a higher serum titer which thus reduces the frequency of the administration of the antibodies and/or reduces the concentration of the antibodies to be administered. Binding to human FcRn in vivo and serum half life of human FcRn high affinity binding polypeptides can be assayed, e.g., in transgenic mice or transfected human cell lines expressing human FcRn, or in primates to which the polypeptides with a variant Fc region are administered. WO 2000/42072 describes antibody variants with improved or diminished binding to FcRns. See also, e.g., Shields et al. J. Biol. Chem. 9(2):6591-6604 (2001).

In other embodiments, Fc alterations may lead to enhanced or reduced ADCC or CDC activity. As in known in the art, CDC refers to the lysing of a target cell in the presence of complement, and ADCC refers to a form of cytotoxicity in which secreted Ig bound onto

FcRs present on certain cytotoxic cells (e.g., Natural Killer cells, neutrophils, and macrophages) enables these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. In the context of the instant invention antibody variants are provided with "altered" FcR binding affinity, which is either enhanced or diminished binding as compared to a parent or unmodified antibody or to an antibody comprising a native sequence FcR. Such variants which display decreased binding may possess little or no appreciable binding, e.g., 0-20% binding to the FcR compared to a native sequence, e.g. as determined by techniques well known in the art. In other embodiments the variant will exhibit enhanced binding as compared to the native immunoglobulin Fc domain. It will be appreciated that these types of Fc variants may advantageously be used to enhance the effective anti-neoplastic properties of the disclosed antibodies. In yet other embodiments, such alterations lead to increased binding affinity, reduced immunogenicity, increased production, altered glycosylation and/or disulfide bonds (e.g., for conjugation sites), modified binding specificity, increased phagocytosis; and/or down regulation of cell surface receptors (e.g. B cell receptor; BCR), etc.

3. Altered Glycosylation

Still other embodiments comprise one or more engineered glycoforms, i.e., a SEZ6 modulator comprising an altered glycosylation pattern or altered carbohydrate composition that is covalently attached to the protein (e.g., in the Fc domain). See, for example, Shields, R. L. *et al.* (2002) *J. Biol. Chem.* 277:26733-26740. Engineered glycoforms may be useful for a variety of purposes, including but not limited to enhancing or reducing effector function, increasing the affinity of the modulator for a target or facilitating production of the modulator. In certain embodiments where reduced effector function is desired, the molecule may be engineered to express an aglycosylated form. Substitutions that may result in elimination of one or more variable region framework glycosylation sites to thereby eliminate glycosylation at that site are well known (see e.g. U.S. P.Ns. 5,714,350 and 6,350,861). Conversely, enhanced effector functions or improved binding may be imparted to the Fc containing molecule by engineering in one or more additional glycosylation sites.

Other embodiments include an Fc variant that has an altered glycosylation composition, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an

antibody having increased bisecting GlcNAc structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Engineered glycoforms may be generated by any method known to one skilled in the art, for example by using engineered or variant expression strains, by co-expression with one or more enzymes (for example N-acetylglucosaminyltransferase III (GnTI11)), by expressing a molecule comprising an Fc region in various organisms or cell lines from various organisms or by modifying carbohydrate(s) after the molecule comprising Fc region has been expressed (see, for example, WO 2012/117002).

4. Additional Processing

The modulators may be differentially modified during or after production, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, $NaBH_4$, acetylation, formylation, oxidation, reduction, metabolic synthesis in the presence of tunicamycin, etc.

Various post-translational modifications also encompassed by the invention include, for example, N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends, attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of prokaryotic host cell expression. Moreover, the modulators may also be modified with a detectable label, such as an enzymatic, fluorescent, radioisotopic or affinity label to allow for detection and isolation of the modulator.

VII. Modulator Characteristics

No matter how obtained or which of the aforementioned forms the modulator takes, various embodiments of the disclosed modulators may exhibit certain characteristics. In selected embodiments, antibody-producing cells (e.g., hybridomas or yeast colonies) may be selected, cloned and further screened for favorable properties including, for example, robust growth, high modulator production and, as discussed in more detail below, desirable

modulator characteristics. In other cases characteristics of the modulator may be imparted or influenced by selecting a particular antigen (e.g., a specific SEZ6 isoform or fragment thereof) or immunoreactive fragment of the target antigen for inoculation of the animal. In still other embodiments the selected modulators may be engineered as described above to enhance or refine immunochemical characteristics such as affinity or pharmacokinetics.

A. Neutralizing Modulators

In certain embodiments, the modulators will comprise "neutralizing" antibodies or derivatives or fragments thereof. That is, the present invention may comprise antibody molecules that bind specific domains, motifs or epitopes and are capable of blocking, reducing or inhibiting the biological activity of SEZ6. More generally the term "neutralizing antibody" refers to an antibody that binds to or interacts with a target molecule or ligand and prevents binding or association of the target molecule to a binding partner such as a receptor or substrate, thereby interrupting a biological response that otherwise would result from the interaction of the molecules.

It will be appreciated that competitive binding assays known in the art may be used to assess the binding and specificity of an antibody or immunologically functional fragment or derivative thereof. With regard to the instant invention an antibody or fragment will be held to inhibit or reduce binding of SEZ6 to a binding partner or substrate (e.g., a neurotrophic ligand) when an excess of antibody reduces the quantity of binding partner bound to SEZ6 by at least about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 97%, 99% or more as measured, for example, by impaired neurotrophic ligand activity or in an *in vitro* competitive binding assay. In the case of antibodies to SEZ6 for example, a neutralizing antibody or antagonist will preferably alter ligand activity by at least about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 97%, 99% or more. It will be appreciated that this modified activity may be measured directly using art-recognized techniques or may be measured by the impact the altered activity has downstream (e.g., oncogenesis, cell survival or pathway activation).

B. Internalizing Modulators

While evidence indicates that SEZ6 or selected isoforms thereof may be present in a soluble form, at least some SEZ6 likely remains associated with the cell surface thereby allowing for internalization of the disclosed modulators. Accordingly, the anti-SEZ6 antibodies of the instant invention may be internalized, at least to some extent, by cells that express SEZ6. For example, an anti-SEZ6 antibody that binds to SEZ6 on the surface of a tumor-initiating cell may be internalized by the tumor-initiating cell. In particularly preferred embodiments such anti-SEZ6 antibodies may be associated with or conjugated to anti-cancer agents such as cytotoxic moieties that kill the cell upon internalization. In particularly preferred embodiments the modulator will comprise an internalizing antibody drug conjugate.

As used herein, a modulator that "internalizes" is one that is taken up (along with any payload) by the cell upon binding to an associated antigen or receptor. As will be appreciated, the internalizing modulator may, in preferred embodiments, comprise an antibody including antibody fragments and derivatives thereof, as well as antibody conjugates. Internalization may occur *in vitro* or *in vivo*. For therapeutic applications, internalization will preferably occur *in vivo* in a subject in need thereof. The number of antibody molecules internalized may be sufficient or adequate to kill an antigen-expressing cell, especially an antigen-expressing cancer stem cell. Depending on the potency of the antibody or antibody conjugate, in some instances, the uptake of a single antibody molecule into the cell is sufficient to kill the target cell to which the antibody binds. For example, certain toxins are so highly potent that the internalization of a few molecules of the toxin conjugated to the antibody is sufficient to kill the tumor cell. Whether an antibody internalizes upon binding to a mammalian cell can be determined by various assays including those described in the Examples below (e.g., Example 15, 17 and 18). Methods of detecting whether an antibody internalizes into a cell are also described in U.S.P.N. 7,619,068 which is incorporated herein by reference in its entirety.

C. Depleting Modulators

In other embodiments the antibodies will comprise depleting antibodies or derivatives or fragments thereof. The term "depleting" antibody refers to an antibody that preferably binds to or associates with an antigen on or near the cell surface and induces, promotes or causes the death or elimination of the cell (e.g., by CDC, ADCC or introduction of a cytotoxic

agent). In some embodiments, the selected depleting antibodies will be associated or conjugated to a cytotoxic agent.

Preferably a depleting antibody will be able to remove, incapacitate, eliminate or kill at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 97%, or 99% of SEZ6 tumorigenic cells in a defined cell population. In some embodiments the cell population may comprise enriched, sectioned, purified or isolated tumor perpetuating cells. In other embodiments the cell population may comprise whole tumor samples or heterogeneous tumor extracts that comprise tumor perpetuating cells. Those skilled in the art will appreciate that standard biochemical techniques as described in the Examples below (e.g., Examples 14 and 15) may be used to monitor and quantify the depletion of tumorigenic cells or tumor perpetuating cells in accordance with the teachings herein.

D. Binning and Epitope Binding

It will further be appreciated the disclosed anti-SEZ6 antibody modulators will associate with, or bind to, discrete epitopes or immunogenic determinants presented by the selected target or fragment thereof. In certain embodiments, epitope or immunogenic determinants include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, may have specific three-dimensional structural characteristics, and/or specific charge characteristics. Thus, as used herein the term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor or otherwise interacting with a molecule. In certain embodiments, an antibody is said to specifically bind (or immunospecifically bind or react) an antigen when it preferentially recognizes its target antigen in a complex mixture of proteins and/or macromolecules. In preferred embodiments, an antibody is said to specifically bind an antigen when the equilibrium dissociation constant (K_D) is less than or equal to 10^{-6}M or less than or equal to 10^{-7}M, more preferably when the equilibrium dissociation constant is less than or equal to 10^{-8}M, and even more preferably when the dissociation constant is less than or equal to 10^{-9}M

More directly the term "epitope" is used in its common biochemical sense and refers to that portion of the target antigen capable of being recognized and specifically bound by a particular antibody modulator. When the antigen is a polypeptide such as SEZ6, epitopes

may generally be formed from both contiguous amino acids and noncontiguous amino acids juxtaposed by tertiary folding of a protein ("conformational epitopes"). In such conformational epitopes the points of interaction occur across amino acid residues on the protein that are linearly separated from one another. Epitopes formed from contiguous amino acids (sometimes referred to as "linear" or "continuous" epitopes) are typically retained upon protein denaturing, whereas epitopes formed by tertiary folding are typically lost upon protein denaturing. In any event an antibody epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

In this respect it will be appreciated that, in certain embodiments, an epitope may be associated with, or reside in, one or more regions, domains or motifs of the SEZ6 protein (e.g., amino acids 1-906 of mature isoform 1). As discussed in more detail herein the extracellular region of the SEZ6 protein comprises a series of generally recognized domains including five Sushi domains and two CUB domains along with an N-terminal domain. For the purposes of the instant disclosure the term "domain" will be used in accordance with its generally accepted meaning and will be held to refer to an identifiable or definable conserved structural entity within a protein that exhibits a distinctive secondary structure content. In many cases, homologous domains with common functions will usually show sequence similarities and be found in a number of disparate proteins (e.g., Sushi domains are reportedly found in a large number of different proteins). Similarly, the art-recognized term "motif" will be used in accordance with its common meaning and shall generally refer to a short, conserved region of a protein that is typically ten to twenty contiguous amino acid residues. As discussed throughout, selected embodiments comprise modulators that associate with or bind to an epitope within specific regions, domains or motifs of SEZ6.

In any event once a desired epitope on an antigen is determined, it is possible to generate antibodies to that epitope, e.g., by immunizing with a peptide comprising the epitope using techniques described in the present invention. Alternatively, during the discovery process, the generation and characterization of antibodies may elucidate information about desirable epitopes located in specific domains or motifs. From this information, it is then possible to competitively screen antibodies for binding to the same epitope. An approach to achieve this is to conduct competition studies to find antibodies that competitively bind with one another, i.e. the antibodies compete for binding to the antigen. A high throughput process

for binning antibodies based upon their cross-competition is described in WO 03/48731. Other methods of binning or domain level or epitope mapping comprising modulator competition or antigen fragment expression on yeast is set forth in Examples 9 and 10 below.

As used herein, the term "binning" refers to methods used to group or classify antibodies based on their antigen binding characteristics and competition. While the techniques are useful for defining and categorizing modulators of the instant invention, the bins do not always directly correlate with epitopes and such initial determinations of epitope binding may be further refined and confirmed by other art-recognized methodology as described herein. However, as discussed and shown in the Examples below, empirical assignment of antibody modulators to individual bins provides information that may be indicative of the therapeutic potential of the disclosed modulators.

More specifically, one can determine whether a selected reference antibody (or fragment thereof) binds to the same epitope or cross competes for binding with a second test antibody (i.e., is in the same bin) by using methods known in the art and set forth in the Examples herein. In one embodiment, a reference antibody modulator is associated with SEZ6 antigen under saturating conditions and then the ability of a secondary or test antibody modulator to bind to SEZ6 is determined using standard immunochemical techniques. If the test antibody is able to substantially bind to SEZ6 at the same time as the reference anti-SEZ6 antibody, then the secondary or test antibody binds to a different epitope than the primary or reference antibody. However, if the test antibody is not able to substantially bind to SEZ6 at the same time, then the test antibody binds to the same epitope, an overlapping epitope, or an epitope that is in close proximity (at least sterically) to the epitope bound by the primary antibody. That is, the test antibody competes for antigen binding and is in the same bin as the reference antibody.

The term "compete" or "competing antibody" when used in the context of the disclosed modulators means competition between antibodies as determined by an assay in which a test antibody or immunologically functional fragment under test prevents or inhibits specific binding of a reference antibody to a common antigen. Typically, such an assay involves the use of purified antigen (e.g., SEZ6 or a domain or fragment thereof) bound to a solid surface or cells bearing either of these, an unlabeled test immunoglobulin and a labeled reference immunoglobulin. Competitive inhibition is measured by determining the amount of label

bound to the solid surface or cells in the presence of the test immunoglobulin. Usually the test immunoglobulin is present in excess and/or allowed to bind first. Antibodies identified by competition assay (competing antibodies) include antibodies binding to the same epitope as the reference antibody and antibodies binding to an adjacent epitope sufficiently proximal to the epitope bound by the reference antibody for steric hindrance to occur. Additional details regarding methods for determining competitive binding are provided in the Examples herein. Usually, when a competing antibody is present in excess, it will inhibit specific binding of a reference antibody to a common antigen by at least 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70% or 75%. In some instance, binding is inhibited by at least 80%, 85%, 90%, 95%, or 97% or more.

Conversely, when the reference antibody is bound it will preferably inhibit binding of a subsequently added test antibody (i.e., a SEZ6 modulator) by at least 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70% or 75%. In some instances binding of the test antibody is inhibited by at least 80%, 85%, 90%, 95%, or 97% or more.

With regard to the instant invention, and as set forth in the Examples 9 and 10 below, it has been determined (via surface plasmon resonance or bio-layer interferometry) that the extracellular domain of SEZ6 defines at least seven bins by competitive binding termed "bin A" to "bin F" and bin U herein.

In this respect, and as known in the art and detailed in the Examples below, the desired binning or competitive binding data can be obtained using solid phase direct or indirect radioimmunoassay (RIA), solid phase direct or indirect enzyme immunoassay (EIA or ELISA), sandwich competition assay, a Biacore™ 2000 system (i.e., surface plasmon resonance – GE Healthcare), a ForteBio® Analyzer (i.e., bio-layer interferometry - ForteBio, Inc.) or flow cytometric methodology. The term "surface plasmon resonance," as used herein, refers to an optical phenomenon that allows for the analysis of real-time specific interactions by detection of alterations in protein concentrations within a biosensor matrix. The term "bio-layer interferometry" refers to an optical analytical technique that analyzes the interference pattern of white light reflected from two surfaces: a layer of immobilized protein on a biosensor tip, and an internal reference layer. Any change in the number of molecules bound to the biosensor tip causes a shift in the interference pattern that can be measured in real-time. In particularly preferred embodiments the analysis (whether surface plasmon resonance, bio-

layer interferometry or flow cytometry) is performed using a Biacore or ForteBio instrument or a flow cytometer (e.g., FACSAria II) as demonstrated in the Examples below.

In order to further characterize the epitopes that the disclosed SEZ6 antibody modulators associate with or bind to, domain-level epitope mapping was performed using a modification of the protocol described by Cochran et al. (J Immunol Methods. 287 (1-2):147-158 (2004) which is incorporated herein by reference). Briefly, individual domains of SEZ6 comprising specific amino acid sequences were expressed on the surface of yeast and binding by each SEZ6 antibody was determined through flow cytometry. The results are discussed below in Example 10 and shown in FIGS. 14A and 14B.

Other compatible epitope mapping techniques include alanine scanning mutants, peptide blots (Reineke (2004) Methods Mol Biol 248:443-63) (herein specifically incorporated by reference in its entirety), or peptide cleavage analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer (2000) Protein Science 9: 487-496) (herein specifically incorporated by reference in its entirety). In other embodiments Modification-Assisted Profiling (MAP), also known as Antigen Structure-based Antibody Profiling (ASAP) provides a method that categorizes large numbers of monoclonal antibodies (mAbs) directed against the same antigen according to the similarities of the binding profile of each antibody to chemically or enzymatically modified antigen surfaces (U.S.P.N. 2004/0101920, herein specifically incorporated by reference in its entirety). Each category may reflect a unique epitope either distinctly different from or partially overlapping with epitope represented by another category. This technology allows rapid filtering of genetically identical antibodies, such that characterization can be focused on genetically distinct antibodies. It will be appreciated that MAP may be used to sort the hSEZ6 antibody modulators of the invention into groups of antibodies binding different epitopes

Agents useful for altering the structure of the immobilized antigen include enzymes such as proteolytic enzymes (e.g., trypsin, endoproteinase Glu-C, endoproteinase Asp-N, chymotrypsin, etc.). Agents useful for altering the structure of the immobilized antigen may also be chemical agents, such as, succinimidyl esters and their derivatives, primary amine-containing compounds, hydrazines and carbohydrazines, free amino acids, etc.

The antigen protein may be immobilized on either biosensor chip surfaces or polystyrene beads. The latter can be processed with, for example, an assay such as multiplex LUMINEX™ detection assay (Luminex Corp.). Because of the capacity of LUMINEX to handle multiplex analysis with up to 100 different types of beads, LUMINEX provides almost unlimited antigen surfaces with various modifications, resulting in improved resolution in antibody epitope profiling over a biosensor assay.

E. Modulator Binding Characteristics

Besides epitope specificity the disclosed antibodies may be characterized using physical characteristics such as, for example, binding affinities. In this regard the present invention further encompasses the use of antibodies that have a high binding affinity for one or more SEZ6 isoforms or, in the case of pan-antibodies, more than one member of the SEZ6 family.

The term "K_D", as used herein, is intended to refer to the dissociation constant of a particular antibody-antigen interaction. An antibody of the invention is said to immunospecifically bind its target antigen when the dissociation constant K_D (k_{off}/k_{on}) is $\leq 10^{-7}$M. The antibody specifically binds antigen with high affinity when the K_D is $\leq 5x10^{-9}$M, and with very high affinity when the K_D is $\leq 5x10^{-10}$M. In one embodiment of the invention, the antibody has a K_D of $\leq 10^{-9}$M and an off-rate of about $1x10^{-4}$/sec. In one embodiment of the invention, the off-rate is $< 1x10^{-5}$/sec. In other embodiments of the invention, the antibodies will bind to SEZ6 with a K_D of between about 10^{-7}M and 10^{-10}M, and in yet another embodiment it will bind with a $K_D \leq 2x10^{-10}$M. Still other selected embodiments of the present invention comprise antibodies that have a disassociation constant or K_D (k_{off}/k_{on}) of less than 10^{-2}M, less than $5x10^{-2}$M, less than 10^{-3}M, less than $5x10^{-3}$M, less than 10^{-4}M, less than $5x10^{-4}$M, less than 10^{-5}M, less than $5x10^{-5}$M, less than 10^{-6}M, less than $5x10^{-6}$M, less than 10^{-7}M, less than $5x10^{-7}$M, less than 10^{-8}M, less than $5x10^{-8}$M, less than 10^{-9}M, less than $5x10^{-9}$M, less than 10^{-10}M, less than $5x10^{-10}$M, less than 10^{-11}M, less than $5x10^{-11}$M, less than 10^{-12}M, less than $5x10^{-12}$M, less than 10^{-13}M, less than $5x10^{-13}$M, less than 10^{-14}M, less than $5x10^{-14}$M, less than 10^{-15}M or less than $5x10^{-15}$M.

In specific embodiments, an antibody of the invention that immunospecifically binds to SEZ6 has an association rate constant or k_{on} (or k_a) rate (SEZ6 (Ab) + antigen (Ag)$^{k}_{on}$←Ab-

Ag) of at least $10^5M^{-1}s^{-1}$, at least $2x10^5M^{-1}s^{-1}$, at least $5x10^5M^{-1}s^{-1}$, at least $10^6M^{-1}s^{-1}$, at least $5x10^6M^{-1}s^{-1}$, at least $10^7M^{-1}s^{-1}$, at least $5x10^7M^{-1}s^{-1}$, or at least $10^8M^{-1}s^{-1}$.

In another embodiment, an antibody of the invention that immunospecifically binds to SEZ6 has a disassociation rate constant or k_{off} (or k_d) rate (SEZ6 (Ab) + antigen (Ag) $^{k}_{off}$←Ab-Ag) of less than $10^{-1}s^{-1}$, less than $5x10^{-1}s^{-1}$, less than $10^{-2}s^{-1}$, less than $5x10^{-2}s^{-1}$, less than $10^{-3}s^{-1}$, less than $5x10^{-3}s^{-1}$, less than $10^{-4}s^{-1}$, less than $5x10^{-4}s^{-1}$, less than $10^{-5}s^{-1}$, less than $5x10^{-5}s^{-1}$, less than $10^{-6}s^{-1}$, less than $5x10^{-6}s^{-1}$ less than $10^{-7}s^{-1}$, less than $5x10^{-7}s^{-1}$, less than $10^{-8}s^{-1}$, less than $5x10^{-8}s^{-1}$, less than $10^{-9}s^{-1}$, less than $5x10^{-9}s^{-1}$ or less than $10^{-10}s^{-1}$.

In other selected embodiments of the present invention anti-SEZ6 antibodies will have an affinity constant or K_a (k_{on}/k_{off}) of at least 10^2M^{-1}, at least $5x10^2M^{-1}$, at least 10^3M^{-1}, at least $5x10^3M^{-1}$, at least 10^4M^{-1}, at least $5x10^4M^{-1}$, at least 10^5M^{-1}, at least $5x10^5M^{-1}$, at least 10^6M^{-1}, at least $5x10^6M^{-1}$, at least 10^7M^{-1}, at least $5x10^7M^{-1}$, at least 10^8M^{-1}, at least $5x10^8M^{-1}$, at least 10^9M^{-1}, at least $5x10^9M^{-1}$, at least $10^{10}M^{-1}$, at least $5x10^{10}M^{-1}$, at least $10^{11}M^{-1}$, at least $5x10^{11}M^{-1}$, at least $10^{12}M^{-1}$, at least $5x10^{12}M^{-1}$, at least $10^{13}M^{-1}$, at least $5x10^{13}M^{-1}$, at least $10^{14}M^{-1}$, at least $5x10^{14}M^{-1}$, at least $10^{15}M^{-1}$ or at least $5x10^{15}M^{-1}$.

Besides the aforementioned modulator characteristics antibodies of the instant invention may further be characterized using additional physical characteristics including, for example, thermal stability (i.e, melting temperature; Tm), and isoelectric points. (See, e.g., Bjellqvist et al., 1993, Electrophoresis 14:1023; Vermeer et al., 2000, Biophys. J. 78:394-404; Vermeer et al., 2000, Biophys. J. 79: 2150-2154 each of which is incorporated by reference).

VIII. Conjugated Modulators

A. Overview

Once the modulators of the invention have been generated and/or fabricated and selected according to the teachings herein they may be linked with, fused to, conjugated to (e.g., covalently or non-covalently) or otherwise associated with pharmaceutically active or diagnostic moieties or biocompatible modifiers. As used herein the term "conjugate" or "modulator conjugate" or "antibody conjugate" will be used broadly and held to mean any biologically active or detectable molecule or drug associated with the disclosed modulators regardless of the method of association. In this respect it will be understood that such conjugates may, in addition to the disclosed modulators, comprise peptides, polypeptides,

proteins, prodrugs which are metabolized to an active agent in vivo, polymers, nucleic acid molecules, small molecules, binding agents, mimetic agents, synthetic drugs, inorganic molecules, organic molecules and radioisotopes. Moreover, as indicated above the selected conjugate may be covalently or non-covalently associated with, or linked to, the modulator and exhibit various stoichiometric molar ratios depending, at least in part, on the method used to effect the conjugation.

Particularly preferred aspects of the instant invention will comprise antibody modulator conjugates or antibody-drug conjugates that may be used for the diagnosis and/or treatment of proliferative disorders. It will be appreciated that, unless otherwise dictated by context, the term "antibody-drug conjugate" or "ADC" or the formula M-[L-D]n shall be held to encompass conjugates comprising both therapeutic and diagnostic moieties. In such embodiments antibody-drug conjugate compounds will comprise a SEZ6 modulator (typically an anti-SEZ6 antibody) as the modulator or cellular binding unit (abbreviated as CBA, M, or Ab herein), a therapeutic (e.g., anti-cancer agent) or diagnostic moiety (D), and optionally a linker (L) that joins the drug and the antigen binding agent. For the purposes of the instant disclosure "n" shall be held to mean an integer from 1 to 20. In a preferred embodiment, the modulator is a SEZ6 mAb comprising at least one CDR from the heavy and light chain variable regions as described above.

Those skilled in the art will appreciate that a number of different reactions are available for the attachment or association of therapeutic or diagnostic moieties and/or linkers to binding agents. In selected embodiments this may be accomplished by reaction of the amino acid residues of the binding agent, e.g., antibody molecule, including the amine groups of lysine, the free carboxylic acid groups of glutamic and aspartic acid, the sulfhydryl groups of cysteine and the various moieties of the aromatic amino acids. One of the most commonly used non-specific methods of covalent attachment is the carbodiimide reaction to link a carboxy (or amino) group of a compound to amino (or carboxy) groups of the antibody. Additionally, bifunctional agents such as dialdehydes or imidoesters have been used to link the amino group of a compound to amino groups of an antibody molecule. Also available for attachment of drugs to binding agents is the Schiff base reaction. This method involves the periodate oxidation of a drug that contains glycol or hydroxy groups, thus forming an aldehyde which is then reacted with the binding agent. Attachment occurs via formation of a

Schiff base with amino groups of the binding agent. Isothiocyanates and azlactones can also be used as coupling agents for covalently attaching drugs to binding agents.

In other embodiments the disclosed modulators of the invention may be conjugated or associated with proteins, polypeptides or peptides that impart selected characteristics (e.g., biotoxins, biomarkers, purification tags, etc.). In certain preferred embodiments the present invention encompasses the use of modulators or fragments thereof recombinantly fused or chemically conjugated (including both covalent and non-covalent conjugations) to a heterologous protein or peptide wherein the protein or peptide comprises at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 amino acids. The construct does not necessarily need to be directly linked, but may occur through amino acid linker sequences. For example, antibodies may be used to target heterologous polypeptides to particular cell types expressing SEZ6, either *in vitro* or *in vivo*, by fusing or conjugating the modulators of the present invention to antibodies specific for particular cell surface receptors to provide bispecific constructs. Moreover, modulators fused or conjugated to heterologous polypeptides may also be used in *in vitro* immunoassays and may be particularly compatible with purification methodology (e.g., his-tags) as is known in the art. See e.g., International publication No. WO 93/21232; European Patent No. EP 439,095; Naramura et al., 1994, Immunol. Lett. 39:91-99; U.S. Pat. No. 5,474,981; Gillies et al., 1992, PNAS 89:1428-1432; and Fell et al., 1991, J. Immunol. 146:2446-2452.

B. Linkers

Besides the aforementioned peptide linkers or spacers, it will be appreciated that several other varieties or types of linker may be used to associate the disclosed modulators with pharmaceutically active or diagnostic moieties or biocompatible modifiers. In some embodiments, the linker is cleavable under intracellular conditions, such that cleavage of the linker releases the drug unit from the antibody in the intracellular environment. In yet other embodiments, the linker unit is not cleavable and the drug is released, for example, by antibody degradation.

The linkers of the ADC are preferably stable extracellularly, prevent aggregation of ADC molecules and keep the ADC freely soluble in aqueous media and in a monomeric state. Before transport or delivery into a cell, the antibody-drug conjugate (ADC) is preferably

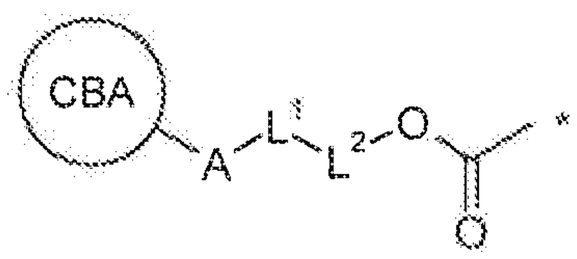
stable and remains intact, i.e. the antibody remains linked to the drug moiety. The linkers are stable outside the target cell and may be cleaved at some efficacious rate inside the cell. An effective linker will: (i) maintain the specific binding properties of the antibody; (ii) allow intracellular delivery of the conjugate or drug moiety; (iii) remain stable and intact, i.e. not cleaved, until the conjugate has been delivered or transported to its targeted site; and (iv) maintain a cytotoxic, cell-killing effect or a cytostatic effect of the PBD drug moiety. Stability of the ADC may be measured by standard analytical techniques such as mass spectroscopy, HPLC, and the separation/analysis technique LC/MS. Covalent attachment of the antibody and the drug moiety requires the linker to have two reactive functional groups, i.e. bivalency in a reactive sense. Bivalent linker reagents which are useful to attach two or more functional or biologically active moieties, such as peptides, nucleic acids, drugs, toxins, antibodies, haptens, and reporter groups are known, and methods have been described their resulting conjugates (Hermanson, G.T. (1996) Bioconjugate Techniques; Academic Press: New York, p 234-242).

To this end certain embodiments of the invention comprise the use a linker that is cleavable by a cleaving agent that is present in the intracellular environment (e.g., within a lysosome or endosome or caveolae). The linker can be, for example, a peptidyl linker that is cleaved by an intracellular peptidase or protease enzyme, including, but not limited to, a lysosomal or endosomal protease. In some embodiments, the peptidyl linker is at least two amino acids long or at least three amino acids long. Cleaving agents can include cathepsins B and D and plasmin, each of which is known to hydrolyze dipeptide drug derivatives resulting in the release of active drug inside target cells. Exemplary peptidyl linkers that are cleavable by the thiol-dependent protease Cathepsin-B are peptides comprising Phe-Leu since Cathepsin-B has been found to be highly expressed in cancerous tissue. Other examples of such linkers are described, for example, in U.S.P.N. 6,214,345 and U.S.P.N. 2012/0078028 each of which incorporated herein by reference in its entirety. In a specific preferred embodiment, the peptidyl linker cleavable by an intracellular protease is a Val-Cit linker, an Ala-Val linker or a Phe-Lys linker such as is described in U.S.P.N. 6,214,345. One advantage of using intracellular proteolytic release of the therapeutic agent is that the agent is typically attenuated when conjugated and the serum stabilities of the conjugates are typically high.

In other embodiments, the cleavable linker is pH-sensitive, i.e., sensitive to hydrolysis at certain pH values. Typically, the pH-sensitive linker hydrolyzable under acidic conditions. For example, an acid-labile linker that is hydrolyzable in the lysosome (e.g., a hydrazone, oxime, semicarbazone, thiosemicarbazone, cis-aconitic amide, orthoester, acetal, ketal, or the like) can be used (See, e.g., U.S.P.N. 5,122,368; 5,824,805; 5,622,929). Such linkers are relatively stable under neutral pH conditions, such as those in the blood, but are unstable at below pH 5.5 or 5.0, the approximate pH of the lysosome.

In yet other embodiments, the linker is cleavable under reducing conditions (e.g., a disulfide linker). A variety of disulfide linkers are known in the art, including, for example, those that can be formed using SATA (N-succinimidyl-S-acetylthioacetate), SPDP (N-succinimidyl-3-(2-pyridyldithio)propionate), SPDB (N-succinimidyl-3-(2-pyridyldithio) butyrate) and SMPT (N-succinimidyl-oxycarbonyl-alpha-methyl-alpha-(2-pyridyl-dithio)toluene). In yet other specific embodiments, the linker is a malonate linker (Johnson et al., 1995, *Anticancer Res.* 15:1387-93), a maleimidobenzoyl linker (Lau et al., 1995, *Bioorg-Med-Chem.* 3(10):1299-1304), or a 3'-N-amide analog (Lau et al., 1995, *Bioorg-Med-Chem.* 3(10):1305-12). In yet other embodiments, the linker unit is not cleavable and the drug is released by antibody degradation. (See U.S. Publication No. 2005/0238649 incorporated by reference herein in its entirety and for all purposes).

More particularly, in preferred embodiments (set forth in U.S.P.N. 2011/0256157 which is incorporated herein by reference in its entirety) compatible linkers will comprise:



where the asterisk indicates the point of attachment to the cytotoxic agent, CBA is a cell binding agent/modulator, L^1 is a linker, A is a connecting group connecting L^1 to the cell binding agent, L^2 is a covalent bond or together with -OC(=O)- forms a self-immolative linker, and L^1 or L^2 is a cleavable linker.

L^1 is preferably the cleavable linker, and may be referred to as a trigger for activation of the linker for cleavage.

The nature of L^1 and L^2, where present, can vary widely. These groups are chosen on the basis of their cleavage characteristics, which may be dictated by the conditions at the site to which the conjugate is delivered. Those linkers that are cleaved by the action of enzymes are preferred, although linkers that are cleavable by changes in pH (e.g. acid or base labile), temperature or upon irradiation (e.g. photolabile) may also be used. Linkers that are cleavable under reducing or oxidising conditions may also find use in the present invention.

L^1 may comprise a contiguous sequence of amino acids. The amino acid sequence may be the target substrate for enzymatic cleavage, thereby allowing release of R^{10} from the N10 position.

In one embodiment, L^1 is cleavable by the action of an enzyme. In one embodiment, the enzyme is an esterase or a peptidase.

In one embodiment, L^2 is present and together with -C(=O)O- forms a self-immolative linker. In one embodiment, L^2 is a substrate for enzymatic activity, thereby allowing release of R^{10} from the N10 position.

In one embodiment, where L^1 is cleavable by the action of an enzyme and L^2 is present, the enzyme cleaves the bond between L^1 and L^2.

L^1 and L^2, where present, may be connected by a bond selected from:

-C(=O)NH-, -C(=O)O-, -NHC(=O)-, -OC(=O)-, -OC(=O)O-, -NHC(=O)O-, -OC(=O)NH-, and -NHC(=O)NH-.

An amino group of L^1 that connects to L^2 may be the N-terminus of an amino acid or may be derived from an amino group of an amino acid side chain, for example a lysine amino acid side chain.

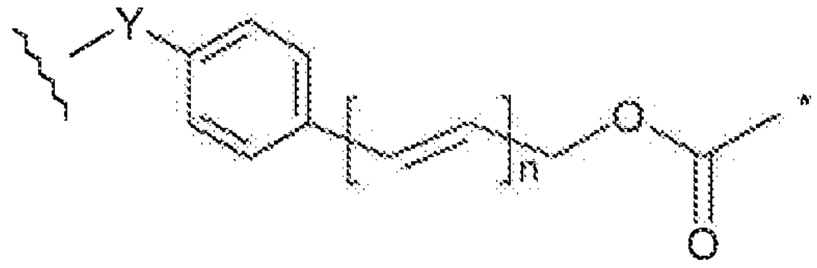
A carboxyl group of L^1 that connects to L^2 may be the C-terminus of an amino acid or may be derived from a carboxyl group of an amino acid side chain, for example a glutamic acid amino acid side chain.

A hydroxyl group of L^1 that connects to L^2 may be derived from a hydroxyl group of an amino acid side chain, for example a serine amino acid side chain.

The term "amino acid side chain" includes those groups found in: (i) naturally occurring amino acids such as alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine; (ii) minor amino acids such as ornithine and

citrulline; (iii) unnatural amino acids, beta-amino acids, synthetic analogs and derivatives of naturally occurring amino acids; and (iv) all enantiomers, diastereomers, isomerically enriched, isotopically labelled (e.g. ^{2}H, ^{3}H, ^{14}C, ^{15}N), protected forms, and racemic mixtures thereof.

In one embodiment, -C(=O)O- and L^2 together form the group:



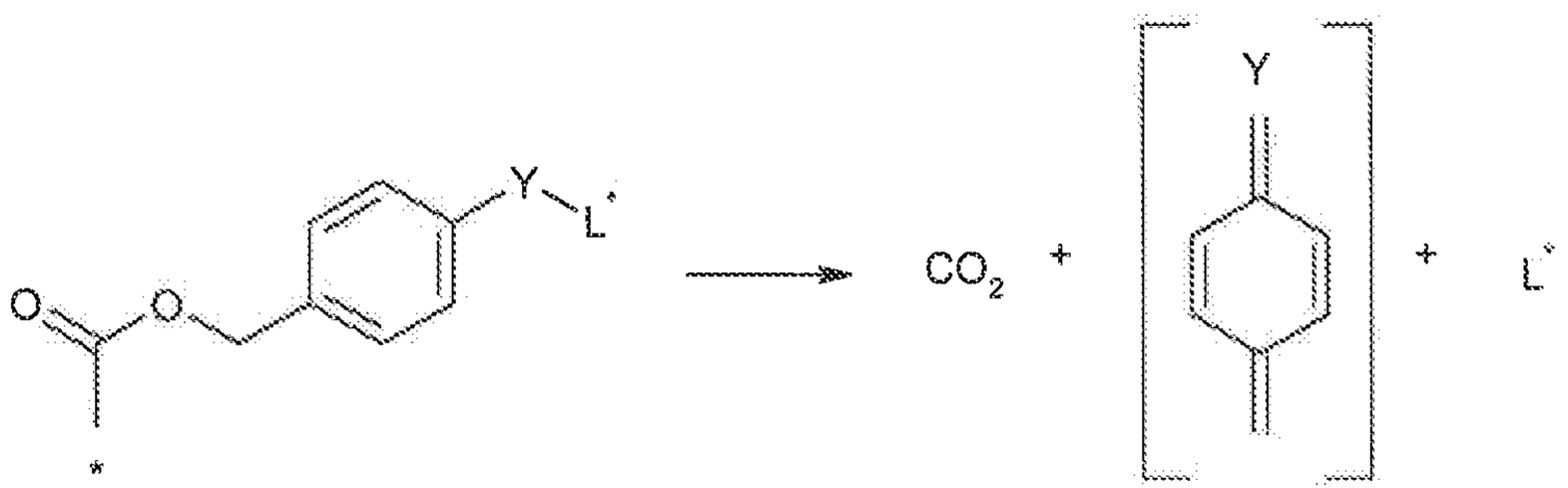
where the asterisk indicates the point of attachment to the drug or cytotoxic agent position, the wavy line indicates the point of attachment to the linker L^1, Y is -N(H)-, -O-, -C(=O)N(H)- or -C(=O)O-, and n is 0 to 3. The phenylene ring is optionally substituted with one, two or three substituents as described herein. In one embodiment, the phenylene group is optionally substituted with halo, NO_2, R or OR.

In one embodiment, Y is NH.

In one embodiment, n is 0 or 1. Preferably, n is 0.

Where Y is NH and n is 0, the self-immolative linker may be referred to as a p-aminobenzylcarbonyl linker (PABC).

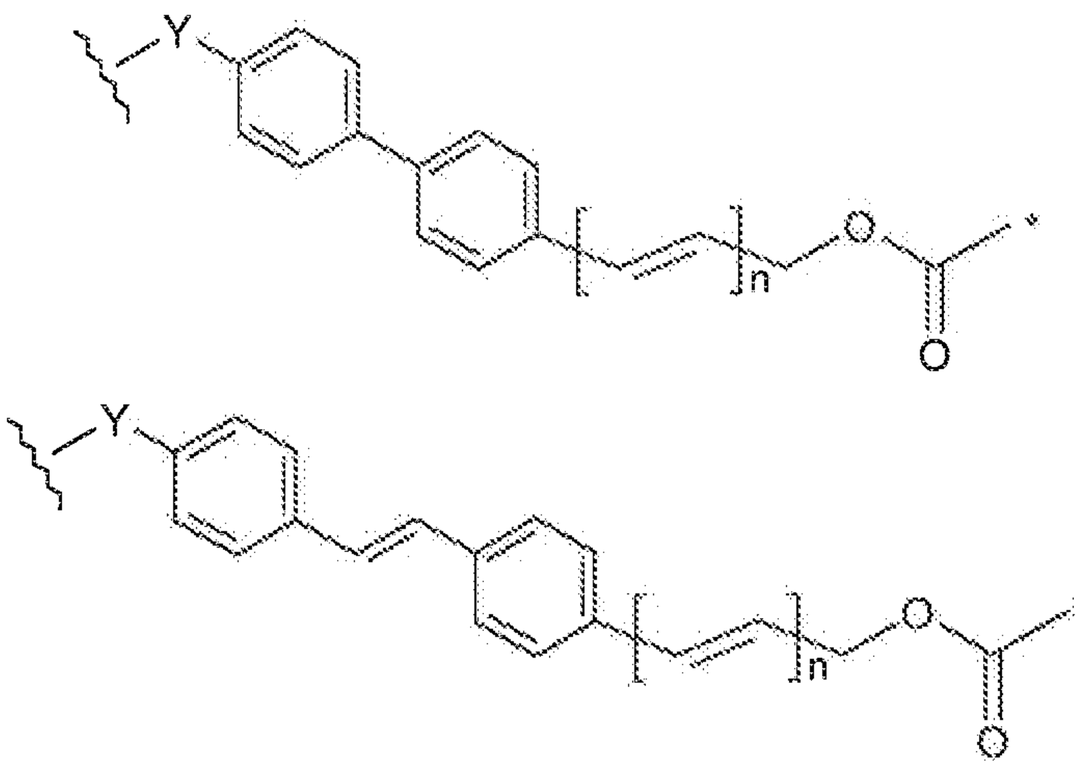
The self-immolative linker will allow for release of the protected compound when a remote site is activated, proceeding along the lines shown below (for n=0):



where L^* is the activated form of the remaining portion of the linker. These groups have the advantage of separating the site of activation from the compound being protected. As described above, the phenylene group may be optionally substituted.

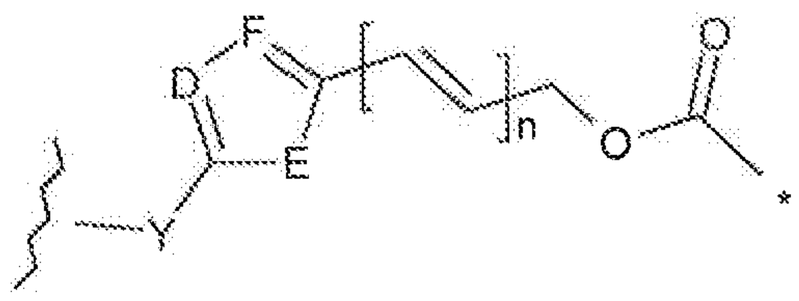
In one embodiment described herein, the group L^* is a linker L^1 as described herein, which may include a dipeptide group.

In another embodiment, -C(=O)O- and L^2 together form a group selected from:



where the asterisk, the wavy line, Y, and n are as defined above. Each phenylene ring is optionally substituted with one, two or three substituents as described herein. In one embodiment, the phenylene ring having the Y substituent is optionally substituted and the phenylene ring not having the Y substituent is unsubstituted. In one embodiment, the phenylene ring having the Y substituent is unsubstituted and the phenylene ring not having the Y substituent is optionally substituted.

In another embodiment, -C(=O)O- and L^2 together form a group selected from:



where the asterisk, the wavy line, Y, and n are as defined above, E is O, S or NR, D is N, CH, or CR, and F is N, CH, or CR.

In one embodiment, D is N.

In one embodiment, D is CH.

In one embodiment, E is O or S.

In one embodiment, F is CH.

In a preferred embodiment, the linker is a cathepsin labile linker.

In one embodiment, L^1 comprises a dipeptide. The dipeptide may be represented as -NH-X_1-X_2-CO-, where -NH- and -CO- represent the N- and C-terminals of the amino acid groups X_1 and X_2 respectively. The amino acids in the dipeptide may be any combination of natural amino acids. Where the linker is a cathepsin labile linker, the dipeptide may be the site of action for cathepsin-mediated cleavage.

Additionally, for those amino acids groups having carboxyl or amino side chain functionality, for example Glu and Lys respectively, CO and NH may represent that side chain functionality.

In one embodiment, the group -X_1-X_2- in dipeptide, -NH-X_1-X_2-CO-, is selected from:

-Phe-Lys-, -Val-Ala-, -Val-Lys-, -Ala-Lys-, -Val-Cit-, -Phe-Cit-, -Leu-Cit-, -Ile-Cit-, -Phe-Arg- and -Trp-Cit- where Cit is citrulline.

Preferably, the group -X_1-X_2- in dipeptide, -NH-X_1-X_2-CO-, is selected from:

-Phe-Lys-, -Val-Ala-, -Val-Lys-, -Ala-Lys-, and -Val-Cit-.

Most preferably, the group -X_1-X_2- in dipeptide, -NH-X_1-X_2-CO-, is -Phe-Lys- or -Val-Ala-.

Other dipeptide combinations may be used, including those described by Dubowchik *et al.*, *Bioconjugate Chemistry*, 2002, 13,855-869, which is incorporated herein by reference.

In one embodiment, the amino acid side chain is derivatised, where appropriate. For example, an amino group or carboxy group of an amino acid side chain may be derivatised.

In one embodiment, an amino group NH_2 of a side chain amino acid, such as lysine, is a derivatised form selected from the group consisting of NHR and NRR'.

In one embodiment, a carboxy group COOH of a side chain amino acid, such as aspartic acid, is a derivatised form selected from the group consisting of COOR, $CONH_2$, CONHR and CONRR'.

In one embodiment, the amino acid side chain is chemically protected, where appropriate. The side chain protecting group may be a group as discussed below in relation to the group R^L. Protected amino acid sequences are cleavable by enzymes. For example, it has been established that a dipeptide sequence comprising a Boc side chain-protected Lys residue is cleavable by cathepsin.

Protecting groups for the side chains of amino acids are well known in the art and are described in the Novabiochem Catalog. Additional protecting group strategies are set out in

Protective Groups in Organic Synthesis, Greene and Wuts.

Possible side chain protecting groups are shown below for those amino acids having reactive side chain functionality:

Arg: Z, Mtr, Tos;

Asn: Trt, Xan;

Asp: Bzl, t-Bu;

Cys: Acm, Bzl, Bzl-OMe, Bzl-Me, Trt;

Glu: Bzl, t-Bu;

Gln: Trt, Xan;

His: Boc, Dnp, Tos, Trt;

Lys: Boc, Z-Cl, Fmoc, Z, Alloc;

Ser: Bzl, TBDMS, TBDPS;

Thr: Bz;

Trp: Boc;

Tyr: Bzl, Z, Z-Br.

In one embodiment, the side chain protection is selected to be orthogonal to a group provided as, or as part of, a capping group, where present. Thus, the removal of the side chain protecting group does not remove the capping group, or any protecting group functionality that is part of the capping group.

In other embodiments of the invention, the amino acids selected are those having no reactive side chain functionality. For example, the amino acids may be selected from: Ala, Gly, Ile, Leu, Met, Phe, Pro, and Val.

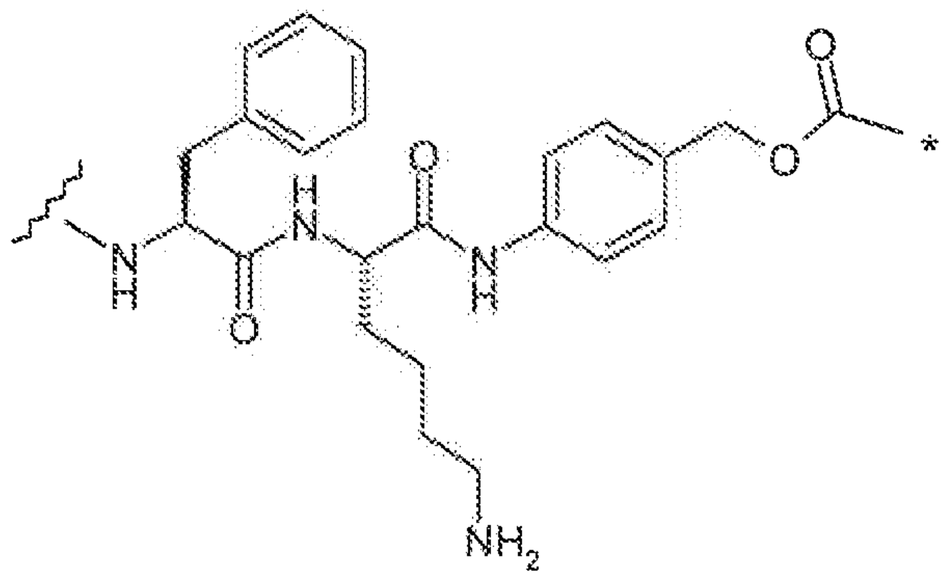
In one embodiment, the dipeptide is used in combination with a self-immolative linker. The self-immolative linker may be connected to $-X_2-$.

Where a self-immolative linker is present, $-X_2-$ is connected directly to the self-immolative linker. Preferably the group $-X_2-CO-$ is connected to Y, where Y is NH, thereby forming the group $-X_2-CO-NH-$.

$-NH-X_1-$ is connected directly to A. A may comprise the functionality -CO- thereby to form an amide link with $-X_1-$.

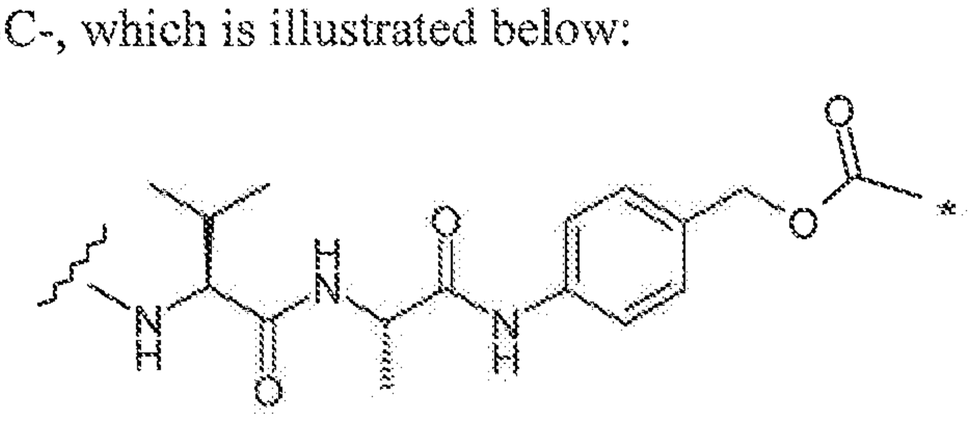
In one embodiment, L^1 and L^2 together with -OC(=O)- comprise the group

NH-X_1-X_2-CO-PABC-. The PABC group is connected directly to the cytotoxic agent. Preferably, the self-immolative linker and the dipeptide together form the group -NH-Phe-Lys-CO-NH-PABC-, which is illustrated below:



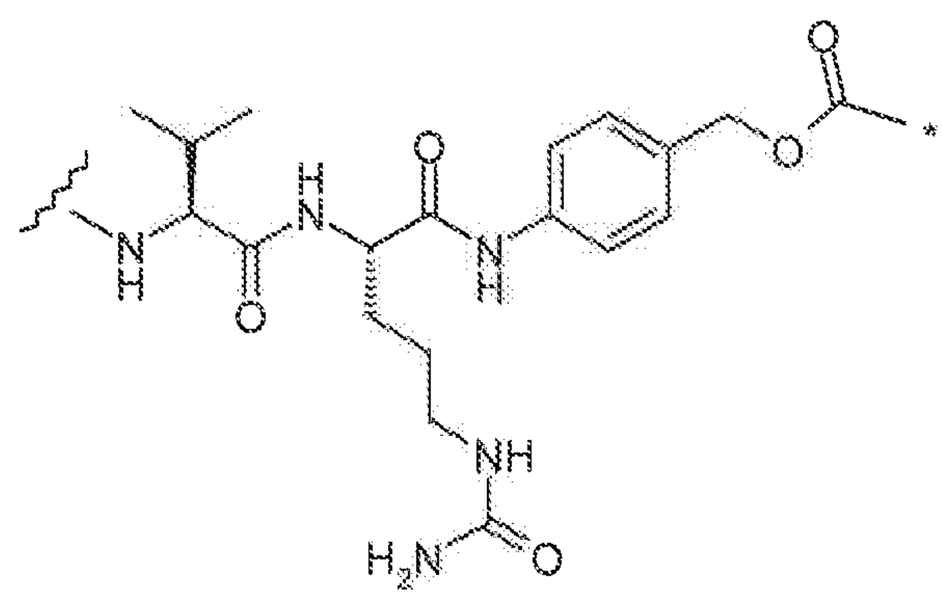
where the asterisk indicates the point of attachment to the selected cytotoxic moiety, and the wavy line indicates the point of attachment to the remaining portion of the linker L^1 or the point of attachment to A. Preferably, the wavy line indicates the point of attachment to A. The side chain of the Lys amino acid may be protected, for example, with Boc, Fmoc, or Alloc, as described above.

Alternatively, the self-immolative linker and the dipeptide together form the group -NH-Val-Ala-CO-NH-PABC-, which is illustrated below:



where the asterisk and the wavy line are as defined above.

Alternatively, the self-immolative linker and the dipeptide together form the group -NH-Val-Cit-CO-NH-PABC-, which is illustrated below:



where the asterisk and the wavy line are as defined above.

In some embodiments of the present invention, it may be preferred that if the drug moiety contains an unprotected imine bond, e.g. if moiety B is present, then the linker does not contain a free amino (H_2N-) group. Thus if the linker has the structure -A-L^1-L^2- then this would preferably not contain a free amino group. This preference is particularly relevant when the linker contains a dipeptide, for example as L^1; in this embodiment, it would be preferred that one of the two amino acids is not selected from lysine.

Without wishing to be bound by theory, the combination of an unprotected imine bond in the drug moiety and a free amino group in the linker can cause dimerisation of the drug-linker moiety which may interfere with the conjugation of such a drug-linker moiety to an antibody. The cross-reaction of these groups may be accelerated in the case the free amino group is present as an ammonium ion (H_3N^+-), such as when a strong acid (e.g. TFA) has been used to deprotect the free amino group.

In one embodiment, A is a covalent bond. Thus, L^1 and the cell binding agent are directly connected. For example, where L^1 comprises a contiguous amino acid sequence, the N-terminus of the sequence may connect directly to the cell binding agent.

Thus, where A is a covalent bond, the connection between the cell binding agent and L^1 may be selected from:

-C(=O)NH-, -C(=O)O-, -NHC(=O)-, -OC(=O)-, -OC(=O)O-, -NHC(=O)O-, -OC(=O)NH-, -NHC(=O)NH-, -C(=O)NHC(=O)-, -S-, -S-S-, -CH_2C(=O)-, and =N-NH-.

An amino group of L^1 that connects to the SEZ6 modulator may be the N-terminus of an amino acid or may be derived from an amino group of an amino acid side chain, for example a lysine amino acid side chain.

A carboxyl group of L^1 that connects to the modulator may be the C-terminus of an amino acid or may be derived from a carboxyl group of an amino acid side chain, for example a glutamic acid amino acid side chain.

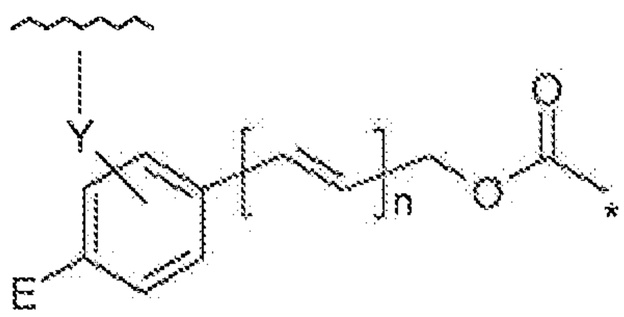
A hydroxyl group of L^1 that connects to the cell binding agent may be derived from a hydroxyl group of an amino acid side chain, for example a serine amino acid side chain.

A thiol group of L^1 that connects to a modulator agent may be derived from a thiol group of an amino acid side chain, for example a serine amino acid side chain.

The comments above in relation to the amino, carboxyl, hydroxyl and thiol groups of L^1

also apply to the cell binding agent.

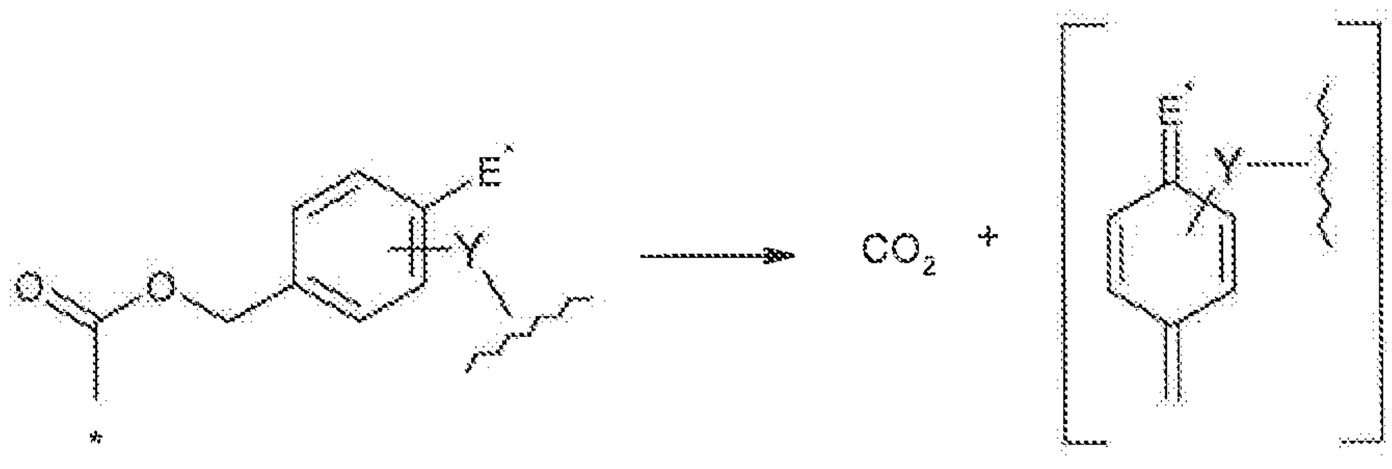
In one embodiment, L^2 together with -OC(=O)- represents:



where the asterisk indicates the point of attachment to the N10 position, the wavy line indicates the point of attachment to L^1, n is 0 to 3, Y is a covalent bond or a functional group, and E is an activatable group, for example by enzymatic action or light, thereby to generate a self-immolative unit. The phenylene ring is optionally further substituted with one, two or three substituents as described herein. In one embodiment, the phenylene group is optionally further substituted with halo, NO_2, R or OR. Preferably n is 0 or 1, most preferably 0.

E is selected such that the group is susceptible to activation, e.g. by light or by the action of an enzyme. E may be $-NO_2$ or glucoronic acid. The former may be susceptible to the action of a nitroreductase, the latter to the action of a β-glucoronidase.

In this embodiment, the self-immolative linker will allow for release of the protected compound when E is activated, proceeding along the lines shown below (for n=0):



where the asterisk indicates the point of attachment to the N10 position, E^* is the activated form of E, and Y is as described above. These groups have the advantage of separating the site of activation from the compound being protected. As described above, the phenylene group may be optionally further substituted.

The group Y may be a covalent bond to L^1.

The group Y may be a functional group selected from:

-C(=O)-, -NH-, -O-, -C(=O)NH-, -C(=O)O-, -NHC(=O)-, -OC(=O)-, -OC(=O)O-, -NHC(=O)O-, -OC(=O)NH-, -NHC(=O)NH-, -NHC(=O)NH, -C(=O)NHC(=O)-, and -S-.

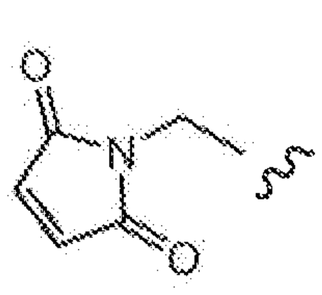
Where L^1 is a dipeptide, it is preferred that Y is -NH- or -C(=O)-, thereby to form an amide bond between L^1 and Y. In this embodiment, the dipeptide sequence need not be a substrate for an enzymatic activity.

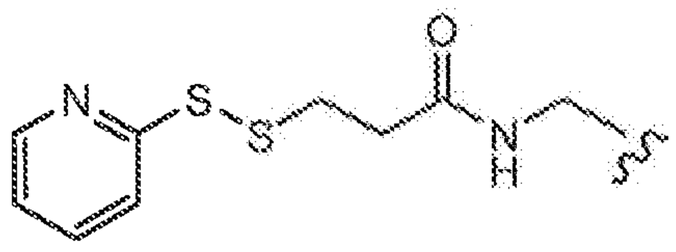
In another embodiment, A is a spacer group. Thus, L^1 and the cell binding agent are indirectly connected.

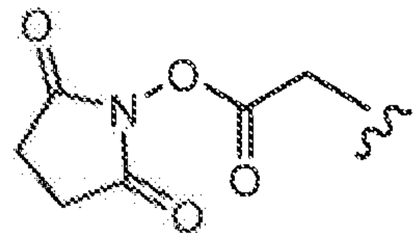
L^1 and A may be connected by a bond selected from:

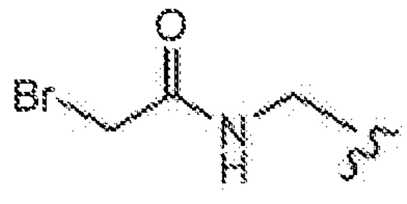
-C(=O)NH-, -C(=O)O-, -NHC(=O)-, -OC(=O)-, -OC(=O)O-, -NHC(=O)O-, -OC(=O)NH-, and -NHC(=O)NH-.

Preferably, the linker contains an electrophilic functional group for reaction with a nucleophilic functional group on the modulator. Nucleophilic groups on antibodies include, but are not limited to: (i) N-terminal amine groups, (ii) side chain amine groups, e.g. lysine, (iii) side chain thiol groups, e.g. cysteine, and (iv) sugar hydroxyl or amino groups where the antibody is glycosylated. Amine, thiol, and hydroxyl groups are nucleophilic and capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) maleimide groups (ii) activated disulfides, (iii) active esters such as NHS (N-hydroxysuccinimide) esters, HOBt (N-hydroxybenzotriazole) esters, haloformates, and acid halides; (iv) alkyl and benzyl halides such as haloacetamides; and (v) aldehydes, ketones, carboxyl, and, some of which are exemplified as follows:







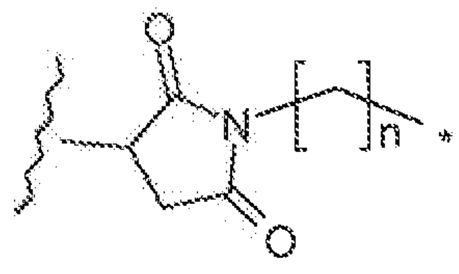


Certain antibodies have reducible interchain disulfides, i.e. cysteine bridges. Antibodies may be made reactive for conjugation with linker reagents by treatment with a reducing agent such as DTT (dithiothreitol). Each cysteine bridge will thus form, theoretically, two reactive thiol nucleophiles. Additional nucleophilic groups can be introduced into antibodies through the reaction of lysines with 2-iminothiolane (Traut's reagent) resulting in conversion of an

amine into a thiol. Reactive thiol groups may be introduced into the antibody (or fragment thereof) by introducing one, two, three, four, or more cysteine residues (e.g., preparing mutant antibodies comprising one or more non-native cysteine amino acid residues). US 7521541 teaches engineering antibodies by introduction of reactive cysteine amino acids.

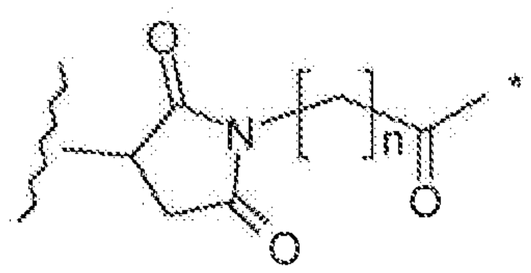
In some embodiments, a linker has a reactive nucleophilic group which is reactive with an electrophilic group present on an antibody. Useful electrophilic groups on an antibody include, but are not limited to, aldehyde and ketone carbonyl groups. The heteroatom of a nucleophilic group of a Linker can react with an electrophilic group on an antibody and form a covalent bond to an antibody unit. Useful nucleophilic groups on a linker include, but are not limited to, hydrazide, oxime, amino, hydroxyl, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide. The electrophilic group on an antibody provides a convenient site for attachment to a Linker.

In one embodiment, the group A is:



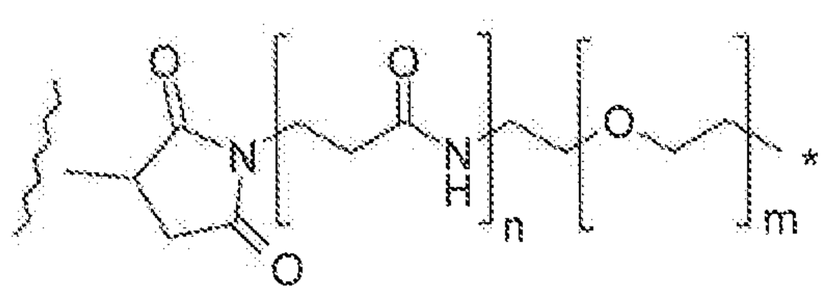
where the asterisk indicates the point of attachment to L^1, the wavy line indicates the point of attachment to the cell binding agent, and n is 0 to 6. In one embodiment, n is 5.

In one embodiment, the group A is:



where the asterisk indicates the point of attachment to L^1, the wavy line indicates the point of attachment to the cell binding agent, and n is 0 to 6. In one embodiment, n is 5.

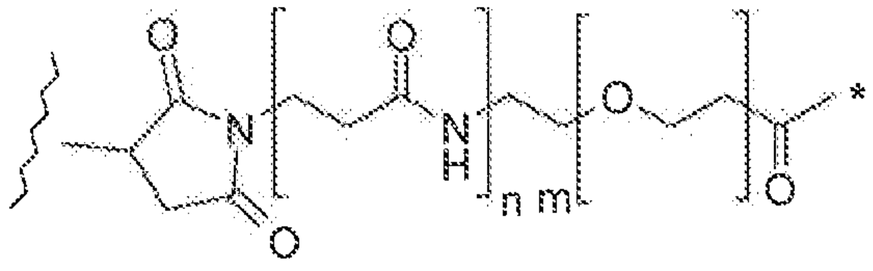
In one embodiment, the group A is:



where the asterisk indicates the point of attachment to L^1, the wavy line indicates the

point of attachment to the cell binding agent, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, and most preferably 4 or 8. In another embodiment, m is 10 to 30, and preferably 20 to 30. Alternatively, m is 0 to 50. In this embodiment, m is preferably 10-40 and n is 1.

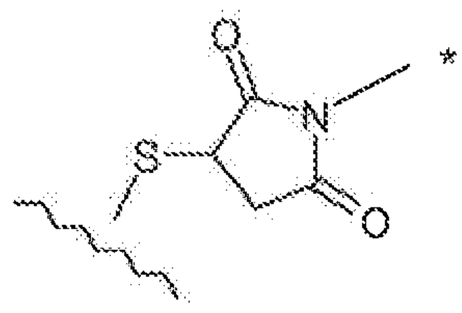
In one embodiment, the group A is:



where the asterisk indicates the point of attachment to L^1, the wavy line indicates the point of attachment to the cell binding agent, n is 0 or 1, and m is 0 to 30. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 8, preferably 4 to 8, and most preferably 4 or 8. In another embodiment, m is 10 to 30, and preferably 20 to 30. Alternatively, m is 0 to 50. In this embodiment, m is preferably 10-40 and n is 1.

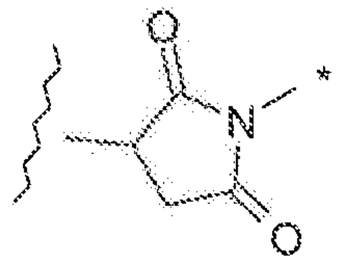
In one embodiment, the connection between the cell binding agent and A is through a thiol residue of the cell binding agent and a maleimide group of A.

In one embodiment, the connection between the cell binding agent and A is:



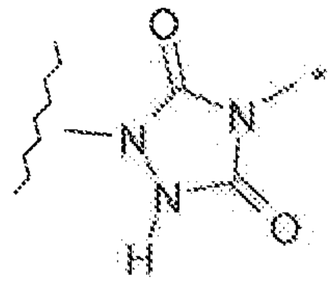
where the asterisk indicates the point of attachment to the remaining portion of A and the wavy line indicates the point of attachment to the remaining portion of the cell binding agent. In this embodiment, the S atom is typically derived from the modulator.

In each of the embodiments above, an alternative functionality may be used in place of the maleimide-derived group shown below:



where the wavy line indicates the point of attachment to the cell binding agent as before, and the asterisk indicates the bond to the remaining portion of the A group.

In one embodiment, the maleimide-derived group is replaced with the group:

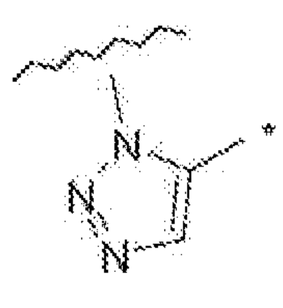


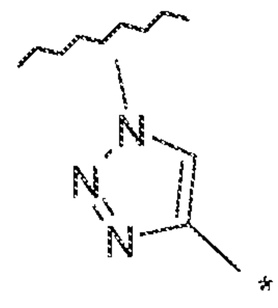
where the wavy line indicates point of attachment to the cell binding agent, and the asterisk indicates the bond to the remaining portion of the A group.

In one embodiment, the maleimide-derived group is replaced with a group, which optionally together with the cell binding agent, is selected from:

-C(=O)NH-, -C(=O)O-, -NHC(=O)-, -OC(=O)-, -OC(=O)O-, -NHC(=O)O-, -OC(=O)NH-, -NHC(=O)NH-, -NHC(=O)NH, -C(=O)NHC(=O)-, -S-, -S-S-, $-CH_2C(=O)-$, $-C(=O)CH_2-$, =N-NH- and -NH-N=.

In one embodiment, the maleimide-derived group is replaced with a group, which optionally together with the cell binding agent, is selected from:





where the wavy line indicates either the point of attachment to the cell binding agent or the bond to the remaining portion of the A group, and the asterisk indicates the other of the point of attachment to the cell binding agent or the bond to the remaining portion of the A group.

Other groups suitable for connecting L^1 to the selected modulator are described in WO 2005/082023.

In another preferred embodiment the modulators of the instant invention may be associated with biocompatible polymers comprising drug linker units. In this respect one such type of compatible polymer comprises Fleximer® polymers (Mersana Therapeutics). Such polymers are reportedly biodegradable, well tolerated and have been clinically validated. Moreover, such polymers are compatible with a number of customizable linker technologies and chemistries allowing for control of pharmacokinetics, localization of drug release and improved biodistribution.

The selected modulators can also be directly conjugated radioisotopes or may comprise macrocyclic chelators useful for conjugating radiometal ions (as described herein). In certain embodiments, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N',N",N"-tetraacetic acid (DOTA) which can be attached to the antibody via a linker molecule. Such linker molecules are commonly known in the art and described in Denardo et al., 1998, Clin Cancer Res. 4:2483; Peterson et al., 1999, Bioconjug. Chem. 10:553; and Zimmerman et al., 1999, Nucl. Med. Biol. 26:943.

More generally, techniques for conjugating therapeutic moieties or cytotoxic agents to modulators are well known. As discussed above moieties can be conjugated to modulators by any art-recognized method, including, but not limited to aldehyde/Schiff linkage, sulphydryl linkage, acid-labile linkage, cis-aconityl linkage, hydrazone linkage, enzymatically degradable linkage (see generally Garnett, 2002, Adv Drug Deliv Rev 53:171). Also see, e.g., Amon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., 1982, Immunol. Rev. 62:119. In preferred embodiments a SEZ6 modulator that is conjugated to a therapeutic moiety or cytotoxic agent may be internalized by a cell upon binding to a SEZ6 molecule associated with the cell surface thereby delivering the therapeutic payload.

C. Biocompatible Modifiers

In selected embodiments the modulators of the invention may be conjugated or otherwise associated with biocompatible modifiers that may be used to adjust, alter, improve or moderate modulator characteristics as desired. For example, antibodies or fusion constructs with increased *in vivo* half-lives can be generated by attaching relatively high molecular weight polymer molecules such as commercially available polyethylene glycol

(PEG) or similar biocompatible polymers. Those skilled in the art will appreciate that PEG may be obtained in many different molecular weight and molecular configurations that can be selected to impart specific properties to the antibody (e.g. the half-life may be tailored). PEG can be attached to modulators or antibody fragments or derivatives with or without a multifunctional linker either through site-specific conjugation of the PEG to the N- or C-terminus of said antibodies or antibody fragments or via epsilon-amino groups present on lysine residues. Linear or branched polymer derivatization that results in minimal loss of biological activity may be used. The degree of conjugation can be closely monitored by SDS-PAGE and mass spectrometry to ensure optimal conjugation of PEG molecules to antibody molecules. Unreacted PEG can be separated from antibody-PEG conjugates by, e.g., size exclusion or ion-exchange chromatography. In a similar manner, the disclosed modulators can be conjugated to albumin in order to make the antibody or antibody fragment more stable in vivo or have a longer half life in vivo. The techniques are well known in the art, see e.g., International Publication Nos. WO 93/15199, WO 93/15200, and WO 01/77137; and European Patent No. 0 413, 622. Other biocompatible conjugates are evident to those of ordinary skill and may readily be identified in accordance with the teachings herein.

D. Diagnostic or Detection Agents

In other preferred embodiments, modulators of the present invention, or fragments or derivatives thereof, are conjugated to a diagnostic or detectable agent, marker or reporter which may be, for example, a biological molecule (e.g., a peptide or nucleotide), a small molecule, fluorophore, or radioisotope. Labeled modulators can be useful for monitoring the development or progression of a hyperproliferative disorder or as part of a clinical testing procedure to determine the efficacy of a particular therapy including the disclosed modulators (i.e. theragnostics) or to determine a future course of treatment. Such markers or reporters may also be useful in purifying the selected modulator, modulator analytics (e.g., epitope binding or antibody binning), separating or isolating TIC or in preclinical procedures or toxicology studies.

Such diagnosis analysis and/or detection can be accomplished by coupling the modulator to detectable substances including, but not limited to, various enzymes comprising for example horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or

acetylcholinesterase; prosthetic groups, such as but not limited to streptavidinlbiotin and avidin/biotin; fluorescent materials, such as but not limited to, umbelliferone, fluorescein, fluorescein isothiocynate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; luminescent materials, such as but not limited to, luminol; bioluminescent materials, such as but not limited to, luciferase, luciferin, and aequorin; radioactive materials, such as but not limited to iodine (^{131}I, ^{125}I, ^{123}I, ^{121}I,), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{115}In, ^{113}In, ^{112}In, ^{111}In,), and technetium (^{99}Tc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{186}Re, ^{188}Re, ^{142}Pr, ^{105}Rh, ^{97}Ru, ^{68}Ge, ^{57}Co, ^{65}Zn, ^{85}Sr, ^{32}P, ^{153}Gd, ^{169}Yb, ^{51}Cr, ^{54}Mn, ^{75}Se, ^{113}Sn, and ^{117}Tin; positron emitting metals using various positron emission tomographies, noradioactive paramagnetic metal ions, and molecules that are radiolabeled or conjugated to specific radioisotopes. In such embodiments appropriate detection methodology is well known in the art and readily available from numerous commercial sources.

As indicated above, in other embodiments the modulators or fragments thereof can be fused or conjugated to marker sequences or compounds, such as a peptide or fluorophore to facilitate purification or diagnostic or analytic procedures such as immunohistochemistry, bio-layer interferometry, surface plasmon resonance, flow cytometry, competitive ELISA, FACs, etc. In preferred embodiments, the marker comprises a his-tag such as that provided by the pQE vector (Qiagen), among others, many of which are commercially available. Other peptide tags useful for purification include, but are not limited to, the hemagglutinin "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., 1984, Cell 37:767) and the "flag" tag (U.S.P.N. 4,703,004).

E. Therapeutic Moieties

As previously alluded to the modulators or fragments or derivatives thereof may also be conjugated, linked or fused to or otherwise associated with a" therapeutic moiety" or "drug" such as an anti-proliferative or anti-cancer agent including, but not limited to, cytotoxic agents, cytostatic agents, anti-angiogenic agents, debulking agents, chemotherapeutic agents, radiotherapy and radiotherapeutic agents, targeted anti-cancer agents, BRMs, therapeutic

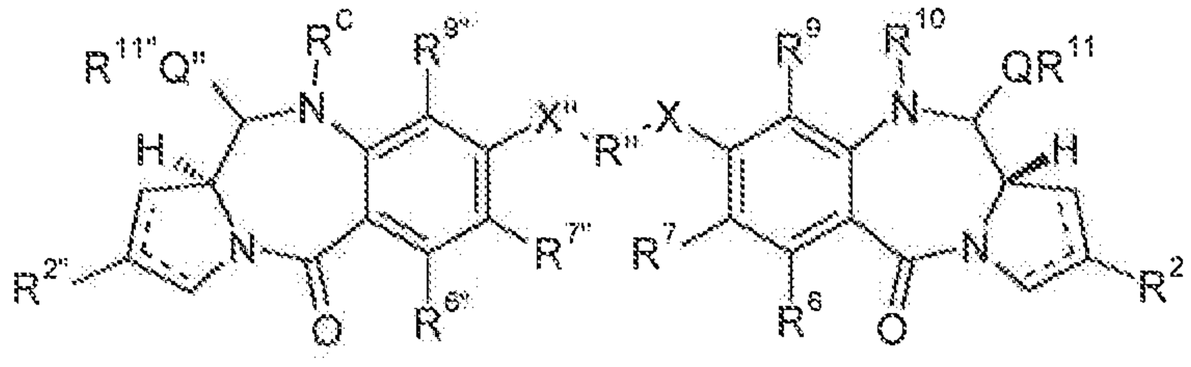
antibodies, cancer vaccines, cytokines, hormone therapies, radiation therapy and anti-metastatic agents and immunotherapeutic agents.

Preferred exemplary anti-cancer agents include cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin, maytansinoids such as DM-1 and DM-4 (Immunogen, Inc.), dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin, epirubicin, and cyclophosphamide and analogs or homologs thereof. Additional compatible cytotoxins comprise dolastatins and auristatins, including monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF) (Seattle Genetics, Inc.), amanitins such as alpha-amanitin, beta-amanitin, gamma-amanitin or epsilon-amanitin (Heidelberg Pharma AG), DNA minor groove binding agents such as duocarmycin derivatives (Syntarga, B.V.) and modified pyrrolobenzodiazepine dimers (Spirogen, Ltd.), splicing inhibitors such as meayamycin analogs or derivatives (e.g., FR901464 as set forth in U.S.P.N. 7,825,267), tubular binding agents such as epothilone analogs and paclitaxel and DNA damaging agents such as calicheamicins and esperamicins. Furthermore, in certain embodiments the SEZ6 modulators of the instant invention may be associated with anti-CD3 binding molecules to recruit cytotoxic T-cells and have them target the tumor initiating cells (BiTE technology; see e.g., Fuhrmann, S. et. al. Annual Meeting of AACR Abstract No. 5625 (2010) which is incorporated herein by reference).

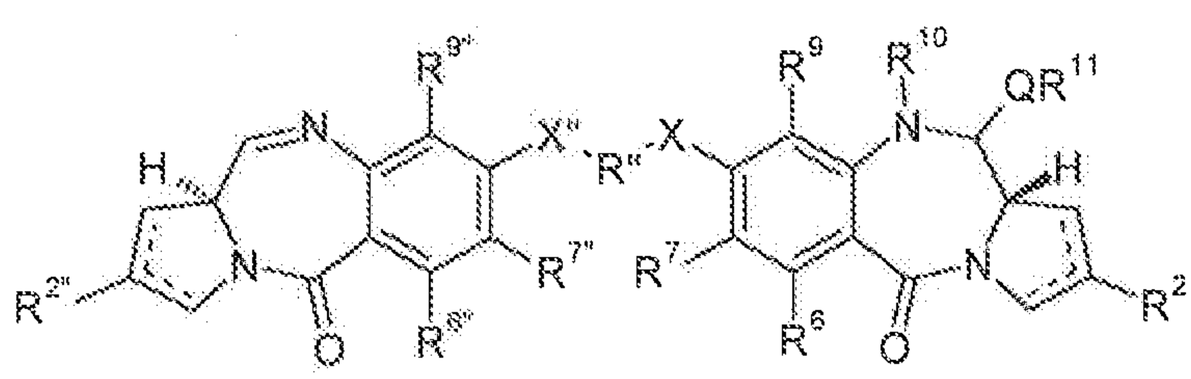
Still additional compatible anti-cancer agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa, chlorambucil, melphalan, carmustine (BCNU) and lomustine (CCNU), busulfan, dibromomannitol, streptozotocin, and cisdichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine). A more extensive list of therapeutic moieties can be found in PCT publication WO 03/075957 and U.S.P.N. 2009/0155255 each of which is incorporated herein by reference.

As indicated above selected embodiments of the instant invention are directed to conjugated SEZ6 modulators such as anti-SEZ6 antibody drug conjugates that comprise pyrrolobenzodiazepine (PBD) as a cytotoxic agent. It will be appreciated that PBDs are alkylating agents that exert anti-tumor activity by covalently binding to DNA in the minor groove and inhibiting nucleic acid synthesis. In this respect PBDs have been shown to have potent antitumor properties while exhibiting minimal bone marrow depression. PBDs compatible with the present invention may be linked to the SEZ6 modulator using any one of several types of linker (e.g., a peptidyl linker comprising a maleimido moiety with a free sulfhydryl) and, in certain embodiments are dimeric in form (i.e., PBD dimers). Compatible PBDs (and optional linkers) that may be conjugated to the disclosed modulators are described, for example, in U.S.P.N.s 6,362,331, 7,049,311, 7,189,710, 7,429,658, 7,407,951, 7,741,319, 7,557,099, 8,034,808, 8,163,736 U.S.P.N. 2011/0256157 and PCT filings WO2011/130613, WO2011/128650 and WO2011/130616 each of which is incorporated herein by reference. Accordingly, in particularly preferred embodiments the modulator will comprise an anti SEZ6 antibody conjugated or associated with one or more PBD dimers (i.e., a SEZ6-PBD ADC).

In particularly preferred embodiments compatible PBDs that may be conjugated to the disclosed modulators are described in U.S.P.N. 2011/0256157. In this disclosure, PBD dimers, i.e. those comprising two PBD moieties may be preferred. Thus, preferred conjugates of the present invention are those having the formula (AB) or (AC):



AB



AC

wherein:

the dotted lines indicate the optional presence of a double bond between C1 and C2 or C2 and C3;

R^2 is independently selected from H, OH, =O, $=CH_2$, CN, R, OR, $=CH\text{-}R^D$, $=C(R^D)_2$, $O\text{-}SO_2\text{-}R$, CO_2R and COR, and optionally further selected from halo or dihalo;

where R^D is independently selected from R, CO_2R, COR, CHO, CO_2H, and halo;

R^6 and R^9 are independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', NO_2, Me_3Sn and halo;

R^7 is independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', NO_2, Me_3Sn and halo;

R^{10} is a linker connected to a modulator or fragment or derivative thereof, as described above;

Q is independently selected from O, S and NH;

R^{11} is either H, or R or, where Q is O, SO_3M, where M is a metal cation;

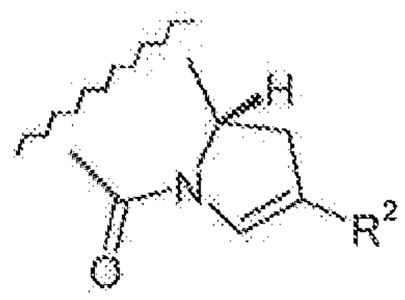
R and R' are each independently selected from optionally substituted $C_{1\text{-}12}$ alkyl, $C_{3\text{-}20}$ heterocyclyl and $C_{5\text{-}20}$ aryl groups, and optionally in relation to the group NRR', R and R' together with the nitrogen atom to which they are attached form an optionally substituted 4-, 5-, 6- or 7-membered heterocyclic ring; and

wherein $R^{2"}$, $R^{6"}$, $R^{7"}$, $R^{9"}$, X", Q" and $R^{11"}$ and are as defined according to R^2, R^6, R^7, R^9, X, Q and R^{11} respectively, and R^C is a capping group.

Double Bond

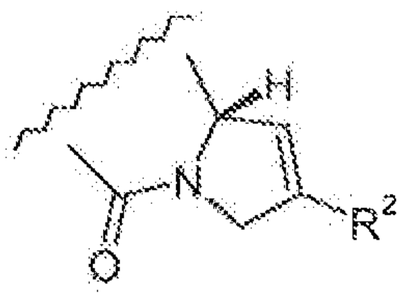
In one embodiment, there is no double bond present between C1 and C2, and C2 and C3.

In one embodiment, the dotted lines indicate the optional presence of a double bond between C2 and C3, as shown below:



In one embodiment, a double bond is present between C2 and C3 when R^2 is C_{5-20} aryl or C_{1-12} alkyl.

In one embodiment, the dotted lines indicate the optional presence of a double bond between C1 and C2, as shown below:



In one embodiment, a double bond is present between C1 and C2 when R^2 is C_{5-20} aryl or C_{1-12} alkyl.

R^2

In one embodiment, R^2 is independently selected from H, OH, =O, $=CH_2$, CN, R, OR, $=CH\text{-}R^D$, $=C(R^D)_2$, O-SO_2-R, CO_2R and COR, and optionally further selected from halo or dihalo.

In one embodiment, R^2 is independently selected from H, OH, =O, $=CH_2$, CN, R, OR, $=CH\text{-}R^D$, $=C(R^D)_2$, O-SO_2-R, CO_2R and COR.

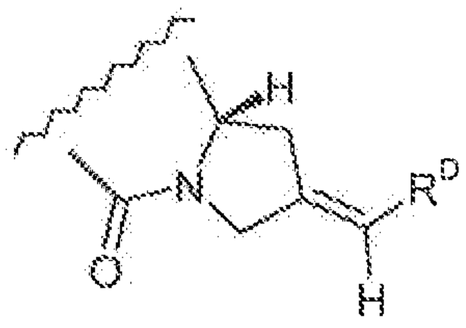
In one embodiment, R^2 is independently selected from H, =O, $=CH_2$, R, $=CH\text{-}R^D$, and $=C(R^D)_2$.

In one embodiment, R^2 is independently H.

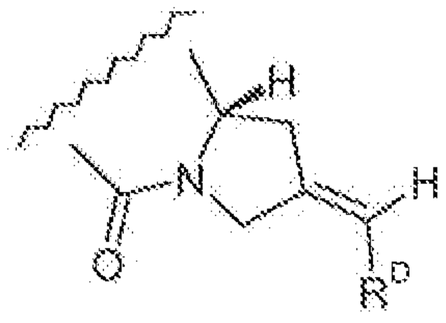
In one embodiment, R^2 is independently =O.

In one embodiment, R^2 is independently $=CH_2$.

In one embodiment, R^2 is independently $=CH\text{-}R^D$. Within the PBD compound, the group $=CH\text{-}R^D$ may have either configuration shown below:



(I)



(III)

In one embodiment, the configuration is configuration (I).

In one embodiment, R^2 is independently $=C(R^D)_2$.

In one embodiment, R^2 is independently $=CF_2$.

In one embodiment, R^2 is independently R.

In one embodiment, R^2 is independently optionally substituted C_{5-20} aryl.

In one embodiment, R^2 is independently optionally substituted C_{1-12} alkyl.

In one embodiment, R^2 is independently optionally substituted C_{5-20} aryl.

In one embodiment, R^2 is independently optionally substituted C_{5-7} aryl.

In one embodiment, R^2 is independently optionally substituted C_{8-10} aryl.

In one embodiment, R^2 is independently optionally substituted phenyl.

In one embodiment, R^2 is independently optionally substituted napthyl.

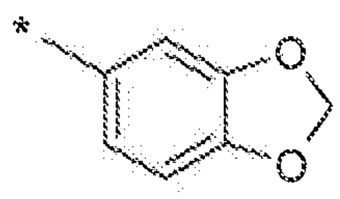
In one embodiment, R^2 is independently optionally substituted pyridyl.

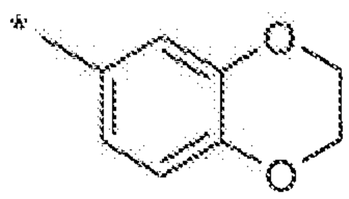
In one embodiment, R^2 is independently optionally substituted quinolinyl or isoquinolinyl.

In one embodiment, R^2 bears one to three substituent groups, with 1 and 2 being more preferred, and singly substituted groups being most preferred. The substituents may be any position.

Where R^2 is a C_{5-7} aryl group, a single substituent is preferably on a ring atom that is not adjacent the bond to the remainder of the compound, i.e. it is preferably β or γ to the bond to the remainder of the compound. Therefore, where the C_{5-7} aryl group is phenyl, the substituent is preferably in the meta- or para- positions, and more preferably is in the para-position.

In one embodiment, R^2 is selected from:





where the asterisk indicates the point of attachment.

Where R^2 is a C_{8-10} aryl group, for example quinolinyl or isoquinolinyl, it may bear any number of substituents at any position of the quinoline or isoquinoline rings. In some embodiments, it bears one, two or three substituents, and these may be on either the proximal and distal rings or both (if more than one substituent).

In one embodiment, where R^2 is optionally substituted, the substituents are selected from those substituents given in the substituent section below.

Where R is optionally substituted, the substituents are preferably selected from:

Halo, Hydroxyl, Ether, Formyl, Acyl, Carboxy, Ester, Acyloxy, Amino, Amido, Acylamido, Aminocarbonyloxy, Ureido, Nitro, Cyano and Thioether.

In one embodiment, where R or R^2 is optionally substituted, the substituents are selected from the group consisting of R, OR, SR, NRR', NO_2, halo, CO_2R, COR, $CONH_2$, CONHR, and CONRR'.

Where R^2 is C_{1-12} alkyl, the optional substituent may additionally include C_{3-20} heterocyclyl and C_{5-20} aryl groups.

Where R^2 is C_{3-20} heterocyclyl, the optional substituent may additionally include C_{1-12} alkyl and C_{5-20} aryl groups.

Where R^2 is C_{5-20} aryl groups, the optional substituent may additionally include C_{3-20} heterocyclyl and C_{1-12} alkyl groups.

It is understood that the term "alkyl" encompasses the sub-classes alkenyl and alkynyl as well as cycloalkyl. Thus, where R^2 is optionally substituted C_{1-12} alkyl, it is understood that the alkyl group optionally contains one or more carbon-carbon double or triple bonds, which may form part of a conjugated system. In one embodiment, the optionally substituted C_{1-12} alkyl group contains at least one carbon-carbon double or triple bond, and this bond is conjugated with a double bond present between C1 and C2, or C2 and C3. In one embodiment, the C_{1-12} alkyl group is a group selected from saturated C_{1-12} alkyl, C_{2-12} alkenyl, C_{2-12} alkynyl and C_{3-12} cycloalkyl.

If a substituent on R^2 is halo, it is preferably F or Cl, more preferably Cl.

If a substituent on R^2 is ether, it may in some embodiments be an alkoxy group, for example, a C_{1-7} alkoxy group (e.g. methoxy, ethoxy) or it may in some embodiments be a C_{5-7} aryloxy group (e.g phenoxy, pyridyloxy, furanyloxy).

If a substituent on R^2 is C_{1-7} alkyl, it may preferably be a C_{1-4} alkyl group (e.g. methyl, ethyl, propyl, butyl).

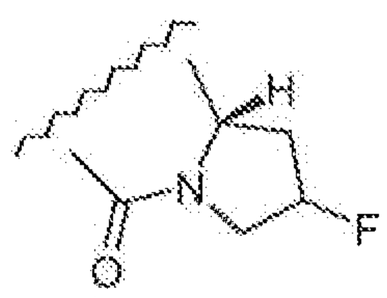
If a substituent on R^2 is C_{3-7} heterocyclyl, it may in some embodiments be C_6 nitrogen containing heterocyclyl group, e.g. morpholino, thiomorpholino, piperidinyl, piperazinyl. These groups may be bound to the rest of the PBD moiety via the nitrogen atom. These groups may be further substituted, for example, by C_{1-4} alkyl groups.

If a substituent on R^2 is bis-oxy-C_{1-3} alkylene, this is preferably bis-oxy-methylene or bis-oxy-ethylene.

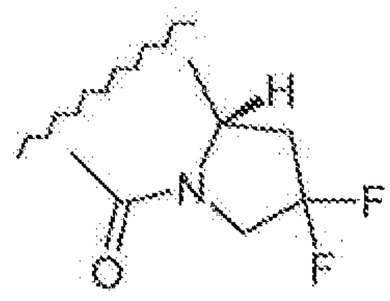
Particularly preferred substituents for R^2 include methoxy, ethoxy, fluoro, chloro, cyano, bis-oxy-methylene, methyl-piperazinyl, morpholino and methyl-thienyl.

Particularly preferred substituted R^2 groups include, but are not limited to, 4-methoxy-phenyl, 3-methoxyphenyl, 4-ethoxy-phenyl, 3-ethoxy-phenyl, 4-fluoro-phenyl, 4-chloro-phenyl, 3,4-bisoxymethylene-phenyl, 4-methylthienyl, 4-cyanophenyl, 4-phenoxyphenyl, quinolin-3-yl and quinolin-6-yl, isoquinolin-3-yl and isoquinolin-6-yl, 2-thienyl, 2-furanyl, methoxynaphthyl, and naphthyl.

In one embodiment, R^2 is halo or dihalo. In one embodiment, R^2 is -F or -F_2, which substituents are illustrated below as (III) and (IV) respectively:



(III)



(IV)

$\underline{R^D}$

In one embodiment, R^D is independently selected from R, CO_2R, COR, CHO, CO_2H, and halo.

In one embodiment, R^D is independently R.

In one embodiment, R^D is independently halo.

$\underline{R^6}$

In one embodiment, R^6 is independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', NO_2, Me_3Sn- and Halo.

In one embodiment, R^6 is independently selected from H, OH, OR, SH, NH_2, NO_2 and Halo.

In one embodiment, R^6 is independently selected from H and Halo.

In one embodiment, R^6 is independently H.

In one embodiment, R^6 and R^7 together form a group -O-$(CH_2)_p$-O-, where p is 1 or 2.

R^7

R^7 is independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', NO_2, Me_3Sn and halo.

In one embodiment, R^7 is independently OR.

In one embodiment, R^7 is independently OR^{7A}, where R^{7A} is independently optionally substituted C_{1-6} alkyl.

In one embodiment, R^{7A} is independently optionally substituted saturated C_{1-6} alkyl.

In one embodiment, R^{7A} is independently optionally substituted C_{2-4} alkenyl.

In one embodiment, R^{7A} is independently Me.

In one embodiment, R^{7A} is independently CH_2Ph.

In one embodiment, R^{7A} is independently allyl.

In one embodiment, the compound is a dimer where the R^7 groups of each monomer form together a dimer bridge having the formula X-R''-X linking the monomers.

R^8

In one embodiment, the compound is a dimer where the R^8 groups of each monomer form together a dimer bridge having the formula X-R''-X linking the monomers.

In one embodiment, R^8 is independently OR^{8A}, where R^{8A} is independently optionally substituted C_{1-4} alkyl.

In one embodiment, R^{8A} is independently optionally substituted saturated C_{1-6} alkyl or optionally substituted C_{2-4} alkenyl.

In one embodiment, R^{8A} is independently Me.

In one embodiment, R^{8A} is independently CH_2Ph.

In one embodiment, R^{8A} is independently allyl.

In one embodiment, R^8 and R^7 together form a group -O-$(CH_2)_p$-O-, where p is 1 or 2.

In one embodiment, R^8 and R^9 together form a group -O-$(CH_2)_p$-O-, where p is 1 or 2.

R^9

In one embodiment, R^9 is independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', NO_2, Me_3Sn- and Halo.

In one embodiment, R^9 is independently H.

In one embodiment, R^9 is independently R or OR.

R and R'

In one embodiment, R is independently selected from optionally substituted C_{1-12} alkyl, C_{3-20} heterocyclyl and C_{5-20} aryl groups. These groups are each defined in the substituents section below.

In one embodiment, R is independently optionally substituted C_{1-12} alkyl.

In one embodiment, R is independently optionally substituted C_{3-20} heterocyclyl.

In one embodiment, R is independently optionally substituted C_{5-20} aryl.

In one embodiment, R is independently optionally substituted C_{1-12} alkyl.

Described above in relation to R^2 are various embodiments relating to preferred alkyl and aryl groups and the identity and number of optional substituents. The preferences set out for R^2 as it applies to R are applicable, where appropriate, to all other groups R, for examples where R^6, R^7, R^8 or R^9 is R.

The preferences for R apply also to R'.

In some embodiments of the invention there is provided a compound having a substituent group -NRR'. In one embodiment, R and R' together with the nitrogen atom to which they are attached form an optionally substituted 4-, 5-, 6- or 7-membered heterocyclic ring. The ring may contain a further heteroatom, for example N, O or S.

In one embodiment, the heterocyclic ring is itself substituted with a group R. Where a further N heteroatom is present, the substituent may be on the N heteroatom.

R"

R″ is a C_{3-12} alkylene group, which chain may be interrupted by one or more heteroatoms, e.g. O, S, N(H), NMe and/or aromatic rings, e.g. benzene or pyridine, which rings are optionally substituted.

In one embodiment, R″ is a C_{3-12} alkylene group, which chain may be interrupted by one or more heteroatoms and/or aromatic rings, e.g. benzene or pyridine.

In one embodiment, the alkylene group is optionally interrupted by one or more heteroatoms selected from O, S, and NMe and/or aromatic rings, which rings are optionally substituted.

In one embodiment, the aromatic ring is a C_{5-20} arylene group, where arylene pertains to a divalent moiety obtained by removing two hydrogen atoms from two aromatic ring atoms of an aromatic compound, which moiety has from 5 to 20 ring atoms.

In one embodiment, R″ is a C_{3-12} alkylene group, which chain may be interrupted by one or more heteroatoms, e.g. O, S, N(H), NMe and/or aromatic rings, e.g. benzene or pyridine, which rings are optionally substituted by NH_2.

In one embodiment, R″ is a C_{3-12} alkylene group.

In one embodiment, R″ is selected from a C_3, C_5, C_7, C_9 and a C_{11} alkylene group.

In one embodiment, R″ is selected from a C_3, C_5 and a C_7 alkylene group.

In one embodiment, R″ is selected from a C_3 and a C_5 alkylene group.

In one embodiment, R″ is a C_3 alkylene group.

In one embodiment, R″ is a C_5 alkylene group.

The alkylene groups listed above may be optionally interrupted by one or more heteroatoms and/or aromatic rings, e.g. benzene or pyridine, which rings are optionally substituted.

The alkylene groups listed above may be optionally interrupted by one or more heteroatoms and/or aromatic rings, e.g. benzene or pyridine.

The alkylene groups listed above may be unsubstituted linear aliphatic alkylene groups.

X

In one embodiment, X is selected from O, S, or N(H).

Preferably, X is O.

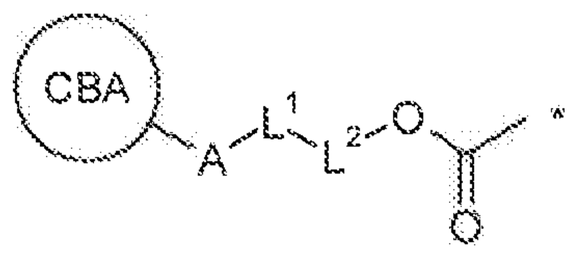
R^{10}

Preferably compatible linkers such as those described above attach a SEZ6 modulator (CBA/Ab/M), to a PBD drug moiety D through covalent bond(s) at the R^{10} position (i.e., N10). The linker is a bifunctional or multifunctional moiety which can be used to link one or more drug moiety (D) and a modulator (preferably an antibody) to form antibody-drug conjugates (ADC). The linker (L) may be stable outside a cell, i.e. extracellular, or it may be cleavable by enzymatic activity, hydrolysis, or other metabolic conditions. Antibody-drug conjugates (ADC) can be conveniently prepared using a linker having reactive functionality for binding to the drug moiety and to the antibody. A cysteine thiol, or an amine, e.g. N-terminus or amino acid side chain such as lysine, of the antibody (Ab) can form a bond with a functional group of a linker or spacer reagent, PBD drug moiety (D) or drug-linker reagent (D-L).

Many functional groups on the linker attached to the N10 position of the PBD moiety may be useful to react with the cell binding agent. For example, ester, thioester, amide, thioamide, carbamate, thiocarbamate, urea, thiourea, ether, thioether, or disulfide linkages may be formed from reaction of the linker-PBD drug intermediates and the cell binding agent.

In another embodiment, the linker may be substituted with groups that modulate aggregation, solubility or reactivity. For example, a sulfonate substituent may increase water solubility of the reagent and facilitate the coupling reaction of the linker reagent with the antibody or the drug moiety, or facilitate the coupling reaction of Ab-L with D, or D-L with Ab, depending on the synthetic route employed to prepare the ADC.

In one preferred embodiment, R^{10} is a group:



where the asterisk indicates the point of attachment to the N10 position, CBA is a cell binding agent/modulator, L^1 is a linker, A is a connecting group connecting L^1 to the cell binding agent, L^2 is a covalent bond or together with -OC(=O)- forms a self-immolative linker, and L^1 or L^2 is a cleavable linker.

L^1 is preferably the cleavable linker, and may be referred to as a trigger for activation of the linker for cleavage.

As discussed in the linker section above the nature of L^1 and L^2, where present, can vary widely. These groups are chosen on the basis of their cleavage characteristics, which may be dictated by the conditions at the site to which the conjugate is delivered. Those linkers that are cleaved by the action of enzymes are preferred, although linkers that are cleavable by changes in pH (e.g. acid or base labile), temperature or upon irradiation (e.g. photolabile) may also be used. Linkers that are cleavable under reducing or oxidizing conditions may also find use in the present invention.

L^1 may comprise a contiguous sequence of amino acids. The amino acid sequence may be the target substrate for enzymatic cleavage, thereby allowing release of R^{10} from the N10 position.

In one embodiment, L^1 is cleavable by the action of an enzyme. In one embodiment, the enzyme is an esterase or a peptidase.

In one embodiment, L^2 is present and together with -C(=O)O- forms a self-immolative linker. In one embodiment, L^2 is a substrate for enzymatic activity, thereby allowing release of R^{10} from the N10 position.

In one embodiment, where L^1 is cleavable by the action of an enzyme and L^2 is present, the enzyme cleaves the bond between L^1 and L^2.

With regard to attaching the chosen linker to a selected PBD the group R^C is removable from the N10 position of certain PBD moieties to leave an N10-C11 imine bond, a carbinolamine, a substituted carbinolamine, where QR^{11} is OSO_3M, a bisulfite adduct, a thiocarbinolamine, a substituted thiocarbinolamine, or a substituted carbinalamine.

In one embodiment, R^C, may be a protecting group that is removable to leave an N10-C11 imine bond, a carbinolamine, a substituted cabinolamine, or, where QR^{11} is OSO_3M, a bisulfite adduct. In one embodiment, R^C is a protecting group that is removable to leave an N10-C11 imine bond.

The group R^C is intended to be removable under the same conditions as those required for the removal of the group R^{10}, for example to yield an N10-C11 imine bond, a carbinolamine and so on. The capping group acts as a protecting group for the intended functionality at the N10 position. The capping group is intended not to be reactive towards a cell binding agent. For example, R^C is not the same as R^L.

Compounds having a capping group may be used as intermediates in the synthesis of

dimers having an imine monomer. Alternatively, compounds having a capping group may be used as conjugates, where the capping group is removed at the target location to yield an imine, a carbinolamine, a substituted cabinolamine and so on. Thus, in this embodiment, the capping group may be referred to as a therapeutically removable nitrogen protecting group, as defined in WO 00/12507.

In one embodiment, the group R^C is removable under the conditions that cleave the linker R^L of the group R^{10}. Thus, in one embodiment, the capping group is cleavable by the action of an enzyme.

In an alternative embodiment, the capping group is removable prior to the connection of the linker R^L to the modulator. In this embodiment, the capping group is removable under conditions that do not cleave the linker R^L.

Where a compound includes a functional group G^1 to form a connection to the cell binding agent, the capping group is removable prior to the addition or unmasking of G^1.

The capping group may be used as part of a protecting group strategy to ensure that only one of the monomer units in a dimer is connected to a cell binding agent.

The capping group may be used as a mask for a N10-C11 imine bond. The capping group may be removed at such time as the imine functionality is required in the compound. The capping group is also a mask for a carbinolamine, a substituted cabinolamine, and a bisulfite adduct, as described above.

In one embodiment, R^C is a carbamate protecting group.

In one embodiment, the carbamate protecting group is selected from:

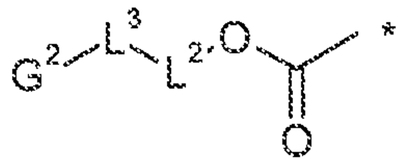
Alloc, Fmoc, Boc, Troc, Teoc, Psec, Cbz and PNZ.

Optionally, the carbamate protecting group is further selected from Moc.

In one embodiment, R^C is a linker group R^L lacking the functional group for connection to the cell binding agent.

This application is particularly concerned with those R^C groups which are carbamates.

In one embodiment, R^C is a group:



where the asterisk indicates the point of attachment to the N10 position, G^2 is a terminating group, L^3 is a covalent bond or a cleavable linker L^1, L^2 is a covalent bond or together with OC(=O) forms a self-immolative linker.

Where L^3 and L^2 are both covalent bonds, G^2 and OC(=O) together form a carbamate protecting group as defined above.

L^1 is as defined above in relation to R^{10}.

L^2 is as defined above in relation to R^{10}.

Various terminating groups are described below, including those based on well known protecting groups.

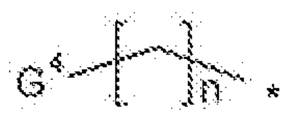
In one embodiment L^3 is a cleavable linker L^1, and L^2, together with OC(=O), forms a self-immolative linker. In this embodiment, G^2 is Ac (acetyl) or Moc, or a carbamate protecting group selected from: Alloc, Fmoc, Boc, Troc, Teoc, Psec, Cbz and PNZ. Optionally, the carbamate protecting group is further selected from Moc.

In another embodiment, G^2 is an acyl group -C(=O)G^3, where G^3 is selected from alkyl (including cycloalkyl, alkenyl and alkynyl), heteroalkyl, heterocyclyl and aryl (including heteroaryl and carboaryl). These groups may be optionally substituted. The acyl group together with an amino group of L^3 or L^2, where appropriate, may form an amide bond. The acyl group together with a hydroxy group of L^3 or L^2, where appropriate, may form an ester bond.

In one embodiment, G^3 is heteroalkyl. The heteroalkyl group may comprise polyethylene glycol. The heteroalkyl group may have a heteroatom, such as O or N, adjacent to the acyl group, thereby forming a carbamate or carbonate group, where appropriate, with a heteroatom present in the group L^3 or L^2, where appropriate.

In one embodiment, G^3 is selected from NH_2, NHR and NRR'. Preferably, G^3 is NRR'.

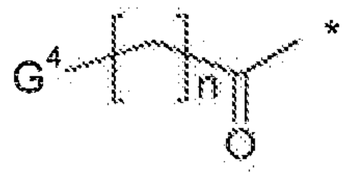
In one embodiment G^2 is the group:



where the asterisk indicates the point of attachment to L^3, n is 0 to 6 and G^4 is selected from OH, OR, SH, SR, COOR, $CONH_2$, CONHR, CONRR', NH_2, NHR, NRR', NO_2, and halo. The groups OH, SH, NH_2 and NHR are protected. In one embodiment, n is 1 to 6, and preferably n is 5. In one embodiment, G^4 is OR, SR, COOR, $CONH_2$, CONHR, CONRR',

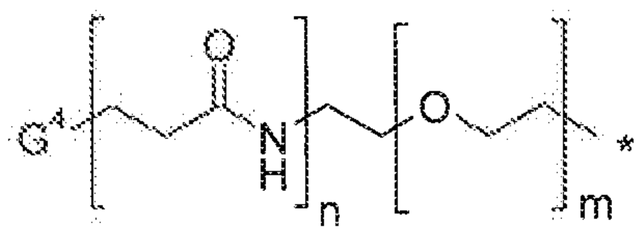
and NRR'. In one embodiment, G^4 is OR, SR, and NRR'. Preferably G^4 is selected from OR and NRR', most preferably G^4 is OR. Most preferably G^4 is OMe.

In one embodiment, the group G^2 is:



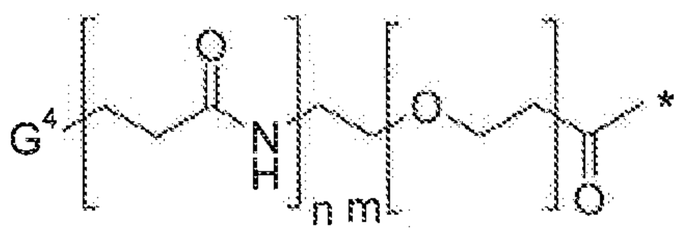
where the asterisk indicates the point of attachment to L^3, and n and G^4 are as defined above.

In one embodiment, the group G^2 is:



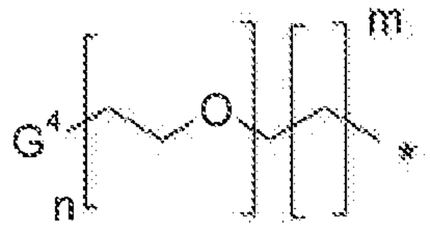
where the asterisk indicates the point of attachment to L^3, n is 0 or 1, m is 0 to 50, and G^4 is selected from OH, OR, SH, SR, COOR, $CONH_2$, CONHR, CONRR', NH_2, NHR, NRR', NO_2, and halo. In a preferred embodiment, n is 1 and m is 0 to 10, 1 to 2, preferably 4 to 8, and most preferably 4 or 8. In another embodiment, n is 1 and m is 10 to 50, preferably 20 to 40. The groups OH, SH, NH_2 and NHR are protected. In one embodiment, G^4 is OR, SR, COOR, $CONH_2$, CONHR, CONRR', and NRR'. In one embodiment, G^4 is OR, SR, and NRR'. Preferably G^4 is selected from OR and NRR', most preferably G^4 is OR. Preferably G^4 is OMe.

In one embodiment, the group G^2 is:



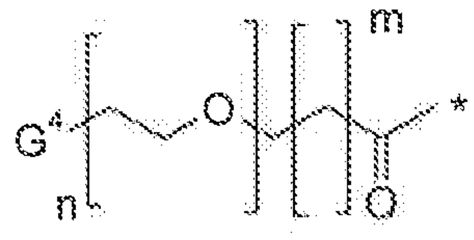
where the asterisk indicates the point of attachment to L^3, and n, m and G^4 are as defined above.

In one embodiment, the group G^2 is:



where n is 1-20, m is 0-6, and G^4 is selected from OH, OR, SH, SR, COOR, $CONH_2$, CONHR, CONRR', NH_2, NHR, NRR', NO_2, and halo. In one embodiment, n is 1-10. In another embodiment, n is 10 to 50, preferably 20 to 40. In one embodiment, n is 1. In one embodiment, m is 1. The groups OH, SH, NH_2 and NHR are protected. In one embodiment, G^4 is OR, SR, COOR, $CONH_2$, CONHR, CONRR', and NRR'. In one embodiment, G^4 is OR, SR, and NRR'. Preferably G^4 is selected from OR and NRR', most preferably G^4 is OR. Preferably G^4 is OMe.

In one embodiment, the group G^2 is:



where the asterisk indicates the point of attachment to L^3, and n, m and G^4 are as defined above.

In each of the embodiments above G^4 may be OH, SH, NH_2 and NHR. These groups are preferably protected.

In one embodiment, OH is protected with Bzl, TBDMS, or TBDPS.

In one embodiment, SH is protected with Acm, Bzl, Bzl-OMe, Bzl-Me, or Trt.

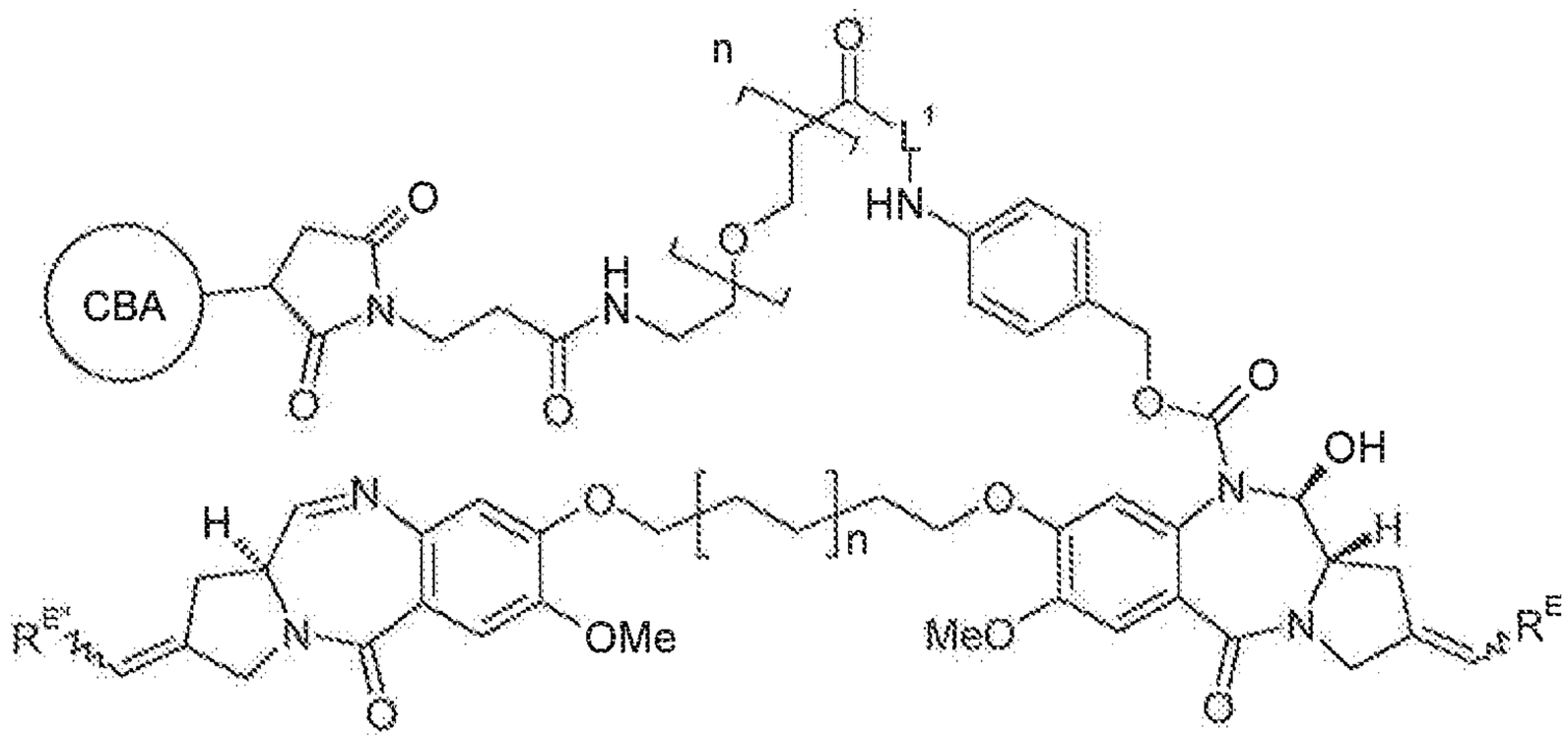
In one embodiment, NH_2 or NHR are protected with Boc, Moc, Z-Cl, Fmoc, Z, or Alloc.

In one embodiment, the group G^2 is present in combination with a group L^3, which group is a dipeptide.

The capping group is not intended for connection to the modulator. Thus, the other monomer present in the dimer serves as the point of connection to the modulator via a linker. Accordingly, it is preferred that the functionality present in the capping group is not available for reaction with a modulator. Thus, reactive functional groups such as OH, SH, NH_2, COOH are preferably avoided. However, such functionality may be present in the capping group if

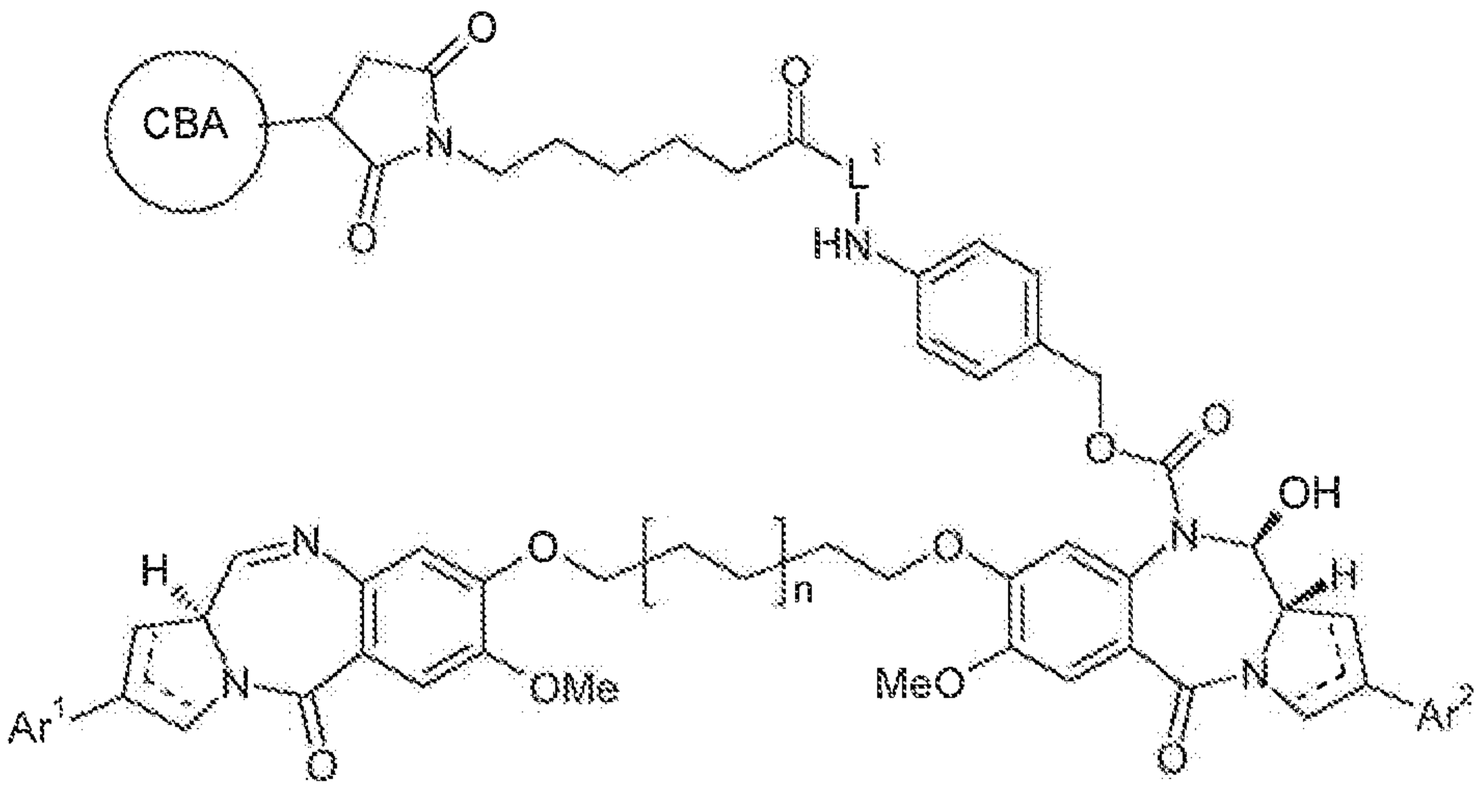
protected, as described above.

Thus, in accordance with the teachings herein one embodiment of the invention comprises a conjugate comprising a compound:



wherein CBA is a cell binding agent/modulator, and n is 0 or 1. L^1 is as previously defined, and R^E and $R^{E"}$ are each independently selected from H or R^D.

In another embodiment, the conjugate comprises a compound:



wherein CBA is a cell binding agent/modulator, L^1 is as previously defined, Ar^1 and Ar^2 are each independently optionally substituted C_{5-20} aryl, and n is 0 or 1.

Those of skill in the art will appreciate that other symmetric and asymmetric PBD dimers and linkers are compatible with the instant invention and could be selected without undue experimentation based on the teachings herein and the prior art.

Another aspect of the invention includes ADCs comprising radioisotopes. Exemplary radioisotopes that may be compatible with such embodiments include, but are not limited to,

iodine (^{131}I, ^{125}I, ^{123}I, ^{121}I,), carbon (^{14}C), copper (^{62}Cu, ^{64}Cu, ^{67}Cu), sulfur (^{35}S), tritium (^{3}H), indium (^{115}In, ^{113}In, ^{112}In, ^{111}In,), bismuth (^{212}Bi, ^{213}Bi), technetium (^{99}Tc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{186}Re, ^{188}Re, ^{142}Pr, ^{105}Rh, ^{97}Ru, ^{68}Ge, ^{57}Co, ^{65}Zn, ^{85}Sr, ^{32}P, ^{153}Gd, ^{169}Yb, ^{51}Cr, ^{54}Mn, ^{75}Se, ^{113}Sn, ^{117}Sn, ^{225}Ac, ^{76}Br, and ^{211}At. Other radionuclides are also available as diagnostic and therapeutic agents, especially those in the energy range of 60 to 4,000 keV. Depending on the condition to be treated and the desired therapeutic profile, those skilled in the art may readily select the appropriate radioisotope for use with the disclosed modulators.

SEZ6 modulators of the present invention may also be conjugated to a therapeutic moiety or drug that modifies a given biological response (e.g., biological response modifiers or BRMs). That is, therapeutic agents or moieties compatible with the instant invention are not to be construed as limited to classical chemical therapeutic agents. For example, in particularly preferred embodiments the drug moiety may be a protein or polypeptide or fragment thereof possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, Onconase (or another cytotoxic RNase), pseudomonas exotoxin, cholera toxin, or diphtheria toxin; a protein such as tumor necrosis factor, α-interferon, β-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF- α, TNF-β, AIM I (see, International Publication No. WO 97/33899), AIM II (see, International Publication No. WO 97/34911), Fas Ligand (Takahashi et al., 1994, J. Immunol., 6:1567), and VEGI (see, International Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, a biological response modifier such as, for example, a lymphokine (e.g., interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), and granulocyte colony stimulating factor ("G-CSF")), or a growth factor (e.g., growth hormone ("GH")). As set forth above, methods for fusing or conjugating modulators to polypeptide moieties are known in the art. In addition to the previously disclosed subject references see, e.g., U.S.P.Ns. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851, and 5,112,946; EP 307,434; EP 367,166; PCT Publications WO 96/04388 and WO 91/06570; Ashkenazi et al., 1991, PNAS USA 88:10535; Zheng et al., 1995, J Immunol 154:5590; and Vil et al., 1992, PNAS USA

89:11337 each of which is incorporated herein by reference. Moreover, as set forth above the association of a modulator with such moieties does not necessarily need to be direct, but may occur through linker sequences. As previously alluded to, such linker molecules are commonly known in the art and described in Denardo et al., 1998, Clin Cancer Res 4:2483; Peterson et al., 1999, Bioconjug Chem 10:553; Zimmerman et al., 1999, Nucl Med Biol 26:943; Garnett, 2002, Adv Drug Deliv Rev 53:171 each of which is incorporated herein.

IX. Diagnostics and Screening

A. Diagnostics

In yet other embodiments, the invention provides *in vitro* or *in vivo* methods for detecting, diagnosing or monitoring proliferative disorders and methods of screening cells from a patient to identify tumorigenic cells including CSCs. Such methods include identifying an individual having cancer for treatment or monitoring progression of a cancer comprising contacting the patient or a sample obtained from a patient (i.e. either *in vivo* or *in vitro*) with a modulator as described herein and detecting presence or absence, or level of association, of the modulator to bound or free target molecules in the sample. In particularly preferred embodiments the modulator will comprise a detectable label or reporter molecule as described herein.

In some embodiments, the association of the modulator, such as an antibody, with particular cells in the sample likely denotes that the sample may contain CSCs, thereby indicating that the individual having cancer may be effectively treated with a modulator as described herein. The methods may further comprise a step of comparing the level of binding to a control. Conversely, when the modulator is a Fc-construct, the binding properties may be exploited and monitored (directly or indirectly, *in vivo* or *in vitro*) when in contact with the sample to provide the desired information.

Exemplary compatible assay methods include radioimmunoassays, enzyme immunoassays, competitive-binding assays, fluorescent immunoassay, immunoblot assays, Western Blot analysis, flow cytometry assays, and ELISA assays. Compatible *in vivo* theragnostics or diagnostics may comprise art-recognized imaging or monitoring techniques such as magnetic resonance imaging, computerized tomography (e.g. CAT scan), positron

tomography (e.g., PET scan) radiography, ultrasound, etc., as would be known by those skilled in the art.

In another embodiment, the invention provides a method of analyzing cancer progression and/or pathogenesis *in vivo*. In another embodiment, analysis of cancer progression and/or pathogenesis *in vivo* comprises determining the extent of tumor progression. In another embodiment, analysis comprises the identification of the tumor. In another embodiment, analysis of tumor progression is performed on the primary tumor. In another embodiment, analysis is performed over time depending on the type of cancer as known to one skilled in the art. In another embodiment, further analysis of secondary tumors originating from metastasizing cells of the primary tumor is analyzed *in-vivo*. In another embodiment, the size and shape of secondary tumors are analyzed. In some embodiments, further *ex vivo* analysis is performed.

In another embodiment, the invention provides a method of analyzing cancer progression and/or pathogenesis *in vivo* including determining cell metastasis or detecting and quantifying the level of circulating tumor cells. In yet another embodiment, analysis of cell metastasis comprises determination of progressive growth of cells at a site that is discontinuous from the primary tumor. In another embodiment, the site of cell metastasis analysis comprises the route of neoplastic spread. In some embodiment, cells can disperse via blood vasculature, lymphatics, within body cavities or combinations thereof. In another embodiment, cell metastasis analysis is performed in view of cell migration, dissemination, extravasation, proliferation or combinations thereof.

Accordingly, in a particularly preferred embodiment the modulators of the instant invention may be used to detect and quantify SEZ6 levels in a patient sample (e.g., plasma or blood) which may, in turn, be used to detect, diagnose or monitor SEZ6 associated disorders including proliferative disorders. In related embodiments the modulators of the instant invention may be used to detect, monitor and/or quantify circulating tumor cells either *in vivo* or *in vitro* (see, for example, WO 2012/0128801 which is incorporated herein by reference). In still other preferred embodiments the circulating tumor cells may comprise cancer stem cells.

In certain examples, the tumorigenic cells in a subject or a sample from a subject may be assessed or characterized using the disclosed modulators prior to therapy or regimen to

establish a baseline. In other examples the sample is derived from a subject that was treated. In some examples the sample is taken from the subject at least about 1, 2, 4, 6, 7, 8, 10, 12, 14, 15, 16, 18, 20, 30, 60, 90 days, 6 months, 9 months, 12 months, or >12 months after the subject begins or terminates treatment. In certain examples, the tumorigenic cells are assessed or characterized after a certain number of doses (e.g., after 2, 5, 10, 20, 30 or more doses of a therapy). In other examples, the tumorigenic cells are characterized or assessed after 1 week, 2 weeks, 1 month, 2 months, 1 year, 2 years, 3 years, 4 years or more after receiving one or more therapies.

In another aspect, and as discussed in more detail below, the present invention provides kits for detecting, monitoring or diagnosing a hyperproliferative disorder, identifying individual having such a disorder for possible treatment or monitoring progression (or regression) of the disorder in a patient, wherein the kit comprises a modulator as described herein, and reagents for detecting the impact of the modulator on a sample.

Yet another aspect of the instant invention comprises the use of labeled SEZ6 for immunohistochemistry (IHC). In this respect SEZ6 IHC may be used as a diagnostic tool to aid in the diagnosis of various proliferative disorders and to monitor the potential response to treatments including SEZ6 modulator therapy. Compatible diagnostic assays may be performed on tissues that have been chemically fixed (including but not limited to: formaldehyde, gluteraldehyde, osmium tetroxide, potassium dichromate, acetic acid, alcohols, zinc salts, mercuric chloride, chromium tetroxide and picric acid) and embedded (including but not limited to: glycol methacrylate, paraffin and resins) or preserved via freezing. As discussed in more detail below such assays could be used to guide treatment decisions and determine dosing regimens and timing.

B. Screening

In certain embodiments, the modulators can also be used to screen for or identify compounds or agents (e.g., drugs) that alter a function or activity of tumorigenic cells or progeny thereof by interacting with an antigen (e.g., genotypic or phenotypic components thereof). Such compounds and agents can be drug candidates that are screened for the treatment of a proliferative disorder, for example. In one embodiment, a system or method includes tumorigenic cells comprising SEZ6 and a compound or agent (e.g., drug), wherein

the cells and compound or agent are in contact with each other. In such embodiments the subject cells may have been identified, monitored and/or enriched using the disclosed modulators.

In yet another embodiment, a method includes contacting, directly or indirectly, tumorigenic cells or progeny thereof with a test agent or compound and determining if the test agent or compound modulates an activity or function of the antigen-associated tumorigenic cells. One example of a direct interaction is physical interaction, while an indirect interaction includes the action of a composition upon an intermediary molecule that, in turn, acts upon the referenced entity (e.g., cell or cell culture). Exemplary activities or functions that can be modulated include changes in cell morphology or viability, expression of a marker, differentiation or de-differentiation, cell respiration, mitochondrial activity, membrane integrity, maturation, proliferation, viability, apoptosis or cell death.

Methods of screening and identifying agents and compounds include those suitable for high throughput screening, which include arrays of cells (e.g., microarrays) positioned or placed, optionally at pre-determined locations or addresses. For example, cells can be positioned or placed (pre-seeded) on a culture dish, tube, flask, roller bottle or plate (e.g., a single multi-well plate or dish such as an 8, 16, 32, 64, 96, 384 and 1536 multi-well plate or dish). High-throughput robotic or manual handling methods can probe chemical interactions and determine levels of expression of many genes in a short period of time. Techniques have been developed that utilize molecular signals (e.g., via fluorophores) and automated analyses that process information at a very rapid rate (see, e.g., Pinhasov *et al.*, Comb. Chem. High Throughput Screen. 7:133 (2004)). For example, microarray technology has been extensively used to probe the interactions of thousands of genes at once, while providing information for specific genes (see, e.g., Mocellin and Rossi, Adv. Exp. Med. Biol. 593:19 (2007)).

Libraries that can be screened include, for example, small molecule libraries, phage display libraries, fully human antibody yeast display libraries (Adimab, LLC), siRNA libraries, and adenoviral transfection vectors.

X. Pharmaceutical Preparations and Therapeutic Uses

A. Formulations and Routes of Administration

Depending on the form of the modulator along with any optional conjugate, the mode of intended delivery, the disease being treated or monitored and numerous other variables, compositions of the invention may be formulated as desired using art-recognized techniques. In some embodiments, the therapeutic compositions of the invention may be administered neat or with a minimum of additional components while others may optionally be formulated to contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries that are well known in the art (see, e.g., Gennaro, *Remington: The Science and Practice of Pharmacy with Facts and Comparisons: Drugfacts Plus*, 20th ed. (2003); Ansel *et al.*, *Pharmaceutical Dosage Forms and Drug Delivery Systems,* 7th ed., Lippencott Williams and Wilkins (2004); Kibbe *et al.*, *Handbook of Pharmaceutical Excipients,* 3rd ed., Pharmaceutical Press (2000)). Various pharmaceutically acceptable carriers, which include vehicles, adjuvants, and diluents, are readily available from numerous commercial sources. Moreover, an assortment of pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are also available. Certain non-limiting exemplary carriers include saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof.

More particularly it will be appreciated that, in some embodiments, the therapeutic compositions of the invention may be administered neat or with a minimum of additional components. Conversely the SEZ6 modulators of the present invention may optionally be formulated to contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries that are well known in the art and are relatively inert substances that facilitate administration of the modulator or which aid processing of the active compounds into preparations that are pharmaceutically optimized for delivery to the site of action. For example, an excipient can give form or consistency or act as a diluent to improve the pharmacokinetics or stability of the modulator. Suitable excipients or additives include, but are not limited to, stabilizing agents, wetting and emulsifying agents, salts for varying osmolarity, encapsulating agents, buffers, and skin penetration enhancers. In certain preferred embodiments the pharmaceutical compositions may be provided in a lyophilized form and reconstituted in, for example, buffered saline prior to administration.

Disclosed modulators for systemic administration may be formulated for enteral, parenteral or topical administration. Indeed, all three types of formulation may be used

simultaneously to achieve systemic administration of the active ingredient. Excipients as well as formulations for parenteral and nonparenteral drug delivery are set forth in Remington, The Science and Practice of Pharmacy 20th Ed. Mack Publishing (2000). Suitable formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form, for example, water-soluble salts. In addition, suspensions of the active compounds as appropriate for oily injection suspensions may be administered. Suitable lipophilic solvents or vehicles include fatty oils, for example, hexylsubstituted poly(lactide), sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension and include, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran. Optionally, the suspension may also contain stabilizers. Liposomes can also be used to encapsulate the agent for delivery into the cell.

Suitable formulations for enteral administration include hard or soft gelatin capsules, pills, tablets, including coated tablets, elixirs, suspensions, syrups or inhalations and controlled release forms thereof.

In general the compounds and compositions of the invention, comprising SEZ6 modulators may be administered *in vivo*, to a subject in need thereof, by various routes, including, but not limited to, oral, intravenous, intra-arterial, subcutaneous, parenteral, intranasal, intramuscular, intracranial, intracardiac, intraventricular, intratracheal, buccal, rectal, intraperitoneal, intradermal, topical, transdermal, and intrathecal, or otherwise by implantation or inhalation. The subject compositions may be formulated into preparations in solid, semi-solid, liquid, or gaseous forms; including, but not limited to, tablets, capsules, powders, granules, ointments, solutions, suppositories, enemas, injections, inhalants, and aerosols. The appropriate formulation and route of administration may be selected according to the intended application and therapeutic regimen.

B. Dosages

Similarly, the particular dosage regimen, i.e., dose, timing and repetition, will depend on the particular individual and that individual's medical history, as well as empirical considerations such as pharmacokinetics (e.g., half-life, clearance rate, etc.). Frequency of administration may be determined and adjusted over the course of therapy, and is based on

reducing the number of proliferative or tumorigenic cells, maintaining the reduction of such neoplastic cells, reducing the proliferation of neoplastic cells, or delaying the development of metastasis. In other embodiments the dosage administered may be adjusted or attenuated to manage potential side effects and/or toxicity. Alternatively, sustained continuous release formulations of a subject therapeutic composition may be appropriate.

In general, the modulators of the invention may be administered in various ranges. These include about 10 μg/kg body weight to about 100 mg/kg body weight per dose; about 50 μg/kg body weight to about 5 mg/kg body weight per dose; about 100 μg/kg body weight to about 10 mg/kg body weight per dose. Other ranges include about 100 μg/kg body weight to about 20 mg/kg body weight per dose and about 0.5 mg/kg body weight to about 20 mg/kg body weight per dose. In certain embodiments, the dosage is at least about 100 μg/kg body weight, at least about 250 μg/kg body weight, at least about 750 μg/kg body weight, at least about 3 mg/kg body weight, at least about 5 mg/kg body weight, at least about 10 mg/kg body weight.

In selected embodiments the modulators will be administered at approximately 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 μg/kg body weight per dose. Other embodiments will comprise the administration of modulators at 200, 300, 400, 500, 600, 700, 800 or 900 μg/kg body weight per dose. In other preferred embodiments the disclosed modulators will be administered at 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 mg/kg. In still other embodiments the modulators may be administered at 12, 14, 16, 18 or 20 mg/kg body weight per dose. In yet other embodiments the modulators may be administered at 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 90 or 100 mg/kg body weight per dose. In accordance with the teachings herein it will be appreciated that the aforementioned dosages are applicable to both unconjugated modulators and modulators conjugated to a cytotoxic agent. One of skill in the art could readily determine appropriate dosages for various conjugated and unconjugated modulators based on preclinical animal studies, clinical observations and standard medical and biochemical techniques and measurements.

With regard to conjugated modulators particularly preferred embodiments will comprise dosages of between about 50 μg/kg to about 5 mg/kg body weight per dose. In this regard conjugated modulators may be administered at 50, 75 or 100 μg/kg or at 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 or 1 mg/kg body weight per dose. In other preferred embodiments the conjugated

modulators of the instant invention may be administered at 1.25, 1.5, 1.75, 2, 2.25, 2.5, 2.75, 3, 3.25, 3.5, 3.75, 4, 4.25, 4.5, 4.75 or 5 mg/kg body weight per dose. In particularly preferred embodiments such conjugated modulator dosages will be administered intravenously over a period of time. Moreover, such dosages may be administered multiple times over a defined course of treatment.

Other dosing regimens may be predicated on Body Surface Area (BSA) calculations as disclosed in U.S.P.N. 7,744,877. As is well known, the BSA is calculated using the patient's height and weight and provides a measure of a subject's size as represented by the surface area of his or her body. In certain embodiments, the modulators may be administered in dosages from 10 mg/m^2 to 800 mg/m^2, from 50 mg/m^2 to 500 mg/m^2 and at dosages of 100 mg/m^2, 150 mg/m^2, 200 mg/m^2, 250 mg/m^2, 300 mg/m^2, 350 mg/m^2, 400 mg/m^2 or 450 mg/m^2.

It will also be appreciated that art recognized and empirical techniques may be used to determine appropriate dosage for conjugated modulators (i.e., ADCs).

In any event, SEZ6 modulators (both conjugated and unconjugated) are preferably administered as needed to subjects in need thereof. Determination of the frequency of administration may be made by persons skilled in the art, such as an attending physician based on considerations of the condition being treated, age of the subject being treated, severity of the condition being treated, general state of health of the subject being treated and the like. Generally, an effective dose of the SEZ6 modulator is administered to a subject one or more times. More particularly, an effective dose of the modulator is administered to the subject once a month, more than once a month, or less than once a month. In certain embodiments, the effective dose of the SEZ6 modulator may be administered multiple times, including for periods of at least a month, at least six months, at least a year, at least two years or a period of several years. In yet other embodiments, several days (2, 3, 4, 5, 6 or 7), several weeks (1, 2, 3, 4, 5, 6, 7 or 8) or several months (1, 2, 3, 4, 5, 6, 7 or 8) or even a year or several years may lapse between administration of the disclosed modulators.

In certain preferred embodiments the course of treatment involving conjugated modulators will comprise multiple doses of the selected drug product (i.e., an ADC) over a period of weeks or months. More specifically, conjugated modulators of the instant invention may administered once every day, every two days, every four days, every week, every ten

days, every two weeks, every three weeks, every month, every six weeks, every two months, every ten weeks or every three months. In this regard it will be appreciated that the dosages may be altered or the interval may be adjusted based on patient response and clinical practices.

Dosages and regimens may also be determined empirically for the disclosed therapeutic compositions in individuals who have been given one or more administration(s). For example, individuals may be given incremental dosages of a therapeutic composition produced as described herein. In selected embodiments the dosage may be gradually increased or reduced or attenuated based respectively on empirically determined or observed side effects or toxicity. To assess efficacy of the selected composition, a marker of the specific disease, disorder or condition can be followed as described previously. In embodiments where the individual has cancer, these include direct measurements of tumor size via palpation or visual observation, indirect measurement of tumor size by x-ray or other imaging techniques; an improvement as assessed by direct tumor biopsy and microscopic examination of the tumor sample; the measurement of an indirect tumor marker (e.g., PSA for prostate cancer) or an antigen identified according to the methods described herein, a decrease in pain or paralysis; improved speech, vision, breathing or other disability associated with the tumor; increased appetite; or an increase in quality of life as measured by accepted tests or prolongation of survival. It will be apparent to one of skill in the art that the dosage will vary depending on the individual, the type of neoplastic condition, the stage of neoplastic condition, whether the neoplastic condition has begun to metastasize to other location in the individual, and the past and concurrent treatments being used.

C. Combination Therapies

Combination therapies may be particularly useful in decreasing or inhibiting unwanted neoplastic cell proliferation, decreasing the occurrence of cancer, decreasing or preventing the recurrence of cancer, or decreasing or preventing the spread or metastasis of cancer. In such cases the modulators of the instant invention may function as sensitizing or chemosensitizing agents by removing the CSCs that would otherwise prop up and perpetuate the tumor mass and thereby allow for more effective use of current standard of care debulking or anti-cancer agents. That is, the disclosed modulators may, in certain embodiments provide an enhanced

effect (e.g., additive or synergistic in nature) that potentiates the mode of action of another administered therapeutic agent. In the context of the instant invention "combination therapy" shall be interpreted broadly and merely refers to the administration of a modulator and one or more anti-cancer agents that include, but are not limited to, cytotoxic agents, cytostatic agents, anti-angiogenic agents, debulking agents, chemotherapeutic agents, radiotherapy and radiotherapeutic agents, targeted anti-cancer agents (including both monoclonal antibodies and small molecule entities), BRMs, therapeutic antibodies, cancer vaccines, cytokines, hormone therapies, radiation therapy and anti-metastatic agents and immunotherapeutic agents, including both specific and non-specific approaches.

There is no requirement for the combined results to be additive of the effects observed when each treatment (e.g., antibody and anti-cancer agent) is conducted separately. Although at least additive effects are generally desirable, any increased anti-tumor effect above one of the single therapies is beneficial. Furthermore, the invention does not require the combined treatment to exhibit synergistic effects. However, those skilled in the art will appreciate that with certain selected combinations that comprise preferred embodiments, synergism may be observed.

In practicing combination therapy, the modulator and anti-cancer agent may be administered to the subject simultaneously, either in a single composition, or as two or more distinct compositions using the same or different administration routes. Alternatively, the modulator may precede, or follow, the anti-cancer agent treatment by, e.g., intervals ranging from minutes to weeks. The time period between each delivery is such that the anti-cancer agent and modulator are able to exert a combined effect on the tumor. In at least one embodiment, both the anti-cancer agent and the modulator are administered within about 5 minutes to about two weeks of each other. In yet other embodiments, several days (2, 3, 4, 5, 6 or 7), several weeks (1, 2, 3, 4, 5, 6, 7 or 8) or several months (1, 2, 3, 4, 5, 6, 7 or 8) may lapse between administration of the modulator and the anti-cancer agent.

The combination therapy may be administered once, twice or at least for a period of time until the condition is treated, palliated or cured. In some embodiments, the combination therapy is administered multiple times, for example, from three times daily to once every six months. The administering may be on a schedule such as three times daily, twice daily, once daily, once every two days, once every three days, once weekly, once every two weeks, once

every month, once every two months, once every three months, once every six months or may be administered continuously via a minipump. The combination therapy may be administered via any route, as noted previously. The combination therapy may be administered at a site distant from the site of the tumor.

In one embodiment a modulator is administered in combination with one or more anti-cancer agents for a short treatment cycle to a subject in need thereof. The invention also contemplates discontinuous administration or daily doses divided into several partial administrations. The modulator and anti-cancer agent may be administered interchangeably, on alternate days or weeks; or a sequence of antibody treatments may be given, followed by one or more treatments of anti-cancer agent therapy. In any event, as will be understood by those of ordinary skill in the art, the appropriate doses of chemotherapeutic agents will be generally around those already employed in clinical therapies wherein the chemotherapeutics are administered alone or in combination with other chemotherapeutics.

In another preferred embodiment the SEZ6 modulators of the instant invention may be used in maintenance therapy to reduce or eliminate the chance of tumor recurrence following the initial presentation of the disease. Preferably the disorder will have been treated and the initial tumor mass eliminated, reduced or otherwise ameliorated so the patient is asymptomatic or in remission. At such time the subject may be administered pharmaceutically effective amounts of the disclosed modulators one or more times even though there is little or no indication of disease using standard diagnostic procedures. In some embodiments, the modulators will be administered on a regular schedule over a period of time, such as weekly, every two weeks, monthly, every six weeks, every two months, every three months every six months or annually. Given the teachings herein, one skilled in the art could readily determine favorable dosages and dosing regimens to reduce the potential of disease recurrence. Moreover such treatments could be continued for a period of weeks, months, years or even indefinitely depending on the patient response and clinical and diagnostic parameters.

In yet another preferred embodiment the modulators of the present invention may be used to prophylactically or as an adjuvant therapy to prevent or reduce the possibility of tumor metastasis following a debulking procedure. As used in the instant disclosure a "debulking procedure" is defined broadly and shall mean any procedure, technique or method that

eliminates, reduces, treats or ameliorates a tumor or tumor proliferation. Exemplary debulking procedures include, but are not limited to, surgery, radiation treatments (i.e., beam radiation), chemotherapy, immunotherapy or ablation. At appropriate times readily determined by one skilled in the art in view of the instant disclosure the disclosed modulators may be administered as suggested by clinical, diagnostic or theragnostic procedures to reduce tumor metastasis. The modulators may be administered one or more times at pharmaceutically effective dosages as determined using standard techniques. Preferably the dosing regimen will be accompanied by appropriate diagnostic or monitoring techniques that allow it to be modified.

Yet other embodiments of the invention comprise administering the disclosed modulators to subjects that are asymptomatic but at risk of developing a proliferative disorder. That is, the modulators of the instant invention may be used in a truly preventative sense and given to patients that have been examined or tested and have one or more noted risk factors (e.g., genomic indications, family history, *in vivo* or *in vitro* test results, etc.) but have not developed neoplasia. In such cases those skilled in the art would be able to determine an effective dosing regimen through empirical observation or through accepted clinical practices.

D. Anti-Cancer Agents

The term "anti-cancer agent" or "anti-proliferative agent" means any agent that can be used to treat a cell proliferative disorder such as cancer, and includes, but is not limited to, cytotoxic agents, cytostatic agents, anti-angiogenic agents, debulking agents, chemotherapeutic agents, radiotherapy and radiotherapeutic agents, targeted anti-cancer agents, BRMs, therapeutic antibodies, cancer vaccines, cytokines, hormone therapies, radiation therapy and anti-metastatic agents and immunotherapeutic agents. It will be appreciated that, in selected embodiments as discussed above, such anti-cancer agents may comprise conjugates and may be associated with modulators prior to administration. In certain embodiments the disclosed anti-cancer agent will be linked to a SEZ6 modulator to provide an ADC as set forth herein.

As used herein the term "cytotoxic agent" means a substance that is toxic to the cells and decreases or inhibits the function of cells and/or causes destruction of cells. Typically, the substance is a naturally occurring molecule derived from a living organism. Examples of

cytotoxic agents include, but are not limited to, small molecule toxins or enzymatically active toxins of bacteria (e.g., Diptheria toxin, Pseudomonas endotoxin and exotoxin, Staphylococcal enterotoxin A), fungal (e.g., α-sarcin, restrictocin), plants (e.g., abrin, ricin, modeccin, viscumin, pokeweed anti-viral protein, saporin, gelonin, momoridin, trichosanthin, barley toxin, Aleurites fordii proteins, dianthin proteins, Phytolacca mericana proteins (PAPI, PAPII, and PAP-S), Momordica charantia inhibitor, curcin, crotin, saponaria officinalis inhibitor, gelonin, mitegellin, restrictocin, phenomycin, neomycin, and the tricothecenes) or animals, (e.g., cytotoxic RNases, such as extracellular pancreatic RNases; DNase I, including fragments and/or variants thereof).

For the purposes of the instant invention a "chemotherapeutic agent" comprises a chemical compound that non-specifically decreases or inhibits the growth, proliferation, and/or survival of cancer cells (e.g., cytotoxic or cytostatic agents). Such chemical agents are often directed to intracellular processes necessary for cell growth or division, and are thus particularly effective against cancerous cells, which generally grow and divide rapidly. For example, vincristine depolymerizes microtubules, and thus inhibits cells from entering mitosis. In general, chemotherapeutic agents can include any chemical agent that inhibits, or is designed to inhibit, a cancerous cell or a cell likely to become cancerous or generate tumorigenic progeny (e.g., TIC). Such agents are often administered, and are often most effective, in combination, e.g., in regimens such as CHOP or FOLFIRI. Again, in selected embodiments such chemotherapeutic agents may be conjugated to the disclosed modulators.

Examples of anti-cancer agents that may be used in combination with (or conjugated to) the modulators of the present invention include, but are not limited to, alkylating agents, alkyl sulfonates, aziridines, ethylenimines and methylamelamines, acetogenins, a camptothecin, bryostatin, callystatin, CC-1065, cryptophycins, dolastatin, duocarmycin, eleutherobin, pancratistatin, a sarcodictyin, spongistatin, nitrogen mustards, antibiotics, enediyne antibiotics, dynemicin, bisphosphonates, esperamicin, chromoprotein enediyne antiobiotic chromophores, aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin,

streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites, erlotinib, vemurafenib, crizotinib,sorafenib, ibrutinib, enzalutamide, folic acid analogues, purine analogs, androgens, anti-adrenals, folic acid replenisher such as frolinic acid, aceglatone, aldophosphamide glycoside, aminolevulinic acid, eniluracil, amsacrine, bestrabucil, bisantrene, edatraxate, defofamine, demecolcine, diaziquone, elformithine, elliptinium acetate, an epothilone, etoglucid, gallium nitrate, hydroxyurea, lentinan, lonidainine, maytansinoids, mitoguazone, mitoxantrone, mopidanmol, nitraerine, pentostatin, phenamet, pirarubicin, losoxantrone, podophyllinic acid, 2- ethylhydrazide, procarbazine, PSK® polysaccharide complex (JHS Natural Products, Eugene, OR), razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, chloranbucil; GEMZAR® gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs, vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; NAVELBINE® vinorelbine; novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (Camptosar, CPT-11), topoisomerase inhibitor RFS 2000; difluorometlhylornithine; retinoids; capecitabine; combretastatin; leucovorin; oxaliplatin; inhibitors of PKC-alpha, Raf, H-Ras, EGFR and VEGF-A that reduce cell proliferation and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators, aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, and anti-androgens; as well as troxacitabine (a 1,3- dioxolane nucleoside cytosine analog); antisense oligonucleotides, ribozymes such as a VEGF expression inhibitor and a HER2 expression inhibitor; vaccines, PROLEUKIN® rIL-2; LURTOTECAN® topoisomerase 1 inhibitor; ABARELIX® rmRH; Vinorelbine and Esperamicins and pharmaceutically acceptable salts, acids or derivatives of any of the above.

In other embodiments the modulators of the instant invention may be used in combination with any one of a number of antibodies (or immunotherapeutic agents) presently in clinical trials or commercially available. To this end the disclosed modulators may be used

in combination with an antibody selected from the group consisting of abagovomab, adecatumumab, afutuzumab, alemtuzumab, altumomab, amatuximab, anatumomab, arcitumomab, bavituximab, bectumomab, bevacizumab, bivatuzumab, blinatumomab, brentuximab, cantuzumab, catumaxomab, cetuximab, citatuzumab, cixutumumab, clivatuzumab, conatumumab, daratumumab, drozitumab, duligotumab, dusigitumab, detumomab, dacetuzumab, dalotuzumab, ecromeximab, elotuzumab, ensituximab, ertumaxomab, etaracizumab, farletuzumab, ficlatuzumab, figitumumab, flanvotumab, futuximab, ganitumab, gemtuzumab, girentuximab, glembatumumab, ibritumomab, igovomab, imgatuzumab, indatuximab, inotuzumab, intetumumab, ipilimumab, iratumumab, labetuzumab, lexatumumab, lintuzumab, lorvotuzumab, lucatumumab, mapatumumab, matuzumab, milatuzumab, minretumomab, mitumomab, moxetumomab, narnatumab, naptumomab, necitumumab, , nimotuzumab, nofetumomabn, ocaratuzumab, ofatumumab, olaratumab, onartuzumab, oportuzumab, oregovomab, panitumumab, parsatuzumab, patritumab, pemtumomab, pertuzumab, pintumomab, pritumumab, racotumomab, radretumab, rilotumumab, rituximab, robatumumab, satumomab, sibrotuzumab, siltuximab, simtuzumab, solitomab, tacatuzumab, taplitumomab, tenatumomab, teprotumumab, tigatuzumab, tositumomab, trastuzumab, tucotuzumab, ublituximab, veltuzumab, vorsetuzumab, votumumab, zalutumumab, CC49, 3F8 and combinations thereof.

Still other particularly preferred embodiments will comprise the use of antibodies approved for cancer therapy including, but not limited to, rituximab, trastuzumab, gemtuzumab ozogamcin, alemtuzumab, ibritumomab tiuxetan, tositumomab, bevacizumab, cetuximab, panitumumab, ofatumumab, ipilimumab and brentuximab vedotin. Those skilled in the art will be able to readily identify additional anti-cancer agents that are compatible with the teachings herein.

E. Radiotherapy

The present invention also provides for the combination of modulators with radiotherapy (i.e., any mechanism for inducing DNA damage locally within tumor cells such as gamma-irradiation, X-rays, UV-irradiation, microwaves, electronic emissions and the like). Combination therapy using the directed delivery of radioisotopes to tumor cells is also contemplated, and may be used in connection with a targeted anti-cancer agent or other

targeting means. Typically, radiation therapy is administered in pulses over a period of time from about 1 to about 2 weeks. The radiation therapy may be administered to subjects having head and neck cancer for about 6 to 7 weeks. Optionally, the radiation therapy may be administered as a single dose or as multiple, sequential doses.

XI. Indications

It will be appreciated that the modulators of the instant invention may be used to diagnose, treat or inhibit the occurrence or recurrence of any SEZ6 associated disorder. Accordingly, whether administered alone or in combination with an anti-cancer agent or radiotherapy, the modulators of the invention are particularly useful for generally treating neoplastic conditions in patients or subjects which may include benign or malignant tumors (e.g., adrenal, liver, kidney, bladder, breast, gastric, ovarian, colorectal, prostate, pancreatic, lung, thyroid, hepatic, cervical, endometrial, esophageal and uterine carcinomas; sarcomas; glioblastomas; and various head and neck tumors); leukemias and lymphoid malignancies; other disorders such as neuronal, glial, astrocytal, hypothalamic and other glandular, macrophagal, epithelial, stromal and blastocoelic disorders; and inflammatory, angiogenic, immunologic disorders and disorders caused by pathogens. Particularly, key targets for treatment are neoplastic conditions comprising solid tumors, although hematologic malignancies are within the scope of the invention. Preferably the "subject" or "patient" to be treated will be human although, as used herein, the terms are expressly held to comprise any mammalian species.

More specifically, neoplastic conditions subject to treatment in accordance with the instant invention may be selected from the group including, but not limited to, adrenal gland tumors, AIDS-associated cancers, alveolar soft part sarcoma, astrocytic tumors, bladder cancer (squamous cell carcinoma and transitional cell carcinoma), bone cancer (adamantinoma, aneurismal bone cysts, osteochondroma, osteosarcoma), brain and spinal cord cancers, metastatic brain tumors, breast cancer, carotid body tumors, cervical cancer, chondrosarcoma, chordoma, chromophobe renal cell carcinoma, clear cell carcinoma, colon cancer, colorectal cancer, cutaneous benign fibrous histiocytomas, desmoplastic small round cell tumors, ependymomas, Ewing's tumors, extraskeletal myxoid chondrosarcoma, fibrogenesis imperfecta ossium, fibrous dysplasia of the bone, gallbladder and bile duct

cancers, gestational trophoblastic disease, germ cell tumors, head and neck cancers, islet cell tumors, Kaposi's Sarcoma, kidney cancer (nephroblastoma, papillary renal cell carcinoma), leukemias, lipoma/benign lipomatous tumors, liposarcoma/malignant lipomatous tumors, liver cancer (hepatoblastoma, hepatocellular carcinoma), lymphomas, lung cancers (small cell carcinoma, adenocarcinoma, squamous cell carcinoma, large cell carcinoma etc.), medulloblastoma, melanoma, meningiomas, multiple endocrine neoplasia, multiple myeloma, myelodysplastic syndrome, neuroblastoma, neuroendocrine tumors, ovarian cancer, pancreatic cancers, papillary thyroid carcinomas, parathyroid tumors, pediatric cancers, peripheral nerve sheath tumors, phaeochromocytoma, pituitary tumors, prostate cancer, posterious unveal melanoma, rare hematologic disorders, renal metastatic cancer, rhabdoid tumor, rhabdomysarcoma, sarcomas, skin cancer, soft-tissue sarcomas, squamous cell cancer, stomach cancer, synovial sarcoma, testicular cancer, thymic carcinoma, thymoma, thyroid metastatic cancer, and uterine cancers (carcinoma of the cervix, endometrial carcinoma, and leiomyoma).

In certain preferred embodiments the proliferative disorder will comprise a solid tumor including, but not limited to, adrenal, liver, kidney, bladder, breast, gastric, ovarian, cervical, uterine, esophageal, colorectal, prostate, pancreatic, lung (both small cell and non-small cell), thyroid, carcinomas, sarcomas, glioblastomas and various head and neck tumors. In other preferred embodiments, and as shown in the Examples below, the disclosed modulators are especially effective at treating small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) (e.g., squamous cell non-small cell lung cancer or squamous cell small cell lung cancer). In one embodiment, the lung cancer is refractory, relapsed or resistant to a platinum based agent (e.g., carboplatin, cisplatin, oxaliplatin, topotecan) and/or a taxane (e.g., docetaxel, paclitaxel, larotaxel or cabazitaxel). Further, in particularly preferred embodiments the disclosed modulators may be used in a conjugated form to treat small cell lung cancer.

With regard to small cell lung cancer particularly preferred embodiments will comprise the administration of conjugated modulators (ADCs). In selected embodiments the conjugated modulators will be administered to patients exhibiting limited stage disease. In other embodiments the disclosed modulators will be administered to patients exhibiting extensive stage disease. In other preferred embodiments the disclosed conjugated modulators will be administered to refractory patients (i.e., those who recur during or shortly after

completing a course of initial therapy). Still other embodiments comprise the administration of the disclosed modulators to sensitive patients (i.e, those whose relapse is longer than 2-3 months after primary therapy. In each case it will be appreciated that compatible modulators may be in a conjugated or unconjugated state depending the selected dosing regimen and the clinical diagnosis.

As discussed above the disclosed modulators may further be used to prevent, treat or diagnose tumors with neuroendocrine features or phenotypes including neuroendocrine tumors. True or canonical neuroendocrine tumors (NETs) arising from the dispersed endocrine system are relatively rare, with an incidence of 2-5 per 100,000 people, but highly aggressive. Neuroendocrine tumors occur in the kidney, genitourinary tract (bladder, prostate, ovary, cervix, and endometrium), gastrointestinal tract (colon, stomach), thyroid (medullary thyroid cancer), and lung (small cell lung carcinoma and large cell neuroendocrine carcinoma). These tumors may secrete several hormones including serotonin and/or chromogranin A that can cause debilitating symptoms known as carcinoid syndrome. Such tumors can be denoted by positive immunohistochemical markers such as neuron-specific enolase (NSE, also known as gamma enolase, gene symbol = ENO2), CD56 (or NCAM1), chromogranin A (CHGA), and synaptophysin (SYP) or by genes known to exhibit elevated expression such as ASCL1. Unfortunately traditional chemotherapies have not been particularly effective in treating NETs and liver metastasis is a common outcome.

While the disclosed modulators may be advantageously used to treat neuroendocrine tumors they may also be used to treat, prevent or diagnose pseudo neuroendocrine tumors (pNETs) that genotypically or phenotypically mimic, resemble or exhibit common traits with canonical neuroendocrine tumors. Pseudo neuroendocrine tumors or tumors with neuroendocrine features are tumors that arise from cells of the diffuse neuroendocrine system or from cells in which a neuroendocrine differentiation cascade has been aberrantly reactivated during the oncogenic process. Such pNETs commonly share certain phenotypic or biochemical characteristics with traditionally defined neuroendocrine tumors, including the ability to produce subsets of biologically active amines, neurotransmitters, and peptide hormones. Histologically, such tumors (NETs and pNETs) share a common appearance often showing densely connected small cells with minimal cytoplasm of bland cytopathology and round to oval stippled nuclei. For the purposes of the instant invention commonly expressed

histological markers or genetic markers that may be used to define neuroendocrine and pseudo neuroendocrine tumors include, but are not limited to, chromogranin A, CD56, synaptophysin, PGP9.5, ASCL1 and neuron-specific enolase (NSE).

Accordingly the modulators of the instant invention may beneficially be used to treat both pseudo neuroendocrine tumors and canonical neuroendocrine tumors. In this regard the modulators may be used as described herein to treat neuroendocrine tumors (both NET and pNET) arising in the kidney, genitourinary tract (bladder, prostate, ovary, cervix, and endometrium), gastrointestinal tract (colon, stomach), thyroid (medullary thyroid cancer), and lung (small cell lung carcinoma and large cell neuroendocrine carcinoma). Moreover, the modulators of the instant invention may be used to treat tumors expressing one or more markers selected from the group consisting of NSE, CD56, synaptophysin, chromogranin A, ASCL1 and PGP9.5 (UCHL1). That is, the present invention may be used to treat a subject suffering from a tumor that is NSE^+ or $CD56^+$ or $PGP9.5^+$ or $ASCL1^+$ or SYP^+ or $CHGA^+$ or some combination thereof.

With regard to hematologic malignancies it will be further be appreciated that the compounds and methods of the present invention may be particularly effective in treating a variety of B-cell lymphomas, including low grade/NHL follicular cell lymphoma (FCC), mantle cell lymphoma (MCL), diffuse large cell lymphoma (DLCL), small lymphocytic (SL) NHL, intermediate grade/follicular NHL, intermediate grade diffuse NHL, high grade immunoblastic NHL, high grade lymphoblastic NHL, high grade small non-cleaved cell NHL, bulky disease NHL, Waldenstrom's Macroglobulinemia, lymphoplasmacytoid lymphoma (LPL), mantle cell lymphoma (MCL), follicular lymphoma (FL), diffuse large cell lymphoma (DLCL), Burkitt's lymphoma (BL), AIDS-related lymphomas, monocytic B cell lymphoma, angioimmunoblastic lymphoadenopathy, small lymphocytic, follicular, diffuse large cell, diffuse small cleaved cell, large cell immunoblastic lymphoblastoma, small, non-cleaved, Burkitt's and non-Burkitt's, follicular, predominantly large cell; follicular, predominantly small cleaved cell; and follicular, mixed small cleaved and large cell lymphomas. See, Gaidono et al., "Lymphomas", IN CANCER: PRINCIPLES & PRACTICE OF ONCOLOGY, Vol. 2: 2131-2145 (DeVita et al., eds., 5.sup.th ed. 1997). It should be clear to those of skill in the art that these lymphomas will often have different names due to changing systems of

classification, and that patients having lymphomas classified under different names may also benefit from the combined therapeutic regimens of the present invention.

The present invention also provides for a preventative or prophylactic treatment of subjects who present with benign or precancerous tumors. Beyond being a SEZ6 associated disorder it is not believed that any particular type of tumor or proliferative disorder should be excluded from treatment using the present invention. However, the type of tumor cells may be relevant to the use of the invention in combination with secondary therapeutic agents, particularly chemotherapeutic agents and targeted anti-cancer agents.

XII. Articles of Manufacture

Pharmaceutical packs and kits comprising one or more containers, comprising one or more doses of a SEZ6 modulator are also provided. In certain embodiments, a unit dosage is provided wherein the unit dosage contains a predetermined amount of a composition comprising, for example, an anti-SEZ6 antibody, with or without one or more additional agents. For other embodiments, such a unit dosage is supplied in single-use prefilled syringe for injection. In still other embodiments, the composition contained in the unit dosage may comprise saline, sucrose, or the like; a buffer, such as phosphate, or the like; and/or be formulated within a stable and effective pH range. Alternatively, in certain embodiments, the composition may be provided as a lyophilized powder that may be reconstituted upon addition of an appropriate liquid, for example, sterile water. In certain preferred embodiments, the composition comprises one or more substances that inhibit protein aggregation, including, but not limited to, sucrose and arginine. Any label on, or associated with, the container(s) indicates that the enclosed composition is used for diagnosing or treating the disease condition of choice.

The present invention also provides kits for producing single-dose or multi-dose administration units of a SEZ6 modulator and, optionally, one or more anti-cancer agents. The kit comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic and contain a pharmaceutically effective amount of the disclosed modulators in a conjugated or unconjugated form. In other preferred embodiments the container(s) comprise a sterile access

port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). Such kits will generally contain in a suitable container a pharmaceutically acceptable formulation of the SEZ6 modulator and, optionally, one or more anti-cancer agents in the same or different containers. The kits may also contain other pharmaceutically acceptable formulations, either for diagnosis or combined therapy. For example, in addition to the SEZ6 modulator of the invention such kits may contain any one or more of a range of anti-cancer agents such as chemotherapeutic or radiotherapeutic drugs; anti-angiogenic agents; anti-metastatic agents; targeted anti-cancer agents; cytotoxic agents; and/or other anti-cancer agents. Such kits may also provide appropriate reagents to conjugate the SEZ6 modulator with an anti-cancer agent or diagnostic agent (e.g., see U.S.P.N. 7,422,739 which is incorporated herein by reference in its entirety).

More specifically the kits may have a single container that contains the SEZ6 modulator, with or without additional components, or they may have distinct containers for each desired agent. Where combined therapeutics are provided for conjugation, a single solution may be pre-mixed, either in a molar equivalent combination, or with one component in excess of the other. Alternatively, the SEZ6 modulator and any optional anti-cancer agent of the kit may be maintained separately within distinct containers prior to administration to a patient. The kits may also comprise a second/third container means for containing a sterile, pharmaceutically acceptable buffer or other diluent such as bacteriostatic water for injection (BWFI), phosphate-buffered saline (PBS), Ringer's solution and dextrose solution.

When the components of the kit are provided in one or more liquid solutions, the liquid solution is preferably an aqueous solution, with a sterile aqueous solution being particularly preferred. However, the components of the kit may be provided as dried powder(s). When reagents or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent. It is envisioned that the solvent may also be provided in another container.

As indicated briefly above the kits may also contain a means by which to administer the antibody and any optional components to an animal or patient, e.g., one or more needles or syringes, or even an eye dropper, pipette, or other such like apparatus, from which the formulation may be injected or introduced into the animal or applied to a diseased area of the body. The kits of the present invention will also typically include a means for containing the

vials, or such like, and other component in close confinement for commercial sale, such as, e.g., injection or blow-molded plastic containers into which the desired vials and other apparatus are placed and retained. Any label or package insert indicates that the SEZ6 modulator composition is used for treating cancer, for example small cell lung cancer.

In other preferred embodiments the modulators of the instant invention may be used in conjunction with, or comprise, diagnostic or therapeutic devices useful in the diagnosis or treatment of proliferative disorders. For example, in on preferred embodiment the compounds and compositions of the instant invention may be combined with certain diagnostic devices or instruments that may be used to detect, monitor, quantify or profile cells or marker compounds involved in the etiology or manifestation of proliferative disorders. For selected embodiments the marker compounds may comprise NSE, CD56, synaptophysin, chromogranin A, and PGP9.5.

In particularly preferred embodiments the devices may be used to detect, monitor and/or quantify circulating tumor cells either *in vivo* or *in vitro* (see, for example, WO 2012/0128801 which is incorporated herein by reference). In still other preferred embodiments, and as discussed above, the circulating tumor cells may comprise cancer stem cells.

XIII. Research Reagents

Other preferred embodiments of the invention also exploit the properties of the disclosed modulators as an instrument useful for identifying, monitoring, isolating, sectioning or enriching populations or subpopulations of tumor initiating cells through methods such as flow cytometry, fluorescent activated cell sorting (FACS), magnetic activated cell sorting (MACS) or laser mediated sectioning. Those skilled in the art will appreciate that the modulators may be used in several compatible techniques for the characterization and manipulation of TIC including cancer stem cells (e.g., see U.S.S.Ns. 12/686,359, 12/669,136 and 12/757,649 each of which is incorporated herein by reference in its entirety).

XIV. Miscellaneous

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall

include pluralities and plural terms shall include the singular. More specifically, as used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a protein" includes a plurality of proteins; reference to "a cell" includes mixtures of cells, and the like. In addition, ranges provided in the specification and appended claims include both end points and all points between the end points. Therefore, a range of 2.0 to 3.0 includes 2.0, 3.0, and all points between 2.0 and 3.0.

Generally, nomenclature used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art. The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g., Abbas *et al.*, Cellular and Molecular Immunology, 6th ed., W.B. Saunders Company (2010); Sambrook J. & Russell D. *Molecular Cloning: A Laboratory Manual*, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2000); Ausubel *et al.*, *Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*, Wiley, John & Sons, Inc. (2002); Harlow and Lane *Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1998); and Coligan *et al.*, *Short Protocols in Protein Science*, Wiley, John & Sons, Inc. (2003). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclature used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Moreover, any section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

XV. SEZ6 References

All references or documents disclosed or cited within this specification are, without limitation, incorporated herein by reference in their entirety. Moreover, any section headings

used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

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XVI. Selected Embodiments of the Invention

In addition to the disclosure and Examples herein, the present invention is directed to selected embodiments specifically set forth immediately below.

PUTATIVE CLAIMS:

1. An isolated SEZ6 modulator.

2. The isolated SEZ6 modulator of claim 1, wherein the SEZ6 modulator comprises a SEZ6 antagonist.

3. The isolated SEZ6 modulator of claim 1, wherein the SEZ6 modulator comprises an antibody or immunoreactive fragment thereof.

4. The isolated SEZ6 modulator of claim 3 wherein the antibody or immunoreactive fragment thereof comprises a monoclonal antibody.

5. The isolated SEZ6 modulator of claim 4 wherein the monoclonal antibody is selected from the group consisting of chimeric antibodies, humanized antibodies and human antibodies.

6. The isolated SEZ6 modulator of claim 4 wherein said monoclonal antibody comprises a neutralizing antibody.

7. The isolated SEZ6 modulator of claim 4 wherein said monoclonal antibody comprises a depleting antibody.

8. The isolated SEZ6 modulator of claim 4 wherein said monoclonal antibody comprises an internalizing antibody.

9. The isolated SEZ6 modulator of claim 8 wherein said monoclonal antibody further comprises a cytotoxic agent.

10. The isolated SEZ6 modulator of claim 4 wherein said monoclonal antibody comprises a light chain variable region having three complementarity determining regions and a heavy chain variable region having three complementarity determining regions wherein the heavy and light chain complementarity determining regions comprise at least one complementarity determining region set forth in FIG. 10A or FIG. 10B, respectively.

11. The isolated SEZ6 modulator of claim 4 wherein said monoclonal antibody comprises a light chain variable region and a heavy chain variable region wherein said light chain

variable region comprises an amino acid sequence having at least 60% identity to an amino acid sequence selected from the group consisting of amino acid sequences as set forth in SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78 SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 102, SEQ ID NO: 104, SEQ ID NO: 106, SEQ ID NO: 108, SEQ ID NO: 110, SEQ ID NO: 112, SEQ ID NO: 114, SEQ ID NO: 116, SEQ ID NO: 118, SEQ ID NO: 120, SEQ ID NO: 122, SEQ ID NO: 124, SEQ ID NO: 126, SEQ ID NO: 128, SEQ ID NO: 130, SEQ ID NO: 132, SEQ ID NO: 134, SEQ ID NO: 136, SEQ ID NO: 138, SEQ ID NO: 140, SEQ ID NO: 142, SEQ ID NO: 144, SEQ ID NO: 146, SEQ ID NO: 148, SEQ ID NO: 150, SEQ ID NO: 152, SEQ ID NO: 154, SEQ ID NO: 156, SEQ ID NO: 158, SEQ ID NO: 160, SEQ ID NO: 162, SEQ ID NO: 164, SEQ ID NO: 166 and SEQ ID NO: 168 and wherein said heavy chain variable region comprises an amino acid sequence having at least 60% identity to an amino acid sequence selected from the group consisting of amino acid sequences as set forth in SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51 , SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 115, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 121, SEQ ID

NO: 123, SEQ ID NO: 125, SEQ ID NO: 127, SEQ ID NO: 129, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137, SEQ ID NO: 139, SEQ ID NO: 141, SEQ ID NO: 143, SEQ ID NO: 145, SEQ ID NO: 147, SEQ ID NO: 149, SEQ ID NO: 151, SEQ ID NO: 153, SEQ ID NO: 155, SEQ ID NO: 157, SEQ ID NO: 159, SEQ ID NO: 161, SEQ ID NO: 163, SEQ ID NO: 165, SEQ ID NO: 167 and SEQ ID NO: 169.

12. An isolated SEZ6 modulator comprising a CDR from any one of the heavy or light chain variable regions set forth in claim 11.

13. An isolated SEZ6 modulator comprising a competing antibody wherein said competing antibody inhibits the binding of an isolated SEZ6 modulator of claim 10 or 11 to SEZ6 by at least about 40%.

14. A nucleic acid encoding an amino acid heavy chain variable region or an amino acid light chain variable region of claim 11.

15. A vector comprising the nucleic acid of claim 14.

16. The isolated SEZ6 modulator of claim 1 comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO: 4 and fragments thereof.

17. The isolated SEZ6 modulator of claim 16 wherein the SEZ6 modulator further comprises at least a portion of an immunoglobulin constant region.

18. The isolated SEZ6 modulator of claim 1 wherein said modulator reduces the frequency of tumor initiating cells upon administration to a subject in need thereof.

19. The isolated SEZ6 modulator of claim 18 wherein the reduction in frequency is determined using flow cytometric analysis of tumor cell surface markers known to enrich for tumor initiating cells.

20. The isolated SEZ6 modulator of claim 18 wherein the reduction in frequency is determined using immunohistochemical detection of tumor cell surface markers known to enrich for tumor initiating cells.

21. The isolated SEZ6 modulator of claim 18 wherein said tumor initiating cells comprise tumor perpetuating cells.

22. The isolated SEZ6 modulator of claim 1 further comprising a cytotoxic agent.

23. A pharmaceutical composition comprising the isolated SEZ6 modulator of claim 1.

24. The pharmaceutical composition of claim 23 wherein said isolated SEZ6 modulator

comprises a monoclonal antibody.

25. The pharmaceutical composition of claim 24 wherein said monoclonal antibody comprises a humanized antibody.

26. The pharmaceutical composition of claim 25 wherein said humanized antibody comprises a cytotoxic agent.

27. The isolated SEZ6 modulator of claim 26 wherein said cytotoxic agent comprises a pyrrolobenzodiazepine.

28. The isolated SEZ6 modulator of claim 26 wherein said cytotoxic agent comprises an auristatin.

29. A method of treating a SEZ6 associated disorder comprising administering a therapeutically effective amount of a SEZ6 modulator to a subject in need thereof.

30. The method of claim 29 wherein said SEZ6 modulator comprises a SEZ6 antagonist.

31. The method of claim 29 wherein said SEZ6 modulator comprises an antibody or immunoreactive fragment thereof.

32. The method of claim 31 wherein the antibody or immunoreactive fragment thereof comprises a monoclonal antibody.

33. The method of claim 32 wherein the monoclonal antibody is selected from the group consisting of chimeric antibodies, humanized antibodies and human antibodies.

34. The method of claim 33 wherein said monoclonal antibody comprises a light chain variable region and a heavy chain variable region wherein said light chain variable region comprises an amino acid sequence having at least 60% identity to an amino acid sequence selected from the group consisting of amino acid sequences as set forth in SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78 SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 102, SEQ ID NO: 104, SEQ ID NO: 106, SEQ ID

NO: 108, SEQ ID NO: 110, SEQ ID NO: 112, SEQ ID NO: 114, SEQ ID NO: 116, SEQ ID NO: 118, SEQ ID NO: 120, SEQ ID NO: 122, SEQ ID NO: 124, SEQ ID NO: 126, SEQ ID NO: 128, SEQ ID NO: 130, SEQ ID NO: 132, SEQ ID NO: 134, SEQ ID NO: 136, SEQ ID NO: 138, SEQ ID NO: 140, SEQ ID NO: 142, SEQ ID NO: 144, SEQ ID NO: 146, SEQ ID NO: 148, SEQ ID NO: 150, SEQ ID NO: 152, SEQ ID NO: 154, SEQ ID NO: 156, SEQ ID NO: 158, SEQ ID NO: 160, SEQ ID NO: 162, SEQ ID NO: 164, SEQ ID NO: 166 and SEQ ID NO: 168 and wherein said heavy chain variable region comprises an amino acid sequence having at least 60% identity to an amino acid sequence selected from the group consisting of amino acid sequences as set forth in SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51 , SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 115, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 121, SEQ ID NO: 123, SEQ ID NO: 125, SEQ ID NO: 127, SEQ ID NO: 129, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137, SEQ ID NO: 139, SEQ ID NO: 141, SEQ ID NO: 143, SEQ ID NO: 145, SEQ ID NO: 147, SEQ ID NO: 149, SEQ ID NO: 151, SEQ ID NO: 153, SEQ ID NO: 155, SEQ ID NO: 157, SEQ ID NO: 159, SEQ ID NO: 161, SEQ ID NO: 163, SEQ ID NO: 165, SEQ ID NO: 167 and SEQ ID NO: 169.

35. The method of claim 34 wherein said monoclonal antibody is a humanized antibody.

36. The method of claim 32 wherein said monoclonal antibody comprises a neutralizing antibody.

37. The method of claim 32 wherein said monoclonal antibody comprises an internalizing antibody.

38. The method of claim 37 wherein said internalizing antibody comprises a cytotoxic agent.

39. The method of claim 38 wherein said cytotoxic agent comprises a pyrrolobenzodiazepine.

40. The method of claim 38 wherein said cytotoxic agent comprises an auristatin.

41. The method of claim 39 wherein said SEZ6 associated disorder comprises a neoplastic disorder.

42. The method of claim 41 wherein said neoplastic disorder comprises a tumor exhibiting neuroendocrine features.

43. The method of claim 42 wherein said tumor exhibiting neuroendocrine features comprises a neuroendocrine tumor.

44. The method of claim 41 wherein the subject is suffering from a neoplastic disorder selected from the group consisting of adrenal cancer, bladder cancer, cervical cancer, endometrial cancer, gastric cancer, kidney cancer, liver cancer, lung cancer, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer and breast cancer.

45. The method of claim 44 wherein the subject is suffering from lung cancer.

46. The method of claim 45 wherein the subject is suffering from small cell lung cancer.

47. The method of claim 41 wherein the subject suffering from said neoplastic disorder exhibits tumors comprising tumor initiating cells.

48. The method of claim 47 further comprising the step of reducing the frequency of tumor initiating cells in said subject.

49. The method of claim 48 wherein the reduction in frequency is determined using flow cytometric analysis of tumor cell surface markers known to enrich for tumor initiating cells or immunohistochemical detection of tumor cell surface markers known to enrich for tumor initiating cells.

50. The method of claim 48 wherein the reduction in frequency is determined using a method from the group consisting of *in vitro* and *in vivo* limiting dilution analysis.

51. The method of claim 50 wherein the reduction in frequency is determined using *in vivo* limiting dilution analysis comprising transplant of live human tumor cells into immunocompromised mice.

52. The method of claim 51 wherein the reduction of frequency is determined using *in*

vivo limiting dilution analysis comprising quantification of tumor initiating cell frequency using Poisson distribution statistics.

53. The method of claim 50 wherein the reduction of frequency is determined using *in vitro* limiting dilution analysis comprising limiting dilution deposition of live human tumor cells into *in vitro* colony supporting conditions.

54. The method of claim 53 wherein the reduction of frequency determined using *in vitro* limiting dilution analysis comprises quantification of tumor initiating cell frequency using Poisson distribution statistics.

55. The method of claim 29 further comprising the step of administering an anti-cancer agent.

56. The method of claim 29 wherein said SEZ6 modulator comprises one or more CDRs from any one of SEQ ID NOS: 20 to 169.

57. The method of claim 29 wherein said SEZ6 modulator comprises a pan-SEZ6 modulator.

58. The method of claim 57 wherein said SEZ6 modulator comprises a cytotoxic agent.

59. A method of reducing the frequency of tumor initiating cells in a subject in need thereof comprising the step of administering a SEZ6 modulator to said subject.

60. The method of claim 59 wherein the tumor initiating cells comprise tumor perpetuating cells.

61. The method of claim 60 wherein said tumor perpetuating cells are selected from cells expressing markers selected from the group consisting of $CD44^+$, $CD324^+$ and $CD133^+$ cells.

62. The method of claim 59 wherein said SEZ6 modulator comprises an antibody.

63. The method of claim 62 wherein said antibody comprises a monoclonal antibody.

64. The method of claim 63 wherein said monoclonal antibody further comprises a cytotoxic agent.

65. The method of claim 59 wherein the subject is suffering from a neoplastic disorder selected from the group consisting of adrenal cancer, bladder cancer, cervical cancer, endometrial cancer, kidney cancer, liver cancer, lung cancer, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer and breast cancer.

66. The method of claim 65 wherein the subject is suffering from lung cancer.

67. The method of claim 66 wherein the subject is suffering from small cell lung cancer.

68. The method of claim 59 wherein the frequency of tumor initiating cells is reduced by at least 10%.

69. The method of claim 59 wherein the reduction in frequency is determined using flow cytometric analysis of tumor cell surface markers known to enrich for tumor initiating cells or immunohistochemical detection of tumor cell surface markers known to enrich for tumor initiating cells.

70. A method of sensitizing a tumor in a subject for treatment with an anti-cancer agent comprising the step of administering a SEZ6 modulator to said subject.

71. The method of claim 70 wherein said SEZ6 modulator comprises an antibody.

72. The method of claim 70 wherein said tumor is a solid tumor.

73. The method of claim 70 wherein said anti-cancer agent comprises a chemotherapeutic agent.

74. The method of claim 70 wherein said anti-cancer agent comprises an immunotherapeutic agent.

75. A method of diagnosing a proliferative disorder in a subject in need thereof comprising the steps of:

i. obtaining a tissue sample from said subject;

ii. contacting the tissue sample with at least one SEZ6 modulator; and

iii. detecting or quantifying the SEZ6 modulator associated with the sample.

76. The method of claim 75 wherein the SEZ6 modulator comprises a monoclonal antibody.

77. The method of claim 76 wherein the monoclonal antibody is operably associated with a reporter.

78. An article of manufacture useful for diagnosing or treating SEZ6 associated disorders comprising a receptacle comprising a SEZ6 modulator and instructional materials for using said SEZ6 modulator to treat or diagnose the SEZ6 associated disorder.

79. The article of manufacture of claim 78 wherein said SEZ6 modulator is a monoclonal antibody.

80. The article of manufacture of claim 78 wherein the receptacle comprises a readable

plate.

81. A method of treating a subject suffering from a neoplastic disorder comprising the step of administering a therapeutically effective amount of at least one internalizing SEZ6 modulator.

82. The method of claim 81 wherein said SEZ6 modulator comprises an antibody.

83. The method of claim 82 wherein said antibody comprises a monoclonal antibody.

84. The method of claim 83 wherein said monoclonal antibody comprises a humanized antibody.

85. The method of claim 83 wherein the monoclonal antibody further comprises a cytotoxic agent.

86. The method of claim 81 further comprising the step of administering an anti-cancer agent.

87. The method of claim 81 wherein the neoplastic disorder comprises a tumor exhibiting neuroendocrine features.

88. The method of claim 81 wherein the neoplastic disorder comprises a tumor exhibiting neural features.

89. The method of claim 81 wherein the neoplastic disorder comprises lung cancer.

90. The method of claim 81 wherein the neoplastic disorder comprises small cell lung cancer.

91. A method of treating a subject suffering from a neoplastic disorder comprising the step of administering a therapeutically effective amount of at least one neutralizing SEZ6 modulator.

92. The method of claim 91 wherein said SEZ6 modulator comprises an antibody.

93. The method of claim 92 wherein said antibody comprises a monoclonal antibody.

94. The method of claim 93 wherein said monoclonal antibody comprises a humanized antibody.

95. The method of claim 94 wherein said humanized antibody further comprises a cytotoxic agent.

96. The method of claim 91 further comprising the step of administering an anti-cancer agent.

97. The method of claim 91 wherein the neoplastic disorder comprises a tumor exhibiting

neural features.

98. The method of claim 91 wherein the neoplastic disorder comprises a tumor exhibiting neuroendocrine features.

99. The method of claim 91 wherein the neoplastic disorder comprises lung cancer.

100. The method of claim 99 wherein the neoplastic disorder comprises small cell lung cancer.

101. A method of identifying, isolating, sectioning or enriching a population of tumor initiating cells comprising the step of contacting said tumor initiating cells with a SEZ6 modulator.

102. The method of claim 101 wherein said SEZ6 modulator comprises an antibody.

103. A SEZ6 modulator comprising a humanized antibody wherein said humanized antibody comprises a light chain variable region and a heavy chain variable region wherein said light chain variable region comprises an amino acid sequence having at least 60% identity to the amino acid sequence selected from the group consisting of SEQ ID NO: 170, SEQ ID NO: 172, SEQ ID NO: 174, SEQ ID NO: 176, SEQ ID NO: 178, SEQ ID NO: 180, SEQ ID NO: 182, SEQ ID NO: 184, SEQ ID NO: 186, SEQ ID NO: 188, SEQ ID NO: 190 and SEQ ID NO: 192 and wherein said heavy chain variable region comprises an amino acid sequence having at least 60% identity to an amino acid sequence selected from the group consisting of amino acid sequences selected from the group consisting of SEQ ID NO: 171, SEQ ID NO: 173, SEQ ID NO: 175, SEQ ID NO: 177, SEQ ID NO: 179, SEQ ID NO: 181, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 187, SEQ ID NO: 189, SEQ ID NO: 191, SEQ ID NO: 193, SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, SEQ ID NO: 197, SEQ ID NO: 198 and SEQ ID NO: 199.

104. A method of inhibiting or preventing metastasis in a subject in need thereof comprising the step of administering a pharmaceutically effective amount of a SEZ6 modulator.

105. The method of claim 104 wherein the subject undergoes a debulking procedure before or after the administration of the SEZ6 modulator.

106. The method of claim 105 wherein said debulking procedure comprises the administration of at least one anti-cancer agent.

107. A method of performing maintenance therapy on a subject in need thereof comprising the step of administering a pharmaceutically effective amount of a SEZ6 modulator.

108. The method of claim 107 wherein said subject was treated for a neoplastic disorder prior to the administration of the SEZ6 modulator.

109. A method of depleting tumor initiating cells in a subject suffering from a proliferative disorder comprising the step of administering a SEZ6 modulator.

110. A method of diagnosing, detecting or monitoring a SEZ6 associated disorder *in vivo* in a subject in need thereof comprising the step of administering a SEZ6 modulator.

111. A method of diagnosing, detecting or monitoring a SEZ6 associated disorder in a subject in need thereof comprising the step of contacting circulating tumor cells with a SEZ6 modulator.

112. The method of claim 111 wherein said contacting step occurs *in vivo*.

113. The method of claim 111 wherein said contacting step occurs *in vitro*.

114. A method of treating a tumor exhibiting neuroendocrine features in a patient in need thereof comprising the step of administering a therapeutically effective amount of a SEZ6 modulator.

115. The method of claim 114 wherein said tumor exhibiting neuroendocrine features is a neuroendocrine tumor.

116. A SEZ6 modulator derived from an antibody selected from the group consisting of SC17.1, SC17.2, SC17.3, SC17.4, SC17.8, SC17.9, SC17.10, SC17.11, SC17.14, SC17.15, SC17.16, SC17.17, SC17.18, SC17.19, SC17.22, SC17.24, SC17.27, SC17.28, SC17.29, SC17.30, SC17.32, SC17.34, SC17.35, SC17.36, SC17.38, SC17.39, SC17.40, SC17.41, SC17.42, SC17.45, SC17.46, SC17.47, SC17.49, SC17.50, SC17.53, SC17.54, SC17.56, SC17.57, SC17.59, SC17.61, SC17.63, SC17.71, SC17.72, SC17.74, SC17.76, SC17.77, SC17.79, SC17.81, SC17.82, SC17.84, SC17.85, SC17.87, SC17.89, SC17.90, SC17.91, SC17.93, SC17.95, SC17.97, SC17.99, SC17.102, SC17.114, SC17.115, SC17.120, SC17121, SC17.122, SC17.140, SC17.151, SC17.156, SC17.161, SC17.166, SC17.187, SC17.191, SC17.193, SC17,199 and SC17.200.

117. An isolated SEZ6 modulator that binds to an epitope associated with the Sushi Domain 1 of SEZ6.

118. The SEZ6 modulator of claim 117 wherein said SEZ6 modulator comprises an antibody or immunoreactive fragment thereof.

119. The SEZ6 modulator of claim 118 wherein said antibody or immunoreactive fragment thereof comprises a monoclonal antibody.

120. The SEZ6 modulator of claim 119 wherein said SEZ6 modulator comprises an ADC.

121. The SEZ6 modulator of claim 120 wherein said ADC comprises a pyrrolobenzodiazepine.

122. The SEZ6 modulator of claim 121 further comprising a linker.

123. An isolated SEZ6 modulator that binds to an epitope associated with the Sushi Domain 2 of SEZ6.

124. The SEZ6 modulator of claim 123 wherein said SEZ6 modulator comprises an antibody or immunoreactive fragment thereof.

125. The SEZ6 modulator of claim 124 wherein said antibody or immunoreactive fragment thereof comprises a monoclonal antibody.

126. The SEZ6 modulator of claim 125 wherein said SEZ6 modulator comprises an ADC.

127. The SEZ6 modulator of claim 126 wherein said ADC comprises a pyrrolobenzodiazepine.

128. The SEZ6 modulator of claim 127 further comprising a linker.

129. An isolated SEZ6 modulator that binds to an epitope associated with the Sushi Domain 3 of SEZ6.

130. The SEZ6 modulator of claim 129 wherein said SEZ6 modulator comprises an antibody or immunoreactive fragment thereof.

131. The SEZ6 modulator of claim 130 wherein said antibody or immunoreactive fragment thereof comprises a monoclonal antibody.

132. The SEZ6 modulator of claim 131 wherein said SEZ6 modulator comprises an ADC.

133. The SEZ6 modulator of claim 132 wherein said ADC comprises a pyrrolobenzodiazepine.

134. The SEZ6 modulator of claim 133 further comprising a linker.

135. An isolated SEZ6 modulator that binds to an epitope associated with the Sushi Domain 4 of SEZ6.

136. The SEZ6 modulator of claim 135 wherein said SEZ6 modulator comprises an antibody or immunoreactive fragment thereof.

137. The SEZ6 modulator of claim 136 wherein said antibody or immunoreactive fragment thereof comprises a monoclonal antibody.

138. The SEZ6 modulator of claim 137 wherein said SEZ6 modulator comprises an ADC.

139. The SEZ6 modulator of claim 138 wherein said ADC comprises a pyrrolobenzodiazepine.

140. The SEZ6 modulator of claim 139 further comprising a linker.

141. An isolated SEZ6 modulator that binds to an epitope associated with the Sushi Domain 5 of SEZ6.

142. The SEZ6 modulator of claim 141 wherein said SEZ6 modulator comprises an antibody or immunoreactive fragment thereof.

143. The SEZ6 modulator of claim 142 wherein said antibody or immunoreactive fragment thereof comprises a monoclonal antibody.

144. The SEZ6 modulator of claim 143 wherein said SEZ6 modulator comprises an ADC.

145. The SEZ6 modulator of claim 144 wherein said ADC comprises a pyrrolobenzodiazepine.

146. The SEZ6 modulator of claim 145 further comprising a linker.

147. An isolated SEZ6 modulator that binds to an epitope associated with the CUB Domain 1 of SEZ6.

148. The SEZ6 modulator of claim 147 wherein said SEZ6 modulator comprises an antibody or immunoreactive fragment thereof.

149. The SEZ6 modulator of claim 148 wherein said antibody or immunoreactive fragment thereof comprises a monoclonal antibody.

150. The SEZ6 modulator of claim 149 wherein said SEZ6 modulator comprises an ADC.

151. The SEZ6 modulator of claim 150 wherein said ADC comprises a pyrrolobenzodiazepine.

152. The SEZ6 modulator of claim 151 further comprising a linker.

153. An isolated SEZ6 modulator that binds to an epitope associated with the CUB Domain 2 of SEZ6.

154. The SEZ6 modulator of claim 153 wherein said SEZ6 modulator comprises an antibody or immunoreactive fragment thereof.

155. The SEZ6 modulator of claim 154 wherein said antibody or immunoreactive fragment thereof comprises a monoclonal antibody.

156. The SEZ6 modulator of claim 155 wherein said SEZ6 modulator comprises an ADC.

157. The SEZ6 modulator of claim 156 wherein said ADC comprises a pyrrolobenzodiazepine.

158. The SEZ6 modulator of claim 157 further comprising a linker.

159. An isolated SEZ6 modulator that binds to an epitope associated with the N-terminal domain of SEZ6.

160. The SEZ6 modulator of claim 159 wherein said SEZ6 modulator comprises an antibody or immunoreactive fragment thereof.

161. The SEZ6 modulator of claim 160 wherein said antibody or immunoreactive fragment thereof comprises a monoclonal antibody.

162. The SEZ6 modulator of claim 161 wherein said SEZ6 modulator comprises an ADC.

163. The SEZ6 modulator of claim 162 wherein said ADC comprises a pyrrolobenzodiazepine.

164. The SEZ6 modulator of claim 163 further comprising a linker.

165. An isolated SEZ6 modulator residing in a bin selected from the group consisting of bin A, bin B, bin C, bin D, bin E, bin F and bin U.

166. An isolated SEZ6 modulator residing in a bin defined by a reference antibody selected from the group consisting of SC17.1, SC17.2, SC17.3, SC17.4, SC17.8, SC17.9, SC17.10, SC17.11, SC17.14, SC17.15, SC17.16, SC17.17, SC17.18, SC17.19, SC17.22, SC17.24, SC17.27, SC17.28, SC17.29, SC17.30, SC17.32, SC17.34, SC17.35, SC17.36, SC17.38, SC17.39, SC17.40, SC17.41, SC17.42, SC17.45, SC17.46, SC17.47, SC17.49, SC17.50, SC17.53, SC17.54, SC17.56, SC17.57, SC17.59, SC17.61, SC17.63, SC17.71, SC17.72, SC17.74, SC17.76, SC17.77, SC17.79, SC17.81, SC17.82, SC17.84, SC17.85, SC17.87, SC17.89, SC17.90, SC17.91, SC17.93, SC17.95, SC17.97, SC17.99, SC17.102, SC17.114, SC17.115, SC17.120, SC17121, SC17.122, SC17.140, SC17.151, SC17.156, SC17.161, SC17.166, SC17.187, SC17.191, SC17.193, SC17.199 and SC17.200.

167. An antibody drug conjugate of the formula M-[L-D]n or a pharmaceutically acceptable salt thereof wherein:

a. M comprises a SEZ6 modulator;

b. L comprises a linker;

c. D is an anti-proliferative agent; and

d. n is an integer from about 1 to about 20.

168. The antibody drug conjugate of claim 167 wherein said SEZ6 modulator comprises an antibody or immunoreactive fragment thereof.

169. The antibody drug conjugate of claim 168 wherein said antibody comprises a monoclonal antibody.

170. The antibody drug conjugate of claim 169 wherein said antibody is derived from an antibody selected from the group consisting of SC17.1, SC17.2, SC17.3, SC17.4, SC17.8, SC17.9, SC17.10, SC17.11, SC17.14, SC17.15, SC17.16, SC17.17, SC17.18, SC17.19, SC17.22, SC17.24, SC17.27, SC17.28, SC17.29, SC17.30, SC17.32, SC17.34, SC17.35, SC17.36, SC17.38, SC17.39, SC17.40, SC17.41, SC17.42, SC17.45, SC17.46, SC17.47, SC17.49, SC17.50, SC17.53, SC17.54, SC17.56, SC17.57, SC17.59, SC17.61, SC17.63, SC17.71, SC17.72, SC17.74, SC17.76, SC17.77, SC17.79, SC17.81, SC17.82, SC17.84, SC17.85, SC17.87, SC17.89, SC17.90, SC17.91, SC17.93, SC17.95, SC17.97, SC17.99, SC17.102, SC17.114, SC17.115, SC17.120, SC17121, SC17.122, SC17.140, SC17.151, SC17.156, SC17.161, SC17.166, SC17.187, SC17.191, SC17.193, SC17.199 and SC17.200.

171. The antibody drug conjugate of claim 169 wherein said antibody is humanized.

172. The antibody drug conjugate of claim 167 wherein the linker comprises a cleavable linker.

173. The antibody drug conjugate of claim 172 wherein said cleavable linker comprises a peptidyl linker.

174. The antibody drug conjugate of claim 167 wherein said anti-proliferative agent comprises a cytotoxic agent.

175. The antibody drug conjugate of claim 174 wherein said cytotoxic agent comprises a pyrrolobenzodiazepine.

176. The antibody drug conjugate of claim 175 wherein said pyrrolobenzodiazepine comprises a pyrrolobenzodiazepine dimer.

177. An isolated SEZ6 modulator residing in a bin selected from the group consisting of bin A, bin B, bin C, bin D, bin E, bin F and bin U.

178. An isolated SEZ6 modulator residing in a bin defined by a reference antibody selected from the group consisting of SC17.1, SC17.2, SC17.3, SC17.4, SC17.8, SC17.9, SC17.10, SC17.11, SC17.14, SC17.15, SC17.16, SC17.17, SC17.18, SC17.19, SC17.22, SC17.24, SC17.27, SC17.28, SC17.29, SC17.30, SC17.32, SC17.34, SC17.35, SC17.36, SC17.38, SC17.39, SC17.40, SC17.41, SC17.42, SC17.45, SC17.46, SC17.47, SC17.49, SC17.50, SC17.53, SC17.54, SC17.56, SC17.57, SC17.59, SC17.61, SC17.63, SC17.71, SC17.72, SC17.74, SC17.76, SC17.77, SC17.79, SC17.81, SC17.82, SC17.84, SC17.85, SC17.87, SC17.89, SC17.90, SC17.91, SC17.93, SC17.95, SC17.97, SC17.99, SC17.102, SC17.114, SC17.115, SC17.120, SC17121, SC17.122, SC17.140, SC17.151, SC17.156, SC17.161, SC17.166, SC17.187, SC17.191, SC17.193, SC17.199 and SC17.200.

179. An antibody drug conjugate of the formula:

M-[L-D]n

or a pharmaceutically acceptable salt thereof wherein

a) M comprises a SEZ6 modulator;

b) L comprises an optional linker;

c) D is a cytotoxic agent selected from the group consisting of auristatins, maytansinoids, amanitins and pyrrolobenzodiazepine dimers.

d) n is an integer from about 1 to about 20.

180. The antibody drug conjugate of claim 179 wherein said SEZ6 modulator comprises an antibody or immunoreactive fragment thereof.

181. The antibody drug conjugate of claim 180 wherein said antibody comprises a monoclonal antibody.

182. The antibody drug conjugate of claim 181 wherein said antibody is derived from an antibody selected from the group consisting of SC17.1, SC17.2, SC17.3, SC17.4, SC17.8, SC17.9, SC17.10, SC17.11, SC17.14, SC17.15, SC17.16, SC17.17, SC17.18,

SC17.19, SC17.22, SC17.24, SC17.27, SC17.28, SC17.29, SC17.30, SC17.32, SC17.34, SC17.35, SC17.36, SC17.38, SC17.39, SC17.40, SC17.41, SC17.42, SC17.45, SC17.46, SC17.47, SC17.49, SC17.50, SC17.53, SC17.54, SC17.56, SC17.57, SC17.59, SC17.61, SC17.63, SC17.71, SC17.72, SC17.74, SC17.76, SC17.77, SC17.79, SC17.81, SC17.82, SC17.84, SC17.85, SC17.87, SC17.89, SC17.90, SC17.91, SC17.93, SC17.95, SC17.97, SC17.99, SC17.102, SC17.114, SC17.115, SC17.120, SC17121, SC17.122, SC17.140, SC17.151, SC17.156, SC17.161, SC17.166, SC17.187, SC17.191, SC17.193, SC17.199 and SC17.200.

183. The antibody drug conjugate of claim 182 wherein said antibody is humanized.

184. The antibody drug conjugate of claim 183 wherein the linker comprises a cleavable linker.

185. The antibody drug conjugate of claim 184 wherein said cleavable linker comprises a peptidyl linker.

186. The antibody drug conjugate of claim 185 wherein said anti-proliferative agent comprises a cytotoxic agent.

187. The antibody drug conjugate of claim 186 wherein said cytotoxic agent comprises a pyrrolobenzodiazepine.

188. The antibody drug conjugate of claim 187 wherein said pyrrolobenzodiazepine comprises a pyrrolobenzodiazepine dimer.

189. A SEZ6 modulator comprising a CDR from any one of SEQ ID NOS: 20-203.

190. The SEZ6 modulator of claim 189 wherein said modulator comprises a plurality of CDRs from any one of SEQ ID NOS: 20-203.

191. A SEZ6 antibody modulator that competes for binding to a SEZ6 protein with a reference antibody selected from the group consisting of SC17.1, SC17.2, SC17.3, SC17.4, SC17.8, SC17.9, SC17.10, SC17.11, SC17.14, SC17.15, SC17.16, SC17.17, SC17.18, SC17.19, SC17.22, SC17.24, SC17.27, SC17.28, SC17.29, SC17.30, SC17.32, SC17.34, SC17.35, SC17.36, SC17.38, SC17.39, SC17.40, SC17.41, SC17.42, SC17.45, SC17.46, SC17.47, SC17.49, SC17.50, SC17.53, SC17.54, SC17.56, SC17.57, SC17.59, SC17.61, SC17.63, SC17.71, SC17.72, SC17.74, SC17.76, SC17.77, SC17.79, SC17.81, SC17.82, SC17.84, SC17.85, SC17.87, SC17.89, SC17.90, SC17.91, SC17.93, SC17.95, SC17.97, SC17.99, SC17.102,

SC17.114, SC17.115, SC17.120, SC17121, SC17.122, SC17.140, SC17.151, SC17.156, SC17.161, SC17.166, SC17.187, SC17.191, SC17.193, SC17.199 and SC17.200wherein binding of the SEZ6 antibody modulator to the SEZ6 protein is inhibited by at least 30%.

192. A SEZ6 modulator that binds to a SEZ6 protein epitope comprising amino acids Q12, P14, I16, E17 and E18 (SEQ ID NO: 401).

193. A SEZ6 modulator that binds to a SEZ6 protein epitope comprising amino acids L73, P74, F75, Q76, P77, D78 and P79 (SEQ ID NO: 402).

194. A method of treating a subject suffering from a proliferative disorder comprising the step of administering a SEZ6 modulator that binds to an epitope contained in a SEZ6 domain selected from the group consisting of the N-terminal domain, the Sushi 1 domain, the Cub 1 domain, the Sushi 2 domain, the Cub 2 domain, the Sushi 3 domain, the Sushi 4 domain and the Sushi 5 domain.

195. A humanized SEZ6 antibody modulator selected from the group consisting of hSC17.16, hSC17.17, hSC17.24, hSC17.28, SC17.34, hSC17.46, SC17.151, SC17.155, SC17.156, SC17.161 and SC17.200.

EXAMPLES

The present invention, thus generally described above, will be understood more readily by reference to the following examples, which are provided by way of illustration and are not intended to be limiting of the instant invention. The examples are not intended to represent that the experiments below are all or the only experiments performed. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1

Identification of Tumors having Neuroendocrine Features and Analysis of Marker Expression using Whole Transcriptome Sequencing

Neuroendocrine tumors (NETs) arising from the dispersed endocrine system are rare, with an incidence of 2-5 per 100,000 people, but highly aggressive. Neuroendocrine tumors

occur in the adrenal gland, kidney, genitourinary tract (bladder, prostate, ovary, cervix, and endometrium), pancreas, gastrointestinal tract (stomach and colon), thyroid (medullary thyroid cancer), and lung (small cell lung carcinoma, large cell neuroendocrine carcinoma, and carcinoid). These tumors may secrete several hormones including serotonin and/or chromogranin A that can cause debilitating symptoms known as carcinoid syndrome. These tumors can be denoted by positive immunohistochemical markers such as neuron-specific enolase (NSE, also known as gamma enolase, gene symbol = ENO2), CD56/NCAM1, and synaptophysin. Traditional chemotherapies have not been successful in treating NETs, and mortality due to metastatic spread is a common outcome. Unfortunately, in most cases surgery is the only potential curative treatment, provided it takes place following early detection and prior to tumor metastasis. In this context work was undertaken to identify novel therapeutic targets associated with tumors comprising neuroendocrine features.

To identify and characterize such tumors as they exist in cancer patients a large non-traditional xenograft (NTX) tumor bank was developed and maintained using art-recognized techniques. The NTX tumor bank, comprising a substantial number of discrete tumor cell lines, was propagated in immunocompromised mice through multiple passages of heterogeneous tumor cells originally obtained from numerous cancer patients afflicted by a variety of solid tumor malignancies. (Note that in some of the Examples and FIGS. herein the passage number of the tested sample is indicated by p0-p# appended to the sample designation where p0 is indicative of an unpassaged sample obtained directly from a patient tumor and p# is indicative of the number of times the tumor has been passaged through a mouse prior to testing.) The continued availability of a large number of discrete early passage NTX tumor cell lines having well defined lineages greatly facilitate the identification and characterization of cells purified from the cell lines. In such work the use of minimally passaged NTX cell lines simplifies *in vivo* experimentation and provides readily verifiable results. Moreover, early passage NTX tumors respond to therapeutic agents such as irinotecan (i.e. Camptosar®) and Cisplatin/Etoposide regimens, which provides clinically relevant insights into underlying mechanisms driving tumor growth, resistance to current therapies and tumor recurrence.

As the NTX tumor cell lines were established, their phenotype was characterized in various ways to examine global gene expression. To identify which NTX lines in the bank

might be NETs, gene expression profiles were generated by whole transcriptome sequencing and/or microarray analysis. Specifically, the data was examined to identify tumors expressing high levels of specific genes known to be elevated in NETs or used as histochemical markers of neuroendocrine differentiation (e.g., ASCL1, NCAM1, CHGA) as well as tumors with changes in NOTCH pathway genes indicative of suppression of NOTCH signaling (e.g., reduced levels of NOTCH receptors, and changes to ligands and effector molecules).

More particularly, upon establishing various NTX tumor cell lines as is commonly done for human tumors in severely immune compromised mice, the tumors were resected after reaching 800 - 2,000 mm^3 and the cells were dissociated and dispersed into suspension using art-recognized enzymatic digestion techniques (see, for example, U.S.P.N. 2007/0292414 which is incorporated herein). The dissociated cell preparations from these NTX lines were then depleted of murine cells, and human tumor cell subpopulations were then further isolated by fluorescence activated cell sorting and lysed in RLTplus RNA lysis buffer (Qiagen). These lysates were then stored at -80°C until used. Upon thawing, total RNA was extracted using a RNeasy isolation kit (Qiagen) following the vendor's instructions and quantified on a Nanodrop spectrophotometer (Thermo Scientific) and a Bioanalyzer 2100 (Agilent Technologies) again using the manufacturer's protocols and recommended instrument settings. The resulting total RNA preparations were suitable for genetic sequencing and gene expression analysis.

Whole transcriptome sequencing using an Applied Biosystems (ABI) SOLiD (Sequencing by Oligo Ligation/Detection) 4.5 or SOLiD 5500xl next generation sequencing system (Life Technologies) was performed on RNA samples from NTX lines. cDNA was generated from total RNA samples using either a modified whole transcriptome (WT) protocol from ABI designed for low input total RNA or Ovation RNA-Seq System V2™ (NuGEN Technologies Inc.). The modified low input WT protocol uses 1.0 ng of total RNA to amplify mRNA at the 3' end which leads to a heavy 3' bias of mapped gene expression, while NuGen's system allows for a more consistent amplification throughout the transcript, and includes amplification of both mRNA and non-polyadenylated transcript cDNA using random hexamers. The cDNA library was fragmented, and barcodes adapters were added to allow pooling of fragment libraries from different samples.

ABI's SOLiD 4.5 and SOLiD 5500xl next generation sequencing platforms enables parallel sequencing of transcriptomes from multiple NTX lines and sorted populations. A cDNA library is constructed from each RNA sample, which is fragmented and barcoded. Barcodes on each fragment library allow multiple samples to be pooled at equal concentrations and run together while ensuring sample specificity. The samples are taken through emulsion PCR using ABI's SOLiD™ EZ Bead™ robotics system, which ensures sample consistency. Paired-end sequencing generates a 50 base read in the 5' to 3' direction and a 25 base read in the 3' to 5' direction for each clonally amplified fragment on a single bead that exists in the pool. In the case of the 5500xl platform, for every set of 8 samples pooled in the method mentioned above, beads are evenly deposited into 6 single channel lanes on a single chip. This will, on average, generate more than 50 million 50 base reads and 50 million 25 base reads for each of the 8 samples and generates a very accurate representation of mRNA transcript level in the tumor cells. Data generated by the SOLiD platform mapped to 34,609 genes as annotated by RefSeq version 47 using NCBI version hg19.2 of the published human genome and provided verifiable measurements of RNA levels in most samples.

The SOLiD platform is able to capture not only expression, but SNPs, known and unknown alternative splicing events, small non-coding RNAs, and potentially new exon discoveries based solely on read coverage (reads mapped uniquely to previously un-annotated genomic locations). Thus, use of this next generation sequencing platform paired with proprietary data analysis and visualization software thus allowed for discovery of differential transcript expression as well as differences and/or preferences for specific splice variants of expressed mRNA transcripts. Sequencing data from the SOLiD platform is nominally represented as a transcript expression value using the metrics RPM (reads per million) and RPKM (read per kilobase per million), enabling basic differential expression analysis as is standard practice.

Whole transcriptome sequencing of four small cell lung cancer (SCLC) tumors (LU73, LU64, LU86 and LU95), one ovarian tumor (OV26) and a large cell neuroendocrine carcinoma (LCNEC; LU37) resulted in the determination of gene expression patterns commonly found in NETs (FIG. 6A). More specifically, these tumors had high expression of several NET markers (ASCL1, NCAM1, CHGA) as well as reduced levels of Notch receptors

and effector molecules (e.g., HES1, HEY1) and elevated markers of Notch suppression (e.g., DLL3 and HES6). In contrast, 4 normal lung samples, 3 lung adenocarcinoma tumors (LU137, LU146 and LU153), and 3 squamous cell lung carcinomas (LU49, LU70 and LU76) all have expression of various Notch receptors and effector molecules, and do not show elevated expression of Notch suppressors such as HES6 and DLL3.

Moreover, as seen in FIG. 6B, an analysis of the whole transcriptome data comparing normal tissue samples to various lung NTX populations having neuroendocrine features, showed that SEZ6 was up-regulated at the mRNA transcript level in four lung cancer populations having neuroendocrine features (LU73, LU64, LU86 and LU95) compared to extremely low or no transcript expression in the normal tissues tested. These results suggest that SEZ6 may play an important role in the tumorigenesis and maintenance of particular cancers (including lung cancers with neuroendocrine features). On this basis, SEZ6 was selected for further analysis as a potential immunotherapeutic target

Example 2

Microarray and RT-PCR Analysis of Gene Expression in Selected NTX Tumors with Neuroendocrine Features

In an effort to identify additional NETs in the aforementioned NTX bank beyond those for which SOLiD whole transcriptome data existed, a larger set of NTX lines was examined using microarray analysis. Specifically, 2-6µg of total RNA samples derived from whole tumors in 46 NTX lines or from 2 normal tissues were analyzed using a OneArray® microarray platform (Phalanx Biotech Group), which contains 29,187 probes designed against 19,380 genes in the human genome. More specifically, RNA samples were obtained (as described in Example 1) from forty-six patient derived whole NTX tumors comprising colorectal (CR), melanoma (SK), kidney (KD), lung (LU), ovarian (OV), endometrial (EM), breast (BR), liver (LIV), or pancreatic (PA) cancers. Normal colorectal (NormCR) and normal pancreas (NormPA) tissues were used as controls. Still more specifically, lung tumors were further subclassified as small cell lung cancers (SCLC), squamous cell cancers (SCC), or large cell neuroendocrine carcinoma (LCNEC). RNA samples were run in triplicate using the manufacturer's protocols and the resulting data was analyzed using standard industry practices for normalizing and transforming the measured intensity values obtained for the

subject gene in each sample. An unbiased Pearson Spearman hierarchical clustering algorithm in the R/BioConductor suite of packages called hclust.2 was used to create a standard microarray dendrogram for these 48 samples. As known in the art R/BioConductor is an open-source, statistical programming language widely used in academia, finance and the pharmaceutical industry for data analysis. Generally the tumors were arranged and clustered based on gene expression patterns, expression intensity, etc.

As shown in FIG. 7A, the dendrogram derived from the 48 samples and across all 19380 genes, clustered NTX lines together based upon their tumor type or tissue of origin. Several tumors typically associated with neuroendocrine phenotypes clustered together on the branch denoted by (1); these included skin cancers, numerous lung cancers and other NETs. Interestingly, a sub-branch, denoted by (2), showed that two large cell lung cancers with neuroendocrine features (LU50.LCNEC and LU37.LCNEC) and a small cell lung cancer (LU102.SCLC) clustered with an ovarian (OV26) and a kidney (KD66) tumor (cluster C) suggesting these later tumors also possessed neuroendocrine phenotypes. Moreover, FIG. 7A shows cluster D which consists of 3 additional SCLC tumors, and to its right is a small cluster (cluster E) containing an additional SCLC tumor (LU100) and a neuroendocrine endometrial tumor (EM6). All of the tumors in clusters D and E are generally understood to possess some neuroendocrine features based on the academic literature and pathology experience in the clinic. The fact that cluster G, comprising SCC, can be found on a completely different branch of the dendrogram of FIG. 7A, indicates that the clustering is not driven exclusively by the organ of origin of the tumor.

Closer inspection of a collection of gene markers associated with NETs (FIG. 7B) shows that they are strongly expressed in tumors comprising clusters C and D, while they are minimally expressed in tumors in Cluster G (squamous cell carcinoma of the lung), suggesting clusters C and D represent NETs or tumors with a neuroendocrine phenotype. More specifically, cluster C NETs highly express ASCL1, CALCA, CHGA, SST and NKX2-1, while cluster D NETs highly express CHGA, ENO2, and NCAM1, and it is the expression of these neuroendocrine phenotype genes that is in part responsible for the clustering of these tumors. An interesting feature is the strong expression of KIT in cluster D, a gene occasionally reported to be associated with neuroendocrine tumors, but clearly linked to oncogenesis in other contexts. This is in contrast to the SCC tumors in cluster G which lack

strong expression any of these genes (FIG. 7B).

Tumors in cluster C show a phenotype consistent with a reduction in Notch signaling: a lack of expression of any Notch receptor, a relative lack of JAG1 and HES1 expression, and strong levels of ASCL1 expression (FIG. 7C). Interestingly, cluster D shows high expression of HES6, a transcription factor that can support ASCL1 activity by antagonizing HES1 activity through heterodimer formation.

In view of the aforementioned results, mRNA expression of HES6 was examined from various NTX lines and normal tissues using an Applied Biosystems 7900HT Machine (Life Technologies) to perform Taqman real-time quantitative RT-PCR (qRT-PCR) in accordance with the manufacturer's protocols. RNA was isolated as described above and checked to ensure quality was suitable for gene expression analysis. RNA from normal tissues was purchased (Agilent Technologies and Life Technologies). 200 ng of RNA was used for cDNA synthesis using the cDNA archive kit (Life Technologies). cDNA was used for qRT-PCR analysis on Taqman Low Density Arrays (TLDA; Life Technologies) which contained the HES6 Taqman assay to measure mRNA levels of HES6.

HES6 mRNA levels are shown for each NTX line or normal tissue sample (single dot on graph) after normalization to endogenous controls. Normalized values are plotted relative to the average expression in the normal tissues of toxicity concern (NormTox). This technique allowed for the rapid identification and characterization of a variety of tumors having neuroendocrine features from the NTX tumor bank through measurement of HES6 and other relevant markers. FIG. 7D illustrates general overexpression of HES6 in the sampled tumors with neuroendocrine features (e.g., LU-SCLC, LU-LCNEC) compared to normal tissues, breast, colon, liver and other selected tumors. Significantly these microarray and qPCR data show that at least some endometrial, kidney and ovarian tumors may exhibit neuroendocrine tumor features (FIGS. 7A and 7D).

The microarray data generated as described above not only showed that the tumors in clusters C, D and E exhibited various neuroendocrine markers, but also showed that the tumors in those clusters expressed markers indicative of neurogenesis, neural commitment, or differentiation towards neural fates (FIG. 7E). Of particular interest, the tumors in Cluster D frequently show a stronger and more consistent upregulation of many of these markers (e.g., BEX1 and BEX4, CD56, NRCAM, SEMA receptors, SOX and ZIC factors) and frequently

reduced hormone upregulation versus other clusters, suggesting a more neural phenotype.

Example 3

Expression of SEZ6 mRNA in Tumors Having Neuroendocrine and Neural Features

Various techniques were used to identify tumors exhibiting neuroendocrine features including whole transcriptome sequencing (Example 1) and microarray and qRT-PCR (Example 2). The data thus generated was further analyzed in order to find potential therapeutic targets that are highly expressed in neuroendocrine tumors when compared to non-neuroendocrine tumors and normal tissues. As discussed in Example 1 it was found that SEZ6, a single pass transmembrane protein that is mainly expressed in the normal brain, has high expression in many neuroendocrine tumors (FIG. 6B).

The microarray data generated in Example 2 showed that tumors located in clusters C, D and E expressed neuroendocrine markers (FIG. 7B) and neural markers (FIG. 7E). Remarkably, the tumors in those same clusters also showed high levels of SEZ6 transcript, suggesting that SEZ6 is associated with tumors having neuroendocrine and neural features (FIG. 7F). This is in line with the known role of SEZ6 in postnatal forebrain development and continued expression in the specific regions of the hippocampus in the adult. SEZ6 is thought to play important roles in cell-cell recognition and signaling. Often developmental pathways are inappropriately expressed in tumors.

In order to determine SEZ6 mRNA expression levels in various sample NTX tumor lines, qRT-PCR was performed using the SEZ6 Taqman assay essentially as described in Example 2 above. FIG. 8A shows SEZ6 expression relative to the average expression in normal tissues and normalized to expression of the endogenous control gene ALAS1. SEZ6 gene expression is elevated more than 10,000,000-fold in neuroendocrine NTX populations versus normal tissues. Five of the SCLC NTX lines shown in FIG. 8A are mRNA samples extracted directly from primary biopsies (p0). The expression of SEZ6 in these unpassaged tumors demonstrates that SEZ6 expression is not an artifact that results from growing human tumors in mice. Three subtypes of NSCLC are also represented in FIG. 8A: LU25 is spindle cell carcinoma, LU50 is a large cell neuroendocrine carcinoma (LCNEC), and LU85 is a squamous cell carcinoma (SCC). KDY66 and OV26, a kidney and ovarian tumor,

respectively, clustered on the microarray with SCLC and LCNEC tumors (FIG 7A), suggesting they also have neuroendocrine features.

To extend the analysis of SEZ6 expression to a wider array of tumor specimens, qRT-PCR was performed using the Fluidigm BioMark™ HD System. Briefly, 1 ng of RNA, prepared as described in Example 1, was converted to cDNA using the cDNA archive kit (Life Technologies). The cDNA was pre-amplified using a SEZ6 specific Taqman assay and was then used to perform qRT-PCR. Expression in normal tissues (NormTox or Norm) was compared to expression in the following NTX lines, where the number in brackets indicates the number of unique NTX lines tested: BR (5), CR (6), KDY (9), OV (16), PA (9), lung adenocarcinoma (LU-Adeno) (7), LCNEC (2), SCC (11) and SCLC (15) (FIG. 8B). SCLC and LCNEC NTX show the highest expression of SEZ6, although some expression of SEZ6 was also seen in OV, PA, CR and LU-Adeno NTX lines compared to normal tissue samples.

"NormTox" represents the following samples of normal tissue: two colon, two kidney, two liver, two lung, two pancreas, two heart, one esophagus, one skeletal muscle, one skin, one small intestine, one spleen, one stomach, and one trachea sample. Another set of normal tissues designated "Norm" represents the following samples of normal tissue: brain, breast, cervix, ovary, peripheral blood mononuclear cells, placenta, prostate, testes, thymus, and thyroid. Most normal tissues have no expression of SEZ6, while low expression is seen in pancreas, colon, liver and lung and high expression in brain. A different SEZ6 specific Taqman assay, using essentially the same method as above, was conducted on various NTX tumor lines. The number of tumor lines that were tested for each type of tumor is denoted as the denominator, whereas the number of tumors that expressed SEZ6 is denoted as the numerator: 1/5 CR, 2/2 GA, 1/1 GB (glioblastoma), 1/1 KDY, 2/6 SK, 2/4 LU-Adeno, 2/2 LCNEC, 3/10 LU-SCC, 10/10 SCLC and 1/2 OV (data not shown).

Taken together, these data suggest that SEZ6 is upregulated in tumors exhibiting neuroendocrine and neural features suggesting it may serve as a therapeutic target for treatment of these types of tumors.

Example 4

Expression of SEZ6 mRNA in Various Tumor and Normal Tissue Specimens using qRT-PCR

To extend the analysis of SEZ6 expression to a wider array of tumor specimens, Taqman qRT-PCR was performed substantially as described in the previous Examples on a TissueScan™ qPCR (Origene Technologies) 384-well array. This array enables comparison of gene expression across 18 different solid tumor types, with multiple patient derived samples for each tumor type and from normal adjacent tissue.

In this regard, FIGS. 9A and 9B show the absolute and relative gene expression levels, respectively, of SEZ6 in whole tumor specimens (grey dots) or normal adjacent tissue (NAT; white dots) from patients with one of eighteen different solid tumor types. More specifically, FIG. 9A shows the absolute mRNA expression level of SEZ6 in various whole tumor specimens or matched normal adjacent tissue. FIG. 9B shows the expression level of SEZ6 as normalized against β-actin and plotted relative to expression in normal adjacent tissue for each tumor type analyzed. Specimens in which SEZ6 was not detected were assigned a Ct value of 50, which represents the last cycle of amplification in the experimental protocol. Each dot represents a single tissue specimen, with the geometric mean value represented as a black line.

Using this Origene Array, overexpression of SEZ6 was seen in a subset of adrenal, liver, lung, ovarian, and pancreatic cancer, many of which may represent neuroendocrine tumors or tumors with poorly differentiated neuroendocrine phenotypes. As shown by the absolute gene expression in FIG. 9A, normal testis and pancreas are the only normal tissues with high expression of SEZ6. This suggests that SEZ6 may play a role in tumorigenesis and/or tumor progression in a wide variety of tumors including but not limited to those with neuroendocrine and neural features.

Example 5

Cloning and Expression of Recombinant SEZ6 Proteins

Human SEZ6

In order to generate and develop all molecular and cellular materials required in the present invention pertaining to human SEZ6, cDNA (FIG. 3A; SEQ ID NO: 5) encoding the complete mature human SEZ6 protein (FIG. 3B, SEQ ID NO: 6) was created as follows. A commercial human SEZ6 cDNA was purchased from Open Biosystems where this cDNA sequence corresponded to NCBI accession BC146292. Sequence alignments showed the protein encoded by BC146292 differed by several residues from that of RefSeq NP_849191 (see residues 414, 415 and 417, FIG. 3C), encoding the endogenous human SEZ6 protein. PCR was used to amplify two separate cDNA fragments from the BC146292 clone, in which the primers used introduced the desired changes into the cDNA at residues 414-417 during the process of overlap PCR to create a cDNA encoding a mature SEZ6 protein with identical sequence to that encoded by NM_178860, the mRNA sequence encoding endogenous human SEZ6 protein. The repaired cDNA clone, termed hSCRx17 (FIG. 3A), was used for all subsequent engineering of constructs expressing the mature human SEZ6 protein or fragments thereof.

In order to generate immunoreactive or immunospecific modulators to the SEZ6 molecule, a chimeric fusion gene was generated in which the ECD portion of the human SEZ6 protein was fused to the human IgG2 Fc domain (FIG. 4A, SEQ ID NO: 8). This was done as follows: cDNA encoding the ECD of SEZ6 was PCR amplified from the hSCRx17 cDNA clone (FIG. 3A), and this PCR product was subsequently subcloned into a CMV driven expression vector in frame and downstream of an IgK signal peptide sequence and upstream of a human IgG2 Fc cDNA, using standard molecular techniques. The cDNA sequence encoding the hSEZ6-Fc fusion protein, termed hSCRx17-Fc ORF, is shown in FIG. 4A; the corresponding protein sequence encoded by hSCRx17-Fc ORF is shown in FIG. 4B (SEQ ID NO: 9). The underlined regions of the sequences correspond to the human IgG2 Fc. The bolded underlined regions correspond to the IgK signal peptide, and the sequences in bold font correspond to the portions of the fusion protein encoded by the cloning restriction sites flanking the SEZ6 ECD.

To generate recombinant hSEZ6 ECD protein a similar PCR-based strategy was used.

The cDNA fragment encoding the ECD of SEZ6 was amplified from the hSCRx17 cDNA clone and subcloned into a CMV driven expression vector in frame and downstream of an IgK signal peptide sequence and in frame upstream of a sequence encoding an 9-Histidine epitope tag (SEQ ID NO: 400).

The CMV-driven expression vector permits high level transient expression in HEK-293T and/or CHO-S cells. Suspension or adherent cultures of HEK-293T cells, or suspension CHO-S cells were transfected with expression constructs encoding either the hSEZ6 ECD-Fc or hSEZ6-ECD-His proteins, using polyethylenimine polymer as the transfecting reagent. Three to five days after transfection, the hSEZ6 ECD-Fc or hSEZ6-ECD-His proteins were purified from clarified cell-supernatants using an AKTA explorer and either MabSelect SuRe™ Protein A (GE Healthcare Life Sciences) or Nickel-EDTA (Qiagen) columns, respectively.

A stable cell line overexpressing recombinant human SEZ6 was constructed using lentiviral vectors to transduce HEK-293T cells as follows: PCR amplification was performed using the hSCRx17 clone as a template in order to produce a cDNA fragment encoding the mature human SEZ6 protein. The fragment that was generated was subcloned in frame downstream of a sequence encoding an IgK signal peptide and DDK epitope tag previously engineered upstream of the multiple cloning site of pCDH-EF1-MCS-T2A-GFP (System Biosciences) using standard molecular cloning techniques. The resulting bicistronic lentiviral vector was used to engineer cell lines overexpressing a human SEZ6-T2A peptide-GFP polypeptide. The T2A sequence promotes ribosomal skipping of a peptide bond condensation, resulting in two independent proteins, in this case SEZ6 and GFP.

Murine SEZ6

A stable cell line overexpressing recombinant murine SEZ6 was engineered essentially as described above for recombinant human SEZ6. HEK-293T cells were transduced with a lentiviral vector expressing murine SEZ6. The vector was engineered essentially as follows. A cDNA fragment (FIG. 5A; SEQ ID NO: 10) encoding the mature murine SEZ6 protein listed as RefSeq NM_021286 in the NCBI database (FIG. 5B; SEQ ID NO: 11) was obtained by PCR amplification from a commercial murine SEZ6 cDNA (Origene; #MC203634) and subcloned downstream of an IgK signal peptide sequence and

DDK epitope tag sequence previously engineered upstream of the multiple cloning site of pCDH-EF1-MCS-IRES-RFP (System Biosciences) using standard molecular cloning techniques. This yielded a bicistronic lentiviral vector that was used to produce a HEK-293T cell line overexpressing murine SEZ6 and RFP.

Rat SEZ6

To generate and develop all molecular and cellular materials required in the present invention pertaining to rat SEZ6 proteins, cDNA (FIG. 5C, SEQ ID NO: 12) encoding the complete mature rat SEZ6 protein (FIG. 5D, SEQ ID NO: 13) was obtained as follows. A cDNA encoding the full length mature rat protein (i.e., the full length protein minus the wild-type signal peptide) was amplified from rat brain marathon-ready cDNA (Clontech #639412). Sequence alignments showed the ECD of the encoded protein to be homologous to the endogenous rat SEZ6 protein listed as RefSeq NP_001099224 in the NCBI database. This cDNA clone, termed rSCRx17 (FIG. 5D), was used for subsequent engineering of constructs expressing the rat SEZ6 protein fragments.

Cynomolgus SEZ6

To generate and develop all molecular and cellular materials required in the present invention pertaining to cynomolgus SEZ6 proteins, cDNA (FIG. 5E, SEQ ID NO: 14) encoding the cynomolgus SEZ6 protein (FIG. 5F, SEQ ID NO: 15) was obtained as follows: A predicted cynomolgus SEZ6 ORF sequence was assembled by bioinformatics analysis in the following way: the ORF of the human SEZ6 gene was obtained from NCBI accession NM_178860 and compared, using the BLAST algorithm, to the whole genome shotgun sequencing contigs in the NCBI database. The BLAST results were then used to assemble a putative cynomolgus SEZ6 ORF. The sequence encoding the predicted wild-type signal peptide of cynomolgus SEZ6 was removed from this BLAST derived sequence, and replaced with a sequence encoding an IgK signal peptide sequence. After codon optimization for production in mammalian cells, this complete hybrid ORF sequence was ordered as a synthetic gene (GeneWiz). This optimized cDNA clone, termed cSCRx17 (FIG. 3E), was used for subsequent engineering of constructs expressing the cynomolgus SEZ6 protein fragments.

Human SEZ6L and SEZ6L2

In the human genome, there are two genes closely related to SEZ6- seizure related 6 homolog-like (SEZ6L) and seizure related 6 homolog like-2 (SEZ6L2). Although the overall percent identity is relatively low between the three proteins (~42%), there are smaller stretches of perfect identity between pairs or all three of the proteins. In order to investigate any possible cross reactivity of the anti-SEZ6 modulators with human SEZ6L and SEZ6L2 proteins, the open reading frames encoding the ECDs of human SEZ6L protein (NP_0066938) and human SEZ6L2 protein (NP_001230261) were codon optimized and synthesized (GeneWiz). These optimized cDNA sequences encoding the ECDs of human SEZ6L or SEZ6L2 proteins are shown in FIGS. 5G and 5I.

Material for cross-reactivity studies

Material was generated in order to study whether the SEZ6 modulators of the invention cross-reacted with rat and/or cynomolgus SEZ6 homologues, or with the closely related human SEZ6L and SEZ6L2 proteins. Chimeric fusion genes were designed in which the ECD portion of either the rat or the cynomolgus SEZ6 protein (underlined in FIGS. 5D and 5F, respectively) was fused to a 9-Histidine epitope tag (SEQ ID NO: 400). Using PCR, the cDNA fragment encoding the ECD of either rat or cynomolgus SEZ6 was amplified from either rSCRx17 or cSCRx17, respectively, and subcloned into a CMV driven expression vector in frame and downstream of an IgK signal peptide sequence and in frame and upstream of a sequence encoding an 9-Histidine epitope tag (SEQ ID NO: 400). Similarly, chimeric fusion genes were designed in which the open reading frame encoding the ECD portion of the human SEZ6L or SEZ6L2 proteins was subcloned into a CMV driven expression vector in frame and downstream of an IgK signal peptide sequence and in frame and upstream of a sequence encoding an 9-Histidine epitope tag (SEQ ID NO: 400). The resultant encoded protein sequences for these fusion proteins are shown in FIGS. 5H and 5J, respectively, with the underlined sequence representing the ECD of the particular protein under consideration.

The rat and cynomolgus SEZ6 ECD-His vectors generated above, were used to produce and purify recombinant rSEZ6-ECD-His protein and cSEZ6-ECD-His protein, respectively, as follows: using art-recognized techniques, suspension or adherent cultures of

HEK-293T cells, or suspension CHO-S cells were transfected with the expression vectors encoding rSEZ6-ECD-His or cSEZ6-ECD-His protein. Polyethylenimine polymer was used as the transfecting reagent. Three to five days after transfection, the rSEZ6-ECD-His or cSEZ6-ECD-His protein was purified from clarified cell-supernatants using AKTA explorer and Nickel-EDTA (Qiagen) columns. Similarly, the human SEZ6L and SEZ6L2 ECD-His vectors were used to produce and purify recombinant human SEZ6L and human SEZ6L2 ECD-His proteins, as described for the rat and cynomolgus homologs.

Example 6

Generation of Anti-SEZ6 Murine Modulators

SEZ6 modulators in the form of murine antibodies were produced in accordance with the teachings herein through inoculation with human SEZ6-Fc. In this regard three strains of mice were used to generate high affinity, murine, monoclonal antibody modulators that can be used to associate with and/or inhibit the action of SEZ6 for the prevention and/or treatment of various proliferative disorders. Specifically, Balb/c, CD-1 and FVB mouse strains were immunized with human recombinant SEZ6-Fc and used to produce Hybridomas.

The SEZ6-Fc antigen was purified from supernatant from CHO-S cells over expressing the construct SEZ6-Fc as set forth in Example 5 (FIGS. 4A and 4B). 10 μg of SEZ6-Fc immunogen was used for the first immunization, followed by 5 μg and 2.5 μg of SEZ6-Fc immunogen for the subsequent three immunizations and five immunizations, respectively. All immunizations were performed with the immunogen emulsified with an equal volume of TITERMAX® Gold (CytRx Corporation) or alum adjuvant. Murine antibodies were generated by immunizing six female mice (two each of: Balb/c, CD-1, FVB) via footpad route for all injections.

Solid-phase ELISA assays were used to screen mouse sera for mouse IgG antibodies specific for human SEZ6. A positive signal above background was indicative of antibodies specific for SEZ6. Briefly, 96 well plates (VWR International, Cat. #610744) were coated with recombinant SEZ6-His at 0.5μg/ml in ELISA coating buffer overnight. After washing with PBS containing 0.02% (v/v) Tween 20, the wells were blocked with 3% (w/v) BSA in PBS, 200 μL/well for 1 hour at room temperature (RT). Mouse serum was titrated (1:100, 1:200, 1:400, and 1:800) and added to the SEZ6 coated plates at 50 μL/well and incubated at

RT for 1 hour. The plates are washed and then incubated with 50 µL/well HRP-labeled goat anti-mouse IgG diluted 1:10,000 in 3% BSA-PBS or 2% FCS in PBS for 1 hour at RT. Again the plates were washed and 40 µL/well of a TMB substrate solution (Thermo Scientific 34028) was added for 15 minutes at RT. After developing, an equal volume of 2N H_2SO_4 was added to stop substrate development and the plates were analyzed by spectrophotometer at OD 450.

Sera-positive immunized mice were sacrificed and draining lymph nodes (popliteal and inguinal, and medial iliac if enlarged) were dissected out and used as a source for antibody producing cells. A single cell suspension of B cells ($228.9x10^6$ cells) was fused with non-secreting P3x63Ag8.653 myeloma cells (ATCC #CRL-1580) at a ratio of 1:1 by electrofusion. Electrofusion was performed using the BTX Hybrimmune™ System, (BTX Harvard Apparatus) as per the manufacturer's directions. After the fusion procedure the cells were resuspended in hybridoma selection medium supplemented with Azaserine (Sigma #A9666), high glucose DMEM medium with sodium pyruvate (Cellgro cat#15-017-CM) containing 15% Fetal Clone I serum (Hyclone), 10% BM Condimed (Roche Applied Sciences), 4 mM L-glutamine, 100 IU Penicillin-Streptomycin and 50 µM 2-mercaptoethanol and then plated in three T225 flasks in 90 mL selection medium per flask. The flasks were then placed in a humidified 37°C incubator containing 5% CO_2 and 95% air for 6-7 days.

After six to seven days of growth the library consisting of the cells grown in bulk in the T225s was plated at 1 cell per well in Falcon 96 well U-bottom plates using the Aria I cell sorter. The selected hybridomas were then grown in 200 µL of culture medium containing 15% Fetal Clone I serum (Hyclone), 10% BM-Condimed (Roche Applied Sciences), 1 mM sodium pyruvate, 4 mM L-glutamine, 100 IU Penicillin-Streptamycin, 50 µM 2-mercaptoethanol, and 100 µM hypoxanthine. Any remaining unused hybridoma library cells were frozen for future library testing. After ten to eleven days of growth supernatants from each well of the plated cells were assayed for antibodies reactive for SEZ6 by ELISA and FACS assays.

For screening by ELISA 96 well plates were coated with SEZ6-Fc at 0.3 µg/mL in PBS overnight at 4°C. The plates were washed and blocked with 3% BSA in PBS/Tween for one hour at 37°C and used immediately or kept at 4°C. Undiluted hybridoma supernatants were incubated on the plates for one hour at RT. The plates were washed and probed with

HRP labeled goat anti-mouse IgG diluted 1:10,000 in 3% BSA-PBS for one hour at RT. The plates were then incubated with substrate solution as described above and read at OD 450. Wells containing immunoglobulin that preferentially bound human SEZ6, as determined by a signal above background, were transferred and expanded.

Selected growth positive hybridoma wells secreting murine immunoglobulin were also screened for human SEZ6 specificity and cynomolgus, rat and murine SEZ6 cross reactivity using a flow cytometry based assay with 293 cells engineered to over-express the selected antigen or constructs fabricated in the previous Example.

For the flow cytometry assays, $50x10^4$ h293 cells transduced respectively with human, cynomolgus, rat or murine SEZ6 were incubated for 30 minutes with 25-100 µL hybridoma supernatant. Cells were washed with PBS, 2% FCS, twice and then incubated with 50 µL of a goat-anti-mouse IgG Fc fragment specific secondary conjugated to DyLight 649 diluted 1:200 in PBS/2%FCS. After 15 minutes of incubation, cells were washed twice with PBS, 2%FCS, and re-suspended in the same buffer with DAPI and analyzed by flow cytometry using a FACSCanto II as per the manufacturer's instructions. Wells containing immunoglobulin that preferentially bound the $SEZ6^+$ GFP^+ cells were transferred and expanded. The resulting hSEZ6 specific clonal hybridomas were cryopreserved in CS-10 freezing medium (Biolife Solutions) and stored in liquid nitrogen. Antibodies that bound with human, cynomolgus, rat or murine SEZ6 cells were noted as cross-reactive (see FIG. 11A).

ELISA and flow cytometry analysis confirmed that purified antibody from most or all of these hybridomas bound SEZ6 in a concentration-dependent manner. Wells containing immunoglobulin that bound SEZ6 GFP cells were transferred and expanded. The resulting clonal hybridomas were cryopreserved in CS-10 freezing medium (Biolife Solutions) and stored in liquid nitrogen.

One fusion was performed and seeded in 48 plates (4608 wells at approximately 40% cloning efficiency). The initial screen yielded sixty-three murine antibodies that associated with human SEZ6. A second screen was subsequently performed and yielded 134 antibodies that associated with human SEZ6.

Example 7

Sequencing of SEZ6 Murine Modulators

Based on the foregoing, a number of exemplary distinct monoclonal antibodies that bind immobilized human SEZ6 or h293-hSEZ6 cells with apparently high affinity were selected for sequencing and further analysis. As shown in a tabular fashion in FIGS. 10A and 10B, sequence analysis of the light chain variable regions (FIG. 10A) and heavy chain variable regions (FIG. 10B) from selected monoclonal antibodies generated in Example 6 confirmed that many had novel complementarity determining regions and often displayed novel VDJ arrangements. Note that the complementarity determining regions set forth in FIGS. 10A and 10B are defined as per Chothia et al., supra.

As a first step in sequencing exemplary modulators, the selected hybridoma cells were lysed in Trizol® reagent (Trizol Plus RNA Purification System, Life Technologies) to prepare the RNA. In this regard between 10^4 and 10^5 cells were resuspended in 1 mL Trizol and shaken vigorously after addition of 200 µL of chloroform. Samples were then centrifuged at 4°C for 10 minutes and the aqueous phase was transferred to a fresh microfuge tube where an equal volume of isopropanol was added. The tubes were again shaken vigorously and allowed to incubate at RT for 10 minutes before being centrifuged at 4°C for 10 minutes. The resulting RNA pellets were washed once with 1 mL of 70% ethanol and dried briefly at RT before being resuspended in 40 µL of DEPC-treated water. The quality of the RNA preparations was determined by fractionating 3 µL in a 1% agarose gel before being stored at - 80°C until used.

The variable region of the Ig heavy chain of each hybridoma was amplified using a 5' primer mix comprising thirty-two mouse specific leader sequence primers, designed to target the complete mouse V_H repertoire, in combination with a 3' mouse Cγ primer specific for all mouse Ig isotypes. A 400 bp PCR fragment of the V_H was sequenced from both ends using the same PCR primers. Similarly a mix of thirty-two 5' Vκ leader sequence primers designed to amplify each of the Vκ mouse families combined with a single reverse primer specific to the mouse kappa constant region were used to amplify and sequence the kappa light chain. The V_H and V_L transcripts were amplified from 100 ng total RNA using reverse transcriptase polymerase chain reaction (RT-PCR).

A total of eight RT-PCR reactions were run for each hybridoma: four for the Vκ light

chain and four for the V gamma heavy chain (γ1). The One Step RT-PCR kit was used for amplification (Qiagen). This kit provides a blend of Sensiscript and Omniscript Reverse Transcriptases, HotStarTaq DNA Polymerase, dNTP mix, buffer and Q-Solution, a novel additive that enables efficient amplification of "difficult" (e.g., GC-rich) templates. Reaction mixtures were prepared that included 3 μL of RNA, 0.5 of 100 μM of either heavy chain or kappa light chain primers (custom synthesized by IDT), 5 μL of 5× RT-PCR buffer, 1 μL dNTPs, 1 μL of enzyme mix containing reverse transcriptase and DNA polymerase, and 0.4 μL of ribonuclease inhibitor RNasin (1 unit). The reaction mixture contains all of the reagents required for both reverse transcription and PCR. The thermal cycler program was set for an RT step 50°C for 30 minutes, 95°C for 15 minutes, followed by 30 cycles of PCR (95°C for 30 seconds, 48°C for 30 seconds, 72°C for one minute). There was then a final incubation at 72°C for 10 minutes.

To prepare the PCR products for direct DNA sequencing, they were purified using the QIAquick™ PCR Purification Kit (Qiagen) according to the manufacturer's protocol. The DNA was eluted from the spin column using 50 μL of sterile water and then sequenced directly from both strands. The extracted PCR products were directly sequenced using specific V region primers. Nucleotide sequences were analyzed using IMGT to identify germline V, D and J gene members with the highest sequence homology. The derived sequences were compared to known germline DNA sequences of the Ig V- and J-regions using V-BASE2 (Retter et al., supra) and by alignment of V_H and V_L genes to the mouse germline database to provide the annotated sequences set forth in FIGS. 10A and 10B.

More specifically, FIG. 10A depicts the contiguous amino acid sequences of seventy-five novel murine light chain variable regions from anti-SEZ6 antibodies (SEQ ID NOS: 20 – 168, even numbers) and eleven humanized light chain variable regions (SEQ ID NOS: 170 – 192, even numbers) derived from representative murine light chains. Similarly, FIG. 10B depicts the contiguous amino acid sequences of seventy-five novel murine heavy chain variable regions (SEQ ID NOS: 21 – 169, odd numbers) from the same anti-SEZ6 antibodies and eleven humanized heavy chain variable regions ((SEQ ID NOS: 171 – 193, odd numbers) from the same murine antibodies providing the humanized light chains. Thus, taken together FIGS. 10A and 10B provide the annotated sequences of sevety-five operable murine anti-SEZ6 antibodies (termed SC17.1, SC17.2, SC17.3, SC17.4, SC17.8, SC17.9, SC17.10,

SC17.11, SC17.14, SC17.15, SC17.16, SC17.17, SC17.18, SC17.19, SC17.22, SC17.24, SC17.27, SC17.28, SC17.29, SC17.30, SC17.32, SC17.34, SC17.35, SC17.36, SC17.38, SC17.39, SC17.40, SC17.41, SC17.42, SC17.45, SC17.46, SC17.47, SC17.49, SC17.50, SC17.53, SC17.54, SC17.56, SC17.57, SC17.59, SC17.61, SC17.63, SC17.71, SC17.72, SC17.74, SC17.76, SC17.77, SC17.79, SC17.81, SC17.82, SC17.84, SC17.85, SC17.87, SC17.89, SC17.90, SC17.91, SC17.93, SC17.95, SC17.97, SC17.99, SC17.102, SC17.114, SC17.115, SC17.120, SC17121, SC17.122, SC17.140, SC17.151, SC17.156, SC17.161, SC17.166, SC17.187, SC17.191, SC17.193, SC17.199 and SC17.200) and eleven humanized antibodies (termed hSC17.16, hSC17.17, hSC17.24, hSC17.28, hSC17.34, hSC17.46, hSC17.151, hSC17.155, hSC17.156, hSC17.161 and hSC17.200). Note that these same designations may refer to the clone that produces the subject antibody and, as such, the use of any particular designation should be interpreted in the context of the surrounding disclosure.

Additionally, hSC17.200vL1 (SEQ ID NO: 192) is a variant of the humanized light chain construct hSC17.200 (SEQ ID NO: 190), hSC17.155vH1 – vH6 (SEQ ID NOS: 193-198) are variants of the heavy chain construct hSC.155 (SEQ ID NO: 184) which is derived from SC17.90 (SEQ ID NO: 127) and that hSC161vH1 (SEQ ID NO: 199) is a variant of the heavy chain construct hSC17.161 (SEQ ID NO: 189). As will be discussed in more detail below these variants were constructed and tested to optimize one or more biochemical properties of the parent antibody.

Further, corresponding nucleic acid sequences of each of the seventy-five exemplary murine modulators and eleven humanized modulators and variants set forth in FIGS. 10A and 10B are included in sequence listing of the instant application (SEQ ID NOS: 220 – 399).

For the purposes of the instant application the SEQ ID NOS of each particular antibody are sequential. Thus mAb SC17.1 comprises SEQ ID NOS: 20 and 21 for the light and heavy chain variable regions respectively. In this regard SC17.2 comprises SEQ ID NOS: 22 and 23, SC17.9 comprises SEQ ID NOS: 24 and 25, and so on. Moreover, corresponding nucleic acid sequences for each antibody amino acid sequence in FIGS. 10A and 10B are appended to the instant application in the sequence listing filed herewith. In the subject sequence listing the included nucleic acid sequences comprise SEQ ID NOS that are two hundred greater than the corresponding amino acid sequence (light or heavy chain). Thus, nucleic acid sequences encoding the light and heavy chain variable region amino acid sequences of mAb SC17.1

(i.e., SEQ ID NOS: 20 and 21) comprise SEQ ID NOS: 220 and 221 in the sequence listing. In this regard nucleic acid sequences encoding all of the disclosed light and heavy chain variable region amino acid sequences, including those encoding the humanized constructs and variants thereof, are numbered similarly and comprise SEQ ID NOS: 220 – 399.

Example 8

Generation of Chimeric and Humanized SEZ6 Modulators

As alluded to above, eleven of the murine antibodies from Example 7 were humanized using complementarity determining region (CDR) grafting. Human frameworks for heavy and light chains were selected based on sequence and structure similarity with respect to functional human germline genes. In this regard structural similarity was evaluated by comparing the mouse canonical CDR structure to human candidates with the same canonical structures as described in Chothia et al. (supra).

More particularly eleven murine antibodies SC17.16, SC17.17, SC17.24, SC17.28, SC17.34, SC17.46, SC17.151, SC17.155, SC17.156, SC17.161 and SC17.200 were humanized using a computer-aided CDR-grafting method (Abysis Database, UCL Business Plc.) and standard molecular engineering techniques to provide hSC17.16, hSC17.17, hSC17.24, hSC17.28, hSC17.34, hSC17.46, hSC17.151, hSC17.155, hSC17.156, hSC17.161 and hSC17.200 modulators. The human framework regions of the variable regions were selected based on their highest sequence homology to the subject mouse framework sequence and its canonical structure. For the purposes of the humanization analysis, the assignment of amino acids to each of the CDR domains is in accordance with Kabat et al. numbering (supra).

Molecular engineering procedures were conducted using art-recognized techniques. To that end total mRNA was extracted from the hybridomas and amplified as set forth in Example 7 immediately above.

From the nucleotide sequence information, data regarding V, D and J gene segments of the heavy and light chains of subject murine antibodies were obtained. Based on the sequence data new primer sets specific to the leader sequence of the Ig V_H and V_K light chain of the antibodies were designed for cloning of the recombinant monoclonal antibody. Subsequently the V-(D)-J sequences were aligned with mouse Ig germ line sequences. The

resulting genetic arrangements for each of the eleven humanized constructs are shown in Table 1 immediately below.

TABLE 1

mAb	human VH	human DH	human JH	FW changes	human VK	human JK	FW changes
hSC17.16	IGHV1-2	IGHD3-16	JH5	none	IGKV-O2	JK1	none
hSC17.17	IGHV1-2	IGHD4-11	JH4	none	IGKV-L6	JK2	none
hSC17.24	VH1-f	IGHD5-12	JH4	48I, 73K	VKB3	JK1	none
hSC17.28	IGHV1-2	IGHD3-16	JH4	none	IGKV-A10	JK4	none
hSC17.34	IGHV1-3	IGHD3-10	JH4	71V	IGKV-L1	JK1	71Y
hSC17.46	IGHV1-2	IGHD4-23	JH4	48I, 69L	IGKV-L11	JK1	87F
hSC17.151	IGHV1-46	IGHD1-14	JH4	none	VKL6	JK2	none
hSC17.155	IGHV1-46	IGHD2-2	JH4	none	VKB3	JK1	none
hSC17.156	IGHV2-26	IGHD4-17	JH4	none	VKO1	JK4	none
hSC17.161	IGHV1-2	IGHD1-14	JH4	none	VKB3	JK2	none
hSC17.200	IGHV5-51	IGHD4-17	JH4	none	IGKV-L6	JK4	none

The humanized antibodies listed in Table 1 correspond to the annotated light and heavy chain sequences set forth in FIGS. 10A and 10B (SEQ ID NOS: 170-191). The corresponding nucleic acid sequences of the light and heavy chain variable regions are set forth in the sequence listing. TABLE 1 further demonstrates that very few framework changes were necessary to maintain the favorable properties of the binding modulators. In this respect framework changes or back mutations were only made in three of the heavy chain variable regions and only two framework modifications were undertaken in the light chain variable regions.

Note that, for some humanized light and heavy chain variable regions (e.g. hSC17.200, hSC17.155 and hSC17.161), conservative amino acid mutations were introduced in the CDRs to address stability concerns while maintaining antigen binding. In each case, the binding affinity of the antibodies with modified CDR's was found to be equivalent to either the corresponding chimeric or murine antibody. The sequences of nine exemplary humanized variant chains (light and heavy,) are listed at the end of FIGS. 10A and 10B (SEQ ID NOS: 192 – 199) where they retain the designation of the humanized parent chain with

notation to indicate they have been altered (e.g. hSC17.200vL1, hSC17.155vH1-6 and hSC17.161vH1).

Following humanization of all selected antibodies by CDR grafting, the resulting light and heavy chain variable region amino acid sequences were analyzed to determine their homology with regard to the murine donor and human acceptor light and heavy chain variable regions. The results, shown in Table 2 below, reveal that the humanized constructs consistently exhibited a higher homology with respect to the human acceptor sequences than with the murine donor sequences. More specifically, the humanized heavy and light chain variable regions generally show a higher percentage homology to a closest match of human germline genes (84%-95%) as compared to the homology of the humanized variable region sequences and the donor hybridoma protein sequences (74%-89%).

TABLE 2

mAb	Homology to Human (CDR acceptor)	Homology to Murine Parent (CDR donor)
hSC17.16 HC	91%	80%
hSC17.16 LC	86%	85%
hSC17.17 HC	93%	80%
hSC17.17 LC	87%	77%
hSC17.24 HC	86%	79%
hSC17.24 LC	93%	89%
hSC17.28 HC	89%	77%
hSC17.28 LC	92%	78%
hSC17.34 HC	85%	83%
hSC17.34 LC	84%	86%
hSC17.46 HC	85%	83%
hSC17.46 LC	84%	80%
hSC17.151 HC	90%	79%
hSC17.151 LC	87%	80%
hSC17.155 HC	90%	80%
hSC17.155 LC	95%	87%
hSC17.156 HC	89%	79%
hSC17.156 LC	86%	93%
hSC17.161 HC	89%	86%
hSC17.161 LC	93%	87%
hSC17.200 HC	90%	74%
hSC17.200 LC	88%	82%

Upon testing, and as will be discussed in Example 9, each of the humanized constructs exhibited favorable binding characteristics roughly comparable to those shown by the murine parent antibodies (Data not shown).

Whether humanized or murine, once the nucleic acid sequences of the variable regions are determined the antibodies of the instant invention may be expressed and isolated using art-recognized techniques. To that end synthetic DNA fragments of the chosen heavy chain (humanized or murine) variable region were cloned into a human IgG1 expression vector. Similarly the variable region light chain DNA fragment (again humanized or murine) was cloned into a human light chain expression vector. The selected antibody was then expressed

by co-transfection of the derived heavy and the light chain nucleic acid constructs into CHO cells.

More particularly, one compatible method of antibody production comprised directional cloning of murine or humanized variable region genes (amplified using PCR) into selected human immunoglobulin expression vectors. All primers used in Ig gene-specific PCRs included restriction sites which allowed direct cloning into expression vectors containing human IgG1 heavy chain and light chain constant regions. In brief, PCR products were purified with Qiaquick PCR purification kit (Qiagen) followed by digestion with AgeI and XhoI (for the heavy chain) and XmaI and DraIII (for the light chain), respectively. Digested PCR products were purified prior to ligation into expression vectors. Ligation reactions were performed in a total volume of 10 µL with 200U T4-DNA Ligase (New England Biolabs), 7.5 µL of digested and purified gene-specific PCR product and 25ng linearized vector DNA. Competent E. coli DH10B bacteria (Life Technologies) were transformed via heat shock at 42°C with 3 µL ligation product and plated onto ampicillin plates (100 µg/mL). The AgeI-EcoRI fragment of the V_H region was than inserted into the same sites of pEE6.4HulgG1 expression vector while the synthetic XmaI-DraIII VK insert was cloned into the XmaI-DraIII sites of the respective pEE12.4Hu-Kappa expression vector.

Cells producing the selected antibody were generated by transfection of HEK 293 cells with the appropriate plasmids using 293fectin. In this respect plasmid DNA was purified with QIAprep Spin columns (Qiagen). Human embryonic kidney (HEK) 293T (ATCC No CRL-11268) cells were cultured in 150mm plates (Falcon, Becton Dickinson) under standard conditions in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% heat inactivated FCS, 100 µg/mL streptomycin, 100 U/mL penicillin G (all from Life Technologies).

For transient transfections cells were grown to 80% confluency. Equal amounts of IgH and corresponding IgL chain vector DNA (12.5 µg of each) was added to 1.5 mL Opti-MEM mixed with 50 µL HEK 293 transfection reagent in 1.5 mL opti-MEM. The mix was incubated for 30 min at room temperature and distributed evenly to the culture plate. Supernatants were harvested three days after transfection, replaced by 20 mL of fresh DMEM supplemented with 10% FBS and harvested again at day 6 after transfection. Culture supernatants were cleared of cell debris by centrifugation at 800×g for 10 min and stored at

4°C. Recombinant chimeric and humanized antibodies were purified with Protein G beads (GE Healthcare) and stored under appropriate conditions.

Example 9

Characteristics of SEZ6 Modulators

Various methods were used to analyze the binding and immunochemical characteristics of selected SEZ6 modulators generated as set forth above. Specifically, a number of the antibody modulators were characterized as to affinity, binning, and cross reactivity with regard to human, cynomolgus, rat and mouse SEZ6 antigen along with SEZ6L and SEZ6L2 proteins by art-recognized methods including flow cytometry. Affinities and kinetic constants k_{on} and k_{off} of the selected modulators were measured using bio-layer interferometry analysis on a ForteBio RED (ForteBio, Inc.) or surface plasmon resonance using a Biacore 2000 each according to the manufacturer's instructions.

The characterization results are set forth in tabular form in FIG. 11A where it may be seen that the selected modulators generally exhibited relatively high affinities in the nanomolar range and, in many cases, were cross-reactive with one or more SEZ6 orthologs. FIG. 12 further lists the empirically determined bin occupied by the subject modulator. Taken together, these data demonstrate the varied binding properties of the disclosed modulators as well as their potential suitability for pharmaceutical development based on their reactivity in animal models.

In this regard flow cytometry was performed using a FACSCanto II as per the manufacturer's instructions in order to confirm that selected SC17 antibody modulators can immunospecifically associate with human SEZ6 and to determine whether the same modulators cross-react with cynomolgus, rat and/or murine SEZ6 in addition to SEZ6L and SEZ6L2. More particularly modulators were tested for cross reactivity to murine SEZ6 and rat SEZ6 by flow cytometry against Neuro2a (ATCC Cat # CCL131), and RIN-m5F (ATCC cat # CRL-11605) cell lines which express mouse SEZ6 and rat SEZ6, respectively. For examining cross reactivity to cynomolgus SEZ6, yeast displaying the extracellular domain of cynomolgus SEZ6 (Boder *et al*, 1997) were used for flow cytometry analysis.

Briefly $1x10^5$ cells per well of Neuro2a, RIN-5mF, or yeast displaying cynomolgus SEZ6 cells were incubated for 30 minutes with 50 µL PBS (2%FCS) buffer with 5 µg/mL

antibody. Cells were washed twice with the same buffer and then incubated with 50 µL per sample DyLight 649 labeled goat-anti-mouse IgG, Fc fragment specific secondary diluted 1:200 in PBS buffer. After incubating for 15 minutes cells were washed twice with the PBS buffer and re-suspended in the same with DAPI for flow cytometry analysis of Neuro2a and Rin-m5F or buffer without DAPI for flow cytometric analysis of yeast cells with cSEZ6. Antibodies that bound to the Neuro2a or RIN-m5F cell lines, or yeast displaying cynomolgus SEZ6 were considered to be cross reactive to murine SEZ6, rat SEZ6, or cynomolgus SEZ6, respectively. FIG. 11A shows the cross reactivity results. Six antibodies were cross reactive for human and mouse SEZ6 (SC17.6, SC17.7, SC17.19, SC17.24, SC17.26 and SC17.42); six for human and rat SEZ6 (SC17.6, SC17.17, SC17.19, SC17.26, SC17.28, SC17.34 and SC17.42); and six for human and cynomolgus SEZ6 (SC17.17, SC17.24, SC17.26, SC17.34, SC17.36 and SC17.45). Note that SC17.6 is duplicative of SC17.16 and exhibits the same binding characteristics.

To verify the cross reactivity data above for rat SEZ6 and to determine the affinity and kinetic constants k_{on} and k_{off} of the selected effectors, either bio-layer interferometry analysis on a ForteBio RED (ForteBio, Inc.) or surface plasmon resonance on a Biacore 2000 (GE Healthcare) were conducted. Affinities were determined to both human recombinant SEZ6-His and rat recombinant SEZ6-His generated in Example 5. As seen in FIG. 11A, a number of the antibodies tested, cross reacted with rat SEZ6. The selected modulators exhibited relatively high affinities for both rat and human SEZ6 in the nanomolar range.

To determine cross reactivity to family member proteins, SEZ6L and SEZ6L2, an ELISA-based assay was used. Plates were coated with SEZ6, SEZ6L, or SEZ6L2 proteins at 0.2 µg/mL in PBS overnight. After washing with PBS containing 0.05% (v/v) Tween 20 (PBST), the wells were blocked with 2% (w/v) BSA in PBS (PBSA), 100 µL/well for 1 hour at room temperature. Antibody was then added at 1 µg/mL in 100 µL PBSA for 1 hour at room temperature. After washing with PBST, 100 µL/well HRP-labeled goat anti-mouse IgG diluted 1:2,000 in PBSA for 1 hour at room temperature. The plates were washed and 100 µL/well of the TMB substrate solution (Thermo Scientific 34028) was added for 15 minutes at room temperature. After developing, an equal volume of 2M H_2SO_4 was added to stop substrate development and analyzed by spectrophotometer at OD 450. FIG. 11A shows that one antibody was cross reactive with SEZ6L (SC17.7) and five were cross-reactive with

SEZ6L2 (SC17.6, SC17.7, SC17.19, SC17.26 and SC17.28). As discussed above, such pan-SEZ6 antibodies are compatible with the teachings herein and may be used in conjunction with the disclosed methods.

Binding characteristics of the following humanized constructs from Example 8, hSC17.16, hSC17.17, hSC17.24, hSC17.28, hSC17.34 and hSC17.46, were analyzed in order to determine whether the CDR grafting process had appreciably altered their binding characteristics. The humanized constructs (CDR grafted) were compared with "traditional" chimeric antibodies comprising the murine parent (or donor) heavy and light chain variable domains and a human constant region substantially equivalent to that used in the humanized constructs. With these constructs surface plasmon resonance was conducted using a Biacore 2000 (GE Healthcare) to identify any subtle changes in rate constants brought about by the humanization process. In all cases, the humanized antibodies had binding affinity equivalent or better than the corresponding murine antibodies (Data not shown).

Antibody binning was determined for various SEZ6 modulators as shown in FIG. 11A. A ForteBio RED was used per manufacturer's instructions to identify competing antibodies that bound to the same or different bins. Briefly, a reference antibody (Ab1) was captured onto an anti-mouse capture chip, a high concentration of non-binding antibody was then used to block the chip and a baseline was collected. Monomeric, recombinant human SEZ6 (described in Example 5) was then captured by the specific antibody (Ab1) and the tip was dipped into a well with either the same antibody (Ab1) as a control or into a well with a different test antibody (Ab2). If additional binding was observed with a new antibody, then Ab1 and Ab2 were determined to be in a different bin. If no further binding occurred, as determined by comparing binding levels with the control Ab1, then Ab2 was determined to be in the same bin. As known in the art this process can be expanded to screen large libraries of unique antibodies using a full row of antibodies representing unique bins in a 96-well plate. In the instant case this binning process showed the screened antibodies bound to at least seven different bins on the SEZ6 protein. Bins A-F are unique bins and the antibodies contained in each of these bins compete with each other (but not with antibodies from other defined bins) for binding to the SEZ6 protein. Bin U contains antibodies that do not compete with antibodies in bins A-F, but may compete for binding with each other.

Example 10

Epitope Mapping of SEZ6 Modulators

In order to characterize the epitopes that the disclosed SEZ6 antibody modulators associate with or bind to, domain-level epitope mapping was performed using a modification of the protocol described by Cochran *et al.* (J Immunol Methods. 287 (1-2):147-158 (2004)) which is incorporated herein by reference). Individual domains of SEZ6 were expressed on the surface of yeast and binding by each SEZ6 antibody was determined through flow cytometry.

Yeast display plasmid constructs were created for the expression of the following constructs: SEZ6 extracellular domain (amino acids 1-904); Sushi Domain 1 (amino acids 336-395), CUB Domain 1 (amino acids 297-508), Sushi Domain 2 (amino acids 511-572), CUB Domain 2 (amino acids 574-685), Sushi Domain 3 (amino acids 690-748), Sushi Domain 4 (amino acids 750-813), Sushi Domain 5 (amino acids 817-878), and Sushi Domain 5 + C-terminus (amino acids 817-904). Additionally, the N terminal domain (amino acids 1-335) was divided into 3 fragments termed N1 (amino acids 1-70), N2 (amino acids 71-169) and N3 (amino acids 169-335), each of which was cloned into the yeast display plasmid. Amino acid numbering does not include the leader peptide. For domain information see generally UniProtKB/Swiss-Prot database entry Q53EL9. These plasmids were transformed into yeast, which were then grown and induced as described in Cochran *et al.* Note that all amino acid numbering is based on mature SEZ6 protein without the 19 aa leader sequence.

To test for binding to a particular construct, 200,000 induced yeast cells expressing the desired construct were washed twice in PBS + 1 mg/mL BSA (PBSA), and incubated in 50 µL of PBSA with chicken anti c-myc (Life Technologies) at 0.1 µg/mL and either 50 nM purified antibody or 1:2 dilution of unpurified supernatant from hybridomas cultured for 7 days. Cells were incubated for 90 minutes on ice and then washed twice in PBSA. Cells were then incubated in 50 µL PBSA with the appropriate secondary antibodies: for murine antibodies, Alexa 488 conjugated anti-chicken, and Alexa 647 conjugated goat anti-mouse (both Life Technologies) were added at 1 µg/mL each, and for humanized or chimeric antibodies, Alexa 647 conjugated anti-chicken (Life Technologies) and R-phycoerythrin conjugated goat anti-human (Jackson Immunoresearch) were added at 1 µg/mL each. After twenty minutes' incubation on ice, cells were washed twice with PBSA and analyzed on a

FACS Canto II.

All modulators bound uniquely to a single domain expressed on yeast cells. In some cases, antibody clones bound specifically to yeast expressing Sushi Domain 5 + C-terminus but not to yeast expressing Sushi Domain 5. These antibody clones were concluded to bind to the C-terminal region only (amino acids 879-904).

Epitopes were classified either as conformational (i.e. discontinuous) or linear. Yeast displaying the SEZ6 ECD construct was heat treated for 30 minutes at 80°C in order to denature the antigen, washed twice in ice-cold PBSA and then subjected to the same staining protocol and flow cytometry analysis as described above. Antibodies that bound to both the denatured and native yeast were classified as binding to a linear epitope, whereas antibodies that bound native yeast but not denatured yeast were classified as conformationally specific.

A summary of the domain-level epitope mapping data of the antibodies tested is presented in TABLE 3 below. Antibodies that bind a linear epitope are underlined and antibodies that bind SEZ6 family members SEZ6L and SEZ6L2 are designated with an asterisk and/or a dagger, respectively.

TABLE 3

Domain	Antibody Clones
N1 (aa 1-70)	SC17.4, SC17.7†*, SC17.9, SC17.56, SC17.81, SC17.101, SC17.114, SC17.120, SC17.134, SC17.151, SC17.162, SC17.SC177, SC17.182, SC17.185, SC17.196, SC17.197, SC17.199
N2 (aa 71-169)	SC17.24, SC17.49, SC17.104, SC17.144, SC17.149, SC17.168, SC17.SC176, SC17.198
N3 (aa 170-335)	SC17.26†, SC17.42, SC17.83, SC17.85, SC17.88, SC17.91, SC17.92, SC17.99, SC17.125, SC17.128, SC17.130, SC17.137, SC17.145, SC17.161, SC17.192, SC17.195
Sushi Domain 1 (aa 336-395)	SC17.34, SC17.36, SC17.46, SC17.75, SC17.82, SC17.87, SC17.97, SC17.116, SC17.129, SC17.SC178, SC17.187, SC17.200
CUB Domain 1 (aa 397-508)	SC17.73, SC17.76, SC17.86, SC17.100, SC17.105, SC17.107, SC17.1SC17, SC17.122, SC17.124, SC17.136, SC17.138, SC17.146, SC17.154, SC17.SC170, SC17.SC174, SC17.189, SC17.201, SC17.202
Sushi Domain 2 (aa 511-572)	SC17.90, SC17.108, SC17.112, SC17.135, SC17.167, SC17.SC173, SC17.SC179, SC17.184, SC17.203, SC17.204
CUB Domain 2 (aa 574-685)	SC17.6†, SC17.28†, SC17.103, SC17.109, SC17.119, SC17.181, SC17.186, SC17.194
Sushi Domain 3 (aa 690-748)	SC17.72, SC17.84, SC17.95, SC17.141, SC17.143, SC17.163
Sushi Domain 4 (aa 750-813)	SC17.SC17, SC17.19†, SC17.93, SC17.102, SC17.121, SC17.140, SC17.156, SC17.159, SC17.166, SC17.SC175, SC17.180, SC17.191, SC17.193
Sushi Domain 5 (aa 817-878)	SC17.74, SC17.106, SC17.142, SC17.190
C terminus (aa 879-904)	SC17.96, SC17.132

An interesting and surprising trend was observed when an *in vitro* cell killing assay was performed using the domain-mapped SEZ6 antibody modulators described in this Example 10. The *in vitro* killing assay, performed essentially as described below in Example 14,

determined the ability of a particular antibody to internalize and kill HEK-293 cells. FIG. 11B is a plot of efficacy of the tested antibodies versus the domains to which they bind. Antibodies that bind to certain domains including: N1, N3, Sushi Domain 1, and Sushi Domain 4, exhibited enhanced *in vitro* killing. The antibodies that associate with Sushi Domain 4, which are very effective at internalizing and killing cells, exhibit a strong correlation with the IGHV1-34 and IKV4-59 murine germline framework regions.

Fine epitope mapping was further performed on selected antibodies to determine the specific amino acids to which they bound. Antibodies that bound to a linear epitope were mapped using the Ph.D.-12 phage display peptide library kit (New England Biolabs E8110S). The antibody selected for epitope mapping was coated onto a Nunc MaxiSorp tube (Nunc) at 50 μg/mL in 3 mL 0.1 M sodium bicarbonate solution, pH 8 and incubated overnight. The tube was blocked with 3% BSA solution in bicarbonate solution. Then, 10^{11} input phage in PBS + 0.1% Tween-20 was allowed to bind, followed by ten consecutive washes with 0.1% Tween-20 to wash away non-binding phage. Remaining phage were eluted with 1 mL 0.2 M glycine for 10 minutes at room temperature with gentle agitation, followed by neutralization with 150 μL 1 M Tris-HCl pH 9. Eluted phage were amplified and panned again with 10^{11} input phage, using 0.5% Tween-20 during the wash steps to increase selection stringency. DNA from 24 plaques of the eluted phage from the second round was isolated using the Qiaprep M13 Spin kit (Qiagen) and sequenced. Binding of clonal phage was confirmed using an ELISA assay, where the mapped antibody or a control antibody was coated onto an ELISA plate, blocked, and exposed to each clone phage. Phage binding was detected using horseradish peroxidase conjugated anti-M13 antibody (GE Healthcare), and the 1-Step Turbo TMB ELISA solution (Pierce). Phage peptide sequences from specifically binding phage were aligned using Vector NTI (Life Technologies) against the antigen ECD peptide sequence to determine the epitope of binding.

Antibodies that bound to a discontinuous epitope were mapped using the technique described by Chao *et al.* (2007). Libraries of SEZ6 ECD mutants were generated with error prone PCR using nucleotide analogues 8-oxo-2'deoxyguanosine-5'-triphosphate and 2'-deoxy-p-nucleoside-5'triphosphate (both from TriLink Bio) for a target mutagenesis rate of one amino acid mutation per clone. These were transformed into a yeast display format. Using the technique described above for domain-level mapping, the library was stained for c-

myc and antibody binding at 50 nM. Using a FACS Aria (BD), clones that exhibited a loss of binding compared to wild type SEZ6 ECD were sorted. These clones were re-grown, and subjected to another round of FACS sorting for loss of binding to the target antibody. Using the Zymoprep Yeast Plasmid Miniprep kit (Zymo Research), individual ECD clones were isolated and sequenced. Where necessary, mutations were reformatted as single-mutant ECD clones using the Quikchange site directed mutagenesis kit (Agilent).

Individual ECD clones were next screened to determine whether loss of binding was due to a mutation in the epitope, or a mutation that caused misfolding. Mutations that involved cysteine, proline, and stop codons were automatically discarded due to the high likelihood of a misfolding mutation. Remaining ECD clones were then screened for binding to a non-competing, conformationally specific antibody. ECD clones that lost binding to non-competing, conformationally specific antibodies were concluded to contain misfolding mutations, whereas ECD clones that retained equivalent binding as wild type SEZ6 ECD were concluded to be properly folded. Mutations in the ECD clones in the latter group were concluded to be in the epitope. Homology models of isolated domains were also constructed using MODELLER to confirm that residues identified to be in the epitope: 1) were localized in close proximity to each other in the folded homology model, and 2) had side chains that were solvent exposed, and not buried, since buried residues would have a higher chance of causing misfolding, and would unlikely be part of the epitope of binding. A summary of antibodies with their epitopes are listed in Table 4.

TABLE 4

Antibody Clone	Epitope	Discontinuous	SEQ ID NO:
SC17.4	Q12, P14, I16, E17, E18	No	401
SC17.17	R762, D781, Q782	Yes	NR
SC17.24	L73, P74, F75, Q76, P77, D78, P79	No	402
SC17.34	T352, S353, H375	Yes	NR
SC17.36	T352, S353, H375, S359	Yes	NR
SC17.46	R343, K389	Yes	NR

NR indicates that no SEQ ID NO was assigned as the epitopes were discontinuous.

In the case of SC17.34, SC17.36 and SC17.46, point mutations were constructed on the isolated domain, Sushi Domain 1, which was determined to be the domain of binding by domain-level epitope mapping. In the case of SC17.46, candidate mutations for screening were not identified in a library-based screen; rather they were identified on the basis of domain mapping, lack of cross reactivity to cynomolgus SEZ6 ECD and rat SEZ6 ECD, and sequence alignments of the different species to identify differences in the species' primary sequence. These candidate mutations were subjected to the same analysis as other antibodies to confirm the epitope of SC17.46.

Example 11

Detection of SEZ6 Surface Expression by Flow Cytometry

Flow cytometry was used to assess the specificity of the anti-SEZ6 antibodies that were generated for detecting the presence of human SEZ6 protein on the surface of engineered HEK-293T cell lines, constructed as described in Example 5. Isotype-stained and fluorescence minus one (FMO) controls were employed to confirm staining specificity. Briefly, HEK-293T transduced with human SEZ6 and GFP (see Example 5) or harvested NTX tumor samples were dissociated and dispersed into suspension using art-recognized enzymatic digestion techniques (see, for example, U.S.P.N. 2007/0292414 which is incorporated herein), were incubated for 30 minutes with an anti-SEZ6 antibody. Cells were washed in PBS (2%FCS) twice and then incubated with 50 µl per sample DyLight 649 labeled goat-anti-mouse IgG, Fc fragment specific secondary diluted 1:200 in PBS buffer. After a 15 minute incubation cells were washed twice with PBS and re-suspended in PBS with DAPI and analyzed by flow cytometry as previously discussed.

As demonstrated by the representative data shown in FIG.12A for SC17.33, the SEZ6 modulator strongly recognized HEK-293T-HuSEZ6 cells. These data demonstrate that modulators were produced that specifically recognized human SEZ6 expressed on the cell surface.

Human SEZ6 protein expression on the surface of selected NTX tumors was assessed by flow cytometry using several exemplary SC17 antibodies. The expression of SEZ6 in LU37, LU86 and KDY66 was tested using the SC17.6 antibody while expression of SEZ6 in LU50, LU100 and LU73 was tested using the SC17.10, SC17.42 and SC17.28 antibodies

respectively. The results are set forth in FIG. 13A. NTX tumors were harvested, dissociated, and co-stained with commercially available anti-mouse CD45, anti-mouse H-2Kd, anti-human EpCAM and one the above-described mouse anti-human SEZ6 antibodies. Data shown in FIG. 13A was generated using cells that did not stain positively for the above mentioned anti-mouse antibodies but did stain positively for anti-human EpCAM. Similar to the HEK-293T-staining experiments described above, isotype-stained and fluorescence minus one (FMO) controls were employed to confirm staining specificity. As seen in FIG. 13A, anti-SEZ6 staining was higher than FMO in all of the human NTX tumor cells, as indicated by the fluorescent profile shift to the right, and by changes in the mean fluorescence intensity (MFI) values, for the lung NTX tumors LU37, LU50 and LU86 and kidney NTX tumor, KDY66. These data suggest that the SEZ6 protein is expressed on the surface of various NTX tumors and therefore amenable to modulation using an anti-SEZ6 antibody.

Example 12

Expression of SEZ6 Protein in Various Tumors

Given the elevated SEZ6 mRNA transcript levels associated with various tumors, work was undertaken to demonstrate a corresponding increase in the expression of SEZ6 protein in NTX tumors. SEZ6 protein expression was detected with (i) an electrochemiluminscence SEZ6 sandwich ELISA assay using the MSD Discovery Platform (Meso Scale Discovery, LLC); and (ii) immunohistochemistry staining.

NTX tumors were excised from mice and flash frozen on dry ice/ethanol. Protein Extraction Buffer (Biochain Institute, Inc.) was added to the thawed tumor pieces and tumors were pulverized using a TissueLyser system (Qiagen). Lysates were cleared by centrifugation (20,000g, 20 minutes, 4°C) and the total protein concentration in each lysate was quantified using bicinchoninic acid. Protein lysates were stored at -80°C until assayed. Normal tissue lysates were purchased from Novus Biologicals.

SEZ6 protein concentrations from the lysate samples were determined by interpolating the values from a standard protein concentration curve that was generated using purified recombinant SEZ6 protein (Example 5). The SEZ6 protein standard curve and protein quantification assay were conducted as follows:

MSD standard plates were coated overnight at 4°C with 30 µL of SC17.17 antibody at

2 μg/mL in PBS. Plates were washed in PBST and blocked in 150 μL MSD 3% Blocker A solution for one hour. Plates were again washed in PBST. The SC17.36 antibody was then conjugated to the MSD sulfo-tag and 25 μL of the tagged SC17.36 was added to the washed plates at 0.5 μg/mL in MSD 1% Blocker A. 25 μL of 10x diluted lysate in MSD 1% Blocker A or serially diluted recombinant SEZ6 standard in MSD 1% Blocker A containing 10% Protein Extraction Buffer was also added to the wells and incubated for two hours. Plates were washed in PBST. MSD Read Buffer T with surfactant was diluted to 1X in water and 150 μL was added to each well. Plates were read on a MSD Sector Imager 2400 using an integrated software analysis program to derive SEZ6 concentrations in NTX samples via interpolation from the standard curve. Values were then divided by total protein concentration to yield nanograms of SEZ6 per milligram of total lysate protein. The resulting concentrations are set forth in FIG. 12B wherein each spot represents SEZ6 protein concentrations derived from a single NTX tumor line. While each spot is derived from a single NTX line, in most cases multiple biological samples were tested from the same NTX line and values were averaged to provide the data point.

FIG. 12B shows that compared to normal tissue lysates, selected kidney, ovarian and LCNEC tumor samples exhibited moderate SEZ6 protein expression whereas the highest SEZ6 protein expression was seen in SCLC tumors. All normal tissue lysates were negative for SEZ6 protein expression with the exception of normal human brain and eye lysate.

Immunohistochemistry (IHC) was performed on PDX tumors to confirm that SEZ6 is expressed on the surface of certain PDX tumors; and in order to determine the location of the SEZ6 protein in the tumor architecture.

IHC was performed on formalin fixed paraffin embedded tissue sections using an indirect detection method which included murine monoclonal primary antibody against SEZ6 (clone 17.140), mouse specific biotin conjugated secondary antibodies, avidin/biotin complex coupled with horse radish peroxidase, and DAB detection (Nakene PK 1968; 16:557-60). When staining xenograft PDX tumors, a mouse IgG blocking agent (Vector Laboratories; catalog no. PK-2200) was used. SC17.140 was validated and confirmed to be appropriate for IHC by showing specific staining on sections of HEK-293T cell pellets overexpressing SEZ6 compared to naïve HEK-293T cell pellets, prepared as known in the art. Specificity was further confirmed by competing signal with a 5 molar excess of purified recombinant SEZ6

on HEK-293T cells overexpressing human SEZ6 and xenograft tumors that were shown by IHC to express SEZ6 (data not shown). FIG. 16 shows SEZ6 expression as measured by IHC in SCLC NTX tumors. Staining intensity was scored to take into account intensity of staining from 0 (negative) to 3 (strong staining). The results show that 64% of the SCLC NTX tumors tested, expressed SEZ6.

These data, combined with the mRNA transcription data for SEZ6 expression set forth above (Example 4), and cell surface protein expression of SEZ6 (Example 11), strongly reinforces the proposition that SEZ6 determinants provide attractive targets for therapeutic intervention.

Example 13

Enrichment of Tumor Initiating Cell Populations

Tumor cells can be divided broadly into two types of cell subpopulations: non-tumorigenic cells (NTG) and tumor initiating cells (TICs). TICs have the ability to form tumors when implanted into immunocompromised mice. Cancer stem cells (CSCs) are a subset of TICs and are able to self-replicate indefinitely while maintaining the capacity for multilineage differentiation. To determine whether SEZ6 expression could be correlated with enhanced tumorigenicity whole transcriptome sequencing, flow cytometry and a tumorigenicity assay were performed, all of which are described below.

Whole transcriptome analysis of SEZ6 expression in various tumor samples was performed as described in Example 1. CSCs were identified on the basis of expression of CD324 which has been shown to be a marker of stem cells in various tumors (see PCT application 2012/031280). The results in FIG. 6A show that SEZ6 mRNA expression was elevated in CSCs compared to NTG cells isolated from two SCLC NTX tumor lines (LU86 and LU95).

Flow cytometry was performed on cells from NTX lung tumors essentially as described in Example 11. LU86, LU117 and LU64 cells were co-stained with CD324, a marker of CSC populations (see PCT application 2012/031280), and the anti-SEZ6 antibody, SC17.10, SC17.28 or SC17.42, respectively to determine if SEZ6 is differentially expressed on these populations. As indicated in FIG. 13B, LU86, LU117 and LU64 cells staining positive for both CD324 and SEZ6 (solid black line) shift further to the right compared to cells staining

positive for SEZ6 alone (dotted black line), indicating that SEZ6 is more highly expressed on CSCs compared to the NTG cell population. The bulk population isotype control is shown as a gray filled histogram (MOPC = IgG1).

To determine whether cell surface SEZ6 expression could be correlated with enhanced ability to generate tumors, a tumorigenicity study was conducted. NTX tumor samples were dissociated and dispersed into suspension using art-recognized enzymatic digestion techniques (see, for example, U.S.P.N. 2007/0292414 which is incorporated herein). The dissociated cell preparations from these NTX lines were stained with fluorescently conjugated antibodies specifically recognizing murine CD45, H2kD, human CD324, and human SEZ6, clone SC17.42. Two subsets of human cells, both identified based on the absence of staining with murine CD45 or H2kD (to deplete the cell preparations of murine cells) were isolated using a FACSAria™ Flow Cytometer (BD Biosciences). One subset was isolated on the basis of a CD324 and SEZ6 co-expression, while the other subset was isolated on the basis of a $CD324^{+}SEZ6^{-}$ phenotype. The distinct marker-enriched subpopulations were subsequently transplanted into female NOD/SCID immunocompromised mice by subcutaneous injection into the mammary fat pad at a dose of approximately 50 cells per mouse.

FIGS. 14A and 14B illustrate the results of such experiments conducted using representative NTX cell lines derived from NSCLC tumors obtained from patients. FIG. 14A is a scatter plot (gated using CD324 and SEZ6) showing the distribution of $mCD45^{-}H2kD^{-}$ subset of the parent tumor and sorted putative tumorigenic cells. FIG. 14B graphically shows the measured tumor volume arising from the implantation of sorted cell subpopulations into immunocompromised mice. Values in parenthesis indicate the number of tumors generated per mice implanted.

Significantly, the data from FIG. 14 show that tumorigenicity was consistently associated with the subpopulation of cells expressing SEZ6 in combination with high levels of CD324. Conversely, these same data demonstrate that tumor cells expressing either no, or low levels of SEZ6 were much less tumorigenic than their high or positive counterparts. Based on the generated data it was surprisingly found that subpopulations of tumor cells expressing the $CD324^{+}SEZ6^{+}$ phenotype generally contain the vast majority of tumorigenic capability and suggest that SEZ6 may provide an effective therapeutic target for tumorigenic cell modulation.

Example 14

SEZ6 Modulators Facilitate Delivery of Cytotoxic Agents to SEZ6-Expressing HEK-293T Cells

To demonstrate that SEZ6 modulators of the instant invention are able to mediate the delivery of a cytotoxic agent to live cells, an *in vitro* cell killing assay was performed using selected SEZ6 antibody modulators bound to a saporin toxin. Saporin kills cells by deactivating ribosomes in the cytoplasm. Thus cell death using the following assay is an indication that the SEZ6 antibodies are able to internalize and deliver cytotoxic agents to the cytoplasm of a target cell.

An anti-Mouse IgG Fab fragment covalently linked to saporin ("Fab-Saporin") (Advanced Targeting Systems, #IT-48) was combined with unlabeled SEZ6 antibodies and incubated with HEK-293T cells expressing human SEZ6 (see Example 5). The ability of the resulting saporin complexes to internalize and kill cells was measured 72 hours later by measuring cell viability.

Specifically, 500 cells per well in DMEM supplemented with 10% fetal bovine serum, were plated into 96 well tissue culture treated plates one day before the addition of antibodies and toxin. HEK-293T cells expressing human SEZ6 were treated with a control (IgG1, IgG2a or IgG2b) or purified murine SEZ6 modulators at a concentration of 100, 50 or 10 pM, together with 2 nM Fab-Saporin. The cells were cultured for three days, after which, viable cell numbers were enumerated using Cell Titer Glo® (Promega) as per manufacturer's instructions. Raw Luminescence Units (RLU) using cultures containing cells with the Saporin Fab fragment were set as 100% reference values and all other counts calculated accordingly (referred to as Normalized RLU or "% live cells"). FIG. 15A shows that many of the SEZ6 modulators tested mediated the killing of HEK-293T cells in a concentration dependent manner. Isotype controls (IgG2a, IgG2b, and IgG1) did not affect cell counts as shown by the results in the first three rows of FIG. 15A (ND = Not Determined).

This assay demonstrates that internalization may occur upon binding of the SEZ6-specific antibody to the cell surface, without the need for additional crosslinking or dimerization.

Example 15

SEZ6 Modulators Mediate Cytotoxicity in Lung Tumor Cells *in vitro*

To corroborate the results of Example 14 and determine whether SEZ6 modulators can mediate toxin internalization and cell killing of human tumor cells (as opposed to engineered cells), mouse lineage-depleted NTX cells were plated and subsequently exposed to anti-SEZ6 antibodies and Fab-saporin.

NTX tumors were dissociated into a single cell suspension and plated on Primaria™ plates (BD Biosciences) in growth factor supplemented serum free media as is known in the art. After culturing the cells for one day at 37˚C/5%CO_2/5%O_2, they were treated with a control (IgG1, IgG2a or IgG2b) or a murine SEZ6 modulator and Fab-saporin as described in Example 14. After seven days, the modulator-mediated saporin cytotoxicity was assessed by quantifying the remaining number of live cells using Cell Titer Glo.

As seen in FIG. 15B a reduction in the number of tumor cells was evident when LU37, a NSCLC tumor and LU80, a SCLC tumor were exposed to SC17.6 (duplicative of SC17.16) and SC17.33 SEZ6 modulators. Similarly when LU100, a SCLC tumor was exposed to four SEZ6 modulators, SC17.6, SC17.19, SC17.33 and SC17.34, at 50 and 500 pM a reduction of tumor cells was effected. In contrast, isotype control antibodies did not impact the number of live cells after treatment.

Not only does this data demonstrate that exemplary antibodies described herein are able to bind SEZ6 antigen on the cell surface and facilitate the delivery of a cytotoxic payload resulting in cell death, but the above data also demonstrated that multiple anti-SEZ6 antibodies can mediate killing of various NTX tumor cells.

Example 16

Preparation of SEZ6 Antibody-Drug Conjugates

Based on the *in vitro* killing assays with saporin in Examples 14 and 15 and to further demonstrate the versatility of the instant invention, anti-SEZ6 antibody drug conjugates were prepared having the M-[L-D] structure as described above. That is, anti-SEZ6 antibody drug conjugates (SEZ6-ADCs) were prepared using covalently linked cytotoxic agents. More specifically, SEZ6-ADCs were prepared comprising a linker as described herein, or in the references immediately below, and selected pyrrolobenzodiazepine (PBD) dimers that were

covalently attached to the disclosed modulators (see, e.g., U.S.P.Ns. 2011/0256157 and 2012/0078028 and U.S.P.N 6,214,345 each of which is incorporated herein by reference in its entirety).

PBD drug-linker combinations were synthesized and purified using art-recognized techniques in view of the cited references. While various PBD dimers and linkers were employed to fabricate the selected drug-linker combinations, each linker unit comprised a terminal maleimido moiety with a free sulfhydryl. Using these linkers, conjugations were prepared via partial reduction of the mAb with tris (2-carboxyethyl)-phosphine (TCEP) followed by reaction of reduced Cys residues with the maleimido-linker payload.

More particularly, the selected SEZ6 antibody modulator was reduced with 1.3 mol TCEP per mol mAb for 2 hr at 37°C in 25 mM Tris HCl pH 7.5 and 5 mM EDTA buffer. The reaction was allowed to cool to 15°C and the linker payload in DMSO was added at a ratio of 2.7 mol/mol mAb followed by an additional amount of DMSO to a final concentration of 6% (v/v). The reaction was allowed to proceed for 1 hour. The unreacted drug-linker was capped by addition of an excess of N-acetyl cysteine. The SEZ6-ADC (or SC17-ADC) was then purified by ion exchange column using an AKTA Explorer FPLC system (G.E. Healthcare) to remove aggregated high molecular weight antibody, co-solvent and small molecules. The eluted ADC was then buffer-exchanged by tangential flow filtration (TFF) into formulation buffer followed by concentration adjustment and addition of a detergent. The final ADC was analyzed for protein concentration (by measuring UV), aggregation (SEC), drug to antibody ratio (DAR) by reverse phase (RP) HPLC, presence of unconjugated antibody by hydrophobic interaction chromatography (HIC) HPLC, non-proteinaceous materials by RP HPLC and *in vitro* cytotoxicity using a SEZ6 expressing cell line.

Using the aforementioned procedure, or substantially similar methodology, a number of ADCs (i.e., M-[L-D]n) comprising various SEZ6 modulators and PBD dimers were generated and tested in a variety of *in vivo* and *in vitro* models. For the purposes of these Examples and the instant disclosures, such ADCs may generally be termed SEZ6-ADCs or SC17-ADCs. Discrete ADCs will be named according to the antibody (e.g., SC17.17) and the specific linker- cytotoxic agent designation ADC1, ADC2, etc. Thus, exemplary modulators compatible with the instant invention may comprise SC17.17-ADC1 or SC17.24-ADC2

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LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 190

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 190

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

CLAIMS:

1. An isolated SEZ6 modulator.

2. The isolated SEZ6 modulator of claim 1, wherein the SEZ6 modulator comprises an antibody or immunoreactive fragment thereof.

3. The isolated SEZ6 modulator of claim 2 wherein the antibody or immunoreactive fragment thereof comprises a monoclonal antibody.

4. The isolated SEZ6 modulator of claim 3 wherein the monoclonal antibody is selected from the group consisting of chimeric antibodies, humanized antibodies and human antibodies.

5. The isolated SEZ6 modulator of claims 3 or 4 wherein said monoclonal antibody or immunoreactive fragment thereof binds to a SEZ6 protein sushi domain or cub domain.

6. The isolated SEZ6 antibody modulator of any of claims 2 to 5 wherein said antibody or immunoreactive fragment thereof comprises a light chain variable region having three complementarity determining regions and a heavy chain variable region having three complementarity determining regions wherein the heavy and light chain complementarity determining regions comprise at least one complementarity determining region set forth in FIG. 10A or FIG. 10B.

7. The isolated SEZ6 antibody modulator of any of claims 2 to 5 that competes for binding to a SEZ6 protein with a reference antibody selected from the group consisting of SC17.1, SC17.2, SC17.3, SC17.4, SC17.8, SC17.9, SC17.10, SC17.11, SC17.14, SC17.15, SC17.16, SC17.17, SC17.18, SC17.19, SC17.22, SC17.24, SC17.27, SC17.28, SC17.29, SC17.30, SC17.32, SC17.34, SC17.35, SC17.36, SC17.38, SC17.39, SC17.40, SC17.41, SC17.42, SC17.45, SC17.46, SC17.47, SC17.49, SC17.50, SC17.53, SC17.54, SC17.56, SC17.57, SC17.59, SC17.61, SC17.63, SC17.71, SC17.72, SC17.74, SC17.76, SC17.77, SC17.79, SC17.81, SC17.82, SC17.84, SC17.85, SC17.87, SC17.89, SC17.90, SC17.91, SC17.93, SC17.95, SC17.97, SC17.99, SC17.102, SC17.114, SC17.115, SC17.120, SC17121, SC17.122, SC17.140, SC17.151, SC17.156, SC17.161, SC17.166, SC17.187, SC17.191, SC17.193, SC17.199 and SC17.200 wherein binding of the SEZ6 antibody modulator to the SEZ6 protein is inhibited by at least 30%.

8. The isolated SEZ6 modulator of any of claims 2 to 7 wherein said antibody comprises an internalizing antibody.

9. The isolated SEZ6 modulator of any of claims 2 to 8 wherein said antibody further comprises a cytotoxic agent.

10. A nucleic acid encoding an amino acid heavy chain variable region or an amino acid light chain variable region of an antibody or immunoreactive fragment thereof of claims 1-8.

11. A host cell comprising the nucleic acid of claim 10.

12. An antibody drug conjugate of the formula:

M-[L-D]n or a pharmaceutically acceptable salt thereof wherein

a) M comprises a SEZ6 modulator;

b) L comprises an optional linker;

c) D is a anti-proliferative agent; and

d) n is an integer from about 1 to about 20.

13. The antibody drug conjugate of claim 12 wherein said SEZ6 modulator comprises an antibody or immunoreactive fragment thereof and said antibody or immunoreactive fragment thereof comprises a light chain variable region having three complementarity determining regions and a heavy chain variable region having three complementarity determining regions wherein the heavy and light chain complementarity determining regions comprise at least one complementarity determining region set forth in FIG. 10A or FIG. 10B.

14. The antibody drug conjugate of claims 12 or 13 wherein said SEZ6 modulator comprises an internalizing antibody.

15. The isolated SEZ6 modulator of any of claims 1-9 or the antibody drug conjugate of claims 12 or 14 for use in a medicine.

16. The isolated SEZ6 modulator of any of claims 1-9 or the antibody drug conjugate of claims 12 or 14 for the treatment of cancer in a patient wherein the cancer is selected from the group consisting of adrenal cancer, bladder cancer, cervical cancer, endometrial cancer, kidney cancer, liver cancer, lung cancer, ovarian cancer, colorectal cancer, pancreatic cancer, prostate cancer and breast cancer.

17. Use of a SEZ6 modulator for the manufacture of a medicament for treating small cell

lung cancer in a subject in need thereof.

18. The medicament of claim 17 wherein said SEZ6 modulator comprises a monoclonal antibody.

19. The medicament of any of claims 17 or 18 wherein said SEZ6 modulator binds to a SEZ6 protein sushi domain or cub domain.

20. The medicament of any of claims 17 to 19 wherein said SEZ6 modulator comprises a cytotoxic agent.

Homo sapiens seizure related 6 homolog (SEZ6), transcript variant 1, mRNA >gi|148839279|ref|NM_178860.4|

(SEQ ID NO: 1)

GATCCCCGGCGCCGTCGCCAGGCGCTGGCCGTGGTGCTGATTCTGTCAGGCGCTGGCGGCGGCAGCGGCGGTGACGGCTGCGG
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AGCCCAGGGCGCGGCCGCAACCAGCACCATGCGCCCGGTAGCCCTGCTGCTCCTGCCCTCGCTGCTGGCGCTCCTGGCTCACG
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GGAATTCCTACAAGAGGGGCTGGAAAAGGGAGATGAGGAGCTGAGGCCAGCACTGCCCTTCCAGCCTGACCCACCTGCACCCT
TCACCCCAAGTCCCCTTCCCCGCCTGGCCAACCAGGACAGCCGCCCTGTCTTTACCAGCCCCACTCCAGCCATGGCTGCGGTA
CCCACTCAGCCCCAGTCCAAGGAGGGACCCTGGAGTCCGGAGTCAGAGTCCCCTATGCTTCGAATCACAGCTCCCCTACCTCC
AGGGCCCAGCATGGCAGTGCCCACCCTAGGCCCAGGGGAGATAGCCAGCACTACACCCCCCAGCAGAGCCTGGACACCAACCC
AAGAGGGTCCTGGAGACATGGGAAGGCCGTGGGTTGCAGAGGTTGTGTCCCAGGGCGCAGGGATCGGGATCCAGGGGACCATC
ACCTCCTCCACAGCTTCAGGAGATGATGAGGAGACCACCACTACCACCACCATCATCACCACCACCATCACCACAGTCCAGAC
ACCAGGCCCTTGTAGCTGGAATTTCTCAGGCCCAGAGGGCTCTCTGGACTCCCCTACAGACCTCAGCTCCCCCACTGATGTTG
GCCTGGACTGCTTCTTCTACATCTCTGTCTACCCTGGCTATGGCGTGGAAATCAAGGTCCAGAATATCAGCCTCCGGGAAGGG
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CCGCAGCCCCACCCACCAAGCGGCCCTGAGGTTCCAGAGCCTCCCGCCACCGGCTGGCCCTGGCACCTTCCATTTCCATTACC
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TTCAAAGGAGCCCGTCTGCATCGCTGCTTGCGGCGGAGTGATCCGCAATGCCACCACCGGCCGCATCGTCTCTCCAGGCTTCC
CGGGCAACTACAGCAACAACCTCACCTGTCACTGGCTGCTTGAGGCTCCTGAGGGCCAGCGGCTACACCTGCACTTTGAGAAG
GTTTCCCTGGCAGAGGATGATGACAGGCTCATCATTCGCAATGGGGACAACGTGGAGGCCCCACCAGTGTATGATTCCTATGA
GGTGGAATACCTGCCCATTGAGGGCCTGCTCAGCTCTGGCAAACACTTCTTTGTTGAGCTCAGTACTGACAGCAGCGGGGCAG
CTGCAGGCATGGCCCTGCGCTATGAGGCCTTCCAGCAGGGCCATTGCTATGAGCCCTTTGTCAAATACGGTAACTTCAGCAGC
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CGAGTGTGTTGACCCCCACGACCCCCAGTGGAATGAGACAGAGCCAGCCTGCCGAGCCGTGTGCAGCGGGGAGATCACAGACT
CGGCTGGCGTGGTACTCTCTCCCAACTGGCCAGAGCCCTACGGTCGTGGGCAGGATTGTATCTGGGGTGTGCATGTGGAAGAG
GACAAGCGCATCATGCTGGACATCCGAGTGCTGCGCATAGGCCCTGGTGATGTGCTTACCTTCTATGATGGGGATGACCTGAC
GGCCCGGGTTCTGGGCCAGTACTCAGGGCCCCGTAGCCACTTCAAGCTCTTTACCTCCATGGCTGATGTCACCATTCAGTTCC
AGTCGGACCCCGGGACCTCAGTGCTGGGCTACCAGCAGGGCTTCGTCATCCACTTCTTTGAGGTGCCCCGCAATGACACATGT
CCGGAGCTGCCTGAGATCCCCAATGGCTGGAAGAGCCCATCGCAGCCTGAGCTAGTGCACGGCACCGTGGTCACTTACCAGTG
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AAAAAAAAAAAAAAAA

FIG. 1A

Homo sapiens seizure related 6 homolog (SEZ6), transcript variant 2, mRNA>gi|148839345|ref|NM_001098635.1|

(SEQ ID NO: 2)

GATCCCCGGCGCCGTCGCCAGGCGCTGGCCGTGGTGCTGATTCTGTCAGGCGCTGGCGGCGGCAGCGGCGGTGACGGCTGCGG
CCCCGCTCCCTCTACCCGGCCGGACCCGGCTCTGCCCCCGCGCCCAAGCCCCACCAAGCCCCCCGCCCTCCCGCCGCGGTCCC
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GACTCTCTTTAGAGGCCCCAACCGTGGGGAAAGGACAAGCCCCAGGCATCGAGGAGACAGATGGCGAGCTGACAGCAGCCCCC
ACACCTGAGCAGCCAGAACGAGGCGTCCACTTTGTCACAACAGCCCCCACCTTGAAGCTGCTCAACCACCACCCGCTGCTTGA
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CCCACTCAGCCCCAGTCCAAGGAGGGACCCTGGAGTCCGGAGTCAGAGTCCCCTATGCTTCGAATCACAGCTCCCCTACCTCC
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ACCAGGCCCTTGTAGCTGGAATTTCTCAGGCCCAGAGGGCTCTCTGGACTCCCCTACAGACCTCAGCTCCCCCACTGATGTTG
GCCTGGACTGCTTCTTCTACATCTCTGTCTACCCTGGCTATGGCGTGGAAATCAAGGTCCAGAATATCAGCCTCCGGGAAGGG
GAGACAGTGACTGTGGAAGGCCTGGGGGGGCCTGACCCACTGCCCCTGGCCAACCAGTCTTTCCTGCTGCGGGGCCAAGTCAT
CCGCAGCCCCACCCACCAAGCGGCCCTGAGGTTCCAGAGCCTCCCGCCACCGGCTGGCCCTGGCACCTTCCATTTCCATTACC
AAGCCTATCTCCTGAGCTGCCACTTTCCCCGTCGTCCAGCTTATGGAGATGTGACTGTCACCAGCCTCCACCCAGGGGGTAGT
GCCCGCTTCCATTGTGCCACTGGCTACCAGCTGAAGGGCGCCAGGCATCTCACCTGTCTCAATGCCACCCAGCCCTTCTGGGA
TTCAAAGGAGCCCGTCTGCATCGCTGCTTGCGGCGGAGTGATCCGCAATGCCACCACCGGCCGCATCGTCTCTCCAGGCTTCC
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CACCAAAGCAGGACACGCCATTTTCCACTGACCACCCTATACCCTGAGGAAAGGGAGACTTTCCTCCGATGTTTATTTAGCTG
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A

FIG. 1B

Homo sapiens seizure protein 6 homolog isoform 1 precursor >gi|148839280|ref|NP_849191.3|

(SEQ ID NO: 3)

MRPVALLLLPSLLALLAHGLSLEAPTVGKGQAPGIEETDGELTAAPTPEQPERGVHFVTTAPTLKLLNHH
PLLEEFLQEGLEKGDEELRPALPFQPDPPAPFTPSPLPRLANQDSRPVFTSPTPAMAAVPTQPQSKEGP
WSPESESPMLRITAPLPPGPSMAVPTLGPGEIASTTPPSRAWTPTQEGPGDMGRPWVAEVVSQGAGI
GIQGTITSSTASGDDEETTTTTTIITTTITTVQTPGPCSWNFSGPEGSLDSPTDLSSPTDVGLDCFFYISVYP
GYGVEIKVQNISLREGETVTVEGLGGPDPLPLANQSFLLRGQVIRSPTHQAALRFQSLPPPAGPGTFHFH
YQAYLLSCHFPRRPAYGDVTVTSLHPGGSARFHCATGYQLKGARHLTCLNATQPFWDSKEPVCIAACGG
VIRNATTGRIVSPGFPGNYSNNLTCHWLLEAPEGQRLHLHFEKVSLAEDDDRLIIRNGDNVEAPPVYDSY
EVEYLPIEGLLSSGKHFFVELSTDSSGAAAGMALRYEAFQQGHCYEPFVKYGNFSSSTPTYPVGTTVEFS
CDPGYTLEQGSIIIECVDPHDPQWNETEPACRAVCSGEITDSAGVVLSPNWPEPYGRGQDCIWGVHVE
EDKRIMLDIRVLRIGPGDVLTFYDGDDLTARVLGQYSGPRSHFKLFTSMADVTIQFQSDPGTSVLGYQQ
GFVIHFFEVPRNDTCPELPEIPNGWKSPSQPELVHGTVVTYQCYPGYQVVGSSVLMCQWDLTWSEDL
PSCQRVTSCHDPGDVEHSRRLISSPKFPVGATVQYICDQGFVLMGSSILTCHDRQAGSPKWSDRAPKCL
LEQLKPCHGLSAPENGARSPEKQLHPAGATIHFSCAPGYVLKGQASIKCVPGHPSHWSDPPPICRAASL
DGFYNSRSLDVAKAPAASSTLDAAHIAAAIFLPLVAMVLLVGGVYFYFSRLQGKSSLQLPRPRPRPYNRITI
ESAFDNPTYETGSLSFAGDERI

FIG. 1C

CA 02865415 2014-08-22

Homo sapiens seizure protein 6 homolog isoform 2 precursor >gi|148839346|ref|NP_001092105.1|

(SEQ ID NO: 4)

MRPVALLLLPSLLALLAHGLSLEAPTVGKGQAPGIEETDGELTAAPTPEQPERGVHFVTTAPT
LKLLNHHPLLEEFLQEGLEKGDEELRPALPFQPDPPAPFTPSPLPRLANQDSRPVFTSPTPAM
AAVPTQPQSKEGPWSPESESPMLRITAPLPPGPSMAVPTLGPGEIASTTPPSRAWTPTQEG
PGDMGRPWVAEVVSQGAGIGIQGTITSSTASGDDEETTTTTTIITTTITTVQTPGPCSWNFS
GPEGSLDSPTDLSSPTDVGLDCFFYISVYPGYGVEIKVQNISLREGETVTVEGLGGPDPLPLA
NQSFLLRGQVIRSPTHQAALRFQSLPPPAGPGTFHFHYQAYLLSCHFPRRPAYGDVTVTSLH
PGGSARFHCATGYQLKGARHLTCLNATQPFWDSKEPVCIAACGGVIRNATTGRIVSPGFPG
NYSNNLTCHWLLEAPEGQRLHLHFEKVSLAEDDDRLIIRNGDNVEAPPVYDSYEVEYLPIEG
LLSSGKHFFVELSTDSSGAAAGMALRYEAFQQGHCYEPFVKYGNFSSSTPTYPVGTTVEFSC
DPGYTLEQGSIIIECVDPHDPQWNETEPACRAVCSGEITDSAGVVLSPNWPEPYGRGQDCI
WGVHVEEDKRIMLDIRVLRIGPGDVLTFYDGDDLTARVLGQYSGPRSHFKLFTSMADVTIQ
FQSDPGTSVLGYQQGFVIHFFEVPRNDTCPELPEIPNGWKSPSQPELVHGTVVTYQCYPGY
QVVGSSVLMCQWDLTWSEDLPSCQRVTSCHDPGDVEHSRRLISSPKFPVGATVQYICDQG
FVLMGSSILTCHDRQAGSPKWSDRAPKCLLEQLKPCHGLSAPENGARSPEKQLHPAGATIH
FSCAPGYVLKGQASIKCVPGHPSHWSDPPPICRAASLDGFYNSRSLDVAKAPAASSTLDAAH
IAAAIFLPLVAMVLLVGGVYFYFSRLQGKSSLQLPRPRPRPYNRITIESAFDNPTYETGETREYE
VSI

FIG. 1D

Alignment of SEZ6 Isoforms

```
                                  1                                                                                80
   hSEZ6v1 (NP_849191)      (1) MRPVALLLLPSLLALLAHGLSLEAPTVGKGQAPGIEETDGELTAAPTPEQPERGVHFVTTAPTLKLLNHHPLLEEFLQEG
hSEZ6v2 (NP_001092105)      (1) MRPVALLLLPSLLALLAHGLSLEAPTVGKGQAPGIEETDGELTAAPTPEQPERGVHFVTTAPTLKLLNHHPLLEEFLQEG
                                 81                                                                               160
   hSEZ6v1 (NP_849191)     (81) LEKGDEELRPALPFQPDPPAPFTPSPLPRLANQDSRPVFTSPTPAMAAVPTQPQSKEGPWSPESESPMLRITAPLPPGPS
hSEZ6v2 (NP_001092105)     (81) LEKGDEELRPALPFQPDPPAPFTPSPLPRLANQDSRPVFTSPTPAMAAVPTQPQSKEGPWSPESESPMLRITAPLPPGPS
                                161                                                                               240
   hSEZ6v1 (NP_849191)    (161) MAVPTLGPGEIASTTPPSRAWTPTQEGPGDMGRPWVAEVVSQGAGIGIQGTITSSTASGDDEETTTTTTIITTTITTVQT
hSEZ6v2 (NP_001092105)    (161) MAVPTLGPGEIASTTPPSRAWTPTQEGPGDMGRPWVAEVVSQGAGIGIQGTITSSTASGDDEETTTTTTIITTTITTVQT
                                241                                                                               320
   hSEZ6v1 (NP_849191)    (241) PGPCSWNFSGPEGSLDSPTDLSSPTDVGLDCFFYISVYPGYGVEIKVQNISLREGETVTVEGLGGPDPLPLANQSFLLRG
hSEZ6v2 (NP_001092105)    (241) PGPCSWNFSGPEGSLDSPTDLSSPTDVGLDCFFYISVYPGYGVEIKVQNISLREGETVTVEGLGGPDPLPLANQSFLLRG
                                321                                                                               400
   hSEZ6v1 (NP_849191)    (321) QVIRSPTHQAALRFQSLPPPAGPGTFHFHYQAYLLSCHFPRRPAYGDVTVTSLHPGGSARFHCATGYQLKGARHLTCLNA
hSEZ6v2 (NP_001092105)    (321) QVIRSPTHQAALRFQSLPPPAGPGTFHFHYQAYLLSCHFPRRPAYGDVTVTSLHPGGSARFHCATGYQLKGARHLTCLNA
                                401                                                                               480
   hSEZ6v1 (NP_849191)    (401) TQPFWDSKEPVCIAACGGVIRNATTGRIVSPGFPGNYSNNLTCHWLLEAPEGQRLHLHFEKVSLAEDDDRLIIRNGDNVE
hSEZ6v2 (NP_001092105)    (401) TQPFWDSKEPVCIAACGGVIRNATTGRIVSPGFPGNYSNNLTCHWLLEAPEGQRLHLHFEKVSLAEDDDRLIIRNGDNVE
                                481                                                                               560
   hSEZ6v1 (NP_849191)    (481) APFVYDSYEVEYLPIEGLLSSGKHFFVELSTDSSGAAAGMALRYEAFQQGHCYEPFVKYGNFSSSTPTYPVGTTVEFSCD
hSEZ6v2 (NP_001092105)    (481) APFVYDSYEVEYLPIEGLLSSGKHFFVELSTDSSGAAAGMALRYEAFQQGHCYEPFVKYGNFSSSTPTYPVGTTVEFSCD
                                561                                                                               640
   hSEZ6v1 (NP_849191)    (561) PGYTLEQGSIIIECVDPHDPQWNETEPACRAVCSGEITDSAGVVLSPNWPEPYGRGQDCIWGVHVEEDKRIMLDIRVLRI
hSEZ6v2 (NP_001092105)    (561) PGYTLEQGSIIIECVDPHDPQWNETEPACRAVCSGEITDSAGVVLSPNWPEPYGRGQDCIWGVHVEEDKRIMLDIRVLRI
                                641                                                                               720
   hSEZ6v1 (NP_849191)    (641) GPGDVLTFYDGDDLTARVLGQYSGPRSHFKLFTSMADVTIQFQSDPGTSVLGYQQGFVIHFFEVPRNDTCPELPEIPNGW
hSEZ6v2 (NP_001092105)    (641) GPGDVLTFYDGDDLTARVLGQYSGPRSHFKLFTSMADVTIQFQSDPGTSVLGYQQGFVIHFFEVPRNDTCPELPEIPNGW
                                721                                                                               800
   hSEZ6v1 (NP_849191)    (721) KSPSQPELVHGTVVTYQCYPGYQVVGSSVLMCQWDLTWSEDLPSCQRVTSCHDPGDVEHSRRLISSPKFPVGATVQYICD
hSEZ6v2 (NP_001092105)    (721) KSPSQPELVHGTVVTYQCYPGYQVVGSSVLMCQWDLTWSEDLPSCQRVTSCHDPGDVEHSRRLISSPKFPVGATVQYICD
                                801                                                                               880
   hSEZ6v1 (NP_849191)    (801) QGFVLMGSSILTCHDRQAGSPKWSDRAPKCLLEQLKPCHGLSAPENGARSPEKQLHPAGATIHFSCAPGYVLKGQASIKC
hSEZ6v2 (NP_001092105)    (801) QGFVLMGSSILTCHDRQAGSPKWSDRAPKCLLEQLKPCHGLSAPENGARSPEKQLHPAGATIHFSCAPGYVLKGQASIKC
                                881                                                                               960
   hSEZ6v1 (NP_849191)    (881) VPGHPSHWSDPPPICRAASLDGFYNSRSLDVAKAPAASSTLDAAHIAAAIFLPLVAMVLLVGGVYFYFSRLQGKSSLQLP
hSEZ6v2 (NP_001092105)    (881) VPGHPSHWSDPPPICRAASLDGFYNSRSLDVAKAPAASSTLDAAHIAAAIFLPLVAMVLLVGGVYFYFSRLQGKSSLQLP
                                961                                994
   hSEZ6v1 (NP_849191)    (961) RPRPRPYNRITIESAFDNPTYETGSLSFAGDERI    (SEQ ID NO: 3)
hSEZ6v2 (NP_001092105)    (961) RPRPRPYNRITIESAFDNPTYETGETREYEVSI-    (SEQ ID NO: 4)
```

FIG. 1E

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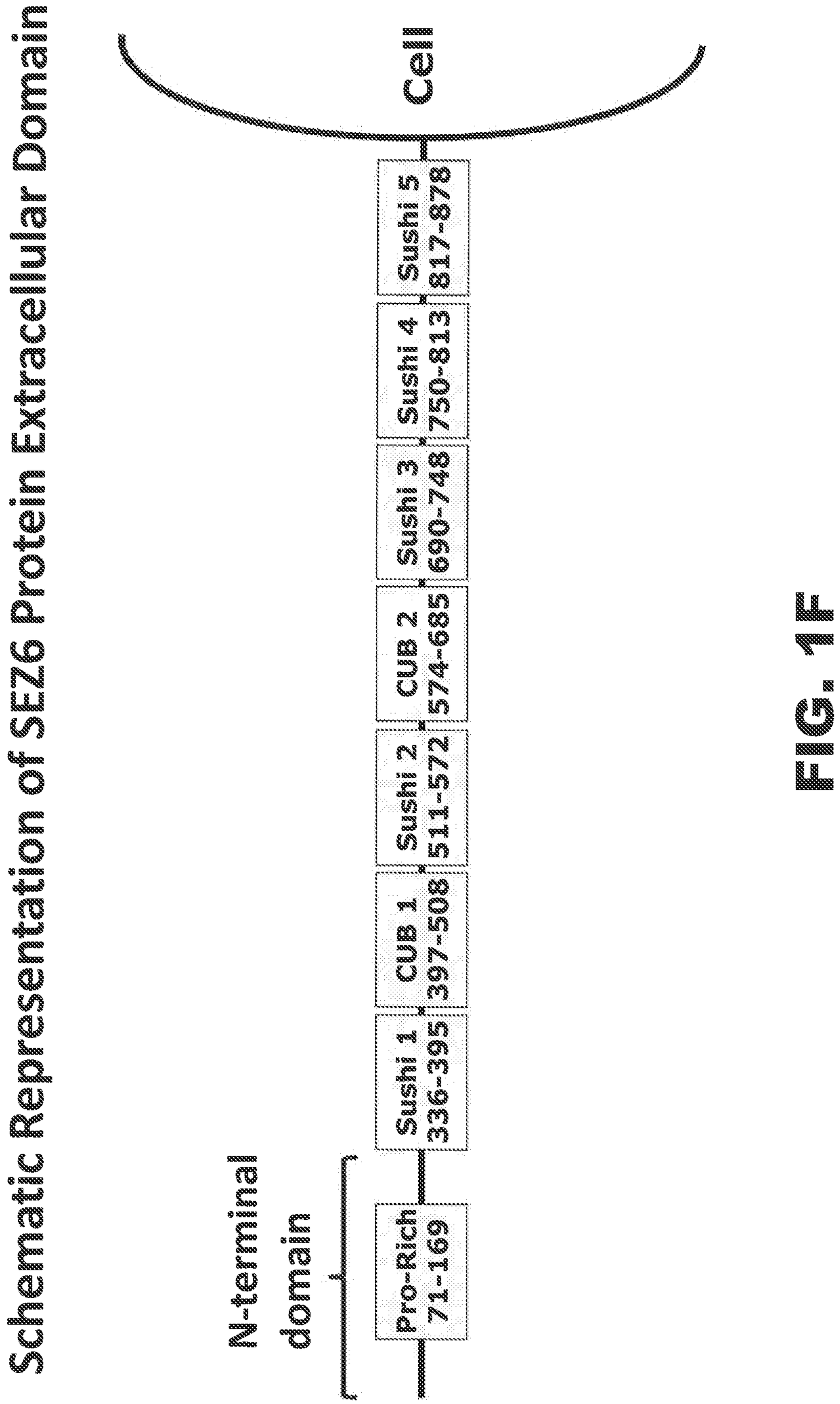
Schematic Representation of SEZ6 Protein Extracellular Domain
N-terminal domain
Pro-Rich 71-169
Sushi 1 336-395
CUB 1 397-508
Sushi 2 511-572
CUB 2 574-685
Sushi 3 690-748
Sushi 4 750-813
Sushi 5 817-878
Cell

FIG. 1F

Percent Identity Between Full Length Mature SEZ6 Proteins from Various Species

SEZ6	rhesus (XP_001110503)	Cynomolgus (herein)	mouse (NP_067261)	rat (NP_001099224)
Human 1 (NP_849191)		97.5%	90.5%	90.4%
Human 2 (NP_001092105)	91.7%			
rhesus (XP_001110503)		93.0%		
mouse (NP_067261)				96.7%

FIG. 2A

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Homo Sapiens SEZ6, SEZ6L, and SEZ6L2 Sequence Accession Numbers - NCBI

		mRNA	protein
SEZ6	1	NM_178860	NP_849191
	2	NM_001098635	NP_001092105
SEZ6L	1	NM_021115	NP_066938
	2	NM_001184773	NP_001171702
	3	NM_001184774	NP_001171703
	4	NM_001184775	NP_001701704
	5	NM_001184776	NP_001171705
	6	NM_001184777	NP_001171706
SEZ6L2	1	NM_012410	NP_036542
	2	NM_201575	NP_963869
	3	NM_001114099	NP_001107571
	4	NM_001114100	NP_001107572
	5	NM_001243332	NP_001230261
	6	NM_001243333	NP_001230262

FIG. 2B

Percent Identity between Various Human SEZ6, SEZ6L and SEZ6L2 Proteins

Homo sapiens	Complete protein SEZ6Lv1 (NP_066938	Complete protein SEZ6L2v5 (NP_001230261)
SEZ6v1 (NP_849191)	42.5%	42.9%
SEZ6Lv1 (NP_066938)		41.1%

Homo sapiens	ECD SEZ6Lv1 (NP_066938)	ECD SEZ6L2v5 (NP_001230261)
SEZ6v1 (NP_849191)	42.4%	43.2%
SEZ6Lv1 (NP_066938)		40.5%

FIG. 2C

>cDNA Sequence of hSCRx17 Clone ORF

(SEQ ID NO: 5)

CTGAGCCTGGAGGCCCCAACCGTGGGGAAAGGACAAGCCCCAGGCATCGAGGAGACAGATGGCGAGCTGACAGCAGCCCCCACACCTGAGCAGCCAGAAC
GAGGCGTCCACTTTGTCACAACAGCCCCCACCTTGAAGCTGCTCAACCACCACCCGCTGCTTGAGGAATTCCTACAAGAGGGGCTGGAAAAGGGAGATGAGG
AGTTGAGGCCAGCACTGCCCTTCCAGCCTGACCCACCTGCACCCTTCACCCCAAGTCCCCTTCCCCGCCTGGCCAACCAGGACAGCCGCCCTGTCTTTACCAGC
CCCACTCCAGCCATGGCTGCGGTACCCACTCAGCCCCAGTCCAAGGAGGGACCCTGGAGTCCGGAGTCAGAGTCCCCTATGCTTCGAATCACAGCTCCCCTAC
CTCCAGGGCCCAGCATGGCAGTGCCCACCCTAGGCCCAGGGGAGATAGCCAGCACTACACCCCCCAGCAGAGCCTGGACACCAACCCAAGAGGGTCCTGGA
GACATGGGAAGGCCGTGGGTTGCAGAGGTTGTGTCCCAGGGCGCGGGGATCGGGATCCAGGGGACCATCACCTCCTCCACAGCTTCAGGAGATGATGAGG
AGACCACCACTACCACCACCATCATCACCACCACCATCACCACAGTCCAGACACCAGGCCCTTGTAGCTGGAATTTCTCAGGCCCAGAGGGCTCTCTGGACTCC
CCTACAGACCTCAGCTCCCCCACTGATGTTGGCCTGGACTGCTTCTTCTACATCTCTGTCTACCCTGGCTATGGCGTGGAAATCAAGGTCCAGAATATCAGCCT
CCGGGAAGGGGAGACAGTGACTGTGGAAGGCCTGGGGGGGCCCGACCCACTGCCCCTGGCCAACCAGTCTTTCCTGCTGCGGGGCCAAGTCATCCGCAGCC
CCACCCACCAAGCGGCCCTGAGGTTCCAGAGCCTCCCGCCACCGGCTGGCCCTGGCACCTTCCATTTCCATTACCAAGCCTATCTCCTGAGCTGCCACTTTCCCC
GTCGTCCAGCTTATGGAGATGTGACTGTCACCAGCCTCCACCCAGGGGGTAGTGCCCGCTTCCATTGTGCCACTGGCTACCAGCTGAAGGGCGCCAGGCATC
TCACCTGTCTCAATGCCACCCAGCCCTTCTGGGATTCAAAGGAGCCCGTCTGCATCGCTGCTTGCGGCGGAGTGATCCGCAATGCCACCACCGGCCGCATCGT
CTCTCCAGGCTTCCCGGGCAACTACAGCAACAACCTCACCTGTCACTGGCTGCTTGAGGCTCCTGAGGGCCAGCGGCTACACCTGCACTTTGAGAAGGTTTCC
CTGGCAGAGGATGATGACAGGCTCATCATTCGCAATGGGGACAACGTGGAGGCCCCACCAGTGTATGATTCCTATGAGGTGGAATACCTGCCCATTGAGGG
CCTGCTCAGCTCTGGCAAACACTTCTTTGTTGAGCTCAGTACTGACAGCAGCGGGGCAGCTGCAGGCATGGCCCTGCGCTATGAGGCCTTCCAGCAGGGCCA
TTGCTATGAGCCCTTTGTCAAATACGGTAACTTCAGCAGCAGCACACCCACCTACCCTGTGGGTACCACTGTGGAGTTCAGCTGCGACCCTGGCTACACCCTG
GAGCAGGGCTCCATCATCATCGAGTGTGTTGACCCCCACGACCCCCAGTGGAATGAGACAGAGCCAGCCTGCCGAGCCGTGTGCAGCGGGGAGATCACAGA
CTCGGCTGGCGTGGTACTCTCTCCCAACTGGCCAGAGCCCTACGGTCGTGGGCAGGATTGTATCTGGGGTGTGCATGTGGAAGAGGACAAGCGCATCATGCT
GGACATCCGAGTGCTGCGCATAGGCCCTGGTGATGTGCTTACCTTCTATGATGGGGATGACCTGACGGCCCGGGTTCTGGGCCAGTACTCAGGGCCCCGTAG
CCACTTCAAGCTCTTTACCTCCATGGCTGATGTCACCATTCAGTTCCAGTCGGACCCCGGGACCTCAGTGCTGGGCTACCAGCAGGGCTTCGTCATCCACTTCT
TTGAGGTGCCCCGCAATGACACATGTCCGGAGCTGCCTGAGATCCCCAATGGCTGGAAGAGCCCATCGCAGCCTGAGCTAGTGCACGGCACCGTGGTCACTT
ACCAGTGCTACCCTGGCTACCAGGTAGTGGGATCCAGTGTCCTCATGTGCCAGTGGGACCTAACTTGGAGTGAGGACCTGCCCTCATGCCAGAGGGTGACTT
CCTGCCACGATCCTGGAGATGTGGAGCACAGCCGACGCCTCATATCCAGCCCCAAGTTTCCCGTGGGGGCCACCGTGCAATATATCTGTGACCAGGGTTTTGT
GCTGATGGGCAGCTCCATCCTCACCTGCCATGATCGCCAGGCTGGCAGCCCCAAGTGGAGTGACCGGGCCCCTAAATGTCTCCTGGAACAGCTCAAGCCATG
CCATGGTCTCAGTGCCCCTGAGAATGGTGCCCGAAGTCCTGAGAAGCAGCTACACCCAGCAGGGGCCACCATCCACTTCTCGTGTGCCCCTGGCTATGTGCTG
AAGGGCCAGGCCAGCATCAAGTGTGTGCCTGGGCACCCCTCGCATTGGAGTGACCCCCCACCCATCTGTAGGGCTGCCTCTCTGGATGGGTTCTACAACAGT
CGCAGCCTGGATGTTGCCAAGGCACCTGCTGCCTCCAGCACCCTGGATGCTGCCCACATTGCAGCTGCCATCTTCTTGCCACTGGTGGCGATGGTGTTGTTGG
TAGGAGGTGTATACTTCTACTTCTCCAGGCTCCAGGGAAAAAGCTCCCTGCAGCTGCCCCGCCCCCGCCCCCGCCCCTACAACCGCATTACCATAGAGTCAGC
GTTTGACAATCCAACTTACGAGACTGGATCTCTTTCCTTTGCAGGAGACGAGAGAATA

FIG. 3A

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> Translation of hSCRx17 clone ORF

(SEQ ID NO: 6)

LSLEAPTVGKGQAPGIEETDGELTAAPTPEQPERGVHFVTTAPTLKLLNHHPLLEEFLQEGLEKGDE
ELRPALPFQPDPPAPFTPSPLPRLANQDSRPVFTSPTPAMAAVPTQPQSKEGPWSPESESPMLRIT
APLPPGPSMAVPTLGPGEIASTTPPSRAWTPTQEGPGDMGRPWVAEVVSQGAGIGIQGTITSST
ASGDDEETTTTTTIITTTITTVQTPGPCSWNFSGPEGSLDSPTDLSSPTDVGLDCFFYISVYPGYGVE
IKVQNISLREGETVTVEGLGGPDPLPLANQSFLLRGQVIRSPTHQAALRFQSLPPPAGPGTFHFHY
QAYLLSCHFPRRPAYGDVTVTSLHPGGSARFHCATGYQLKGARHLTCLNATQPFWDSKEPVCIAA
CGGVIRNATTGRIVSPGFPGNYSNNLTCHWLLEAPEGQRLHLHFEKVSLAEDDDRLIIRNGDNVE
APPVYDSYEVEYLPIEGLLSSGKHFFVELSTDSSGAAAGMALRYEAFQQGHCYEPFVKYGNFSSST
PTYPVGTTVEFSCDPGYTLEQGSIIIECVDPHDPQWNETEPACRAVCSGEITDSAGVVLSPNWPEP
YGRGQDCIWGVHVEEDKRIMLDIRVLRIGPGDVLTFYDGDDLTARVLGQYSGPRSHFKLFTSMAD
VTIQFQSDPGTSVLGYQQGFVIHFFEVPRNDTCPELPEIPNGWKSPSQPELVHGTVVTYQCYPGY
QVVGSSVLMCQWDLTWSEDLPSCQRVTSCHDPGDVEHSRRLISSPKFPVGATVQYICDQGFVL
MGSSILTCHDRQAGSPKWSDRAPKCLLEQLKPCHGLSAPENGARSPEKQLHPAGATIHFSCAPGY
VLKGQASIKCVPGHPSHWSDPPPICRAASLDGFYNSRSLDVAKAPAASSTLDAAHIAAAIFLPLVA
MVLLVGGVVYFYFSRLQGKSSLQLPRPRPRPYNRITIESAFDNPTYETGSLSFAGDERI

FIG. 3B

Alignment of SEZ6 Variants

```
                          1                                                                  80
 hSEZ6 BC146292     (1) MRPVALLLLPSLLALLAHGLSLEAPTVGKGQAPGIEETDGELTAAPTPEQPERGVHFVTTAPTLKLLNHHPLLEEFLQEG
hSEZ6 NP_849191     (1) MRPVALLLLPSLLALLAHGLSLEAPTVGKGQAPGIEETDGELTAAPTPEQPERGVHFVTTAPTLKLLNHHPLLEEFLQEG
                         81                                                                 160
 hSEZ6 BC146292    (81) LEKGDEELRPALPFQPDPPAPFTPSPLPRLANQDSRPVFTSPTPAMAAVPTQPQSKEGPWSPESESPMLRITAPLPPGPS
hSEZ6 NP_849191    (81) LEKGDEELRPALPFQPDPPAPFTPSPLPRLANQDSRPVFTSPTPAMAAVPTQPQSKEGPWSPESESPMLRITAPLPPGPS
                        161                                                                 240
 hSEZ6 BC146292   (161) MAVPTLGPGEIASTTPPSRAWTPTQEGPGDMGRPWVAEVVSQGAGIGIQGTITSSTASGDDEETTTTTTIITTTITTVQT
hSEZ6 NP_849191   (161) MAVPTLGPGEIASTTPPSRAWTPTQEGPGDMGRPWVAEVVSQGAGIGIQGTITSSTASGDDEETTTTTTIITTTITTVQT
                        241                                                                 320
 hSEZ6 BC146292   (241) PGPCSWNFSGPEGSLDSPTDLSSPTDVGLDCFFYISVYPGYGVEIKVQNISLREGETVTVEGLGGPDPLPLANQSFLLRG
hSEZ6 NP_849191   (241) PGPCSWNFSGPEGSLDSPTDLSSPTDVGLDCFFYISVYPGYGVEIKVQNISLREGETVTVEGLGGPDPLPLANQSFLLRG
                        321                                                                 400
 hSEZ6 BC146292   (321) QVIRSPTHQAALRFQSLPPPAGPGTFHFHYQAYLLSCHFPRRPAYGDVTVTSLHPGGSARFHCATGYQLKGARHLTCLNA
hSEZ6 NP_849191   (321) QVIRSPTHQAALRFQSLPPPAGPGTFHFHYQAYLLSCHFPRRPAYGDVTVTSLHPGGSARFHCATGYQLKGARHLTCLNA
                        401          ** *                                                  480
 hSEZ6 BC146292   (401) TQPFWDSKEPVCIGECPGVIRNATTGRIVSPGFPGNYSNNLTCHWLLEAPEGQRLHLHFEKVSLAEDDDRLIIRNGDNVE
hSEZ6 NP_849191   (401) TQPFWDSKEPVCIAACGGVIRNATTGRIVSPGFPGNYSNNLTCHWLLEAPEGQRLHLHFEKVSLAEDDDRLIIRNGDNVE
                        481                                                                 560
 hSEZ6 BC146292   (481) APPVYDSYEVEYLPIEGLLSSGKHFFVELSTDSSGAAAGMALRYEAFQQGHCYEPFVKYGNFSSSTPTYPVGTTVEFSCD
hSEZ6 NP_849191   (481) APPVYDSYEVEYLPIEGLLSSGKHFFVELSTDSSGAAAGMALRYEAFQQGHCYEPFVKYGNFSSSTPTYPVGTTVEFSCD
                        561                                                                 640
 hSEZ6 BC146292   (561) PGYTLEQGSIILECVDPHDPQWNETEPACRAVCSGEITDSAGVVLSPNWPEPYGRGQDCIWGVHVEEDKRIMLDIRVLRI
hSEZ6 NP_849191   (561) PGYTLEQGSIILECVDPHDPQWNETEPACRAVCSGEITDSAGVVLSPNWPEPYGRGQDCIWGVHVEEDKRIMLDIRVLRI
                        641                                                                 720
 hSEZ6 BC146292   (641) GPGDVLTFYDGDDLTARVLGQYSGPRSHFKLFTSMADVTIQFQSDPGTSVLGYQQGFVIHFFEVPRNDTCPELPEIPNGW
hSEZ6 NP_849191   (641) GPGDVLTFYDGDDLTARVLGQYSGPRSHFKLFTSMADVTIQFQSDPGTSVLGYQQGFVIHFFEVPRNDTCPELPEIPNGW
                        721                                                                 800
 hSEZ6 BC146292   (721) KSPSQPELVHGTVVTYQCYPGYQVVGSSVLMCQWDLTWSEDLPSCQRVTSCHDPGDVEHSRRLISSPKFFVGATVQYICD
hSEZ6 NP_849191   (721) KSPSQPELVHGTVVTYQCYPGYQVVGSSVLMCQWDLTWSEDLPSCQRVTSCHDPGDVEHSRRLISSPKFFVGATVQYICD
                        801                                                                 880
 hSEZ6 BC146292   (801) QGFVLMGSSILTCHDRQAGSPKWSDRAPKCLLEQLKPCHGLSAPENGARSPEKQLHPAGATIHFSCAPGYVLKGQASIKC
hSEZ6 NP_849191   (801) QGFVLMGSSILTCHDRQAGSPKWSDRAPKCLLEQLKPCHGLSAPENGARSPEKQLHPAGATIHFSCAPGYVLKGQASIKC
                        881                                                                 960
 hSEZ6 BC146292   (881) VPGHPSHWSDPPPICRAASLDGFYNSRSLDVAKAPAASSTLDAAHIAAAIFLPLVAMVLLVGGVYFYFSRLQGKSSLQLP
hSEZ6 NP_849191   (881) VPGHPSHWSDPPPICRAASLDGFYNSRSLDVAKAPAASSTLDAAHIAAAIFLPLVAMVLLVGGVYFYFSRLQGKSSLQLP
                        961                 994
 hSEZ6 BC146292   (961) RPRPRPYNRITIESAFDNPTYETGSLSFAGDERI          (SEQ ID NO: 7)
hSEZ6 NP_849191   (961) RPRPRPYNRITIESAFDNPTYETGSLSFAGDERI          (SEQ ID NO: 3)
```

FIG. 3C

WO 2013/126810
PCT/US2013/027476
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>hSCRx17-Fc ORF

(SEQ ID NO: 8)

ATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTTCCCGGGTCCACTGGTGAC**GGCGCGCCTGGATCC**CTGAGCCTGGAGGCCCCAACCGTGGGGAAAGG
ACAAGCCCCAGGCATCGAGGAGACAGATGGCGAGCTGACAGCAGCCCCCACACCTGAGCAGCCAGAACGAGGCGTCCACTTTGTCACAACAGCCCCCACCTTGAAGCTGCT
CAACCACCACCCGCTGCTTGAGGAATTCCTACAAGAGGGGCTGGAAAAGGGAGATGAGGAGTTGAGGCCAGCACTGCCCTTCCAGCCTGACCCACCTGCACCCTTCACCCC
AAGTCCCCTTCCCCGCCTGGCCAACCAGGACAGCCGCCCTGTCTTTACCAGCCCCACTCCAGCCATGGCTGCGGTACCCACTCAGCCCCAGTCCAAGGAGGGACCCTGGAGT
CCGGAGTCAGAGTCCCCTATGCTTCGAATCACAGCTCCCCTACCTCCAGGGCCCAGCATGGCAGTGCCCACCCTAGGCCCAGGGGAGATAGCCAGCACTACACCCCCCAGCA
GAGCCTGGACACCAACCCAAGAGGGTCCTGGAGACATGGGAAGGCCGTGGGTTGCAGAGGTTGTGTCCCAGGGCGCGGGGATCGGGATCCAGGGGACCATCACCTCCTCC
ACAGCTTCAGGAGATGATGAGGAGACCACCACTACCACCACCATCATCACCACCACCATCACCACAGTCCAGACACCAGGCCCTTGTAGCTGGAATTTCTCAGGCCCAGAGG
GCTCTCTGGACTCCCCTACAGACCTCAGCTCCCCCACTGATGTTGGCCTGGACTGCTTCTTCTACATCTCTGTCTACCCTGGCTATGGCGTGGAAATCAAGGTCCAGAATATCA
GCCTCCGGGAAGGGGAGACAGTGACTGTGGAAGGCCTGGGGGGGCCCGACCCACTGCCCCTGGCCAACCAGTCTTTCCTGCTGCGGGGCCAAGTCATCCGCAGCCCCACC
CACCAAGCGGCCCTGAGGTTCCAGAGCCTCCCGCCACCGGCTGGCCCTGGCACCTTCCATTTCCATTACCAAGCCTATCTCCTGAGCTGCCACTTTCCCCGTCGTCCAGCTTAT
GGAGATGTGACTGTCACCAGCCTCCACCCAGGGGGTAGTGCCCGCTTCCATTGTGCCACTGGCTACCAGCTGAAGGGCGCCAGGCATCTCACCTGTCTCAATGCCACCCAGC
CCTTCTGGGATTCAAAGGAGCCCGTCTGCATCGCTGCTTGCGGCGGAGTGATCCGCAATGCCACCACCGGCCGCATCGTCTCTCCAGGCTTCCCGGGCAACTACAGCAACAA
CCTCACCTGTCACTGGCTGCTTGAGGCTCCTGAGGGCCAGCGGCTACACCTGCACTTTGAGAAGGTTTCCCTGGCAGAGGATGATGACAGGCTCATCATTCGCAATGGGGAC
AACGTGGAGGCCCCACCAGTGTATGATTCCTATGAGGTGGAATACCTGCCCATTGAGGGCCTGCTCAGCTCTGGCAAACACTTCTTTGTTGAGCTCAGTACTGACAGCAGCG
GGGCAGCTGCAGGCATGGCCCTGCGCTATGAGGCCTTCCAGCAGGGCCATTGCTATGAGCCCTTTGTCAAATACGGTAACTTCAGCAGCAGCACACCCACCTACCCTGTGGG
TACCACTGTGGAGTTCAGCTGCGACCCTGGCTACACCCTGGAGCAGGGCTCCATCATCATCGAGTGTGTTGACCCCCACGACCCCCAGTGGAATGAGACAGAGCCAGCCTGC
CGAGCCGTGTGCAGCGGGGAGATCACAGACTCGGCTGGCGTGGTACTCTCTCCCAACTGGCCAGAGCCCTACGGTCGTGGGCAGGATTGTATCTGGGGTGTGCATGTGGA
AGAGGACAAGCGCATCATGCTGGACATCCGAGTGCTGCGCATAGGCCCTGGTGATGTGCTTACCTTCTATGATGGGGATGACCTGACGGCCCGGGTTCTGGGCCAGTACTC
AGGGCCCCGTAGCCACTTCAAGCTCTTTACCTCCATGGCTGATGTCACCATTCAGTTCCAGTCGGACCCCGGGACCTCAGTGCTGGGCTACCAGCAGGGCTTCGTCATCCACT
TCTTTGAGGTGCCCCGCAATGACACATGTCCGGAGCTGCCTGAGATCCCCAATGGCTGGAAGAGCCCATCGCAGCCTGAGCTAGTGCACGGCACCGTGGTCACTTACCAGT
GCTACCCTGGCTACCAGGTAGTGGGATCCAGTGTCCTCATGTGCCAGTGGGACCTAACTTGGAGTGAGGACCTGCCCTCATGCCAGAGGGTGACTTCCTGCCACGATCCTGG
AGATGTGGAGCACAGCCGACGCCTCATATCCAGCCCCAAGTTTCCCGTGGGGGCCACCGTGCAATATATCTGTGACCAGGGTTTTGTGCTGATGGGCAGCTCCATCCTCACC
TGCCATGATCGCCAGGCTGGCAGCCCCAAGTGGAGTGACCGGGCCCCTAAATGTCTCCTGGAACAGCTCAAGCCATGCCATGGTCTCAGTGCCCCTGAGAATGGTGCCCGA
AGTCCTGAGAAGCAGCTACACCCAGCAGGGGCCACCATCCACTTCTCGTGTGCCCCTGGCTATGTGCTGAAGGGCCAGGCCAGCATCAAGTGTGTGCCTGGGCACCCCTCG
CATTGGAGTGACCCCCCACCCATCTGTAGGGCTGCCTCTCTGGATGGGTTCTACAACAGTCGCAGCCTGGATGTTGCCAAGGCACCTGCTGCCTCCAGCACCCTGGATGCTG
CCCAC**CTGGCCGGCCACAGATCT**GTCGAGTGCCCACCGTGCCCAGCACCACCTGTGGCAGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCC
CGGACCCCTGAGGTCACGTGCGTGGTGGTGGACGTGAGCCACGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCC
ACGGGAGGAGCAGTTCAACAGCACGTTCCGTGTGGTCAGCGTCCTCACCGTTGTGCACCAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCT
CCCAGCCCCCATCGAGAAAACCATCTCCAAAACCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCAGGGAGGAGATGACCAAGAACCAGGTCAGCCT
GACCTGCCTGGTCAAAGGCTTCTACCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCATGCTGGACTCCGACG
GCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAA
GAGCCTCTCCCTGTCTCCGGGTTGA

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FIG. 4A

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>hSCRx17-Fc protein

(SEQ ID NO: 9)

METDTLLLWVLLLWVPGSTGDGAPGSLSLEAPTVGKGQAPGIEETDGELTAAPTPEQPERGVHFVTTAPTLKLLN
HHPLLEEFLQEGLEKGDEELRPALPFQPDPPAPFTPSPLPRLANQDSRPVFTSPTPAMAAVPTQPQSKEGPWSPES
ESPMLRITAPLPPGPSMAVPTLGPGEIASTTPPSRAWTPTQEGPGDMGRPWVAEVVSQGAGIGIQGTITSSTASG
DDEETTTTTTIITTTITTVQTPGPCSWNFSGPEGSLDSPTDLSSPTDVGLDCFFYISVYPGYGVEIKVQNISLREGETV
TVEGLGGPDPLPLANQSFLLRGQVIRSPTHQAALRFQSLPPPAGPGTFHFHYQAYLLSCHFPRRPAYGDVTVTSLH
PGGSARFHCATGYQLKGARHLTCLNATQPFWDSKEPVCIAACGGVIRNATTGRIVSPGFPGNYSNNLTCHWLLEA
PEGQRLHLHFEKVSLAEDDDRLIIRNGDNVEAPPVYDSYEVEYLPIEGLLSSGKHFFVELSTDSSGAAAGMALRYEA
FQQGHCYEPFVKYGNFSSSTPTYPVGTTVEFSCDPGYTLEQGSIIIECVDPHDPQWNETEPACRAVCSGEITDSAG
VVLSPNWPEPYGRGQDCIWGVHVEEDKRIMLDIRVLRIGPGDVLTFYDGDDLTARVLGQYSGPRSHFKLFTSMA
DVTIQFQSDPGTSVLGYQQGFVIHFFEVPRNDTCPELPEIPNGWKSPSQPELVHGTVVTYQCYPGYQVVGSSVLM
CQWDLTWSEDLPSCQRVTSCHDPGDVEHSRRLISSPKFPVGATVQYICDQGFVLMGSSILTCHDRQAGSPKWSD
RAPKCLLEQLKPCHGLSAPENGARSPEKQLHPAGATIHFSCAPGYVLKGQASIKCVPGHPSHWSDPPPICRAASLD
GFYNSRSLDVAKAPAASSTLDAAH**LAGHRS**VECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPR
EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQ
GNVFSCSVMHEALHNHYTQKSLSLSPG

FIG. 4B

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>cDNA sequence encoding mature murine SEZ6

(SEQ ID NO: 10)

CTCTCCTCAGAGGCTCCGATCACGGGGGAAGGTCATGCCACGGGCATCAGGGAGACGGATGGGGAGCTGACCGCAGCCCCTACACCTGAGCAGTCAGACC
GAGGCGTCCACTTCGTCACCACAGCCCCTACCCTCAAGCTGCTCAACCACCACCCACTTCTGGAAGAATTTCTTCAAGAGGGGCTAGAAAGAGAGGAAGCGC
CGCAGCCTGCACTGCCCTTCCAGCCGGACTCACCTACACACTTTACTCCAAGCCCCCTCCCCCGCCTCACCAACCAGGACAACCGCCCCGTCTTTACCAGTCCG
ACTCCAGCCGTGGCTGCAGCACCCACCCAGCCCCACTCCAGGGAGAAACCTTGGAACCTAGAATCCAAACCCCCTGAGCTTTCTATCACATCGTCCCTTCCTCC
AGGGCCGAGTATGGCAGTGCCCACACTGCTCCCAGAGGACAGACCCAGTACTACACCCCCTAGCCAAGCATGGACTCCAACTCAGGAGGGTCCTGGAGACA
TGGACAGACCTTGGGTTCCAGAGGTCATGTCTAAGACCACAGGGCTTGGTGTCGAGGGAACCATTGCCACCTCCACAGCTTCAGGGGATGACGAAGAGACC
ACTACCACCATCATTACCACTACTGTCACCACAGTTCAGCCACCAGGCCCCTGTAGCTGGAATTTCTCAGGCCCAGAGGGCTCTCTGGATTCCCCCACGGCCCC
CAGCTCACCCTCTGATGTTGGCCTGGACTGTTTCTACTATATCTCTGTCTACCCTGGATATGGAGTAGAGATCAAGGTGGAGAACATCAGCCTTCAGGAAGGG
GAGACCATCACCGTGGAGGGCCTGGGGGGCCCCGATCCACTGCCCTTGGCTAACCAGTCGTTCCTGCTGAGGGGCCAGGTCATCCGCAGCCCCACCCACCA
AGCAGCCCTGAGGTTCCAGAGCCTCCCGCTACCCGCTGGGCCTGGCACTTTCCATTTCCGCTACCAAGCCTATCTCCTGAGCTGCCACTTTCCCCGACGTCCAG
CGTATGGAGATGTGACTGTCACCAGTCTCCACCCAGGAGGCAGCGCCCACTTCCATTGTGCCACTGGCTACCAGCTCAAGGGTGCCAGGTTCCTCACCTGTCT
CAATGCCACCCAGCCCTTTTGGGATTCCCAAGAGCCTGTTTGCATTGCTGCTTGTGGTGGAGTGATTCGGAATGCCACCACTGGCCGCATTGTCTCTCCTGGC
TTCCCGGGGAACTACAGCAACAACCTCACCTGCCACTGGTTGCTAGAGGCTCCAGAGAGCCAGCGGCTGCACCTGCACTTTGAAAAGGTCTCCCTGGCAGAA
GACGACGACAGGCTCATCATCCGCAATGGAAATAACGTGGAGGCCCCGCCGGTGTACGACTCCTATGAGGTGGAATACCTGCCCATTGAGGGCCTGCTCAG
CTCTGGCAGACACTTCTTCGTGGAGTTCAGTACTGACAGCAGTGGGGCAGCTGCAGGCATGGCCCTGCGCTATGAGGCCTTCCAGCAAGGACATTGCTATGA
GCCCTTTGTCAAATACGGCAACTTCAGCAGCAGTGCACCGTCCTACCCTGTGGGTACAACTGTGGAGTTCAGCTGTGACCCTGGCTACACCCTGGAGCAGGG
CTCCATCATCATCGAATGCGTCGACCTCCACGACCCCCAGTGGAATGAGACAGAGCCAGCCTGCCGAGCCGTGTGCAGCGGGGAGATCACAGACTCTGCAG
GCGTGGTGCTCTCTCCAAACTGGCCGGAGCCTTATGGCCGAGGGCAGGACTGCATCTGGGGTGTGCATGTGGAGGAGGACAAGCGCATCATGCTGGACAT
CCGAGTGCTGCGCATAGGCTCTGGGGATGTACTGACCTTCTACGATGGGGATGACCTCACAGCCCGGGTCCTGGGCCAATACTCAGGGCCCCGTGGCCACTT
CAAGCTCTTTACCTCCATGGCCGATGTCACCATCCAGTTCCAGTCAGACCCTGGGACCTCGGCGCTGGGTTACCAGCAAGGATTTGTCATCCACTTCTTTGAG
GTTCCCCGCAACGACACATGTCCAGAGCTACCCGAGATCCCCAACGGCTGGAAGAACCCATCACAGCCTGAGCTGGTGCACGGCACGGTGGTCACCTATCAG
TGCTACCCTGGTTACCAGGTGGTGGGATCCAGTATTCTCATGTGCCAGTGGGACCTAAGCTGGAGTGAGGACCTGCCTTCATGCCAGAGAGTGACATCTTGC
CATGACCCAGGGGATGTGGAGCACAGCCGACGCCTCATATCCAGCCCCAAGTTTCCCGTGGGAGCAACTGTGCAATATGTCTGTGACCAGGGTTTTGTGCTG
ACGGGGAGTGCCATTCTCACCTGCCATGATCGGCAAGCAGGCAGTCCCAAGTGGAGTGACAGGGCCCCCAAGTGTCTCTTGGAACAATTCAAGCCGTGCCA
TGGCCTCAGCGCCCCGGAGAATGGTGCCCGCAGCCCTGAGAAGCGGCTTCACCCAGCAGGGGCCACCATCCACTTCTCCTGTGCCCCTGGTTATGTGCTGAA
GGGCCAGGCCAGCATCAAATGCGTGCCTGGACACCCCTCGCATTGGAGTGACCCACCACCCATCTGTAGGGCTGCCTCTCTGGATGGGTTCTACAACGGCCG
TAGCCTGGATGTTGCCAAGGCACCTGCCGCCTCCAGTGCCCTGGACGCTGCTCACCTGGCTGCTGCCATCTTCCTACCATTGGTGGCCATGGTGTTGCTGGTG
GGAGGAGTGTACCTCTATTTTTCCAGATTCCAGGGGAAAAGTCCCCTGCAACTTCCCCGAACTCATCCTCGCCCCTATAACCGCATCACGGTAGAGTCAGCAT
TTGACAATCCAACTTATGAGACTGGATCTCTTTCCTTTGCAGGAGACGAGAGAATATGA

FIG. 5A

>Translation of mSCRx17 clone ORF

(SEQ ID NO: 11)

LSSEAPITGEGHATGIRETDGELTAAPTPEQSDRGVHFVTTAPTLKLLNHHPLLEEFLQEGLEREEAPQPALPFQPDSPTHFT
PSPLPRLTNQDNRPVFTSPTPAVAAAPTQPHSREKPWNLESKPPELSITSSLPPGPSMAVPTLLPEDRPSTTPPSQAWTPT
QEGPGDMDRPWVPEVMSKTTGLGVEGTIATSTASGDDEETTTTIITTTVTTVQPPGPCSWNFSGPEGSLDSPTAPSSPSD
VGLDCFYYISVYPGYGVEIKVENISLQEGETITVEGLGGPDPLPLANQSFLLRGQVIRSPTHQAALRFQSLPLPAGPGTFHFR
YQAYLLSCHFPRRPAYGDVTVTSLHPGGSAHFHCATGYQLKGARFLTCLNATQPFWDSQEPVCIAACGGVIRNATTGRIVS
PGFPGNYSNNLTCHWLLEAPESQRLHLHFEKVSLAEDDDRLIIRNGNNVEAPPVYDSYEVEYLPIEGLLSSGRHFFVEFSTD
SSGAAAGMALRYEAFQQGHCYEPFVKYGNFSSSAPSYPVGTTVEFSCDPGYTLEQGSIIIECVDLHDPQWNETEPACRAV
CSGEITDSAGVVLSPNWPEPYGRGQDCIWGVHVEEDKRIMLDIRVLRIGSGDVLTFYDGDDLTARVLGQYSGPRGHFKLF
TSMADVTIQFQSDPGTSALGYQQGFVIHFFEVPRNDTCPELPEIPNGWKNPSQPELVHGTVVTYQCYPGYQVVGSSILMC
QWDLSWSEDLPSCQRVTSCHDPGDVEHSRRLISSPKFPVGATVQYVCDQGFVLTGSAILTCHDRQAGSPKWSDRAPKCLL
EQFKPCHGLSAPENGARSPEKRLHPAGATIHFSCAPGYVLKGQASIKCVPGHPSHWSDPPPICRAASLDGFYNGRSLDVAK
APAASSALDAAHLAAAIFLPLVAMVLLVGGVYLYFSRFQGKSPLQLPRTHPRPYNRITVESAFDNPTYE TGSLSFAGDERI

FIG. 5B

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>cDNA sequence of rSCRx17 clone ORF

(SEQ ID NO: 12)

ctctcctcagaggctccaatcacgggggaaggtcaagccacgggcatcagggagatggatggggagctgaccgcagcccctacacctgagcagtcagaccgaggcgtccacttcgtcaccac
agcccctaccctcaagctactcaaccaccacccacttctggaggaatttcttcaagaggggctagaagggagaggaggaagctccgaggccggcactgcccttccagccagactcacctacac
cctttactccaagccccctttcccccgcctcaccaaccaggacaaccgccctgtctttaccagtccgacgccagctgtagctgcggcacccacgcagccccactccagaaagaaaccctggaacc
cagagtcagagccccccggagctttacatcacatctcccctccctccagggccgagtatggcagtgcccacactgcacccagaggacagacccagcactacacccccccagccaagcatggact
ccaacccaggagggtcctggagacatgggcagaccttgggttccagagatcatgtctaagaccacagggcttggtatcgagggggaccattgccacctccacagcttcaggggatgacgaaga
gaccaccaccaccaccatcattaccaccgtcaccacaattcagccaccaggcccctgtagctggaatttctcaggcccggaggggctctctggattcccctgcggtcccccagcgtcccctctgatg
ttggcctggactgtctctactacatctctgtctaccctggatatggagtcgagatcaaggtgaagaacatcagccttcaggaaggagagaccataaccgtggagggcctgggggggcctgacc
cactgcccttggctaaccagtctttcctgctgagggccaggtcatccgcagcccccacccaccaggcagccgtgaggttccaaagccttccacttcccgctggacctggtactttccatttccact
accaagcctatctcctgagctgccactttcctcggcgtccagcttatggagatgtgactgtcaccagcctccacccaggaggcagcgcccgcttccactgtgccactggctaccagctaaagggt
gccaggttcctcacctgtctcaatgccacccagccctttgggattcccaagagcctgtctgcattgctgcttgtggaggagtgattcggaatgccaccactggccgcattgtctctcctggctttcc
cgggaactacagcaacaacctcacctgccactggctgctagaagcccccgagagccagcggctgcacctgcactttgaaaaggtctccctggcagaagatgacgacaggctcatcatccgta
acgggaataacgtggaggcccccgccagtgtatgactcctatgaggtggagtacctgcccattgagggcctgctcagttctggcagacacttcttcgtggagttcagtactgacagcagcgggggc
agccgcaggcatggcactgcgctatgaggccttccagcaaggacattgctatgagccctttgtcaaatacggtaacttcagcagcagcgcaccgtcctaccctgtgggtacgactgtggagttc
agctgtgaccctggctacaccctggagcagggttccatcatcatcgaatgcgtcgacctccgtgacccccagtggaatgagacagaaccagcctgccgagccgtgtgcagcgggggagatcac
agactctgcaggcgtggtgctctctccaaactggccggagccttatggccgagggcaggactgcatctgggatgtgcatgtggaggaggacaagcgcatcatgctggacatccgagtgctgcg
cataggctctggggatgtactgaccttctacgatggggatgacctgacagcccgggtcctgggccaatactcagggccccgtggccacttcaagctctttacctccatggctgatgtcaccattc
agttccagtcagaccctgggacgtcggcgctgggttaccagcaaggatttgtcatccacttctttgaggtgccccgcaatgacacatgtccagagcttcccgagatccccaacggctggaagaa
cccatcacagcctgagctggtgcatggcacggtggtcacctatcagtgctaccccggttaccaggtggtgggatccagtattctcatgtgccagtgggacctgagctggagtgaggacctgccct
catgccagagagtgacatcctgccatgacccaggggatgtggagcacagccgacgcctcatatccagcctcaagtttcctgtgggagcaactgtgcagtatatctgtgaccagggttttgtgctc
acgggtagcgccatccttacttgccatgatcgtcaagcgggcagtcccaagtggagtgacagggccccaagtgtctcttggaacagttcaaaccatgtcatggcctcagtgccccctgagaatg
gtgcccgcagccctgagaagaggctccacccagcaggggccaccattcacttctcctgtgccctggttatgtgctgaagggccaggccagcatcaaatgcgtgcctggacaccccctcacattg
gagtgatcctccacccatctgtagggctgcttctctggatgggttctacaacggccgtagcctggatgttgccaaggcacctgccacctccagtgccctggatgctgcccacatggcagctgcca
tctttctaccattggtggccatggtgttgctggtgggaggagtgtacctctatttctccagactccagggaaaaagtcctctgcagcttcccggaactcatcctcgcccctataaccgtatcacggt
agagtcagcatttgacaatccaacttatgagaccggatctctttcctttgcaggagacgagagaata

FIG. 5C

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>Translation of rSCRx17 clone ORF

(SEQ ID NO: 13)

LSSEAPITGEGQATGIREMDGELTAAPTPEQSDRGVHFVTTAPTLKLLNHHPLLEEFLQEGLEGREEAPRPALPFQPDSPTPFTPS
PLPRLTNQDNRPVFTSPTPAVAAAPTQPHSRKKPWNPESEPPELYITSPLPPGPSMAVPTLHPEDRPSTTPPSQAWTPTQEGPG
DMGRPWVPEIMSKTTGLGIEGTIATSTASGDDEETTTTTIITTVTTIQPPGPCSWNFSGPEGSLDSPAVPSVPSDVGLDCLYYISV
YPGYGVEIKVKNISLQEGETITVEGLGGPDPLPLANQSFLLRGQVIRSPTHQAAVRFQSLPLPAGPGTFHFHYQAYLLSCHFPRRP
AYGDVTVTSLHPGGSARFHCATGYQLKGARFLTCLNATQPFWDSQEPVCIAACGGVIRNATTGRIVSPGFPGNYSNNLTCHWL
LEAPESQRLHLHFEKVSLAEDDDRLIIRNGNNVEAPPVYDSYEVEYLPIEGLLSSGRHFFVEFSTDSSGAAAGMALRYEAFQQGH
CYEPFVKYGNFSSSAPSYPVGTTVEFSCDPGYTLEQGSIIIECVDLRDPQWNETEPACRAVCSGEITDSAGVVLSPNWPEPYGRG
QDCIWGVHVEEDKRIMLDIRVLRIGSGDVLTFYDGDDLTARVLGQYSGPRGHFKLFTSMADVTIQFQSDPGTSALGYQQGFVI
HFFEVPRNDTCPELPEIPNGWKNPSQPELVHGTVVTYQCYPGYQVVGSSILMCQWDLSWSEDLPSCQRVTSCHDPGDVEHS
RRLISSLKFPVGATVQYICDQGFVLTGSAILTCHDRQAGSPKWSDRAPKCLLEQFKPCHGLSAPENGARSPEKRLHPAGATIHFSC
APGYVLKGQASIKCVPGHPSHWSDPPPICRAASLDGFYNGRSLDVAKAPATSSALDAAHMAAAIFLPLVAMVLLVGGVYLYFSR
LQGKSPLQLPGTHPRPYNRITVESAFDNPTYETGSLSFAGDERI

FIG. 5D

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>cDNA sequence of cSCRx17 clone ORF

(SEQ ID NO: 14)

atggagacagacacactcctgctatgggtactgctgctctgggttccaggttccactggtgacggcgcgccactcagcagcgaagctcccacaatgggcaagggacaggcccccggaattga
agaaaccgatggcgaactcaccgctgcccctaccccctgagcaacccgaaaggggagtgcactttgtgaccaccgctcccaccctgaagctgctcaatcaccaccccctcctggaggagtttct
gcaggaaggcctggaaaaaggcgacgaggaactcagacctgcccctgcccttccaaacccgaccctcctaccccctttacacctagcccctctccctagactggccaaccaagactccagacctgt
gttcaccagccctacacctgctacagctgccgtccctacccaacctcaatccaaggagggaccttggagcctcgagagcgagcctcccgtgctgaagaatcacagctcctctccctcctggcccctt
ccatggctgtccccacactcggacctggcgaaaggcccagcacaacacccccctccagagcctggacccctacacaagaaggcccctggcgacatgggaaggcccttgggtccctgaagtcgtg
agccaaggcgccggcatcggaatccagggaaccatcgccagctccacagccagcggagacgatgaggaaacaaccaccacaaccaccatcatcaccaccacaatcacaacagtccagacc
cccggcccttgcagctggaatttttccggccctgagggatccctggattcccccacagatctgtcctcccctcctgacgtgggcctcgactgtttcttctatatctccgtgtatcctggctacggcgt
cgaaatcaaagtccagaacatctccctgagggagggcgaaacagtcaccgtggaaggactggcggacccgctcctctgcctctcgccaaccaatccttcctcctcaggggccaagtgattag
atcccccacacatcaagctgctctcaggttccaaagcctccctcccccgctggacccggaaccttcacttccactaccaagcctatctcctcagctgccatttcccccacaggcccgcttatgg
agatgtcacagtcacctccctgcatcctggcggctccgctagattccactgcgctaccggataccaactcaagggcgccaggcatctgacatgtctcaatgctacccagcccttctgggacagcc
aaggagcccgtctgcattgccgcttgcggaggcgtcatcagaaatgccaccaccggcagaatcgtgagccccggcttccctggcaactactccaacaacctgacatgccactggctgctggaa
gctcctgaggccagagactgcatctgcacttcgagaaggtcagcctggccgaagatgacgacagactcatcatcaggaacggcgacaacgtggaggctccccccgtctatgattcctacgag
gtcgagtacctccccatcgagggactgctgtcctccggcaagcatttttttcgtggagctgtccacagattccagcggagctgccgccggaatggctctcaggtacgaggctttccaacagggcca
ctgttacgagccctttgtgaagtacggcaacttctccagctccgctcctacctaccccgtcggcacaaccgtcgaatttagctgcgaccctggatacacactcgagcaaggctccatcatcatcg
agtgtgtcgaccccacgaccccaatggaacgagacagagcccgcctgtagggccgtgtgtagcggagagattaccgactccgccggagtggtgctctccctaattggcctgaaccctacg
gcagaggacaagattgtatttggggcgtccatgtcgaggaggacaagaggattatgctcgacgtgagggtgctgaggattggacctggcgacgtgctcacattctatgacggcgacgatctca
ccgccagagtcctgggacaatactccggccctcacagccacttcaagctgttcaccagcatggctgacgtgaccatccagttccagtccgatcctggaacatccgtgctgggataccagcaggg
cttcgtcatccacttcttcgaggtccccaggaacgacacctgccccgaactgcccgagattcccaacggctggaaatccccctcccaacctgatctgtgcacggcaccgtcgtcacctaccaat
gctaccctggataccaagtcgtcggcagcagcgtgctgatgtgccaatgggacctcacctggagcgaggatctgccctcctgccagagagtcacctcctgccacgatcccggcgatgtggaac
actccaggaggctgattagctcccccaagttcctgtcggagccaccgtgcaatacatctgcgaccagggctttgtgctgaccggaaccagcatcctcacatgccacgacaggcaagctggatc
ccccaagtggtccgatagggcccccaaatgcctcctggaacagctgaagccttgtcatggcctcagcgctcctgaaaacggcgctaggagccccgaaaagaggctccaccctgccggagcca
ccatccacttttcctgtgccccccggatacgtgctgaaggggccaggcctccattaagtgcgtgcccggacatccttcccactggtccgaccccctcccatctgtaaagccgcctccctggacggat
tctataacagcagaagcctggacgtcgctaaggcccctgctgcttcctccaccctggatgctgctcacatcgctgctgccatctttctgcccctcgtcgccatggtgctgctggtgggaggcgtct
acttctacttctccaggctgcagggaaagagctccctgcaactgcctaggacaagacccaggccctacaataggatcacagtcgagagcgccttcgacaaccccacatacgagacaggatcc
ctgagctttgccggagacgagagaatt

FIG. 5E

>Translation of cSCRx17 clone ORF

(SEQ ID NO: 15)

METDTLLLWVLLLWVPGSTGDGAPLSSEAPTMGKGQAPGIEETDGELTAAPTPEQPER
GVHFVTTAPTLKLLNHHPLLEEFLQEGLEKGDEELRPALPFQPDPPTPFTPSPLPRLANQ
DSRPVFTSPTPATAAVPTQPQSKEGPWSLESEPPVLRITAPLPPGPSMAVPTLGPGERPS
TTPPSRAWTPTQEGPGDMGRPWVPEVVSQGAGIGIQGTIASSTASGDDEETTTTTTIITT
TITTVQTPGPCSWNFSGPEGSLDSPTDLSSPPDVGLDCFFYISVYPGYGVEIKVQNISLR
EGETVTVEGLGGPAPLPLANQSFLLRGQVIRSPTHQAALRFQSLPPPAGPGTFHFHYQAY
LLSCHFPHRPAYGDVTVTSLHPGGSARFHCATGYQLKGARHLTCLNATQPFWDSKEPVCI
AACGGVIRNATTGRIVSPGFPGNYSNNLTCHWLLEAPEGQRLHLHFEKVSLAEDDDRLII
RNGDNVEAPPVYDSYEVEYLPIEGLLSSGKHFFVELSTDSSGAAAGMALRYEAFQQGHCY
EPFVKYGNFSSSAPTYPVGTTVEFSCDPGYTLEQGSIIIECVDPHDPQWNETEPACRAVC
SGEITDSAGVVLSPNWPEPYGRGQDCIWGVHVEEDKRIMLDVRVLRIGPGDVLTFYDGDD
LTARVLGQYSGPHSHFKLFTSMADVTIQFQSDPGTSVLGYQQGFVIHFFEVPRNDTCPEL
PEIPNGWKSPSQPDLVHGTVVTYQCYPGYQVVGSSVLMCQWDLTWSEDLPSCQRVTSCHD
PGDVEHSRRLISSPKFPVGATVQYICDQGFVLTGTSILTCHDRQAGSPKWSDRAPKCLLE
QLKPCHGLSAPENGARSPEKRLHPAGATIHFSCAPGYVLKGQASIKCVPGHPSHWSDPPP
ICKAASLDGFYNSRSLDVAKAPAASSTLDAAHIAAAIFLPLVAMVLLVGGVYFYFSRLQG
KSSLQLPRTRPRPYNRITVESAFDNPTYETGSLSFAGDERI

FIG. 5F

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>cDNA sequence of human SEZ6L ECD

(SEQ ID NO: 16)

ctcgagagggatgctctgcctgagggagatgcttcccctctcggaccttatctgctgcccagcggagctcctgagaggggatcccccggaaaaggagcatcccgaagaaagagtggtcacagct
ccccctagctccagccagagcgctgaggtgctgggagaactggtcctcgacggaacagccccttccgcccatcacgatattcctgccctcagccctctcctccccgaggaagctaggcctaaaac
acgccctcccccctaaaaagaagctgccttccctcaagcaggtcaattccgccaggaagcagctcagacccaaggccacctccgctgctacagtccaaagagctggatcccagcctgccagc
cagggactcgatctgctcagcagctccacagaaaaacctggacctcctggcgatcctgaccctattgtggccagcgaagaagctagcgaagtcccctctgtggctggacaggaaggagtccgct
gtccccaccacacccgctcctctccagatcagcccccttcacctcccagccttatgtcgctcatacactgcctcagaggcctgagcctggcgaacctggacctgacatggctcaggaggctcctca
ggaggacaccagccctatggccctgatggataagggcgagaatgaactgaccggaagcgccagcgaggaaagccaggagaccaccaccagcacaatcatcaccaccaccgtcatcaccac
cgaacaggcccccgctctgtgttccgtgtcctttttccaaccccgagggctacattgacagcagcgattacccctgctccctctcaacaacttcctcgagtgcacctacaatgtgaccgtgtacac
cggctacggagtcgaactccaggtgaagtccgtgaacctctccgatggcgaactgctctccattaggggcgtcgatggccctacactcaccgtcctggctaaccaaaccctgctcgtcgaaggc
caggtgattaggtcccccaccaacaccatctccgtctacttcaggacctttcaagacgacggactgggaaccttccaactgcattaccaggccttcatgctgtcctgtaatttccccaggagaccc
gactccggagacgtcaccgtcatggatctgcactccggaggcgtggcccactttcattgtcacctcggctacgagctccagggcgccaagatgctgacatgcatcaacgccagcaaacctcact
ggtccagccaggagcctatctgtagcgctccttgcggcggagccgtgcacaatgctacaattggcagagtgctcagcccttcctaccctgaaaacaccaacggctcccagttctgcatctggac
aatcgaggcccccgaaggccaaaagctgcacctgcactttgagaggctcctgctccacgacaaagacaggatgaccgtgcactccggccagaccaataagtccgccctcctgtatgacagcc
tgcagacagagtccgtccctttttgaaggcctgctgtccgagggcaataccatcaggattgagttcacatccgaccaagccagggctgctagcaccttcaacattaggtttgaggctttcgaaaag
ggacactgctacgagccctatattcagaatggcaatttcacaacctccgaccccacctacaatatcggcacaattgtggagtttacctgcgaccctggacacagcctggagcagggacctgcca
tcatcgaatgcatcaacgtcagggaccccctactggaacgacacagaacctctgtgtagggctatgtgcggaggcgaactgagcgctgtggctggagtcgtgctctcccctaactggcccgaac
cctatgtggagggcgaaggattgcatctggaagatccacgtcggcgaggaaaaaaggatctttctggacatccagttcctgaatctctccaacagcgacatcctgaccatctacgacggagatga
ggtcatgccccacattctgggccagtatctcggaaactccggccccccaaaagctctactcctccacccccgacctcacaatccaattccacagcgatcctgctggcctcatctttggaaaggggac
aaggctttatcatgaattacatcgaggtcagcagaaacgacagctgctccgacctgcctgagatccagaacggatggaagaccacctcccacaccgagctcgtcagggagctaggatcaca
taccagtgcgaccccggatacgacatcgtcggctccgataccctgacatgccagtgggatctgagctggagctccgaccccccctttttgtgagaagatcatgtactgcaccgaccccggcgaag
tcgatcatagcaccaggctcatcagcgatcctgtgctgctcgtcggcacaaccatccaatacacctgtaaccccggattcgtgctcgaaggatcctccctgctcacctgttacagcagggaaacc
ggcacccccatttggacatccaggctgcctcactgcgtgtccgaagagagcctggcttgcgataatcccggcctgcctgagaacggataccagattctgtacaaaaggctgtacctccccggcg
agtccctgaccttcatgtgctacgaaggattcgagctcatgggcgaagtcaccatcaggtgcatcctcggccagcccctcccactggaacggacctctccccgtctgtaaggtcaatcaggattcc
ttcgagcacgctctggaagtcgctgaggctgccgccgagacaagcctggaaggcggc

FIG. 5G

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>human SEZ6L ECD protein

(SEQ ID NO: 17)

METDTLLLWVLLLWVPGSTGDHGAPLERDALPEGDASPLGPYLLPSGAPERGSPGKEHPEERVVT
APPSSSQSAEVLGELVLDGTAPSAHHDIPALSPLLPEEARPKHALPPKKKLPSLKQVNSARKQLRPK
ATSAATVQRAGSQPASQGLDLLSSSTEKPGPPGDPDPIVASEEASEVPLWLDRKESAVPTTPAPLQI
SPFTSQPYVAHTLPQRPEPGEPGPDMAQEAPQEDTSPMALMDKGENELTGSASEESQETTTSTII
TTTVITTEQAPALCSVSFSNPEGYIDSSDYPLLPLNNFLECTYNVTVYTGYGVELQVKSVNLSDGELL
SIRGVDGPTLTVLANQTLLVEGQVIRSPTNTISVYFRTFQDDGLGTFQLHYQAFMLSCNFPRRPDS
GDVTVMDLHSGGVAHFHCHLGYELQGAKMLTCINASKPHWSSQEPICSAPCGGAVHNATIGRV
LSPSYPENTNGSQFCIWTIEAPEGQKLHLHFERLLLHDKDRMTVHSGQTNKSALLYDSLQTESVPF
EGLLSEGNTIRIEFTSDQARAASTFNIRFEAFEKGHCYEPYIQNGNFTTSDPTYNIGTIVEFTCDPGH
SLEQGPAIIECINVRDPYWNDTEPLCRAMCGGELSAVAGVVLSPNWPEPYVEGEDCIWKIHVGEE
KRIFLDIQFLNLSNSDILTIYDGDEVMPHILGQYLGNSGPQKLYSSTPDLTIQFHSDPAGLIFGKGQG
FIMNYIEVSRNDSCSDLPEIQNGWKTTSHTELVRGARITYQCDPGYDIVGSDTLTCQWDLSWSSD
PPFCEKIMYCTDPGEVDHSTRLISDPVLLVGTTIQYTCNPGFVLEGSSLLTCYSRETGTPIWTSRLPH
CVSEESLACDNPGLPENGYQILYKRLYLPGESLTFMCYEGFELMGEVTIRCILGQPSHWNGPLPVC
KVNQDSFEHALEVAEAAAETSLEGGLAGHHHHHHHHHH

FIG. 5H

>cDNA sequence of human SEZ6L2 ECD

(SEQ ID NO: 18)

ctgcctctcaaagaggaagagattctccccgagccccggatccgagacacccacagtggcttccgaagccctcgctgaactgctgcacggagccctcctgagaaaggggacctgaaatgggcta
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aggagacaaccaccaccattattaccaccaccaccgtgacaaccacagtgaccagccctgtcctgtgcaacaacaacatcagcgaaggcgaaggctatgtggaatcccctgacctgggctcc
cctgtgtccagaacactcggcctcctggattgcacatactccattcacgtgtaccccggctacggaatcgaggattcaggtgcagaccctgaatctgtcccaggaggaggaactgctggtgctgg
ctggcggaggaagccctggcctcgctcctagactcctcgctaactcctccatgctcggcgaaggccaggtcctcagatcccctaccaacaggctgctcctgcacttccagagccccagagtgcc
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gacgaggacaacgacaggctcatggtcagaagcggcggaagccctctcagccctgtgatttacgacagcgacatggacgatgtgcctgagagggggcctcatctccgatgcccaaagcctgta
cgtggaactcctctccgagacccccgctaaccccctcctcctgagcctcagattcgaggcttcgaggaggacagatgtttcgctcctttctggcccatggcaacgtgaccacaaccgaccccg
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ccccgaactgcctcctcccgagtggggatggaggacagcttcccatggcgacctgatcaggggaaccgtgctgacatatcagtgtgaacccggctacgagctgctgggaagcgatatcctgac
ctgtcagtgggatctctcctggagcgctgctccccctgcctgtcagaaaatcatgacctgcgctgaccctggagagatcgctaacggccacaggaccgcttccgacgctggatttcccgtgggct
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tgacaattacctgtgtgcccggccatccttcccagtggaccagccagccccctctctgtaaggtcgcctacgaagagctgctcgacaataggaagctggaggtcacccagaccaccgacccttc
cagacaactggaaggcggc

>human SEZ6L2 ECD protein

(SEQ ID NO: 19)

METDTLLLWVLLLWVPGSTGDGAPLPLKEEEILPEPGSETPTVASEALAELLHGALLRRGPEMGYL
PGSDRDPTLATPPAGQTLAVPSLPRATEPGTGPLTTAVTPNGVRGAGPTAPELLTPPPGTTAPPPPS
PASPGPPLGPEGGEEETTTTIITTTTVTTTVTSPVLCNNNISEGEGYVESPDLGSPVSRTLGLLDCTY
SIHVYPGYGIEIQVQTLNLSQEEELLVLAGGGSPGLAPRLLANSSMLGEGQVLRSPTNRLLLHFQSP
RVPRGGGFRIHYQAYLLSCGFPPRPAHGDVSVTDLHPGGTATFHCDSGYQLQGEETLICLNGTRPS
WNGETPSCMASCGGTIHNATLGRIVSPEPGGAVGPNLTCRWVIEAAEGRRLHLHFERVSLDEDN
DRLMVRSGGSPLSPVIYDSDMDDVPERGLISDAQSLYVELLSETPANPLLLSLRFEAFEEDRCFAPF
LAHGNVTTTDPEYRPGALATFSCLPGYALEPPGPPNAIECVDPTEPHWNDTEPACKAMCGGELSE
PAGVVLSPDWPQSYSPGQDCVWGVHVQEEKRILLQVEILNVREGDMLTLFDGDGPSARVLAQL
RGPQPRRRLLSSGPDLTLQFQAPPGPPNPGLGQGFVLHFKEVPRNDTCPELPPPEWGWRTASH
GDLIRGTVLTYQCEPGYELLGSDILTCQWDLSWSAAPPACQKIMTCADPGEIANGHRTASDAGFP
VGSHVQYRCLPGYSLEGAAMLTCYSRDTGTPKWSDRVPKCALKYEPCLNPGVPENGYQTLYKHH
YQAGESLRFFCYEGFELIGEVTITCVPGHPSQWTSQPPLCKVAYEELLDNRKLEVTQTTDPSRQLEG
GLAGHHHHHHHHH

FIG. 5J

WO 2013/126810

PCT/US2013/027476

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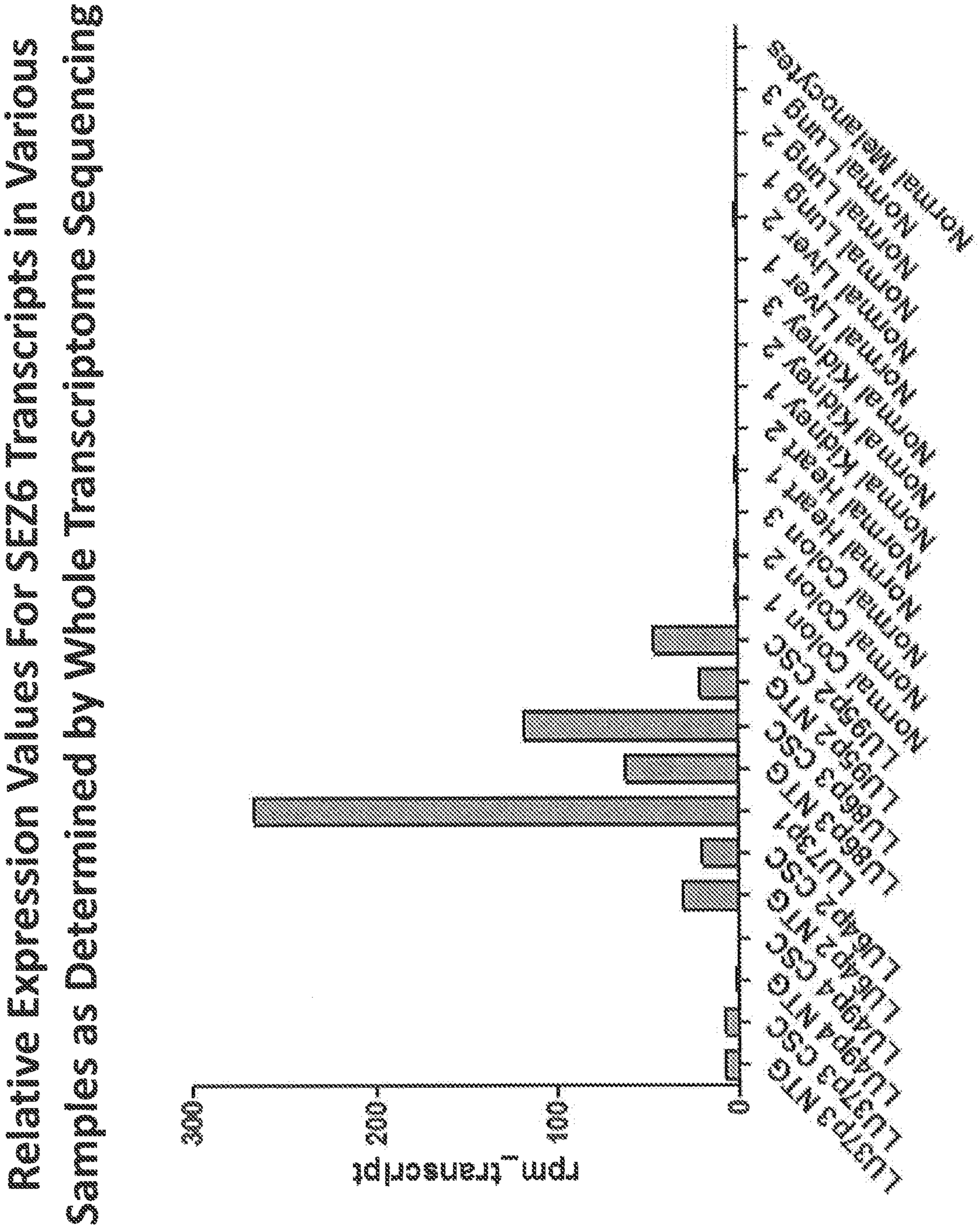
Relative Expression Values for mRNA Transcripts Associated With Neuroendocrine Tumors in NTX Lines using Whole Transcriptome Sequencing

	DLL1	DLL3	DLL4	NOTCH1	NOTCH2	NOTCH3	NOTCH4	ASCL1	NCAM1	CHGA	HES1	HES6	HEY1
LU37p3 - LCNEC	86.2	93.7	4.5	0.1	14.4	7.8	0.7	1553.1	72.0	94.0	2.7	55.1	5.6
LU64p2 - SCLC	10.9	34.3	10.8	8.2	0.2	0.7	5.7	418.4	57.8	729.5	6.3	2605.8	2.0
LU73p1 - SCLC	176.1	227.6	16.6	4.2	0.0	33.0	75.7	3623.1	77.5	515.0	34.5	3270.6	33.9
LU86p3 - SCLC	4.7	11.9	12.2	18.7	176.8	14.5	0.5	0.4	294.9	17.7	13.9	285.2	9.9
LU95p2 - SCLC	2.4	16.0	1.6	2.1	0.4	8.5	12.2	273.2	171.5	18.2	2.8	72.6	9.3
LU137p0 -LU_Ad	1.8	0.0	4.3	10.6	1455.1	27.5	0.4	0.0	0.0	0.0	54.6	1.5	1.7
LU146p0 - LU_Ad	0.0	0.0	0.3	5.6	56.8	37.9	3.9	0.0	0.8	0.0	42.8	0.8	0.3
LU153p0 - LU_Ad	0.8	0.0	5.7	8.1	195.1	8.0	6.5	0.2	4.4	0.0	4.8	0.2	1.4
LU49p4 – LU_SCC	2.6	0.7	0.0	7.6	104.2	0.0	1.3	0.0	0.0	0.0	62.1	4.3	0.0
LU70p4 - LU_SCC	4.7	0.0	0.8	12.6	123.2	1.8	0.1	0.0	0.0	0.3	42.3	2.1	0.0
LU76p5 - LU_SCC	0.8	0.0	4.8	20.0	32.5	0.1	0.0	3.4	0.3	0.3	23.0	0.8	0.0
OV26p3 - OV	34.2	65.4	15.7	0.0	101.0	17.4	0.6	2353.7	128.8	23.1	4.3	35.1	7.1
OV100p0 - OV	0.0	0.5	0.4	3.6	154.2	16.3	0.5	0.0	2.0	0.0	1.0	0.0	17.3
OV45p3 - OV	1.7	1.9	0.1	14.9	53.2	84.5	2.7	0.0	60.4	0.1	14.6	2.4	29.2
OV55p5 - OV	0.3	0.2	0.0	31.0	139.8	71.7	1.4	0.0	11.4	0.0	19.4	1.9	6.7
OV72METp0 - OV	0.0	0.1	0.2	1.6	303.1	46.8	0.2	0.3	34.5	0.1	17.1	1.9	2.0
OV91METp0 - OV	0.3	1.6	0.1	10.5	340.1	345.3	2.3	0.0	3.9	0.0	31.7	1.2	1.0
Normal Lung 1	1.7	0.0	5.7	8.2	85.9	33.1	11.4	0.4	3.4	0.0	13.8	0.1	11.4
Normal Lung 2	17.0	0.1	8.8	24.0	81.5	54.0	62.8	5.3	4.6	0.4	23.2	2.4	43.0
Normal Lung 3	26.9	0.2	41.6	146.0	25.6	239.3	21.7	0.8	1.8	1.3	11.9	8.1	40.2
Normal Lung 4	0.2	0.0	6.0	11.8	81.5	40.4	15.8	0.0	1.2	0.0	11.4	0.3	14.1
Normal Ovary	0.3	0.0	5.1	7.8	250.9	44.1	5.1	0.6	125.5	0.2	8.5	0.7	0.4

FIG. 6A


Relative Expression Values For SEZ6 Transcripts in Various Samples as Determined by Whole Transcriptome Sequencing
rpm_transcript
300
200
100
0
Normal Cerebellum 1
Normal Cerebellum 2
Normal Cerebellum 3
Normal Cerebellum 4
Normal Cerebellum 5
Normal Cerebellum 6
Normal Cerebellum 7
Normal Cerebellum 8
Normal Cerebellum 9
Normal Cerebellum 10
Normal Cerebellum 11
Normal Cerebellum 12
Normal Cerebellum

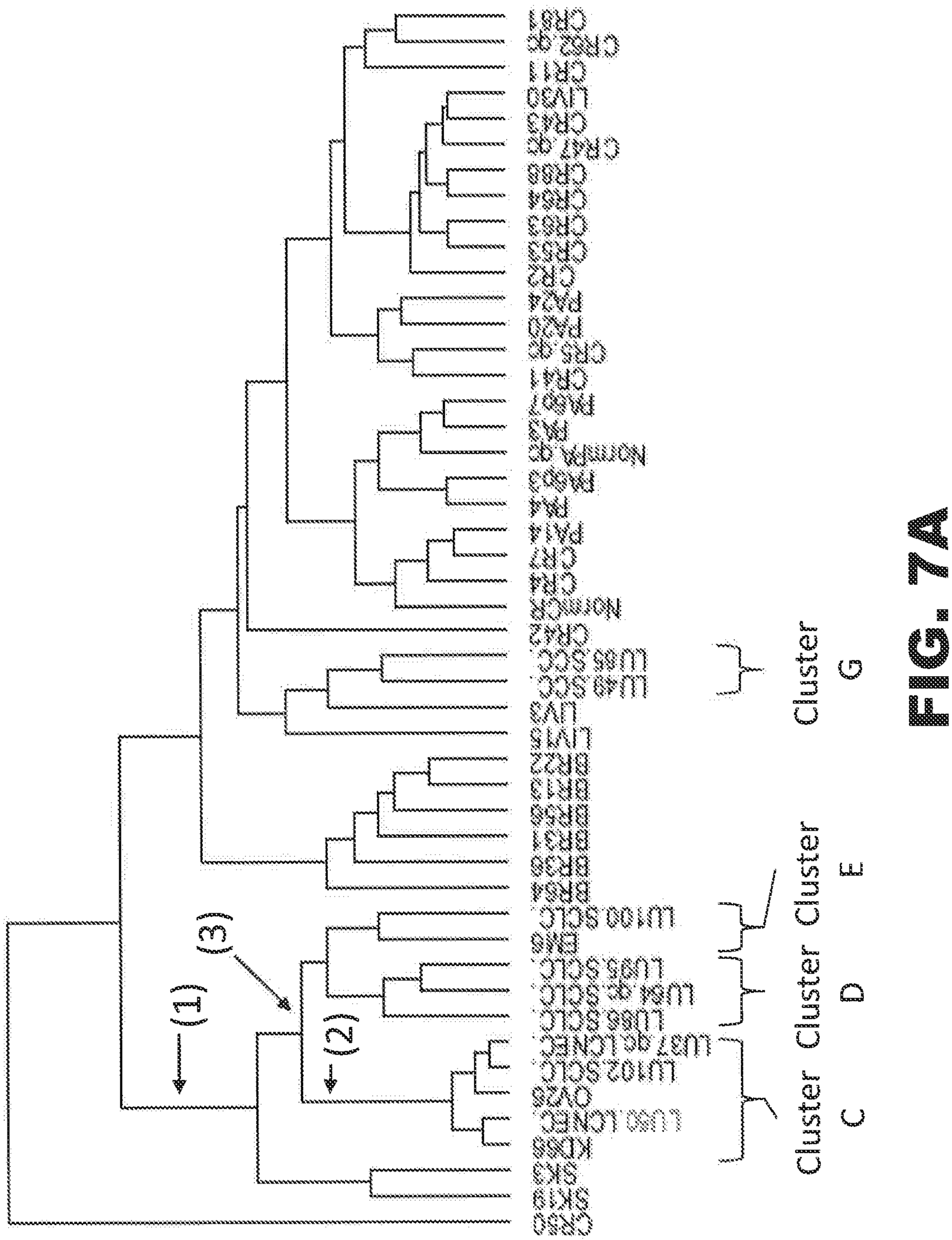

Microarray Data Showing Clusters
of Tumors with Neuroendocrine Features
(1)
(2)
(3)
Cluster A
Cluster B
Cluster C
Cluster D
Cluster E
Cluster F
Cluster G

FIG. 7A

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Average Normalized Intensity Values for Common Markers of Neuroendocrine Phenotype

			Cluster C					Cluster D			Cluster E		Cluster G	
	Gene Symbol	Median (48 samples)	KD66	LU50(LCNEC)	OV26	LU102(SCLC)	LU37-qc(LCNEC)	LU86(SCLC)	LU64-qc(SCLC)	LU95(SCLC)	EM6	LU100(SCLC)	LU49(SCC)	LU85(SCC)
Achaete-scute complex homolog 1	ASCL1	9	6589	8238	9382	[illegible]	9664	11	3390	[illegible]	14	261	8	5
Calcitonin	CALCA	73	[illegible]	8352	[illegible]	[illegible]	[illegible]	70	24	2477	53	45	52	39
CGRP	CALCB	143	2534	1147	1547	2584	2757	[illegible]	433	1264	309	135	65	81
CD117, Kit receptor	KIT	343	5978	4907	3561	6416	6254	[illegible]	[illegible]	[illegible]	712	948	34	301
Chromogranin A	CHGA	53	6167	8902	[illegible]	8206	7408	7950	[illegible]	[illegible]	9726	4378	1249	2364
Chromogranin B	CHGB	22	1615	2152	1516	1456	1242	2365	1362	4151	4656	1833	32	24
Dopa decaroxylase	DDC	2441	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1498	4234	9297	85	334	134	286
Gamma (Neural) Enolase	ENO2	1910	2054	1881	1911	1573	1262	4043	4737	[illegible]	6394	1824	2472	2241
GDNF family receptor alpha 1	GFRA1	9	263	29	37	146	133	90	4	6	77	47	9	4
CD56	NCAM1	82	551	875	801	999	727	2618	2519	3296	361	2899	425	106
PGP9.5	UCHL1	415	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	9749	[illegible]	7789	4249	122	8251
Proopiomelanocortin	POMC	94	751	427	590	750	657	66	560	5204	68	84	213	160
Somatostatin	SST	67	[illegible]	1316	[illegible]	5869	[illegible]	35	9	62	1992	3613	90	28
Somatostatin Receptor 5	SSTR5	613	733	906	875	636	633	907	344	622	916	662	918	401
Synaptophysin	SYP	19	15	15	9	17	15	27	53	91	49	19	11	19
TTF1	NKX2-1	18	3137	3508	2734	5180	3315	1258	3585	2229	27	311	85	8

FIG. 7B

Average Normalized Intensity Values for Selected Genes in the Notch Pathway and ASCL1

		Cluster C					Cluster D			Cluster G	
	Median (48 samples)	KD66	LU50(LCNEC)	OV26	LU102(SCLC)	LU37(LCNEC)	LU86(SCLC)	LU64(SCLC)	LU95(SCLC)	LU49(SCC)	LU85(SCC)
ASCL1	9	6589	8238	9382	12169	9664	11	3390	10298	8	5
DLL1	51	348	565	406	497	179	218	98	514	29	120
DLL3	350	4584	3985	6232	5884	5233	1686	3137	5814	601	492
DLL4	614	601	445	592	301	280	763	198	673	357	469
HES1	670	128	129	160	92	82	551	137	335	2665	2024
HES6	117	196	361	481	416	279	5456	2716	3535	28	33
HEY1	89	86	101	116	103	77	1660	680	2502	3776	231
HEYL	87	157	132	128	148	132	2645	102	267	333	80
JAG1	630	159	114	110	95	111	743	521	311	9131	678
JAG2	125	335	529	398	420	247	324	513	611	159	153
NOTCH1	666	34	23	41	17	14	1039	381	202	4720	438
NOTCH2	26	6	11	12	16	12	105	11	1	37	5
NOTCH3	101	13	27	91	81	72	302	37		1474	322
NOTCH4	14	6	7	13	9	5	14	15	69	14	7
RBPJ	1289	1891	2255	1933	2717	2278	4502	2678	5167	1226	1029

FIG. 7C

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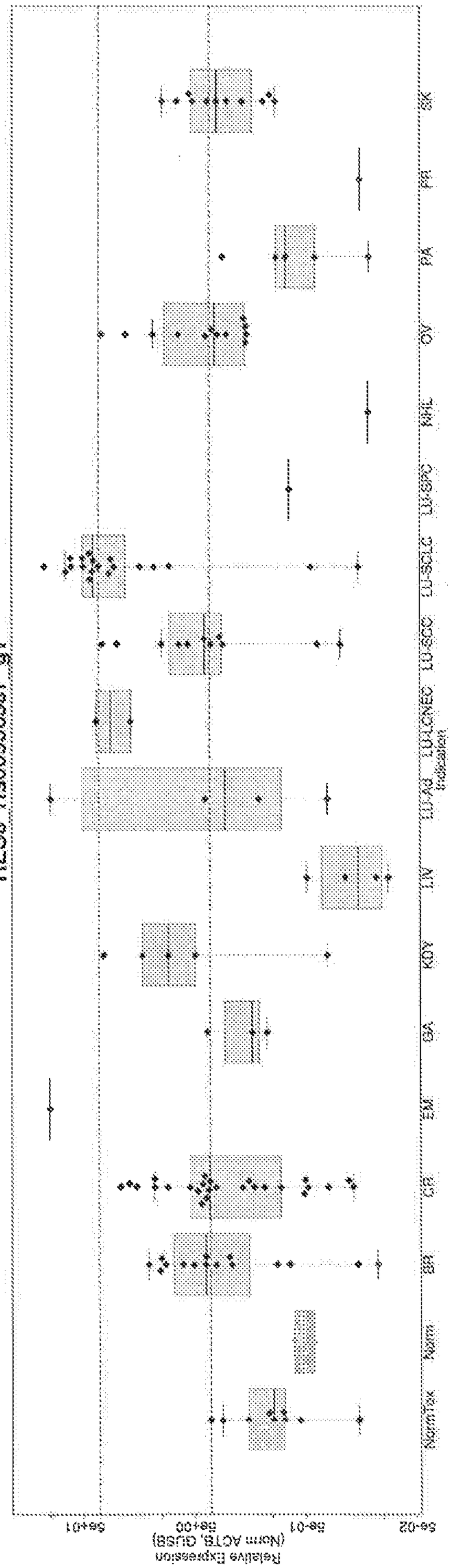

Expression of HES6 mRNA in Neuroendocrine Tumors Derived by qRT-PCR
HES6 Hs00936587_g1
Relative Expression (Norm ACTB, GUSB)
5e-02
5e-01
5e+00
5e+01
NORMAL
PANC
LUNG
COLO
BRCA
OVAR
MEL
GIST
KIDY
GBM
LIV
LU-AD
LU-LCNEC
LU-SCC
LU-SCLC
LU-BRC
MBL
Histology

FIG. 7D

Levels of Selected Genes Linked To Neural Phenotypes

	Gene Symbol	Median (48 samples)	Cluster C					Cluster D			Cluster E		Cluster G	
			KD66	LU50 (LCNEC)	OV26	LU102 (SCLC)	LU37 (LCNEC)	LU86 (SCLC)	LU64 (SCLC)	LU95 (SCLC)	EM6	LU100 (SCLC)	LU49 (SCC)	LU85 (SCC)
Achaete-scute complex homolog 1	ASCL1	9	6529	2138	5887	12140	9664	11	3390	6299	14	261	8	5
Brain-expressed X-linked protein 1	BEX1	62	2393	4403	3249	5982	3653	6545	5164	4554	1424	81	764	50
Brain-expressed X-linked protein 3	NGFRAP1	8484	3367	4346	5975	12692	5212	14046	15695	16941	12287	12165	4500	12766
Brain-expressed X-linked protein 4	BEX4	36	8	8	36	14	6	2108	9743	6528	2113	197	521	408
CD56	NCAM1	82	551	875	601	999	727	2618	2519	3296	361	2693	425	106
Claudin 11	CLDN11	7	670	739	2374	5820	3373	548	126	213	14	108	18	3
Cytokeratin 10	KRT10	2624	3096	2503	2855	5373	5057	2731	1642	4671	3756	1026	12106	2330
Cytokeratin 17	KRT17	5858	5436	7664	9874	12215	8933	2600	3701	5971	63	304	5283	5662
Cytokeratin 19	KRT19	19604	2091	2432	3214	2435	2532	445	317	7074	877	816	20560	15081
Cytokeratin 7	KRT7	123	249	348	396	515	313	23	9	333	13	54	18	600
Delta-like 1	DLL1	51	348	565	406	497	179	218	98	514	146	162	29	120
Delta-like 3	DLL3	350	4584	7001	6555	6555	5555	1686	3137	6814	758	676	601	492
Hairy and enhancer of split 1	HES1	670	128	129	160	92	82	551	137	335	118	528	2665	2024
Hairy and enhancer of split 6	HES6	117	196	361	481	416	279	5476	2716	2535	1634	694	28	33
Islet 1	ISL1	48	18	23	22	33	16	5067	10055	9744	121	872	34	28
LIM homeobox 1	LHX2	21	9	12	29	8	1	2746	6672	10032	6249	1663	161	29
Neurogenic differentiation 1	NEUROD1	8	44	11	35	62	91	331	135	799	4917	1475	6	2
Neurogenic differentiation 2	NEUROD2	59	49	40	52	32	25	808	231	416	3019	450	61	65
Neuronal cell adhesion molecule	NRCAM	66	721	797	870	1156	1409	3981	4359	4579	1331	1619	5191	659
NK2 homeobox 2	NKX2-2	7	4	7	7	10	4	9725	206	1162	63	301	0	247
Rhombotin-3	LMO3	145	6975	15823	7103	6932	6566	45	2252	7445	182	9882	72	271
Secretagogin	SCGN	77	1950	2620	2468	1701	3052	1164	9356	12272	147	939	42	20
Secretogranin II	SCG2	23	445	299	423	1122	1265	37	693	4085	1002	3050	108	51
Secretogranin III	SCG3	26	1552	1961	1723	1870	1692	2331	3164	10229	5825	3721	54	19
Semaphorin 4C	SEMA4C	1647	941	651	781	1123	742	9648	5952	6341	2769	3426	5134	957
Semaphorin 6C	SEMA6C	118	323	318	279	241	220	1313	1859	619	512	432	1106	107
Seizure related 6 homolog	SEZ6	60	153	143	135	75	66	1087	435	2324	135	690	51	61
Seizure related 6 homolog like	SEZ6L	113	43	25	53	42	26	48	64	116	108	114	18	119
Seizure related 6 homolog like2	SEZ6L2	1464	1136	1667	1852	1336	1089	2317	3026	5631	3793	4933	1491	3247
SRY (sex determining region Y)-box 11	SOX11	13	426	360	361	597	768	3754	6124	981	10	1880	11	17
SRY (sex determining region Y)-box 2	SOX2	8	171	175	270	396	313	2816	74	3267	0	2937	1376	348
SRY (sex determining region Y)-box 4	SOX4	5404	5693	5641	4070	3872	5340	12552	14688	15570	12175	12442	2455	1367
Synaptosomal-associated protein, 25kDa	SNAP25	10	1037	1040	1027	1108	1040	1112	921	568	618	1612	37	27
Synaptotagmin IV	SYT4	26	1418	1237	2115	3958	3076	1150	1061	4320	2775	2488	11	10
TTF-1	NKX2-1	18	3137	3508	2734	5190	3315	1758	3585	2229	27	311	85	8
Zic family member 2	ZIC2	163	25	18	29	10	5	7583	5553	3440	697	279	93	227

FIG. 7E

WO 2013/126810
PCT/US2013/027476
CA 02865415 2014-08-22

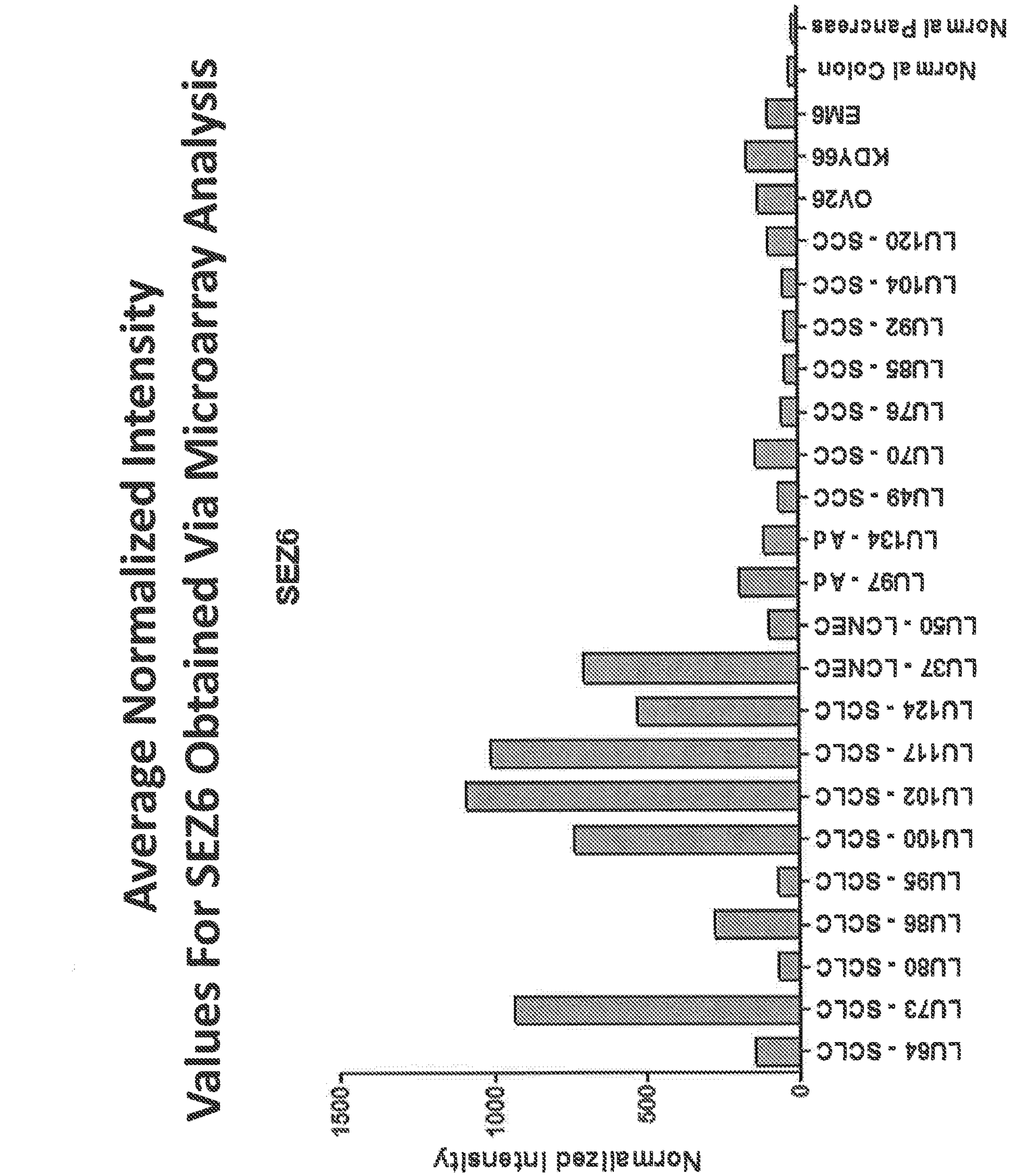

Average Normalized Intensity Values For SEZ6 Obtained Via Microarray Analysis
SEZ6
Normalized Intensity
0
500
1000
LU84 - SCLC
LU73 - SCLC
LU80 - SCLC
LU86 - SCLC
LU95 - SCLC
LU100 - SCLC
LU102 - SCLC
LU117 - SCLC
LU124 - SCLC
LU37 - LCNEC
LU50 - LCNEC
LU97 - AD
LU134 - AD
LU49 - SCC
LU70 - SCC
LU76 - SCC
LU85 - SCC
LU92 - SCC
LU104 - SCC
LU120 - SCC
OV26
KDY66
EM6
Normal Colon
Normal Pancreas

FIG. 7F

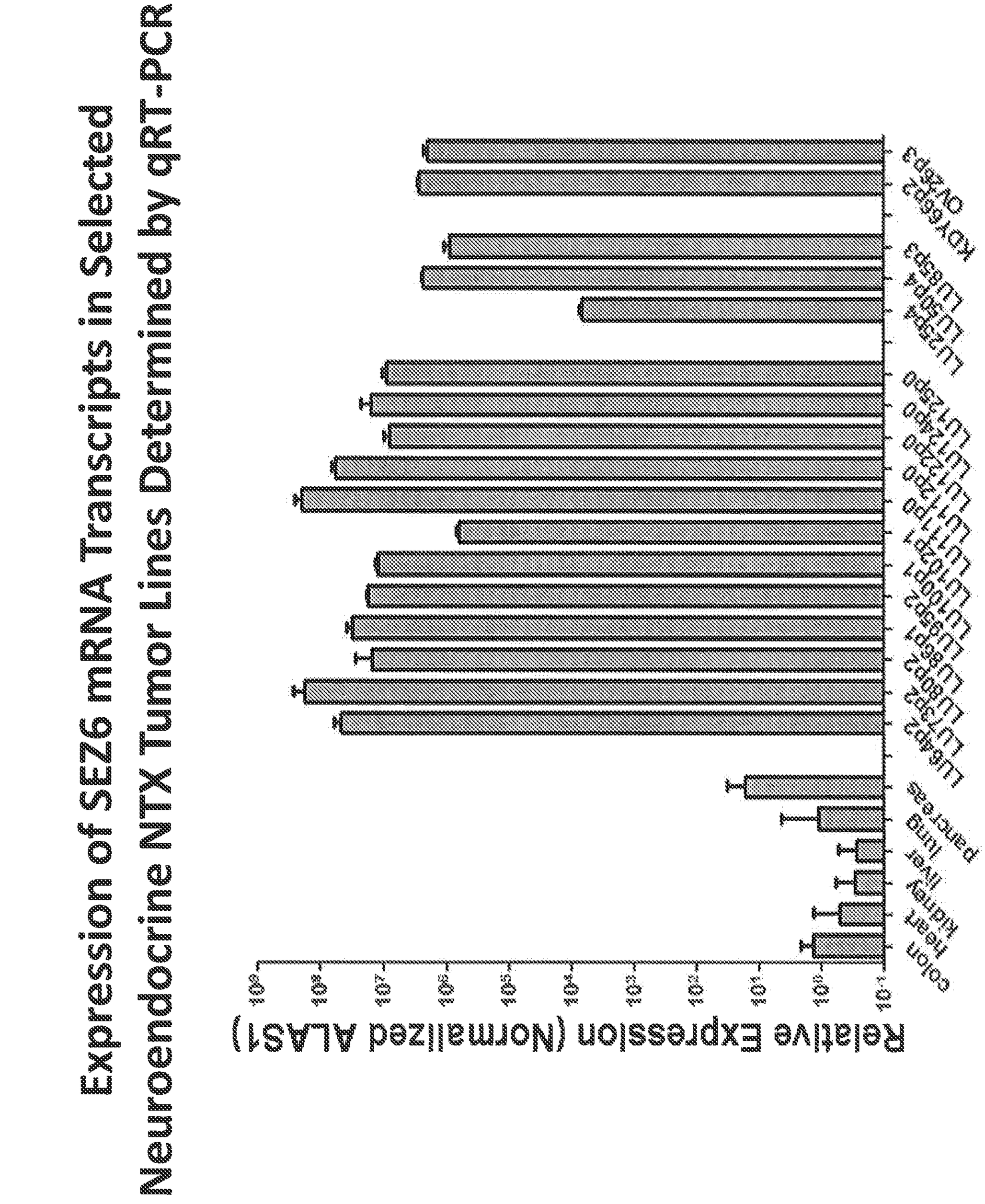

Neuroendocrine NTX Tumor Lines Determined by qRT-PCR
Expression of SEZ6 mRNA Transcripts in Selected
Relative Expression (Normalized ALAS1)
Colon
Heart
Kidney
Liver
Lung
pancreas
LU130A
LU168P2
LU168P1
LU139P2
LU139P1
LU149P2
LU149P1
LU128P2
LU128P1
LU120P2
LU120P1
LU122P2
LU122P1
LU126P2
LU126P1
LU130P2
LU130P1
LU135P2
LU135P1
LU139P2
LU139P1
LU149P2
LU149P1
LU168P2
LU168P1
LU130A

FIG. 8A

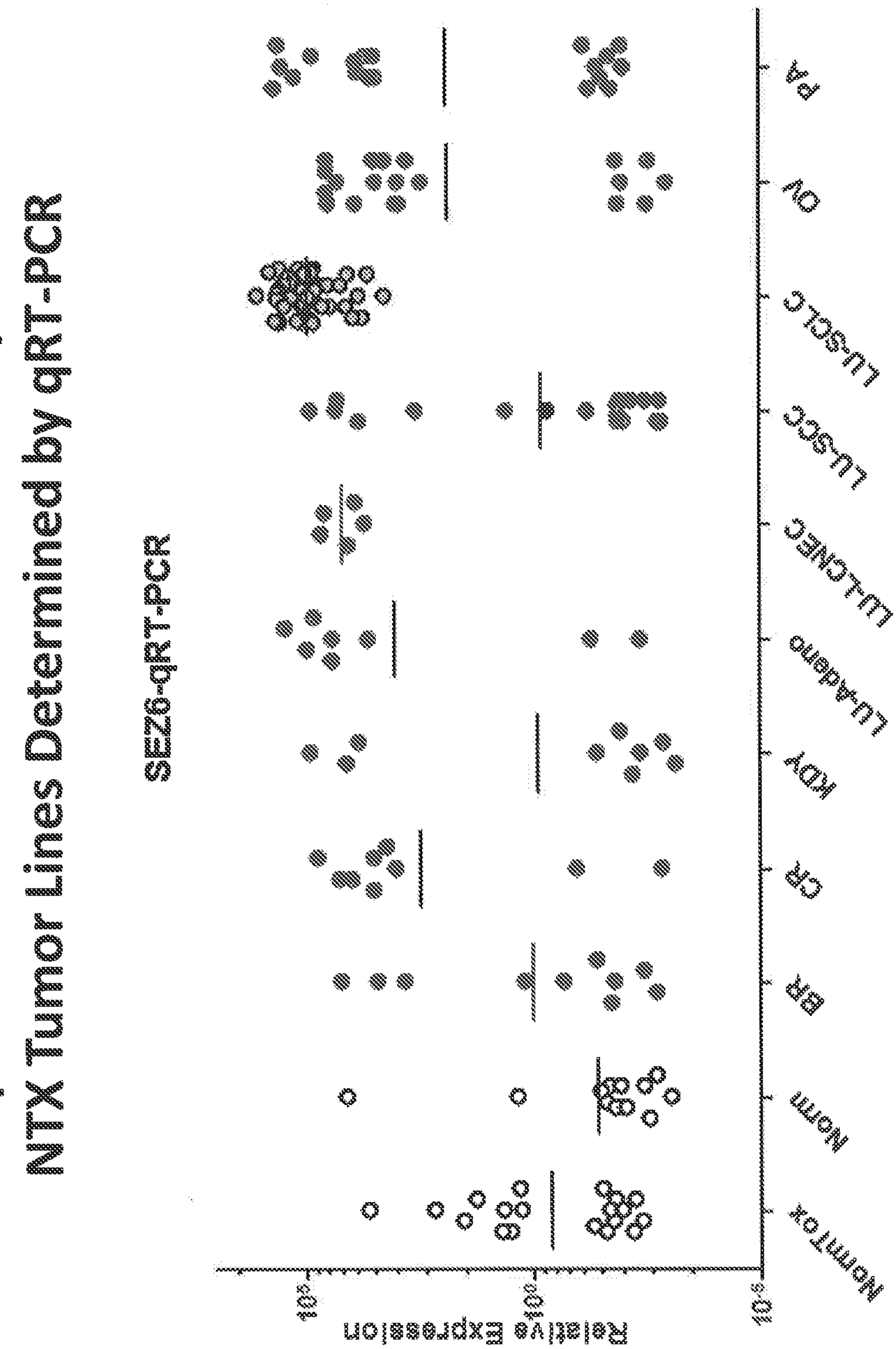

Expression of SEZ6 mRNA Transcripts in NTX Tumor Lines Determined by qRT-PCR
SEZ6-qRT-PCR
Relative Expression
NormTox
Norm
BR
CR
Kidney
Urinary
Uterine
Lung
Ovary

FIG. 8B

FIG. 9A

Absolute and Normalized SEZ6 mRNA Expression in Various Whole Tumor Samples Using qRT-PCR

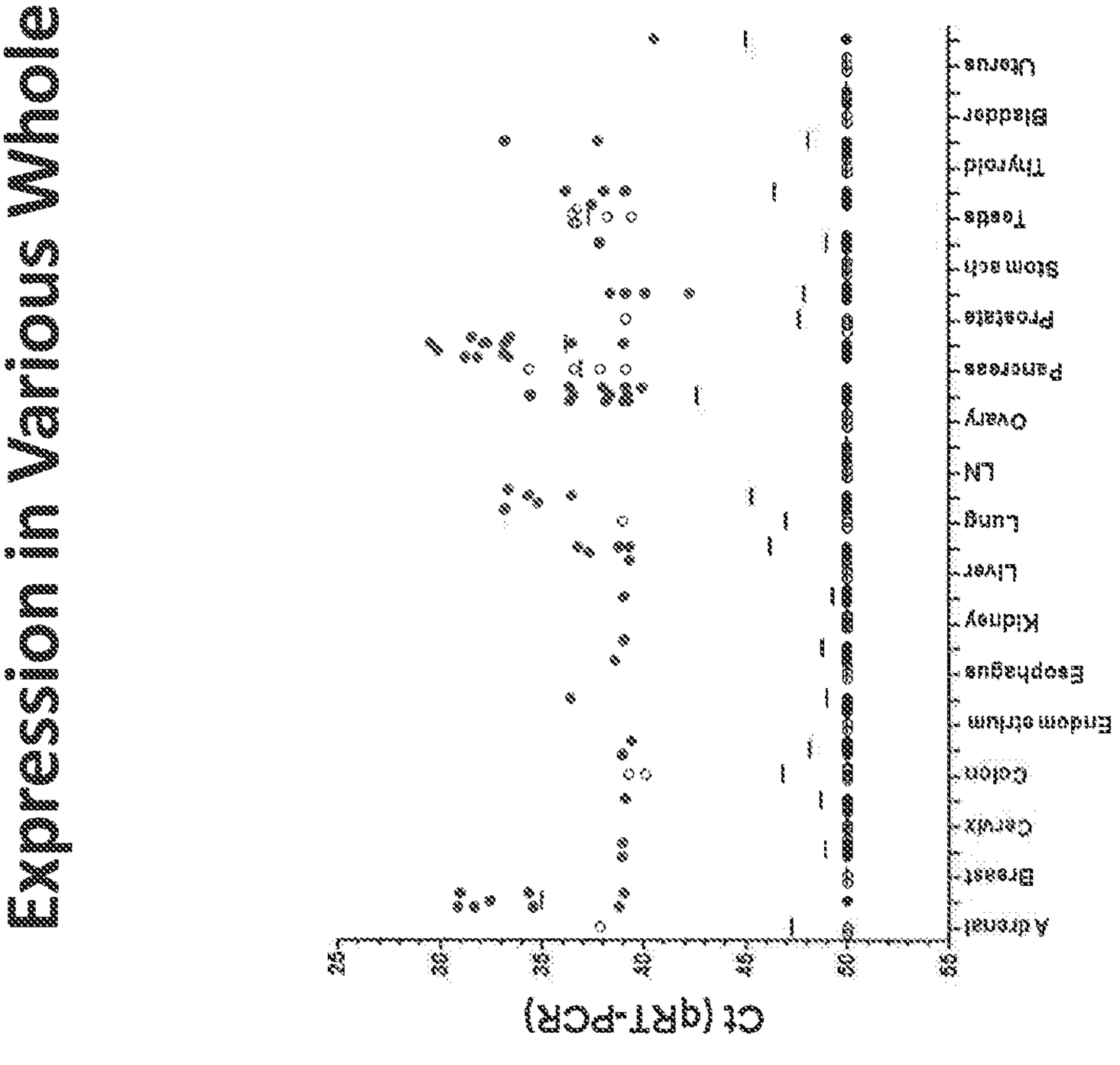
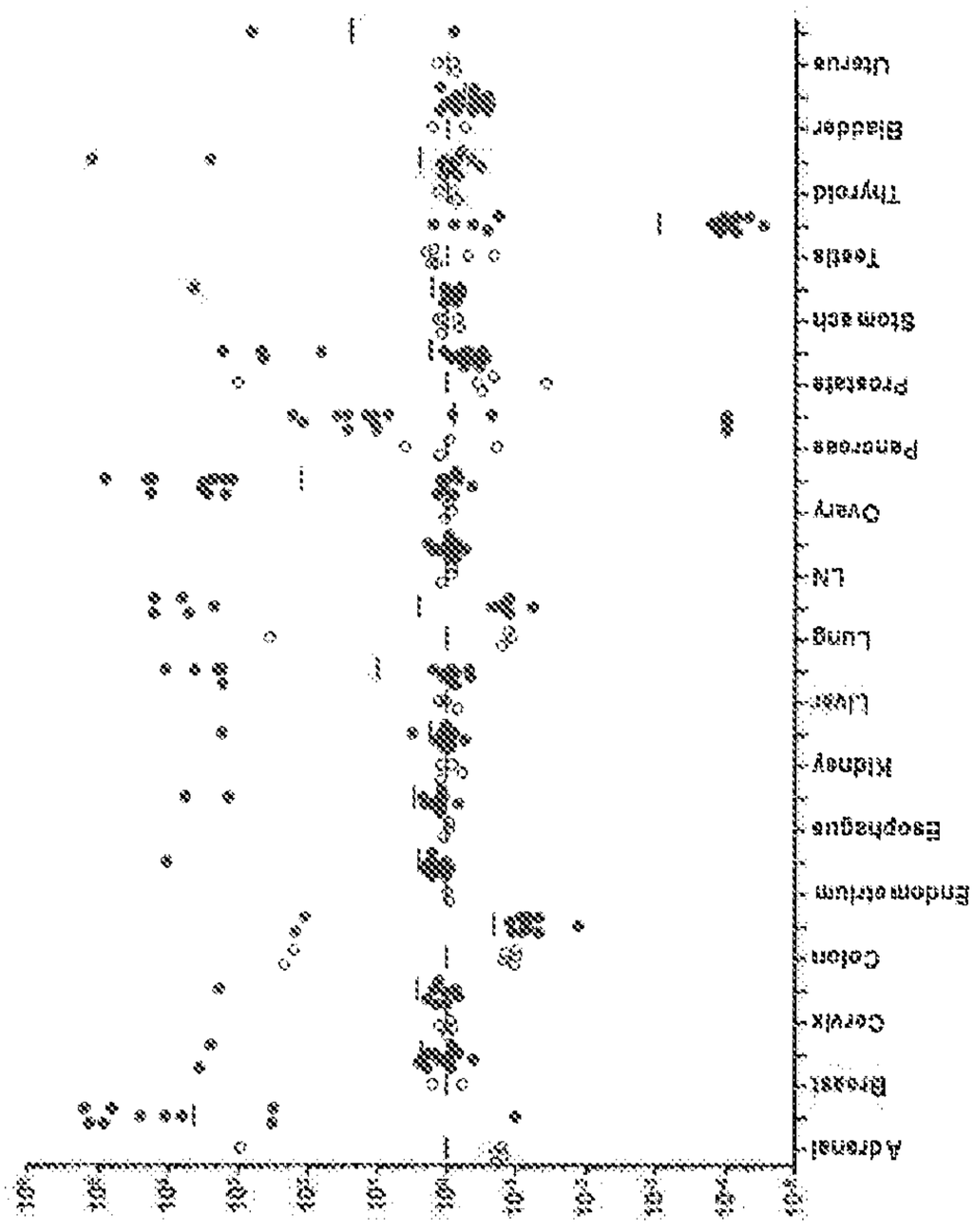


FIG. 9B



Protein Sequences of Exemplary Murine SEZ6 Modulator Light Chain Variable Regions

mAb	FW1	CDRL1	FW2	CDRL2	FW3	CDRL3	SEQ ID NO.
SC17.1	QIVLTQSPALMSASPGEKVSLTC	SANSTVSF	MYWYQQKPRSSPTPWIY	LTSNLAS	GVPARFSGSGSGTSYSLTISSMEAEDAATYYC	QQWSSNSPITFGAGTKLELK	20
SC17.2	DIVMSQSPSSLAVSVGEKVTMSC	KSSQSLLYSSNQKSY	LAWYQQKPGQSPKLLIY	WASTRES	GVPDRFTGSGSGTDFTLTISSVQAEDLAVYYC	KQSYNLRTFGGGTKLEIK	22
SC17.9	DIVMSQSPSSLAVSVGEKVTMSC	KSSQSLLYSSNQKNY	LAWYQQKPGQSPKLLIY	WASTRES	GVPDRFTGSGSGTDFTLTISSVKAEDLAVYFC	QQYYNYPYTFGGGTKLK	24
SC17.16	DIQMTQSPASLSVSVGETVTITC	RASANINSN	LVWYQQKQGKSPQLLVY	AATNLAD	GVPSRFSGSGSGTQYSLKINSLQSEDFGNYYC	QHFWGTPRTFGGGTKLEIK	26
SC17.38	DIVVTQSPASLAVSLGQRATISC	RASESVEYYGTSL	MQWFQQKPGQPPKLLIY	AASNVES	GVPARFSGSGSGTDFSLNIHPVEEDDIAMYFC	QQDRKVPWTFGGGTKLEIK	28
SC17.3	DIVMSQSPSSLAVSVGEKVTMSC	KSSQSLLYSSNQKNY	LAWYQQKPGQSPKLLIY	WASTRES	GVPDRFTGSGSGTDFTLTISSVKAEDLAVYYC	QQYYSYPTFGGGTKLEIK	30
SC17.4	DIKMTQSPSSMYASLGERVTITC	KASQDINSY	LTWFQQKPGKSPKTLIY	RANRLID	GVPSRFSGSGSGQDYSLTISSLDYEDMGIYYC	LQYDDFPWTFGGGTKLEIK	32
SC17.8	DVVMTQTPLSLPVSLGDQASISC	RSSQSLVHSNGDTY	LHWYLQKPGQSPKLLIY	KVSNRFS	GVPDRFSGSGSGTDFTLKISRVEAEDLGLYFC	SQSTLIPYTFGGGTKLDIKR	34
SC17.10	DIVMSQSPSSLAVSVGEKVTMSC	KSSQSLLYSSNQKNY	LAWYQQKPGQSPKLLIY	WASTRES	GVPDRFTGSGSGTDFTLTISSVKTEDLALYYC	QQYYWFPYTFGGGTKLEIK	36
SC17.11	ENVLTQSPAIMSASPGEKVTMTC	RASSSVSSSY	LHWYQQKSGASPKLWIY	STSNLAS	GVPARFSGSGSGTSYSLTISSVEAEDAATYYC	QQYSDYPFTFGSGTKLVIK	38
SC17.14	DVLMTQTPLSLPVSLGDQASISC	RSSQSIVHSNGNTY	LEWFLQKPGQSPKLLIY	KVSNRFS	GVPDRFSGSGSGTDFTLKISRVEAEDLGVYYC	FQGSHVPYTFGGGTKLEIKR	40
SC17.15	QIVLTQSPALMSASPGEKVTMTC	SASSSVNY	MYWYQQKPRSSPKPWIY	LTSNLAS	GVPVRFSGSGSGTSYSLTISSMEAEDAATYYC	QQWSNNPPTFGSGTKLELK	42
SC17.17	QIVLTQSPAIMSASPGEKVTMTC	SASSSVSY	MHWYQQKSGTSPKRWIY	DTSKLPS	GVPARFSGSGSGTSYSLTISSMEAEDAATYYC	QQWSSTPPTFGAGTKLELK	44
SC17.18	DIQMTQSSSYLSVSLGGRVTITC	KASDHINNW	LAWYQQKPGNAPRLLIS	GATSLET	GVPSRFSGSGSGKDYTLSITSLQTEDVATYYC	QQYWSIPLTFGAGTKLELK	46
SC17.19	DIVLTQSPASLAVSLGQRAAISC	KPSQSVDYDGDSY	MNWYQQKPGQPPKLLIY	AASNLES	GIPARFSGSGSGTDFTLNIHPVEEEDAATYYC	HQINDDPWTFGGGTKLK	48
SC17.22	DVVLTQTPLSLPVSLGDQASISC	RSSQSIVHINRHTY	LGWYLQKPGQSLKLLIY	GVSNRFS	GVPDRFSGSGSGTDFTLKISRVEAEDMGVYYC	FQGTHVPFTFGSGTKLEIK	50
SC17.24	DIVMSQSPSSLAVSVGEKVTMSC	KSSQSLLYSSNQKSY	LAWYQQKPGQSPKLLIY	WASTRES	GVPDRFTGSGSGTDFTLTISSVQAEDLAVYYC	KQSYNLRTFGGGTKLEIK	52
SC17.27	DVVMTQTPLTLSVTIGQPASISCK	SSQSLLESDGKTY	LNWLLQRPGQSPKRLIY	LVSKLDS	GVPDRFTGSGSGTDFTLKISRVEAEDLGVYYC	WQGIQHPRTFGGGTKLEIK	54
SC17.28	DILLTQSPAILSVSPGEGVSFSC	RASQSIGTS	IHWYQQRTNGSPRLLIK	YASESIS	GIPSRFSGSGSGTDFTLRINSLESEDIADYYC	QQSNSWPLTFGAGTKLELK	56
SC17.29	DIVMTQSHKFMSTSVGDRVSITC	KASQDVGTD	VAWYQQKPGQSPKLLIY	WASTRHT	GVPDRFTGSGSGTDFTLTISNVQSEDLADYFC	QQYSSYPYTFGGGTKLEIKR	58
SC17.30	ENVLTQSPAIVSASPGEKVTMTC	RASSSVISSY	LHWYQQKSGASPKLWIY	STSNLAS	GVPARFSGSASGTSYSLTISSVEAEDAATYYC	QQYSGYPLTFGAGTKLELK	60

FIG. 10A

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Protein Sequences of Exemplary Murine SEZ6 Modulator Light Chain Variable Regions

mAb	FW1	CDRL1	FW2	CDRL2	FW3	CDRL3	SEQ ID NO.
SC17.32	DIQMTQSPASLSASVGETVTMTC	RASGNIHNY	LVWYQQKQGKSPQLLVY	NAKTLAD	GVPSRFSGSGSGTQYSLKINSLQPEDFGSYYC	QHFWSTPPTFGGGTKLEIK	62
SC17.34	DIKMTQSPSSMYASLGERVTITC	KASQDINSY	LSWFQQKPGKSPKTLIY	RANRLVD	GVPSRFSGSGSGQDYSLTISSLEYEDMGIYYC	LQYDEFPPTFGGGTKLEIK	64
SC17.35	ENVLTQSPAIMSASPGEKVTLTC	RASSSMSSSY	LHWYQQKSGASPKLWIY	STSNLAS	GVPARFSGSGSGTSYSLTISSVEAEDAATYYC	QQYSAYPFTFGSGTKLEIK	66
SC17.36	QIVLTQSPALMSASPGEKVTMTC	SASSSVSY	MYWYQQKPRSSPKPWIY	LTSNLAS	GVPARFSGSGSGTSYSLTISSMEAEDAATYYC	QQWSSNPPTFGGGTKLEIKR	68
SC17.39	DVLMTQTPLSLPVSLGDQASISC	RSSQSLVHRNGNTY	FHWYLQKPGQSPKLLIY	KVSNRFS	GVPDRFSGSGSGTDFTLKISRVEAEDLGVYFC	SQSTYVPWTFGGGTKLEIK	70
SC17.40	DVVMTQTPLSRPVTLGDQASISC	RSSQSLVHSNGNTY	LHWYLQKPGQSPKLLIY	KVSNRFS	GVPDRFSGSGSGTDFTLKISRVEAEDLGVYFC	SQNTHVPFTFGSGTKLEIK	72
SC17.41	QIVLTQSPALMSASPGEKVTMTC	SASSSVSY	MYWYQQKPRSSPKPWIY	LTSNLAS	GVPTRFSGSGSGTSYSLTISSMGAEDAATYYC	QQWNTNPPTFGAGTKLELK	74
SC17.42	ENVLTQSPAIMSASPGEKVTMTC	SASSSVNY	MYWYQQKSSTSPKLWIY	DTSKLTS	GVPGRFSGSGSGNSYSLTISNMEAEDVATYYC	FQGSGYPLTFGSGTKLEIK	76
SC17.45	ENVLTQSPAIMSASPGEKVTMTC	SASSSVNY	MYWYQQKSSTSPKLWIY	DTSKLT	GVPGRFSGSGSGNSYSLTISNMEAEDVATYYC	FQGSGYPLTFGSGTKLEIK	78
SC17.46	SFVMTQTPKFLLVSAGDRVTITC	KASQSVNND	VAWYQQKPGQSPKLLIY	YASNRYT	GVPDRFTGSGYGTDFTFTISTVQAEDLAVYFC	QQDYSSPRTFGGGTKLEIK	80
SC17.47	QIVLTQSPAIMSASPGEKVSMTC	SASSSVSY	MHWYQQKSGTSPKRWIY	DTSKLAS	GVPARFSGSGSGSSYSLTISSMEAEDAATYYC	QQWSSTPPTFGGGTKLEIKR	82
SC17.49	DVVMTQTPLTLSVTIGQPASISC	KSSQSLLESDGKTY	LNWLLQRPGQSPKRLIY	LVSKLDS	GVPDRFTGSGSGTDFTLKISRVEAEDLGVYYC	WQGIQHPRTFGGGTKLEIK	84
SC17.50	DIVLTQSPASLAASLGQRATISC	RASQSVSTSSYSY	MHWYQQKPGQPPKLLIK	YASNLES	GVPARFSGSGSGTDFTLNIHPVEEEDTATYYC	QHSWEIPWTFGGGTKLEIK	86
SC17.53	DIVLTQSPASLAASLGQRATISC	RASQSVSTSSYSY	MHWYQQKPGHPPKLLIR	YASNLES	GVPARFSGSGSGTDFTLNIHPVEEEDTATYYC	QHSWEIPYTFGGGTKLEIKR	88
SC17.54	DVVLTQTPLTLSVTIGQPASISC	KSSQSLLYSDGKTY	LNWLLQRPGQSPKRLIY	LVSKLDS	GVPDRFTGSGSGTDFTLKISRVEAEDLGLYYC	WQGTHFPWTFGGGTKLEIK	90
SC17.56	DIVMSQSPSSLAVSVGEKVTMSC	KSSQSLLYSSNQKNY	LAWYQQKPGQSPKLLIY	WASTRES	GVPDRFTGSGSGTDFTLTISSVKAEDLAVYFC	QQYYNYPYTFGGGTKLEIKR	92
SC17.57	QIVLTQSPAIMSASLGERVTMTC	TASSSVSSSY	LHWYQQKPGSSPKLWIY	STSNLAS	GVPPRFSGSGSGTSYSLTISSMEAEDAATYYC	HQYHRSPPTFGGGTKLEIK	94
SC17.59	DIQMTQSPASLSASVGETVTITC	RASGNLHNY	LAWYQQKQGKSPQLLVY	NAKTLAD	GVPSRFSGSGSGTQYSLKINSLQPEDFGTYFC	QHFWSIPPTFGGGTKLEIKR	96
SC17.61	QIVLTQSPAIMSASPGEKVTISC	SASSSVSY	IYWYQQKPGSSPKPWIY	RTSNLAS	GVPARFSGSGSGTSYSLTISSMEAEDAATYYC	QQYHSYPWTFGGGTKLEIK	98
SC17.63	SIVMTQTPKFLLVSAGDRVAITC	KASQSVSND	VAWYQQKPGQSPTLLISY	ASNRYT	GVPDRFTGSGYGTDFTFTISTVQAEDLAVYFC	QQGYSSPFTFGGGTKLEIKR	100

FIG. 10A (Cont.)

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Protein Sequences of Exemplary Murine SEZ6 Modulator Light Chain Variable Regions

mAb	FW1	CDRL1	FW2	CDRL2	FW3	CDRL3	SEQ ID NO.
SC17.71	DIQMTQSPASLSASVGETVTIAC	RASGNIHNY	LTWYQQRQGKSPQLLVY	NAKTLAV	GVPSRFSGSGSGTQYSLKINSLQPEDFGSYYC	QHFWNTPPTFGGGTKLEIK	102
SC17.72	DIQMTQTTSSLSASLGDRVTISC	SASQGISNY	LNWYQQKPDGTVKLLIY	YTSSLHS	GVPSKFSGSGSGTDYSLTISNLEPEDIATYYC	QQYSKLPYTFGGGTKLEIKR	104
SC17.74	DIQMTQSSSYLSVSLGGRVTITC	KASDHINNW	LAWYQQKPGNAPRLLIS	GATSLET	GVPSRFSGSGSGKDYTLSITSLQTEDVATYYC	QQYWSTPPTFGAGTKLELK	106
SC17.76	DIVITQDELSNPVTSGESVSISC	RSSKSLLYKDGKTY	LNWFLQRPGQSPQLLIY	LMSTRAS	GVSDRFSGSGSGTDFTLEISRVKAEDVGVYYC	QQLVEYPRTFGGGTKLEIK	108
SC17.77	DIQMTQSPASLSASVGETVTITC	RASGNIHNY	LAWYQQKQGKSPQLLVY	NAKALAD	GVPSRFSGSGSGTQYSLKINSLQPEDFGSYYC	QHFWSIPPTFGGGTKLEIK	110
SC17.79	DIQMTQSPASLSASVGETVTITC	RASGNIHNY	LAWYQQKQGKSPQLLVY	NAKTLAD	GVPSRFSGSGSGTQYSLRINSLQPEDFGSYYC	QHFWSTPPTFGGGTKVEIK	112
SC17.81	DIVMSQSPSSLTVSVGEKVTLSC	KSSQSLLYSTNQKIY	LAWYQQKPGQSPKLLIY	WASTRES	GVPDRFTGSGSGTDFTLAISNVKAEDLAVYYC	QQYYSYPYTFGGGTKLEIKR	114
SC17.82	QIVLTQSPAIMSASLGEEITLTC	SASSSVSY	MHWYQQKSGTSPKLLIY	STSNLAS	GVPSRFSGSGSGTFYSLTISSVEAEDAADYYC	HQWSSFTFGSGTKLEIK	116
SC17.84	QIVLTQSPAIMSASPGEKVTMTC	SASSSISY	MHWYQQKSGTSPKRWIY	DTSKLAS	GVPARFSGSGSGTSYSLTISNMEAEDAATYYC	QQWSSTPPTFGGGTKLEIKR	118
SC17.85	DIVMTQAAFSNPVTLGTSASISC	RSSKSLLHSNGITY	LYWYLQKPGQSPQLLIY	QMSNLAS	GVPERFSSSGSGSDFTLRISRVEAEDVGVYYC	AQNLEHPTFGGGTKLEIK	120
SC17.87	DVVMTQTPLSLPVSLGDQASISC	RSSQSLVHSNGNTY	LHWYLQKPGQSPKLLIS	KVSNRFS	GVPDRFSGSGSGTDFTLKISRVEAEDLGVYFC	SQSTHVPPMFGGGTRLEIK	122
SC17.89	DVLMTQTPLSLPVSLGDQASISC	RSSQSIVHSNGNTY	LEWYLQKPGQSPKLLIY	KVSNRFS	GVPDRFSGSGSGTDFTLKISRVEAEDLGVYYC	FQGSHVPFTFGSGTKLEIK	124
SC17.90	DIVMSQSPSSLAVSVGEKVTMSC	KSSQSLLYSSNQKNY	LAWYQQKPGQSPKLLIY	WASTRKS	GVPDRFTGSGSGTDFTLTISSVKAEDLAVYYC	HQYYSYPLTFAAGTKLELK	126
SC17.91	DVVMTQTPLSLPVSLGDQASISC	RSSQSLVHSNGNTY	LLWYLQKPGQSPKLLIY	KVSNRFS	GVPDRFSGSGSGTDFALKISRVEAEDLGVYFC	SQSTHVPWTFGGGTKLEIK	128
SC17.93	DIVMSQSPSSLAVSVGEKVTMTC	KSSQSLLYSSNQKNY	LAWYQQKPGQSPKLLIY	WASTRES	GVPDRFIGSGSGTDFTLTISSVKAEDLAIYYC	QQYYRYPLTFGAGTKLELK	130
SC17.95	DIQMTQTTSSLSASLGDRVTISC	SASQGINNY	LNWYQQKPDGTVTLLIY	YTSSLHS	GVPSRFSGSGSGTDYSLTISNLEPEDIATYYC	QQYSKLPWTFGGGTKLEIK	132
SC17.97	DVVMTQTPLSLPVSLGDQASISC	RSSQSLVHNNGNTY	LHWYLQKPGQSPNLLIY	KVSNRFS	GVPDRFSGSGSGTDFTLKISIVEAEDLGLYFC	SQSTHVPRTFGGGTKLEIK	134
SC17.99	DIVMSQSPSSLAVSVGEKVTMNC	ESSQSLLYSSNQKNY	LAWYQQKPGQSPKLLIY	WASTRDS	GVPDRFTGSGSGTDFTLTISSVRAEDPAVYYC	QQYYSYPLTFGAGTKLELK	136
SC17.102	QIVLTQSPAIMSASPGEKVTMTC	SASSSVSY	MHWYQQKSGTSPKRWIY	DTSKLAS	GVPPRFSGRGSGTSYSLTISSMEAEDAATYYC	QHWSSNPPTFGAGTKLEMK	138

FIG. 10A (Cont.)

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Protein Sequences of Exemplary Murine SEZ6 Modulator Light Chain Variable Regions

mAb	FW1	CDRL1	FW2	CDRL2	FW3	CDRL3	SEQ ID NO.
SC17.114	DVVMTQSPLSLPVSLGDQASISC	RSSQSLVHSNGNTY	LHWYLQKPGQSPKLLIY	KVSSRFS	GVPDRFSGSGSGTDFTLKISRVEAEDLGVYFC	SQSTHVPFTFGSGTKLEIK	140
SC17.115	DIVMTQSPLSLPVSLGDQASISC	RSSQSLVHSNGNTY	LHWYLQKPGQSPKLLIY	RVSNRFS	GVPDRFSGSGSGTDFTLTISRVEAEDLGVYFC	SQSTHLPRTFGGGTKLEIK	142
SC17.120	DIVLTQSPASLAVSLGQRATISC	RASESVDSYGNSF	MHWYQQKPGQPPKVLIY	RASNLES	GIPARFSGSGSRTDFTLTINPVEDEDVATYYC	QQSNEDPYTFGGGTKLEIKR	144
SC17.121	QIVLTQSPAIMSASPGEKVTMTC	SASSSVSY	MHWYQQKSGTSPKRWIY	DTSKLAS	GVPARFSGSGSGTSYSLTISSMETEDAATYYC	QQWSNTPPTFGSVTKLEIK	146
SC17.122	DIVITQDDLSNPVTSGESVSISC	RSSKSLLYKDGKTY	LNWFLQRPGQSPQLLIY	LMSTRAS	GVSDRFSGSGSGTDFTLEISRVKAEDVGVYYC	QQLVEYPRTFGGGTKLEIK	148
SC17.140	QIVLTQSPAIMSASPGEKVTMTC	SASSSVSY	MHWYQQKSGTSPKRWIY	DTSKLAS	GVPARFSGSGSGTSYSLTISSMEAEDAATYYC	QQWSSTPPTFGSGTKLEIK	150
SC17.151	DIVLTQFPASLAVSLGQRATIPC	RASESVDSYGNSF	MHWFQQKPGQPPKLLIY	RASNLES	EIPARFSGSGSGTDFTLTINPVEADDVATYYC	QQSHEDPYTFGGGTKMEIKR	152
SC17.156	DVVMTQSPLSLPVSLGDQASISC	RSSQSIVHSNGNTY	LEWYLQKPGQSPKLLIY	KVSNRFS	GVPDRFSGSGSGTDFTLKISRVEAEDLGVYYC	FQGSHVPPTFGGGTKLEIK	154
SC17.161	DIVMSQSPSSLAVSVGEKVTMNC	ESSQSLLYNSNQKNY	LAWYQQKPGQSPKLLIY	WASTRDS	GVPDRFTGSGSGTDFTLTISSVRADDPAVYYC	QQYFNYPLTFGAGTKLELK	156
SC17.166	QIVLTQSPAIMSASPGEKVTMTC	SASSSISY	MHWYQQKSGTSPKRWIY	DTSKLAS	GVPARFSGSGSGTSYSLTISNMEAEDAATYYC	QQWSSTPPTFGGGTKLEIKR	158
SC17.187	DIKMTQSPSSMYASLGERVTLTC	KASQDINSY	LSWFQQKPGKSPETLIY	RANRLID	GVPSRFSGSGSGQDYSLTISSLEYEDMGIYYC	LQYDEFPPTFGGGTKLEIK	160
SC17.191	QIVLTQSPAIMSASPGEKVTMTC	SASSSVSY	MHWYQQKSGTSPKRWIY	DTSKLAS	GVPARFTGSGSGTSYSLTISSMEAEDAATYYC	QQWSSSPPTFGAGTKLELK	162
SC17.193	DLVLTQSPASLAVSLGQRATISC	RASESVSTSGYSY	MHWYQQKPGQPPKLLIY	LASNLES	GVPARFSGSGSGTDFTLNIHPVEEEDATTYYC	QHSRELPYTFGGGTKLEIKR	164
SC17.199	DIVLTQSPASLAVSLGQRATISC	RASESVDSYGNSF	MHWYQQKPGQPPKPLIY	RASNLES	GIPARFSGSGSRTDFTLTINPVEADDVATYYC	QQSNEDPYTFGGGTKLEIKR	166
SC17.200	DIVLTQSPASLAVSLGQRATIFC	RASQSVDYNGISY	MHWFQQKPGQPPKLLIY	AASNVQS	GIPARFSGSGSGTDFTLNIHPVEEEDAATFYC	QQSIEDPPTFGGGTKLEIK	168

FIG. 10A (Cont.)

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Protein Sequences of Exemplary Humanized SEZ6 Modulator Light Chain Variable Regions

mAb	FW1	CDRL1	FW2	CDRL2	FW3	CDRL3	SEQ ID NO.
hSC17.16	DIQMTQSPSSLSASVGDRVTITCR	ASANINSN	LVWYQQKPGKAPKLLIY	AATNLAD	GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC	QHFWGTPRTFGGGTKLEIK	170
hSC17.17	EIVLTQSPATLSLSPGERATLSC	SASSSVSY	MHWYQQKPGQAPRLLIY	DTSKLPS	GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC	QQWSSTPPTFGQGTKLEIK	172
hSc17.24	DIVMTQSPDSLAVSLGERATINC	KSSQSLLYSSNQKSY	LAWYQQKPGQPPKLLIY	WASTRES	GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC	KQSYNLRTFGGGTKVEIKR	174
hSC17.28	EIVLTQSPDFQSVTPKEKVTITC	RASQSIGTS	IHWYQQKPDQSPKLLIK	YASESIS	GVPSRFSGSGSGTDFTLTINSLEAEDAATYYC	QQSNSWPLTFGQGTKLEIK	176
hSC17.34	DIQMTQSPSSLSASVGDRVTITC	KASQDINSY	LSWFQQKPGKAPKSLIY	RANRLVD	GVPSRFSGSGSGTDYTLTISSLQPEDFATYYC	LQYDEFPPTFGQGTKLEIK	178
hSC17.46	AIQMTQSPSSLSASVGDRVTITC	KASQSVNND	VAWYQQKPGKAPKLLIY	YASNRYT	GVPSRFSGSGSGTDFTLTISSLQPEDFATYFC	QQDYSSPRTFGQGTKLEIK	180
hSC17.151	EIVLTQSPATLSLSPGERATLSC	RASESVDSYGNSF	MHWYQQKPGQAPRLLIY	RASNLES	GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC	QQSHEDPYTFGQGTKLEIK	182
hSC17.155	DIVMTQSPDSLAVSLGERATINC	KSSQSLLYSSNQKNY	LAWYQQKPGQPPKLLIY	WASTRKS	GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC	HQYYSYPLTFGQGTKLEIK	184
hSC17.156	DIVMTQTPLSLPVTPGEPASISC	RSSQSIVHSNGNTY	LEWYLQKPGQSPQLLIY	KVSNRFS	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYY	CFQGSHVPPTFGGGTKLEIK	186
hSC17.161	DIVMTQSPDSLAVSLGERATINC	ESSQSLLYNSNQKNY	LAWYQQKPGQPPKLLIY	WASTRES	GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC	QQYFNYPLTFGQGTKLEIKR	188
hSC17.200	EIVLTQSPATLSLSPGERATLSC	RASQSVDYNGISY	MHWYQQKPGQAPRLLIY	AASNVQS	GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC	QQSIEDPPTFGGGTKVEIK	190
hSC17.200vL1	EIVLTQSPATLSLSPGERATLSC	RASQSVDYDGISY	MHWYQQKPGQAPRLLIY	AASNVQS	GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC	QQSIEDPPTFGGGTKVEIK	192

FIG. 10A (Cont.)

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Protein Sequences of Exemplary Murine SEZ6 Modulator Heavy Chain Variable Regions

mAb	FW1	CDRH1	FW2	CDRH2	FW3	CDRH3	SEQ ID NO.
SC17.1	SDVQLQDSGPGLVKPSQSLSVTCTVT	GYSITWGY	YWNWIRQFPGNKLEWMGN	IHNSGGTN	YNPSLKSRISITRDTSKNQFFLQLNSVTTEDTATYYC	ATTNWDYFDYWGQGTTLTVSS	21
SC17.2	QVQLQQSDAELVKPGASVKISCKVS	GYTFTDHT	IHWMKQRPEQGLEWIGY	IYPRDGST	KYNEEFKGKATLTADKSSSTAYMQLNSLTSEDSAVYFC	ARSYSNYFDYWGQGTTLTVSS	23
SC17.9	QVQLQQPGAEIVRPGASVKLSCKAS	GYTFTDYW	MNWVKQRPGQGLEWIGA	IDPSDSYT	SYNPKFKGKATLTVDTSSSSAYMQLSSLTSEDSAVYFC	ARRGTPGKPLVYWGQGTLVTVSA	25
SC17.16	EVQLQQSGPELMKPGASVKMSCKAS	GYTFTDYN	MYWVKQNQGKSLEWIGE	INPNNGGT	AYNQKFRGKATLTVDKSSSTAYMELRSLTSEDSAVYYC	ARYDKGFDYWGQGTTLTVSS	27
SC17.38	QVTLKESGPGILQPSQTLSLTCSFS	GFSLNTSGMS	VGWVRQPSGRGLEWLAP	IWWNGDK	YYNPALKSRLTISKDTSNNQVFLKIASVVTADTATYFC	ARIRQYYYAMDYWGQGTSVTVSS	29
SC17.3	QVQLQQPGAELVKPGASVKLSCKAS	GYTFPSYW	IHCVKQRPGQGLEWIGV	INPSNGRT	NYNEKFKNKATLTVDKSSSTAYMQLSSLTSEDSAVYYC	VRGGTGYTMDYWGQGTSVTVSS	31
SC17.4	QIQLVQSGPELKKPGETVKISCKAS	GYTFTDYS	MHWVKQAPGKGLKWLGW	INTETGEP	TYSEDFKGRFAFSLETSASTAYLQINNLKNEDTATYFC	VKNKGWFAYWGQGTLVTVSA	33
SC17.8	QVHLQQSGTEVMKPGASVKISCKAT	GYTFSSYW	IEWIKQRPGHGLEWIGE	ILPGSGNT	NNNEKFKGKATITADTSSNIAYIQLSSLTSEDSAVYYC	AGGPAAYWGQGTLVTVSA	35
SC17.10	EVQLQQSGAELVKPGASVKLSCTAS	GFNIKDTY	MHWVKQRPEQGLEWIGR	IDPANVNT	KYDPKFQGKATITADTSSNTAYLQLSSLTSEDTAVYYC	VRGNVYWGQGTLVTVSA	37
SC17.11	EVQLQQSGPELVKPGALVMMSCKAS	GYTFTDYY	MHWVKQSHGQSLEWIGEV	IPYNDET	FYNRKFKDKATLTVDKSSSTAYMELRSLTSEDSAIYYC	ARRHRYDGFRYAIDYWGQGTSVTVSS	39
SC17.14	EVQLQQSGPVLVKPGASVKMSCKAS	GYTITDYN	MNWVKQSHGKSLEWIGV	INPYNGNT	RYNQMFKGKATLTVDKSSSTAYMELNSLTSEDSAVYYC	TRWGTTVVGANWGQGTTLTVSS	41
SC17.15	DVKLVESGGGLVKLGGSLKLSCAAS	GFTFSSYA	MSWVRQTPEKRLEWVAT	ITSGGGNT	YYPDSVKGRFTISRDNAKNTLYLQMSSLKSEDTAMYYC	ARRDYYGSSYVMFAYWGQGTLVTVSA	43
SC17.17	EVQLQQSGPEVMKPGASVKMSCKAS	GYTFTDYN	MHWVKQNQGKSLEWIGE	INPNIGGT	GYNQKFKGKATLTVHKSSSTAYMELRSLTSEDSAVYYC	ARTYSYYSYEFAYWGQGTLVTVSA	45
SC17.18	QVTLKESGPGILQPSQTLSLTCSFS	GFSLSTSTMG	VGWIRQPSGKGLEWLAD	IWWDDSK	YYNPSLKSRLTISKDTSSNQVFLKITSVDTADTATYYC	ARKGRTARATRGFAYWGHGTLVTVSA	47
SC17.19	SDVQLQESGPGLVKPSQSLSVTCTVT	GYSITSSYT	WNWIRQFPGNKLEWMGY	IHYSGST	NYNPSLRSRISITRDTSKNQFFLQLNSVTTEDTATYYC	ARSRYYYDAYGFAYWGQGTLVTVSA	49
SC17.22	QIQMMQSGPELKKPGETVKISCKAS	GYSFTNYG	MNWVKQAPGKGLKWMGW	INTYTGEP	TYADDFKGRFAFSLETSASTAYLQINNLKNEDMATYFC	TRGYYGSSYDALDYWGQGTSVTVSS	51
SC17.24	QVQLQQSDAELVKPGASVKISCKVS	GYTFTDHT	IHWMKQRPEQGLEWIGY	IYPRDGST	KYNEEFKGKATLTADKSSSTAYMQLNSLTSEDSAVYFC	ARSYSNYFDYWGQGTTLTVSS	53
SC17.27	QVQLQQSGAELVRPGASVTLSCKAS	GYTFTDYE	MHWVKQTPVHGLEWIGG	IDPETGGTA	YNQKFKGKATLTADKSSSTAYMELRSLTSEDSAVYFC	TRWFSYWGPGTLVTVSA	55
SC17.28	QVHLPQSRPELVKPGASVKISCKAS	GYGFTRSY	IHWVKQRPGQGLEWIGY	ISSGSGGT	TYNQKFKGKASLTADNPSSTAYMHLSSLTSEDSAIYFC	ARGGVRYFDVWGAGTTVTVSS	57
SC17.29	EVQLQQSGPELMKPGASVKMSCKAS	GYTFTDYN	MHWVKQNQGKSLEWIGE	INPHNGGT	GYNQKFKGKATLTVDKSSSTSYMELRSLTSEDSAVYYC	AGGYPAFDYWGQGTTLTVSS	59
SC17.30	EVKLVESEGGLVQPGSSMKLSCTAS	GFTFSDYY	MAWVRQVPEKGLEWVAN	INYDGSST	YYLDSLKSRFIISRDNAKNILYLQMSSLKSEDTATYYC	ARDDYYGSSPSYWYFDVWGAGTTVTVSS	61

FIG. 10B

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Protein Sequences of Exemplary Murine SEZ6 Modulator Heavy Chain Variable Regions

mAb	FW1	CDRH1	FW2	CDRH2	FW3	CDRH3	SEQ ID NO.
SC17.32	EVKLEESGGGLVQPGGSMKLSCVAS	GFTFSNYW	MSWVRQSPEKGLEWVAE	IRLKSNNYAT	HYAESVKGRFTISRDDSKSSVFLQMNNLRTEDTGIYYC	TRHYYYAMDYWGQGTSVTVSS	63
SC17.34	EVQLQQSGPELVKPGSSVKISCKAS	GYTFTDYN	MDWVKQSHGKRLEWIGY	IYPDNGGA	GYNQKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYC	SRSITTAWFAYWGQGTLVTVSA	65
SC17.35	EVQLQQSGPELVKPGALVKMSCKAS	GYTFTDYY	IHWVKQSHGKSLEWIGE	INPYNGET	FYNQKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYC	ARRGWYLTGYAMDYWGQGTSVTVSS	67
SC17.36	EVQLQESGPSLVKPSQSQSLTCSVT	GDSITSDY	WNWIRKFPGKKVEYMGY	INYSGST	YYNPSLKSRISITRDTSKNQYYLQLNSVTSEDTATYYC	ARTSYYNKFLPFAYWGQGTLVTVSA	69
SC17.39	EVQLVESGGGLVQPGGSRKLSCAAS	GFTFSSYG	MHWVRQAPEKGLEWVAY	ISSNDGTI	YYADTVRGRFTISRDNAKNTLFLQMTSLRSEDTAMYYC	ARPSNWVFDYWGQGTTLTVSS	71
SC17.40	QVQLQQPGAEIVRPGASVKLSCKAS	GYTFTDYW	MNWVKQRPGQGLEWIGT	IDPSDSYT	RYNQKFKGKATLTVDTSFSSAYMQLSSLTSEDSAVYFC	ASGGRGFGYWGQGTPVTVSV	73
SC17.41	DVKLVESGGGLVKLGGSLKLSCAAS	GFTFSSYA	MSWVRQTPEKRLEWVAT	ISSGGGNT	YYPDSVKGRFTISRDNAKNTLYLQMSSLKSEDTAMYYC	ARRDYYGTSYVMFAYWGQGTLVTVSA	75
SC17.42	DVKLVESGGGLVRPGGSLKLSCAAS	GFTFSRYT	MSWVRQTPEKRLEWAAT	INSGGSNT	YYPDSVKGRFTISRDNAKNTLFLQMSSLKSEDTAMYYC	TNGNHWGQGTTLTVSS	77
SC17.45	QVQLQQPGSVLVRPGDSVKLSCKAS	GYTFTSYW	MHWVKQSPGQGLEWIGE	IHPHSGST	NYNEKFKGKATLTVDTSSSTAYVDLSSLTSEDSAVYYC	VGGHYDYWGQGTTLTVSS	79
SC17.46	QVQLQQPGAELVKPGASMKLSCKAS	GYTFTSYW	INWVKQRPGQGLEWIGN	IFPDTTTT	NYNEKFKSKATLTVDTSSSTAYMQLSSLTSDDSAVYYC	AREYYDGTYDAMDYWGQGTSVTVS	81
SC17.47	EVQLQQSGPELVKPGASVKISCKAS	GYSFTDYY	MRWVKQSPEKSLEWIGE	INPSTGGTT	TYNQNFKAKATLTVDKSSSTAYMQLKSLTSEDSAVYYC	ARGGYFLYYFDYWGQGTTLTVSS	83
SC17.49	QVQLQQSGAELVRPGASVTLSCKAS	GYTFTDYE	MHWVKQTPVHGLEWIGG	IDPETGGT	AYNQKFKGKATLTADKSSSTAYMELRSLTSEDSAVYFC	TRWFSYWGPGTLVTVSA	85
SC17.50	EVQLVESGGGLVKPGGSLKLSCAAS	GFTFSDYG	MHWVRQAPEKGLEWVAY	ISSGSRTI	YYADTVKGRFTISRDNAKNTLFLQMTSLRSEDTAMYYC	ARVYYGSTYGYFDVWGTGTTVTVSS	87
SC17.53	EVQLQQSGPELVKPGASVKISCKAS	GYTFTDYN	MHWVKQSHGKRLEWIGY	IHPYNGGS	GYNQKFKRKATLTVDNSSNTTYMELRSLTSEDSAVYYC	ARSYDYDTWFGYWGQGTLVTVRA	89
SC17.54	EVKLEESGGGLVQPGGSMKLSCVAS	GFTFSNYW	INWVRQSPEKGLEWVAE	IRMKSNNYAT	HYAESVKGRFTISRDDSKSCVYLQMNNLRPEDTGIYYC	TRGGYWGQGTTLTVSS	91
SC17.56	QIQLVQSGPELKKPGETVKISCKAS	GYTFTNYG	MNWVKQAPGKGLKWMAW	INTYTGEP	TYADDFKGRFAFSLETSASTASLQIINLKNEDTATYFC	ARIGDSSPSDYWGQGTTLTVS	93
SC17.57	QIQLVQSGPELKKPGETVKISCKAS	DYTFTDFS	IHWVRQSPGKGLKWMGW	INTETGEPT	VAEDFKGRFAFSLETSASTAFLQIYNLKNEDSATYFC	ARGRYYGHDYAMDYWGQGTSVTVSS	95
SC17.59	EVKLEESGGGLVQPGGSMKLSCVAS	GFTFSNYW	MNWVRQSPEKGLEWVAE	IRLKSNNYAT	HYAESVKGRFTISRDDSKSSVYLQMNNLRAEDTGIYYC	TRLWDFAMDYWGQGTSVTVSS	97
SC17.61	QVTLKESGPGILQPSQTLSLTCSFS	GFSLSTFGMG	VGWIRQPSGKGLEWLAQ	IWWDDYK	YYNPALKSRLTISKDTSKNQVFLKIANVDTADTATYYC	ARIGYYSGSSRCWYFDVWGTGSTVTVSS	99
SC17.63	QVQLQQSDAELVKPGASVKISCKAA	GYTFTDLT	IHWVKQRPEQGLEWIGY	IYPGDSNT	KYNEKFKGKATLTADKSSSTAYMQLNSLTSEDSVVYFC	ARMITPYYFDYWGQGTTLTVS	101

FIG. 10B (Cont.)

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Protein Sequences of Exemplary Murine SEZ6 Modulator Heavy Chain Variable Regions

mAb	FW1	CDRH1	FW2	CDRH2	FW3	CDRH3	SEQ ID NO.
SC17.71	EVKLEESGGGLVQPGGSMKLSCVAS	GIIFSNYW	MNWVRQSPEKGLEWVAE	IRLKSNNYST	HYAESVKGRFTISRDDSKSSVYLQMNNLRAEDTGIYYC	TRHYYYAMDYWGQGTSVTVSS	103
SC17.72	EVQLVESGGGLVKPGGSLKLSCAAS	GFTFSSYG	MSWVRQTPEKRLEWVAA	INSNGGST	YYPDTVKGRLTISRDNGKNTLYLQMSSLRSEDTALYYC	VRDDGYYVFFAYWGQGTLVTVSA	105
SC17.74	QVQLKQSGPGLVAPSQSLSITCTVS	GFSLTSYG	VDWVRQSPGKGLEWLGV	IWGGGST	NYNSALKSRLSITKDNSKSQVFLKMNSLQTDDTAMYYC	ASGDYDGSLWFAYWGQGTLVTVSA	107
SC17.76	EVQLVESGGDLVKPGGSLKLSCVAS	GFTFSSYG	MSWVRQTPDKRLEWVAT	ISSGGTFT	YYPDSVKGRFTVSRDNAKNTLYLQMSSLKSEDTAMYYC	SRHGWGWGQGTLVTVSA	109
SC17.77	EVKLEESGGGLVQPGGSMKLSCVAS	GFTFSNYW	MNWVRQSPEKGLEWVAE	IRLKSNNYAT	HYAESVKGRFTISRDDSKSSVYLQMNNLRVEDTAIYYC	TRHYDYAMDYWGQGTSVTVSS	111
SC17.79	EVKLEESGGGLVQPGGSMKLSCVAS	GFTFSDYW	MNWVRQSPEKGLELVAE	IRLISNNYAT	HYAESVKGRFTISRDDSKSSVYLQMNNLRAEDTGIYYC	TRHYYYALDYWGQGTSVTVSS	113
SC17.81	EVQLQQSGAELVKPGASVKLSCTAS	GFNINDTY	YHWLKQRPEQGLEWIGR	IDPANVNT	KYDPKFQGKATLTADTSSNTAYLQLSSLTSEDTAVYYC	GRGNAYWGQGTLVTVSA	115
SC17.82	EVQLQQSGPELVKPGASVKMSCKAS	GYTFTDSY	MNWVKQSHGKSLEWIGR	VNPNNGGA	SYNHKFKGKATLTVDKSLSTAYMRLNSLTSEDSAVYYC	SRSGDLYYYAMDYWGQGTSVTVSS	117
SC17.84	EVQLQQSGPELMKPGASVKMSCKAS	GYIFTDYN	MHWVKQNQGKSLEWIGE	VNPNTGGI	GYNQKFKGKATLTVDKSSSTAYMDLRSLTSEDSAVYYC	ARDGNYCFDYWGQGTTLTVSS	119
SC17.85	EVQLVESGGDLVKPGGSLKLSCAAS	GFTFSNYG	MSWVRQTPDKRLEWVAT	ISTGGTYT	YYPDSVKGRFTISRDNAKNTLYLQMSSLKSEDTAMYYC	VGQSYSDYVSFAYWGQGTQVTVSA	121
SC17.87	EVQLQQSGAELLKPGASVKLSCTAS	GLNIKDYY	IHWVYQRPEQGLEWIGR	IDPESDNT	LYDPKFQGKASITADTSSNTAYLQLSSLTSEDTAVYYC	TTNTPFAYWGQGTLVTVST	123
SC17.89	QVQLQQSGAELVRPGTSVKVSCKTS	GYAFTNYL	IEWVKQRPGQGLEWIGV	INPGSGGT	NYNEKFKVKATLTADKSSSTAYMQLTSLTSDDSAVYFC	TRRDGYFFPWFAYWGQGTLVTVSA	125
SC17.90	QVQLQQPGSVLVRPGASVKLSCKAS	GYTFTSYW	MHWVKQRPGQGLEWIGE	IHPNNGST	NYNEKFKGKATLTVDTSSSTAYVDLSSLTSEDSAVYYC	ARWTLFTYWGQGTLVTVSA	127
SC17.91	EVQLVESGGGLVKPGGSRKLSCAAS	GFTFSDYG	MHWVRQAPEKGLEWVAY	ISRGSSTI	HYADTVKGRFTISRDNAKNTLFLQMTSLRSEDTAMYYC	ARPFNWYFDVWGAGTTVTVSS	129
SC17.93	QVQLQQPGAELVKPGASVMLSCKAS	GYTFTSYW	VHWVKQRPGQGLEWIGV	INPRNGRN	NYNEKFKTKATLTVDKSSSTAYMQLSSPTSEDSAVYYC	AREDYDGGDYAMDYWGQGTSVTVSS	131
SC17.95	EVELQQSGPELVKPGASVKISCKTS	GNTYTEYT	MQWVKLSHGKSLEWIGG	INPNNGIT	SYNQKFKGKATLTVDKSSSTAYMELRSLKSEDSAVYYC	ARAGLGNYVWAMDYWGQGASVTVSS	133
SC17.97	QVQLPQSGAELAKPGASVKISCKAS	GFTFTSYW	MHWVKQRPGQGLEWIGY	INPSTDYT	EYNQKFKDKATLTADKSSSTAYMQLGSLTSEDSAVYYC	ARSSYGSSPFDYWGQGSTLTVSS	135
SC17.99	EVKLEESGGGLVQPGGSMKLSCAAS	GFTFSDAW	MDWVRQSPEKGLEWVAE	IRSKANNHAT	YYAESVKGRFTISRDDSKSSAYLQMNSLRAEDTGIYYC	VSTGTSYWGQGTLVTVSA	137
SC17.102	EVQLQQSGPELMKPGASVKMSCKAS	GDTFTDYN	IHWVKQNQGKSLEWIGE	VNPNIGGI	GYNQKFKGKATLTVDKSSSTAYMELRSLTSEDSAVYYC	AMGRWYFDVWGAGTTVTVSS	139

FIG. 10B (Cont.)

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Protein Sequences of Exemplary Murine SEZ6 Modulator Heavy Chain Variable Regions

mAb	FW1	CDRH1	FW2	CDRH2	FW3	CDRH3	SEQ ID NO.
SC17.114	EVQLQQSGPEMVKPGASVKISCKA	GYTFTDYY	MHWVKQSHGKSLEWIGR	VNTNNGGT	SYDQKFEGKATLTVDKSSSTAYMELNSLTSEDSAVYYC	VIPAWFAYWGQGTLVTVSA	141
SC17.115	QVQLQQSGSVLVRPGASVKLSCKAS	GYTFTSY	MHWVKQRPGQGLEWIGE	IHPNSGNT	NYNEKFKGKATLTVDTSSSTAYVDLSSLTSEDSAVYYC	AGGNYDYWGQGTTLTVSS	143
SC17.120	EVQLEQSGTVLARPGASVKMSCKAS	GYTFTSYW	MHWVKQRPGQGLEWIGA	FYPGNSGT	YYNQKFKDKAKLTAVTSASTAYMELSSLTNEDSAVYYC	SRSGSGRFAYWGQGTLVTVSA	145
SC17.121	EVQLQQSGPELMKPGASVKMSCKAS	GYTFTDHN	IHWVKQHQGKSLEWIGE	INPNTGGT	GYNQKFQGKATMTVDKSSSTAYMELRSLTSEDSAVYYC	VRGLYFFDYWGQGTTLTVSS	147
SC17.122	EVHLVESGGDLVKPGGSLKLSCAAS	GFTFSSYG	MSWVRQTPDKRLEWVAT	ISSGGTYT	YYPDSVKGRFTISRDNAKNTLYLQMSSLKSEDTAMYYC	SRHGWGWGQGTLVTVSA	149
SC17.140	EVQLQQSGPELMKPGASVKMSCKAS	GYTFTDYN	MHWVKQNQGKSLEWIGE	INPNTGGT	GYNQKFKGKATLTVDKFSSTAFIELRSLTSEDSAIYYC	TRGGYDHYWYFDVWGAGTTVTVSS	151
SC17.151	EVQLQQSGTVLARPGASVKMSCKA	GYTFTSYW	MHWVKQRPGQGLEWIGA	IYPGKNDT	TYNQKFKGKAKLTAVTSASTLYMELSSLTNEDSAVYYC	TRSGKGYFAYWGQGTLVTVSA	153
SC17.156	QVTLKESGPGILQPSQTLSLTCSFS	GFSLSTSGMGV	VSWIRKTSGKGLEWLAH	IFWDDDK	WYNPSLKSRLTISKATSSNQVFLILTSVDTADTATYYC	ATFYGLYFAYWGQGTTLTVSS	155
SC17.161	EVKLEESGGGLVQPGGSMKLSCAAS	GFTFSDAW	MDWVRQSPEKGLEWVAE	IRSKPNNHAT	YYAESVKGRFTISRDDSKSSAYLQMNSLRAEDTGIYYC	VSTGTSYWGQGTLVTVSA	157
SC17.166	EVQLQQSGPELMKPGASVKMSCKAS	GYIFTDYN	MHWVKQNQGKSLEWIGE	VNPNTGGI	GYNQKFKGKATLTVDKSSSTAYMDLRSLTSEDSAVYYC	ARDGNYCFDYWGQGTTLTVSS	159
SC17.187	EVHLQQSGPELVNPGSSVKISCKAA	GYTFTDYN	MDWVKQSHGKRLEWIGN	IYPNNGGA	GYNQNFKDKATLTVDKSSSTAYMELRSLTSEDSAVYYC	ARSITAAWFAYWGQGTLVTVSA	161
SC17.191	EVQLQQSGPELMKPGASVKMSCKAS	GYTFTDYN	MHWVKQNQGKSLEWIGE	INPNTGGT	GYNQKFKDKATLTVDKSSSTAYMELRSLTSEDSAVYYC	ARIPSLRRYYFDYWGQGTTLTVSS	163
SC17.193	QVTLKESGPGILQPSQTLSLTCSFS	GFSLITYGIG	VGWIRQPSGKGLEWLAH	IWWNDNK	YYNTALKSRLTISKDTSNNQVFLKIANVDTADTATYYC	ARMVYYDYDGGFAYWGQGTLVTVSA	165
SC17.199	EVQLQQSGTVLARPGASVRMSCKAS	GYTFTSYW	MHWVKQRPGQGLEWIGA	IYPGNSDT	SYNHKFKGKAKLTAVTSASTAYMELSSLTNEDSAVYYC	TRSGTGWFAYWGQGTLVTVSA	167
SC17.200	QVQLQQSGPELVKPGASVKISCKAS	GYAFSSSW	INWVKQRPGQGLEWIGR	IYPGEGDT	NYSGNFEGKATLTADKSSTTAYMQLSSLTSVDSAVYFC	TRGLVMDYWGQGTALTVSS	169

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FIG. 10B (Cont.)

Protein Sequences of Exemplary Humanized SEZ6 Modulator Heavy Chain Variable Regions

mAb	FW1	CDRH1	FW2	CDRH2	FW3	CDRH3	SEQ ID NO.
hSC17.16	QVQLVQSGAEVKKPGASVKVSCKAS	GYTFTDYN	MYWVRQAPGQGLEWMGE	INPNNGGT	AYNQKFRGKVTMTRDTSISTAYMELSRLRSDDTAVYYC	ARYDKGFDYWGQGTTVTVSS	171
hSC17.17	QVQLVQSGAEVKKPGASVKVSCKAS	GYTFTDYN	MHWVRQAPGQGLEWMGE	INPNIGGT	GYNQKFKGRVTMTRDTSISTAYMELSRLRSDDTAVYYC	ARTYSYYSYEFAYWGQGTLVTVSS	173
hSC17.24	EVQLVQSGAEVKKPGATVKISCKVS	GYTFTDHT	IHWVRQAPGKGLEWIGY	IYPRDGST	KYNEEFKGRVTITADTSTDTAYMELSSLRSEDTAVYYC	ARSYSNYFDYWGQGTTVTVSS	175
hSC17.28	QVQLVQSGAEVKKPGASVKVSCKAS	GYTFTRSY	IHWVRQAPGQGLEWMGY	ISSGSGGT	TYNQKFKGRVTSTRDTSISTAYMELSRLRSDDTAVYYC	ARGGVRYFDVWGQGTTVTVSS	177
hSC17.34	QVQLVQSGAEVKKPGASVKVSCKAS	GYTFTDYN	MDWVRQAPGQRLEWIGY	IYPDNGGA	GYNQKFKGRVTITVDTSASTAYMELSSLRSEDTAVYYC	SRSITTAWFAYWGQGTLVTVSS	179
hSC17.46	QVQLVQSGAEVKKPGASVKVSCKAS	GYTFTSYW	INWVRQAPGQGLEWIGN	IFPDTTTT	NYNEKFKGRVTLTRDTSISTAYMELSRLRSDDTAVYYC	AREYYDGTYDAMDYWGQGTLVTVSS	181
hSC17.151	QVQLVQSGAEVKKPGASVKVSCKAS	GYTFTSYW	MHWVRQAPGQGLEWMGA	IYPGKSDT	TYNQKFKGRVTMTRDTSTSTVYMELSSLRSEDTAVYYC	ARSGKGYFAYWGQGTLVTVSS	183
hSC17.155	QVQLVQSGAEVKKPGASVKVSCKAS	GYTFNSYW	MHWVRQAPGQGLEWMGE	IHPNNGST	NYNEKFKGRVTMTRDTSTSTVYMELSSLRSEDTAVYYC	ARWTLFTYWGQGTLVTVSS	185
hSC17.156	QVTLKESGPVLVKPTETLTLTCTVS	GFSLSTSGMG	VSWIRQPPGKALEWLAH	IFWDDDK	WYNPSLKSRLTISKDTSKSQVVLTMTNMDPVDTATYYC	ATFYGLYFAYWGQGTLVTVSS	187
hSC17.161	QVQLVQSGAEVKKPGASVKVSCKAS	GFTFSDAW	MDWVRQAPGQRLEWMGE	IRSKPNNHAT	YYAESVKGRVTITRDTSASTAYMELSSLRSEDTAVYYC	ARTGTSYWGQGTLVTVSS	189
hSC17.200	EVQLVQSGAEVKKPGESLKISCKGS	GYSFTSSW	INWVRQMPGKGLEWMGR	IYPGEGDT	NYSGNFEGQVTISADKSISTAYLQWSSLKASDTAMYYC	TRGLVMDYWGQGTLVTVS	191
hSC17.155vH1	QVQLVQSGAEVKKPGASVKVSCKAS	GYTFDSYW	MHWVRQAPGQGLEWMGE	IHPNNGST	NYNEKFKGRVTMTRDTSTSTVYMELSSLRSEDTAVYYC	ARWTLFTYWGQGTLVTVSS	193
hSC17.155vH2	QVQLVQSGAEVKKPGASVKVSCKAS	GYTFTSYW	MHWVRQAPGQGLEWMGE	IHPNNGST	NYNEKFKGRVTMTRDTSTSTVYMELSSLRSEDTAVYYC	ARWTLFTYWGQGTLVTVSS	194
hSC17.155vH3	QVQLVQSGAEVKKPGASVKVSCKAS	GYTFNYYW	MHWVRQAPGQGLEWMGE	IHPNNGST	NYNEKFKGRVTMTRDTSTSTVYMELSSLRSEDTAVYYC	ARWTLFTYWGQGTLVTVSS	195
hSC17.155vH4	QVQLVQSGAEVKKPGASVKVSCKAS	GYTFNSYW	MHWVRQAPGQGLEWMGE	IHPNDGST	NYNEKFKGRVTMTRDTSTSTVYMELSSLRSEDTAVYYC	ARWTLFTYWGQGTLVTVSS	196
hSC17.155vH5	QVQLVQSGAEVKKPGASVKVSCKAS	GYTFNSYW	MHWVRQAPGQGLEWMGE	IHPNGGST	NYNEKFKGRVTMTRDTSTSTVYMELSSLRSEDTAVYYC	ARWTLFTYWGQGTLVTVSS	197
hSC17.155vH6	QVQLVQSGAEVKKPGASVKVSCKAS	GYTFNSYW	MHWVRQAPGQGLEWMGE	IHPNSGST	NYNEKFKGRVTMTRDTSTSTVYMELSSLRSEDTAVYYC	ARWTLFTYWGQGTLVTVSS	198
hSC17.161vH1	EVQLVESGGGLVQPGGSLRLSCAAS	GFTFSDAW	MDWVRQAPGKGLEWVGE	IRSKPNNHAT	YYAESVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYC	ARTGTSYWGQGTLVTVSS	199

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FIG. 10B (Cont.)

SEZ6 Modulator Characteristics

Clone	Bin	Hu Affinity (Kd, nM)	Mouse XR	Rat XR (Kd, nM)	Cyno XR	SEZ6L XR	SEZ6L2 XR
SC17.3	D	N.D.	No	No	N.D.	No	No
SC17.4	A	N.D.	No	No	N.D.	No	No
SC17.6	B	<1	Yes	Yes (<1)	N.D.	No	Yes
SC17.7	A	N.D.	Yes	No	No	Yes	Yes
SC17.17	U	1.8	No	Yes (5.0)	Yes	No	No
SC17.19	E	5.3	Yes	Yes	N.D.	No	Yes
SC17.24	C	5.1	Yes	No	Yes	No	No
SC17.26	F	62.0	Yes	Yes (17.4)	Yes	No	Yes
SC17.28	U	22.1	No	Yes (31)	N.D.	No	Yes
SC17.34	A	15.1	No	Yes (79.5)	Yes	No	No
SC17.36	A	6.3	No	No	Yes	No	No
SC17.42	A	2.7	Yes	Yes (3.0)	No	No	No
SC17.45	A	17.4	No	No	Yes	No	No
SC17.46	E	N.D.	No	No	No	No	No
SC17.49	U	23.1	No	No	N.D.	No	No

FIG. 11A

WO 2013/126810 PCT/US2013/027476
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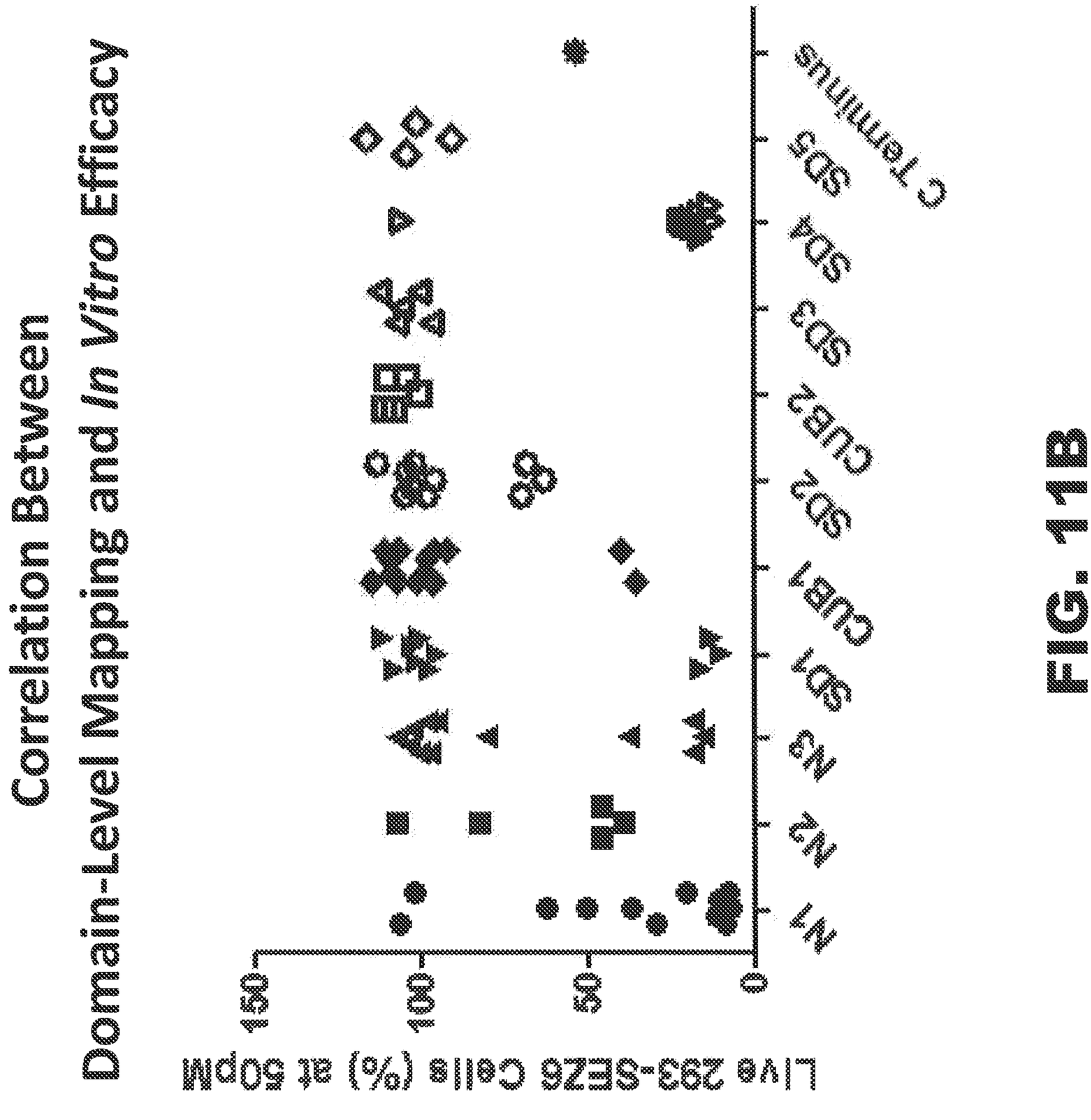

Correlation Between
Domain-Level Mapping and In Vitro Efficacy
LLV6 293-SEZ6 Cells (%) at 50pM
0
8
100
1000
N1
N2
N3
N4
N5
N6
N7
N8
C Terminal Domains

FIG. 11B

Detection of SEZ6 Surface Expression on Engineered HEK-293 Cells

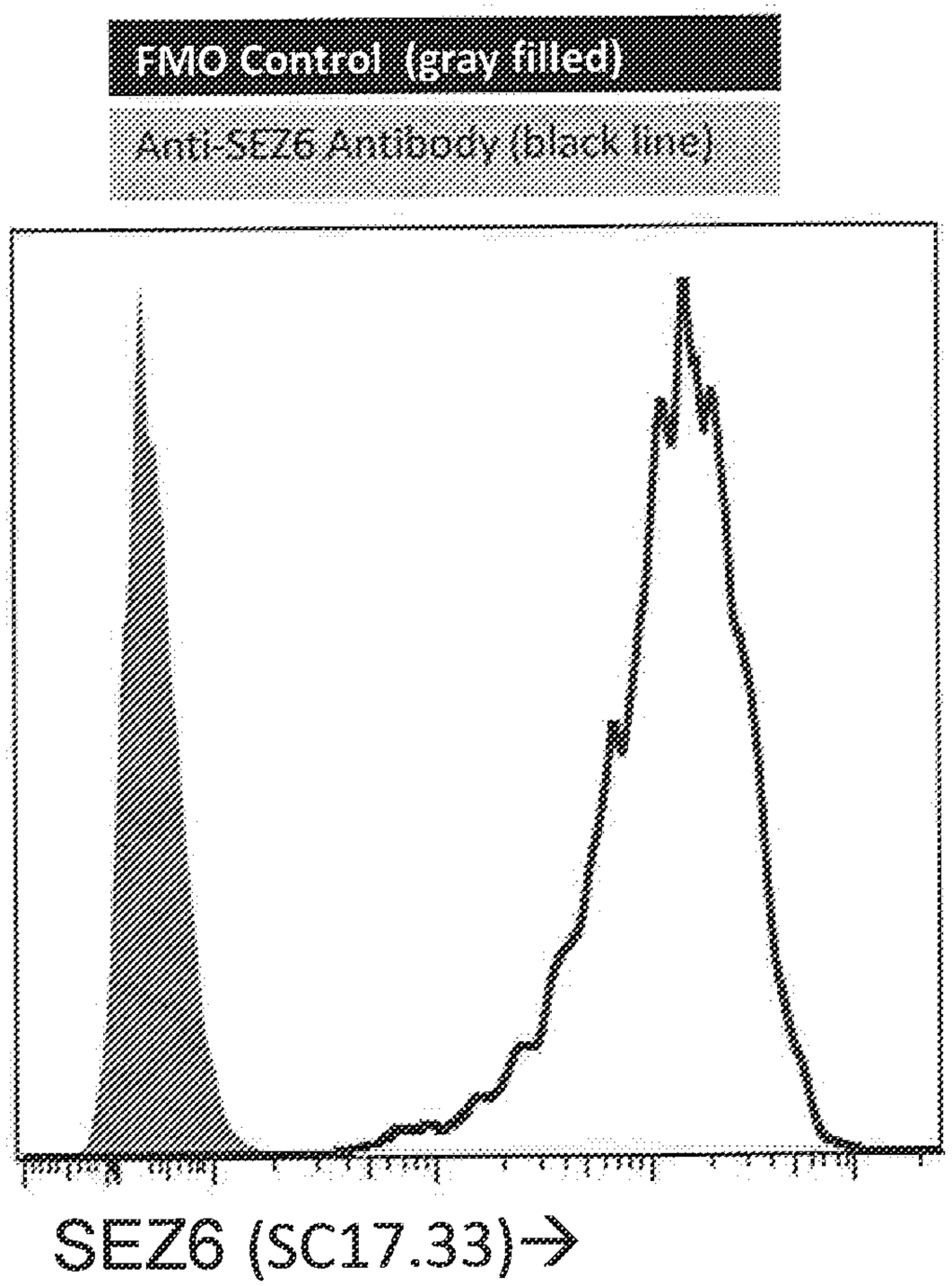


FIG. 12A

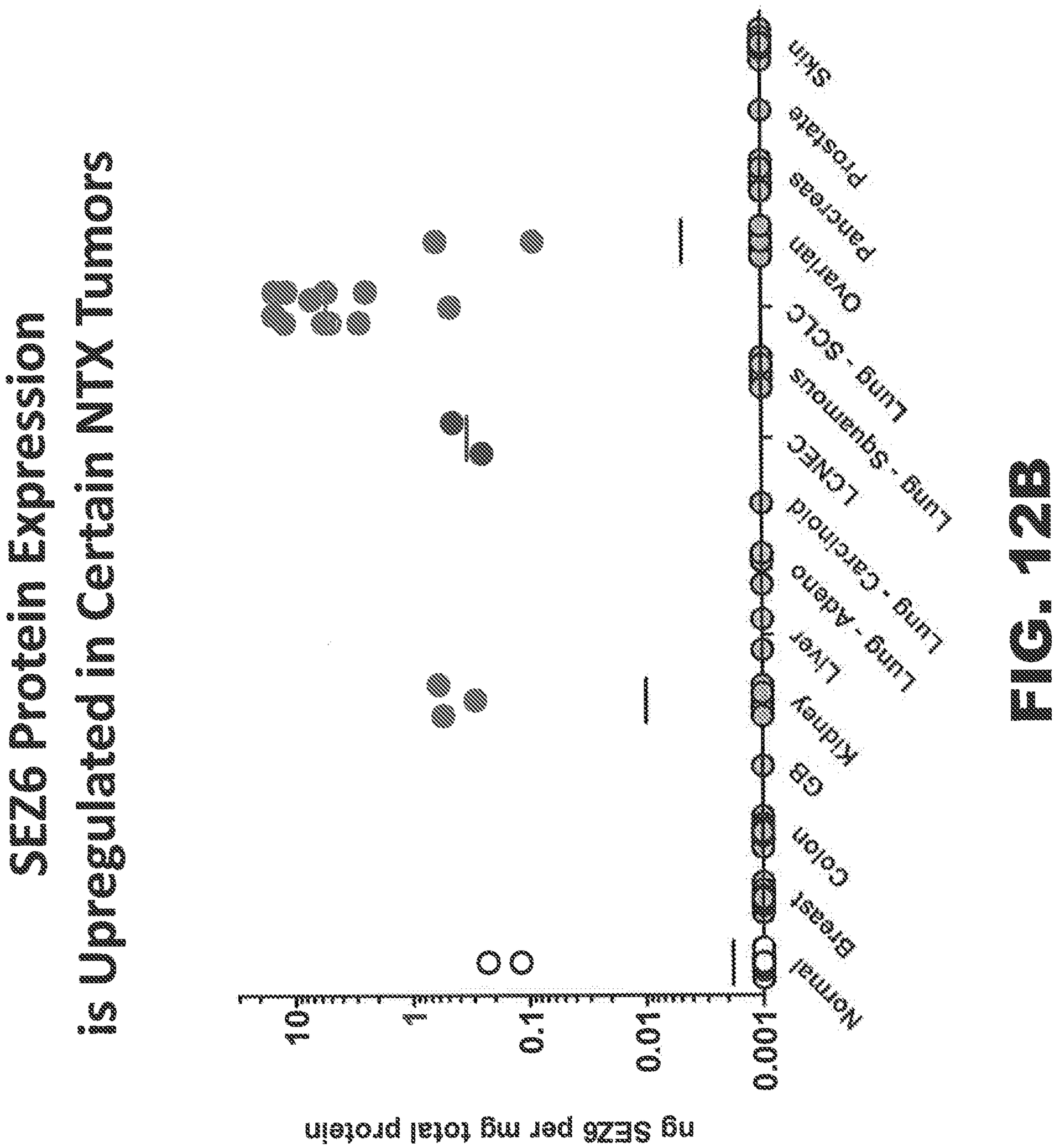
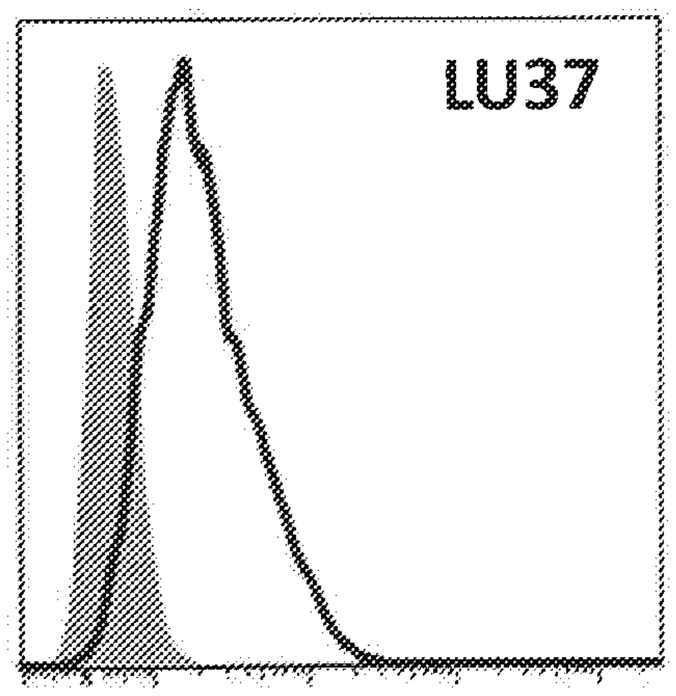
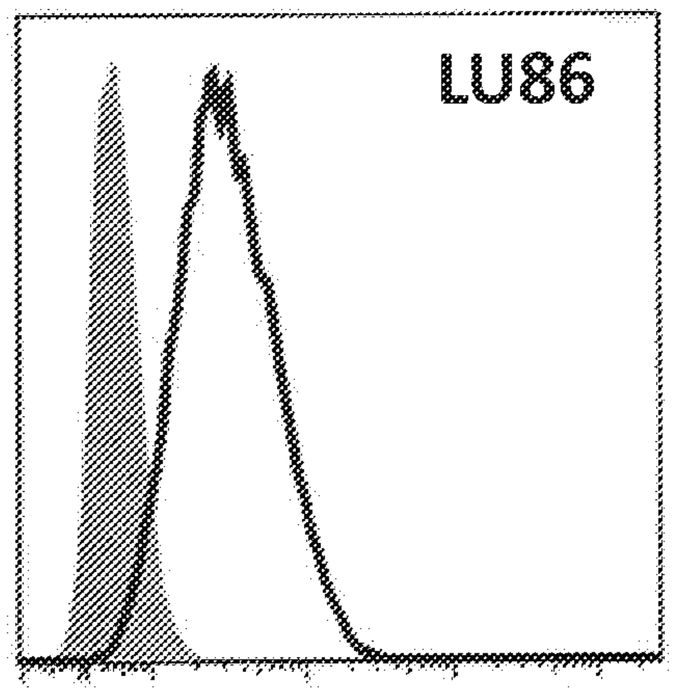

SEZ6 Protein Expression
is Upregulated in Certain NTX Tumors
ng SEZ6 per mg total protein
0.01
0.1
1
10
100
Normal
Breast
Colon
GB
Kidney
Liver
Lung - Adeno
Lung - Carcinoid
LCNEC
Lung - Squamous
Lung - SCLC
Ovarian
Pancreas
Prostate

FIG. 12B

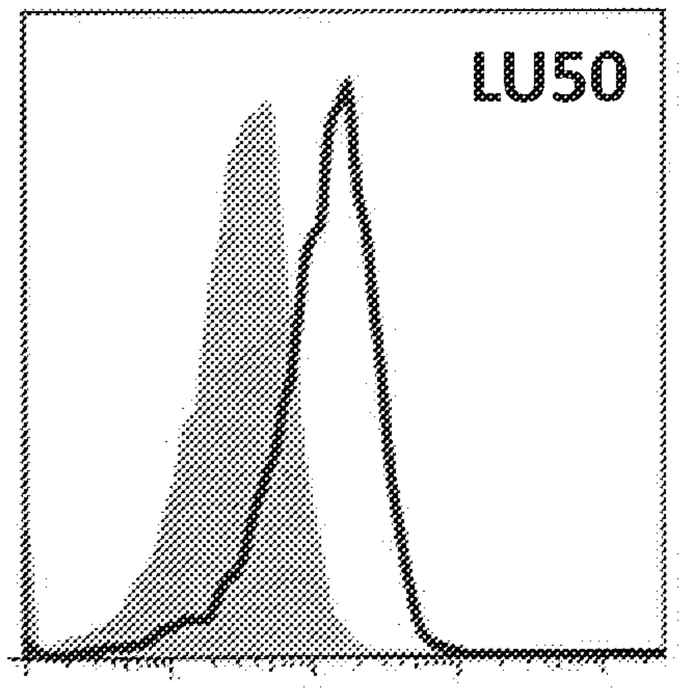
Selected SEZ6 Modulators Detect Surface Expression of SEZ6 in NTX Tumors



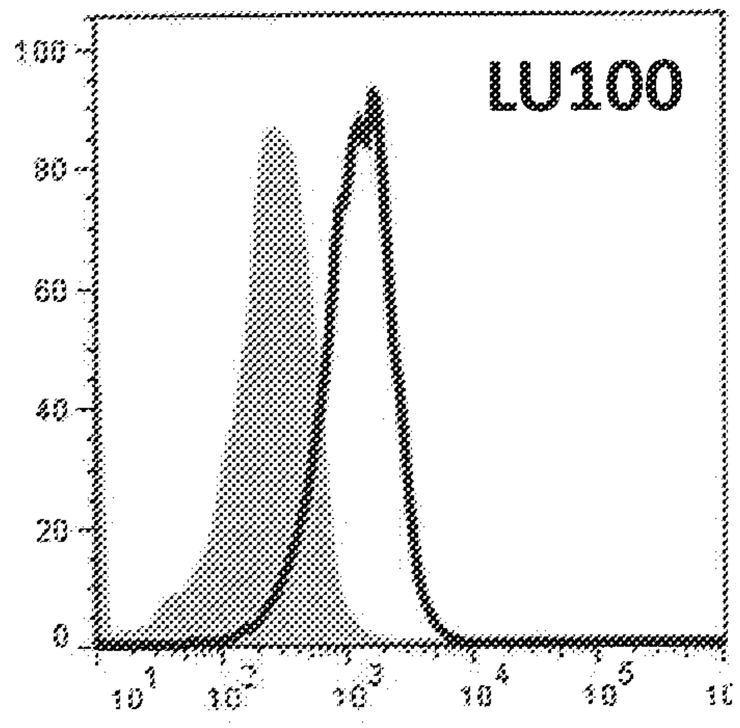
SEZ6 (SC17.6)→



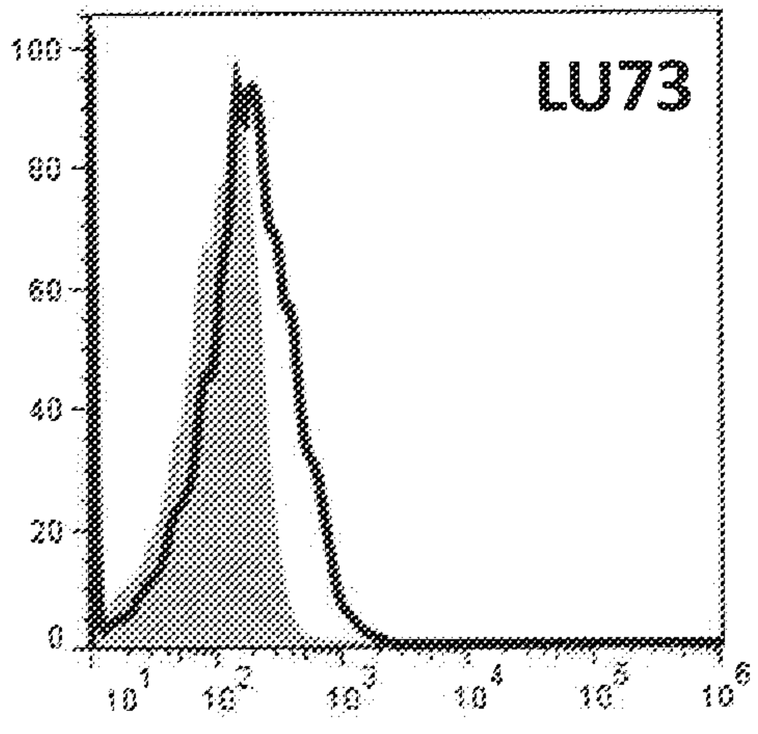
SEZ6 (SC17.6)→



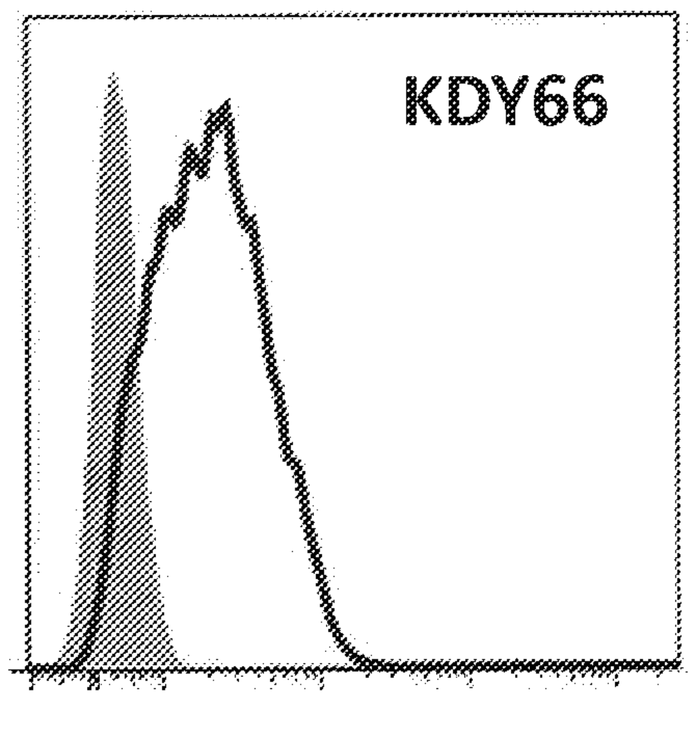
SEZ6 (SC17.10)→



SEZ6 (SC17.42)→



SEZ6 (SC17.28)→



SEZ6 (SC17.6)→

FMO (gray filled)

Anti-SEZ6 Antibody (black line)

Tumor Line	FMO (MFI)	Tumor (MFI)
LU37	29.4	199
LU86	95.1	810
LU50	37.3	300
LU100	289	1400
LU73	112	237
KDY66	22.6	192

FIG. 13A

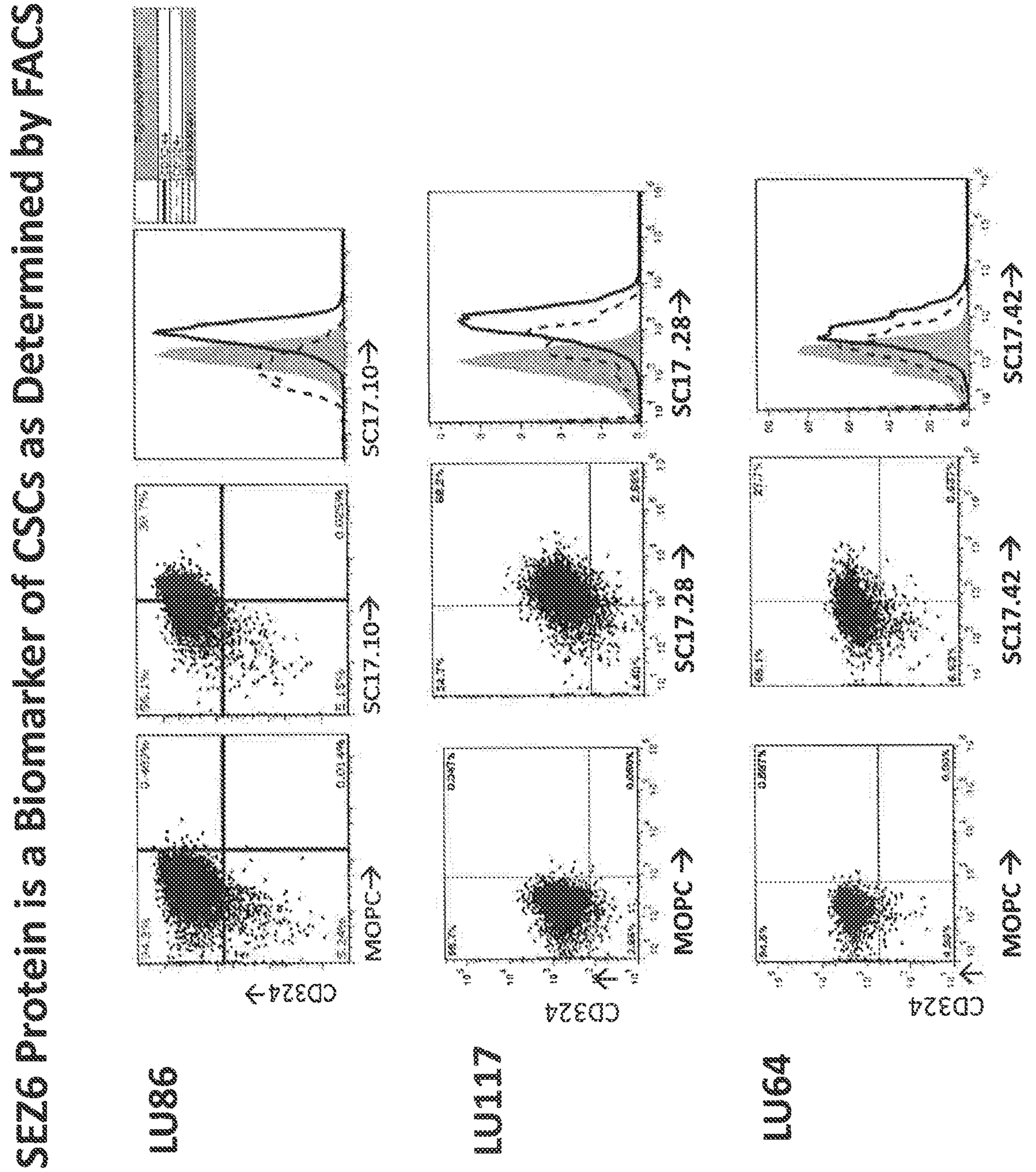

SE26 Protein is a Biomarker of CSCs as Determined by FACS
LU86
LU117
LU64
CD324
MOPC
SC17.10
SC17.28
SC17.42

FIG. 13B

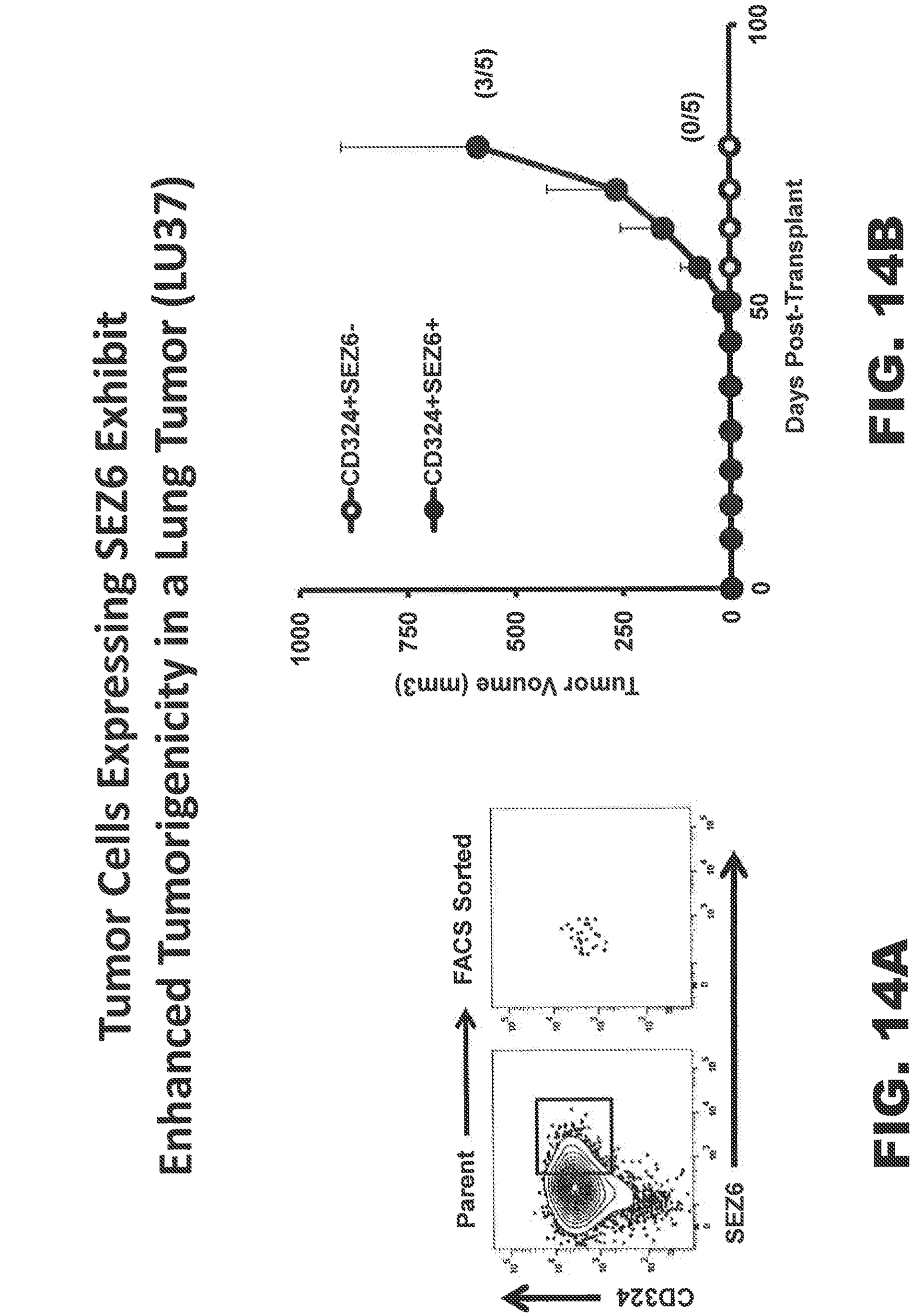


FIG. 14A

FIG. 14B

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SEZ6 Modulators Facilitate Delivery of Cytotoxic Agents to SEZ6-Expressing HEK-293T Cells

Clone	100pM	50pM	10pM
IgG1	103.1	ND	94.3
IgG2a	97.3	100.4	94.7
IgG2b	102.3	ND	96.9
SC17.1	26.3	ND	70.1
SC17.3	16.9	ND	38.5
SC17.4	18.7	ND	34.7
SC17.6	8.8	ND	14.3
SC17.7	23.9	ND	49.4
SC17.8	15.2	ND	22.8
SC17.9	13.8	ND	24.4
SC17.10	56.6	ND	92.2
SC17.11	23.8	ND	64.5
SC17.12	112.4	ND	111.7
SC17.13	107.0	ND	103.4
SC17.14	34.9	ND	71.8
SC17.15	95.1	ND	110.6
SC17.17	11.3	12.8	21.4
SC17.18	59.3	ND	89.6
SC17.19	9.2	ND	14.4
SC17.21	36.4	ND	62.7
SC17.22	46.9	ND	68.9
SC17.23	13.3	ND	38.0
SC17.24	49.5	ND	77.5
SC17.25	58.7	ND	88.8
SC17.26	13.4	ND	15.6
SC17.27	21.7	ND	33.6
SC17.28	12.8	ND	17.9

Clone	100pM	50pM	10pM
SC17.29	112.4	ND	103.3
SC17.30	22.6	ND	50.0
SC17.31	47.0	ND	87.4
SC17.32	104.7	ND	122.9
SC17.33	10.4	ND	17.6
SC17.34	8.8	ND	16.7
SC17.35	15.9	ND	41.8
SC17.36	9.8	ND	12.9
SC17.37	78.4	ND	92.2
SC17.38	104.9	ND	104.8
SC17.39	121.7	ND	121.8
SC17.40	9.6	ND	11.3
SC17.41	60.3	ND	98.1
SC17.42	58.5	ND	91.7
SC17.43	21.5	ND	36.8
SC17.44	47.3	ND	86.3
SC17.45	11.1	ND	12.6
SC17.46	12.7	16.3	23.6
SC17.47	15.0	ND	39.2
SC17.49	21.3	ND	39.3
SC17.50	102.7	ND	109.9
SC17.51	105.4	ND	102.1
SC17.52	8.2	ND	8.6
SC17.53	111.6	ND	99.9
SC17.54	77.0	ND	99.6
SC17.55	11.0	ND	28.9
SC17.56	12.4	ND	16.5

Clone	100pM	50pM	10pM
SC17.57	111.7	ND	103.0
SC17.58	102.3	ND	95.1
SC17.59	111.4	ND	114.5
SC17.60	110.8	ND	101.2
SC17.61	114.0	ND	113.7
SC17.72	ND	101.3	ND
SC17.73	ND	96.4	ND
SC17.74	ND	105.1	ND
SC17.75	ND	108.3	ND
SC17.76	ND	99.6	ND
SC17.81	ND	62.8	ND
SC17.82	ND	97.7	ND
SC17.83	ND	95.4	ND
SC17.84	ND	107.8	ND
SC17.86	ND	111.7	ND
SC17.87	ND	100.9	ND
SC17.88	ND	97.8	ND
SC17.89	ND	17.0	ND
SC17.90	ND	105.4	ND
SC17.91	ND	104.0	ND
SC17.92	ND	107.4	ND
SC17.93	ND	106.3	ND
SC17.95	ND	113.0	ND
SC17.96	ND	53.5	ND
SC17.97	ND	111.9	ND
SC17.99	ND	18.8	ND
SC17.100	ND	97.0	ND

FIG. 15A

SEZ6 Modulators Facilitate Delivery of Cytotoxic Agents to SEZ6-Expressing HEK-293T Cells

Clone	100pM	50pM	10pM
SC17.101	ND	50.7	ND
SC17.102	ND	13.6	ND
SC17.103	ND	111.4	ND
SC17.104	ND	39.4	ND
SC17.105	ND	115.2	ND
SC17.106	ND	116.6	ND
SC17.107	ND	36.0	ND
SC17.108	ND	104.7	ND
SC17.109	ND	108.3	ND
SC17.112	ND	102.7	ND
SC17.114	ND	11.4	ND
SC17.115	ND	10.7	ND
SC17.116	ND	95.4	ND
SC17.117	ND	107.4	ND
SC17.119	ND	111.0	ND
SC17.120	ND	9.1	ND
SC17.121	ND	16.2	ND
SC17.122	ND	107.1	ND
SC17.124	ND	109.9	ND
SC17.125	ND	98.7	ND
SC17.129	ND	102.9	ND
SC17.130	ND	80.4	ND
SC17.133	ND	108.2	ND
SC17.134	ND	37.3	ND
SC17.135	ND	99.0	ND

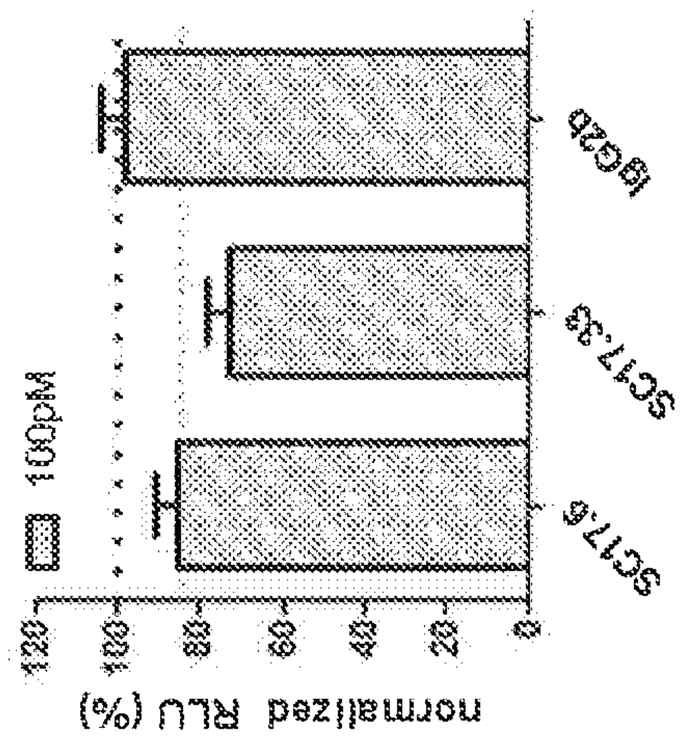
Clone	100pM	50pM	10pM
SC17.136	ND	92.9	ND
SC17.137	ND	15.3	ND
SC17.138	ND	107.7	ND
SC17.140	ND	22.5	ND
SC17.142	ND	101.4	ND
SC17.143	ND	97.0	ND
SC17.145	ND	100.8	ND
SC17.146	ND	96.7	ND
SC17.147	ND	108.9	ND
SC17.149	ND	45.9	ND
SC17.151	ND	8.1	ND
SC17.154	ND	102.0	ND
SC17.155	ND	15.3	ND
SC17.156	ND	19.5	ND
SC17.161	ND	18.3	ND
SC17.162	ND	30.1	ND
SC17.163	ND	106.5	ND
SC17.166	ND	19.2	ND
SC17.167	ND	103.4	ND
SC17.168	ND	46.0	ND
SC17.169	ND	109.7	ND
SC17.170	ND	108.9	ND
SC17.173	ND	68.9	ND
SC17.175	ND	15.9	ND
SC17.176	ND	82.8	ND

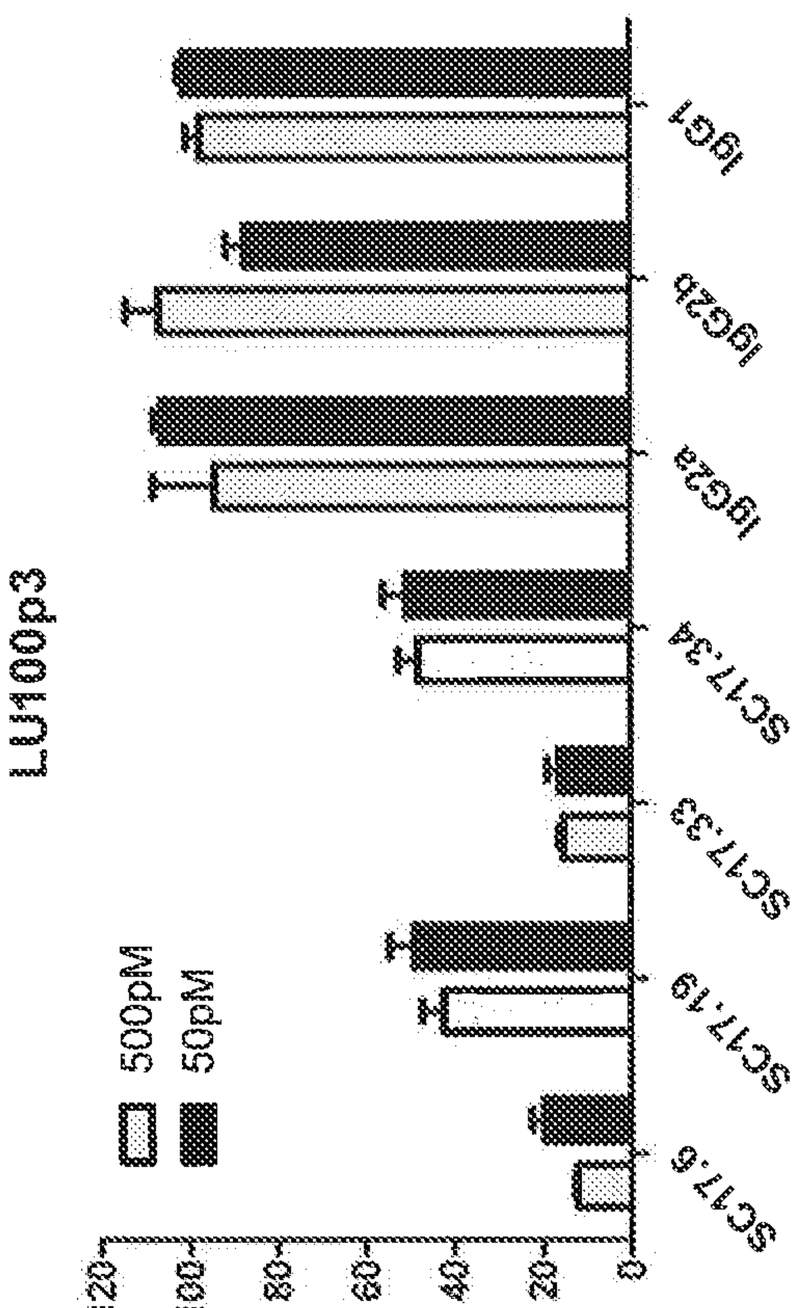
Clone	100pM	50pM	10pM
SC17.177	ND	102.5	ND
SC17.178	ND	102.1	ND
SC17.179	ND	70.2	ND
SC17.180	ND	21.7	ND
SC17.181	ND	101.2	ND
SC17.182	ND	10.9	ND
SC17.184	ND	63.2	ND
SC17.186	ND	105.1	ND
SC17.187	ND	13.5	ND
SC17.188	ND	21.2	ND
SC17.189	ND	98.8	ND
SC17.190	ND	91.3	ND
SC17.191	ND	17.3	ND
SC17.192	ND	103.6	ND
SC17.193	ND	21.1	ND
SC17.195	ND	38.0	ND
SC17.196	ND	106.8	ND
SC17.197	ND	7.0	ND
SC17.198	ND	108.0	ND
SC17.199	ND	21.0	ND
SC17.200	ND	9.9	ND
SC17.201	ND	40.1	ND
SC17.203	ND	96.2	ND
SC17.204	ND	114.1	ND

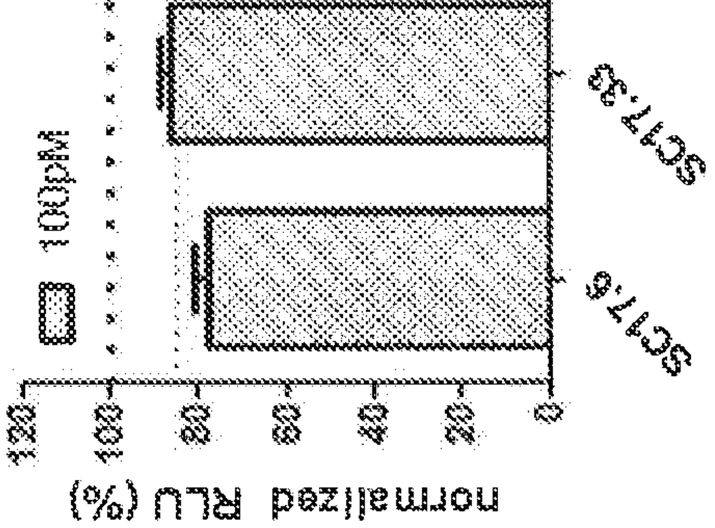
FIG. 15A (Cont.)

Selected SEZ6 Modulators Mediate Killing of NSCLC NTX Tumor Cells

FIG. 15B







IHC Measurements of SEZ6 Expression in Various Tumors

position	sex	age	organ	pathology	stage	Clone 140
A1	M	50	Lung	Small cell carcinoma	I	0
A2	F	51	Lung	Small cell carcinoma	I	1
A3	M	75	Lung	Small cell carcinoma	I	0
A4	F	65	Lung	Small cell carcinoma	I	0
A5	M	56	Lung	Small cell carcinoma	I	1
A6	M	53	Lung	Small cell carcinoma	II	1
A7	M	61	Lung	Small cell carcinoma	I	0
A8	M	43	Lung	Small cell carcinoma	I	1
A9	M	56	Lung	Small cell carcinoma	I	1
B1	F	62	Lung	Small cell carcinoma	I	0
B2	M	55	Lung	Small cell carcinoma	I	0
B3	M	61	Lung	Small cell carcinoma	I	0
B4	M	54	Lung	Small cell carcinoma	II	1
B5	F	66	Lung	Small cell carcinoma	II	1
B6	M	52	Lung	Small cell carcinoma	I	2
B7	M	54	Lung	Small cell carcinoma	II	1
B8	M	62	Lung	Small cell carcinoma	II	1
B9	F	40	Lung	Small cell carcinoma	II	1
C1	M	62	Lung	Small cell carcinoma	II	0
C2	M	58	Lung	Small cell carcinoma	II	1
C3	F	60	Lung	Small cell carcinoma	II	1
C4	M	58	Lung	Small cell carcinoma	II	1

position	sex	age	organ	pathology	stage	Clone 140
C5	F	67	Lung	Small cell carcinoma	II	0
C6	M	35	Lung	Small cell carcinoma	II	1
C7	M	39	Lung	Small cell carcinoma	II	1
C8	F	55	Lung	Small cell carcinoma	II	0
C9	F	52	Lung	Small cell carcinoma	II	1
D1	M	59	Lung	Small cell carcinoma	II	0
D2	M	59	Lung	Small cell carcinoma	II	3
D3	F	53	Lung	Small cell carcinoma	II	1
D4	M	61	Lung	Small cell carcinoma	IIIa	1
D5	M	70	Lung	Small cell carcinoma	IIIa	0
D6	M	60	Lung	Small cell carcinoma	II	1
D7	M	28	Lung	Small cell carcinoma	II	0
D8	M	34	Lung	Small cell carcinoma	II	2
D9	M	44	Lung	Small cell carcinoma	IIIa	0
E1	F	49	Lung	Small cell carcinoma	IIIa	3
E2	F	52	Lung	Small cell carcinoma	IIIa	0
E3	M	44	Lung	Small cell carcinoma	IIIb	3
E4	F	55	Lung	Small cell carcinoma	IIIa	1
E5	-	-	Blank	Empty core	-	XXXX
E6	M	41	Lung	Small cell carcinoma	IIIb	1
E7	M	73	Lung	Small cell carcinoma	IIIa	1
E8	M	51	Lung	Small cell carcinoma	IIIb	0
E9	M	16	Lung	Small cell carcinoma	IIIb	1

Tissue Type	0	1	2	3	Number of Cases
Primary SCLC	36%	52%	5%	7%	N=44

FIG. 16

WO 2013/126810 PCT/US2013/027476 CA 02865415 2014-08-22

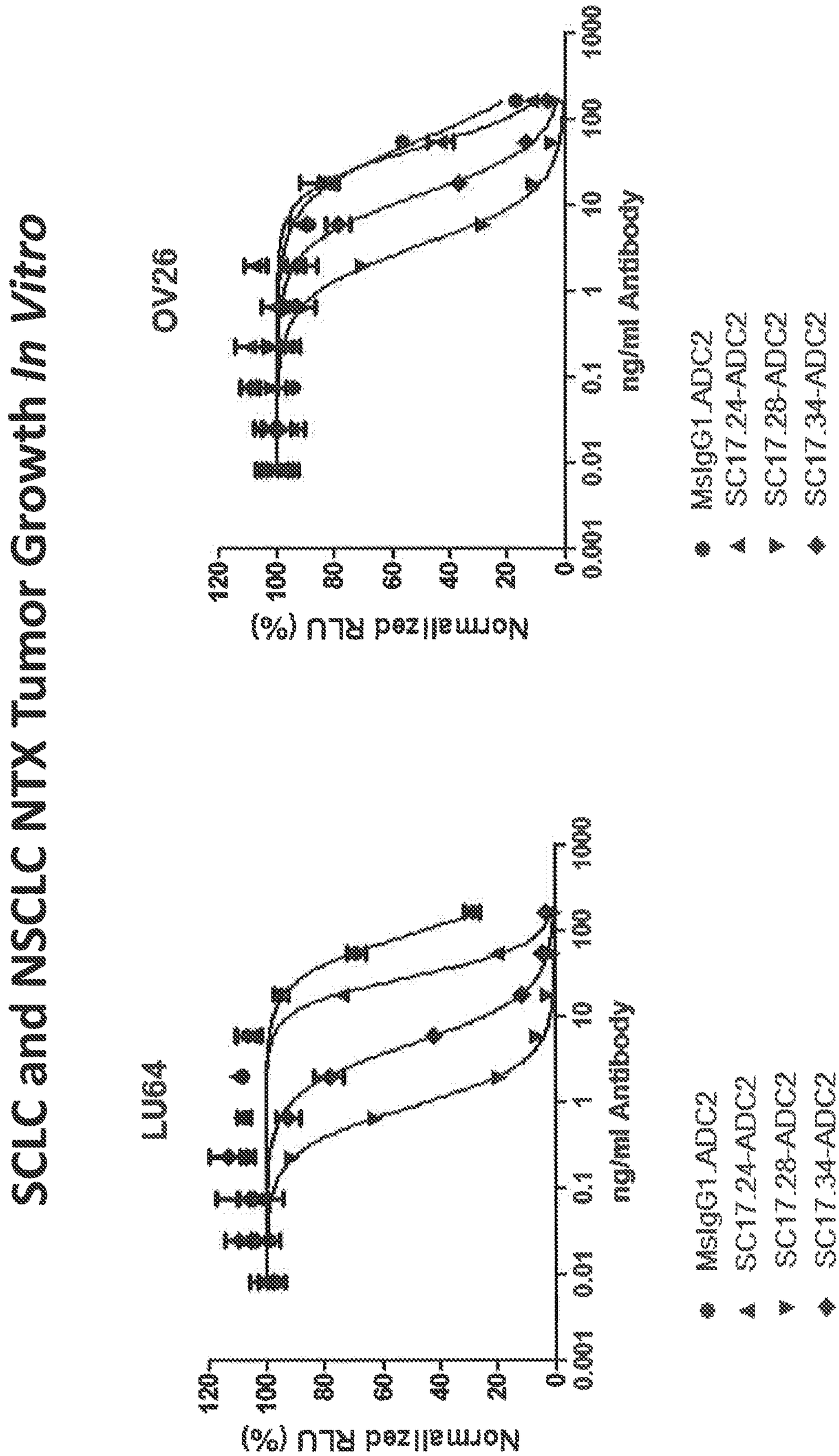

Murine SEZ6 ADC Modulators Suppress SCLC and NSCLC NTX Tumor Growth In Vitro
OV26
LU64
Normalized RLU (%)
ng/ml Antibody
MsIgG1-ADC2
SC17.24-ADC2
SC17.28-ADC2
SC17.34-ADC2

FIG. 17A

Mouse SEZ6 ADC Modulators Suppress SCLC and NSCLC NTX Tumor Growth *In Vivo*

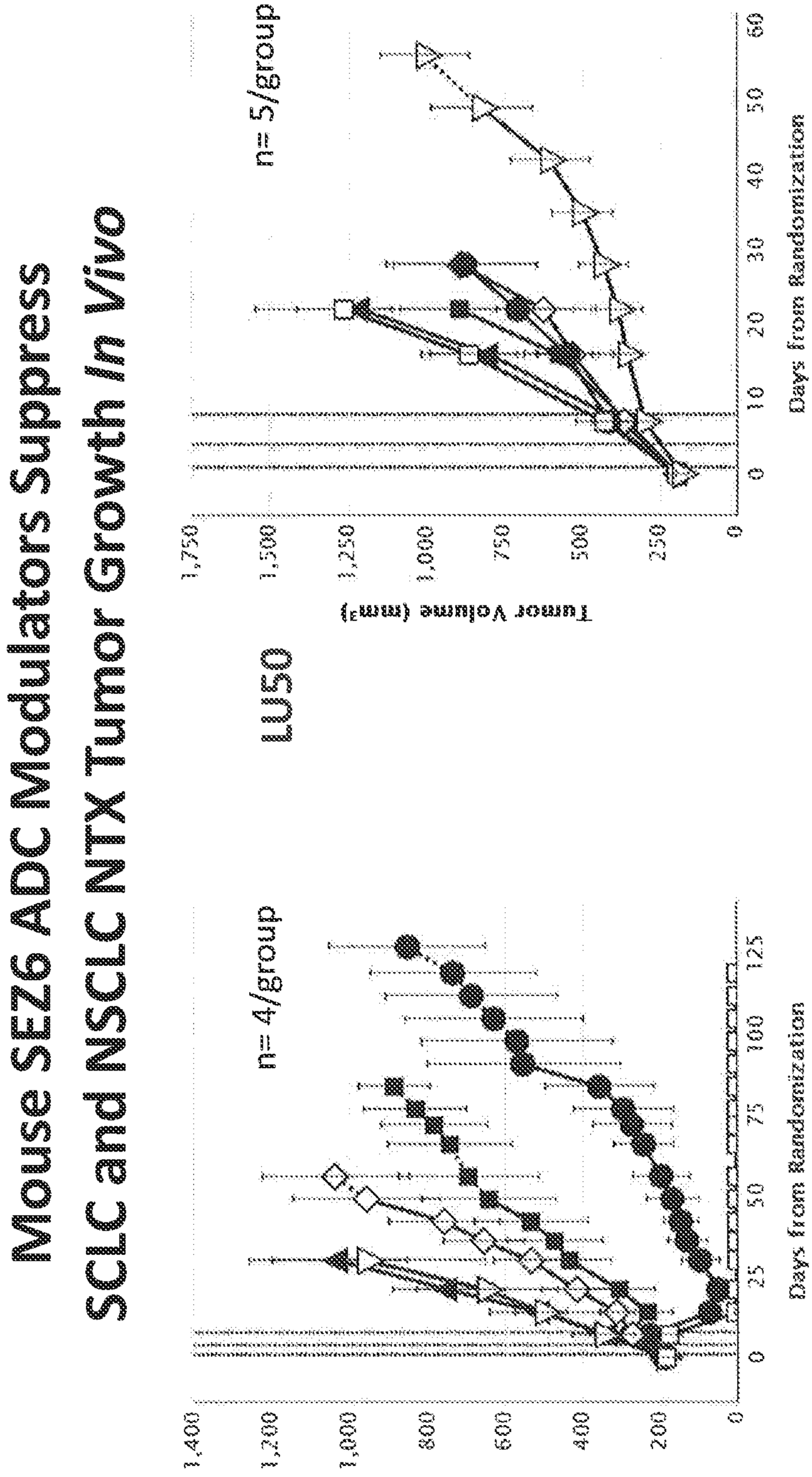


FIG. 17B

Humanized SEZ6 ADC Modulators Suppress SCLC NTX Tumor Growth *In Vivo*

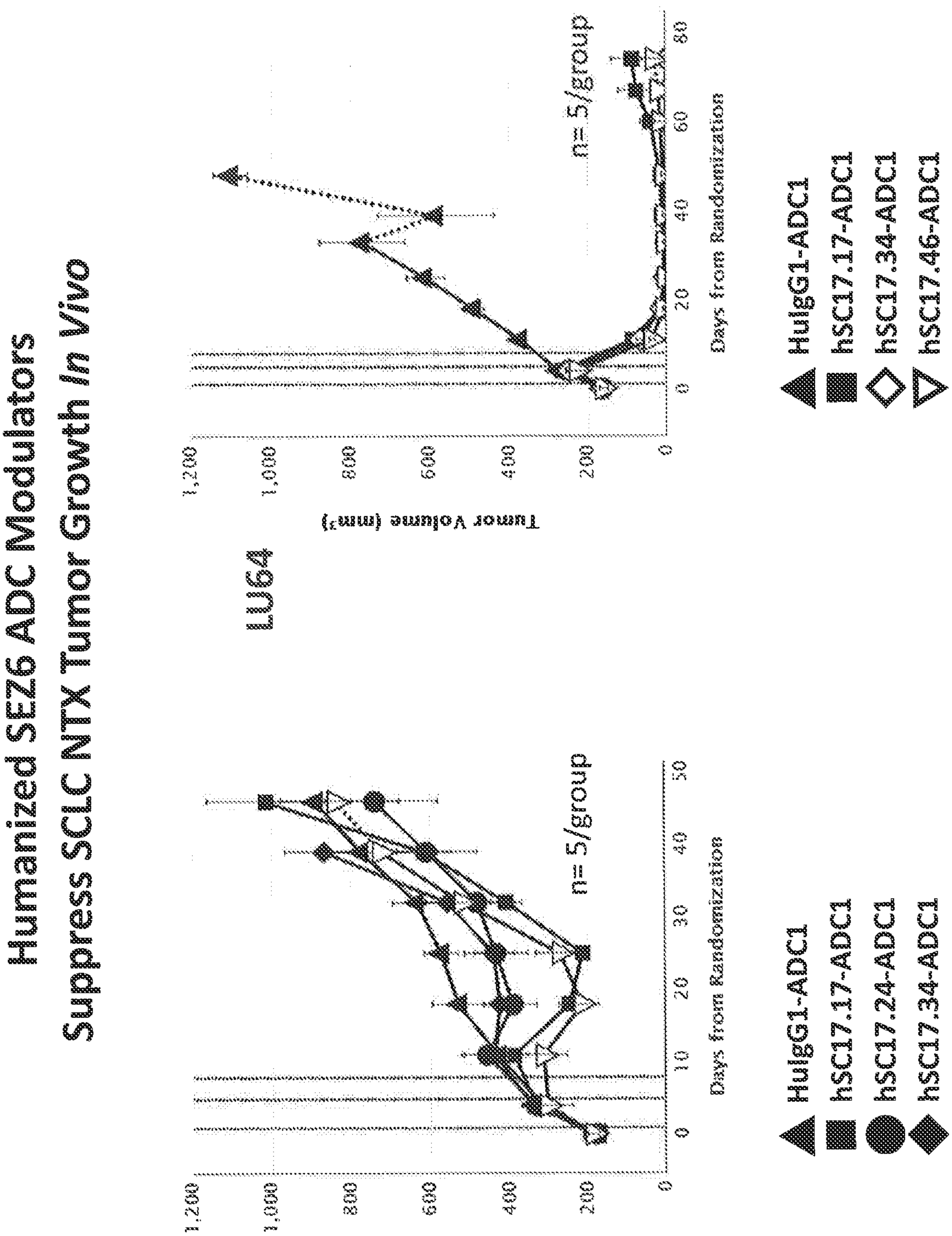
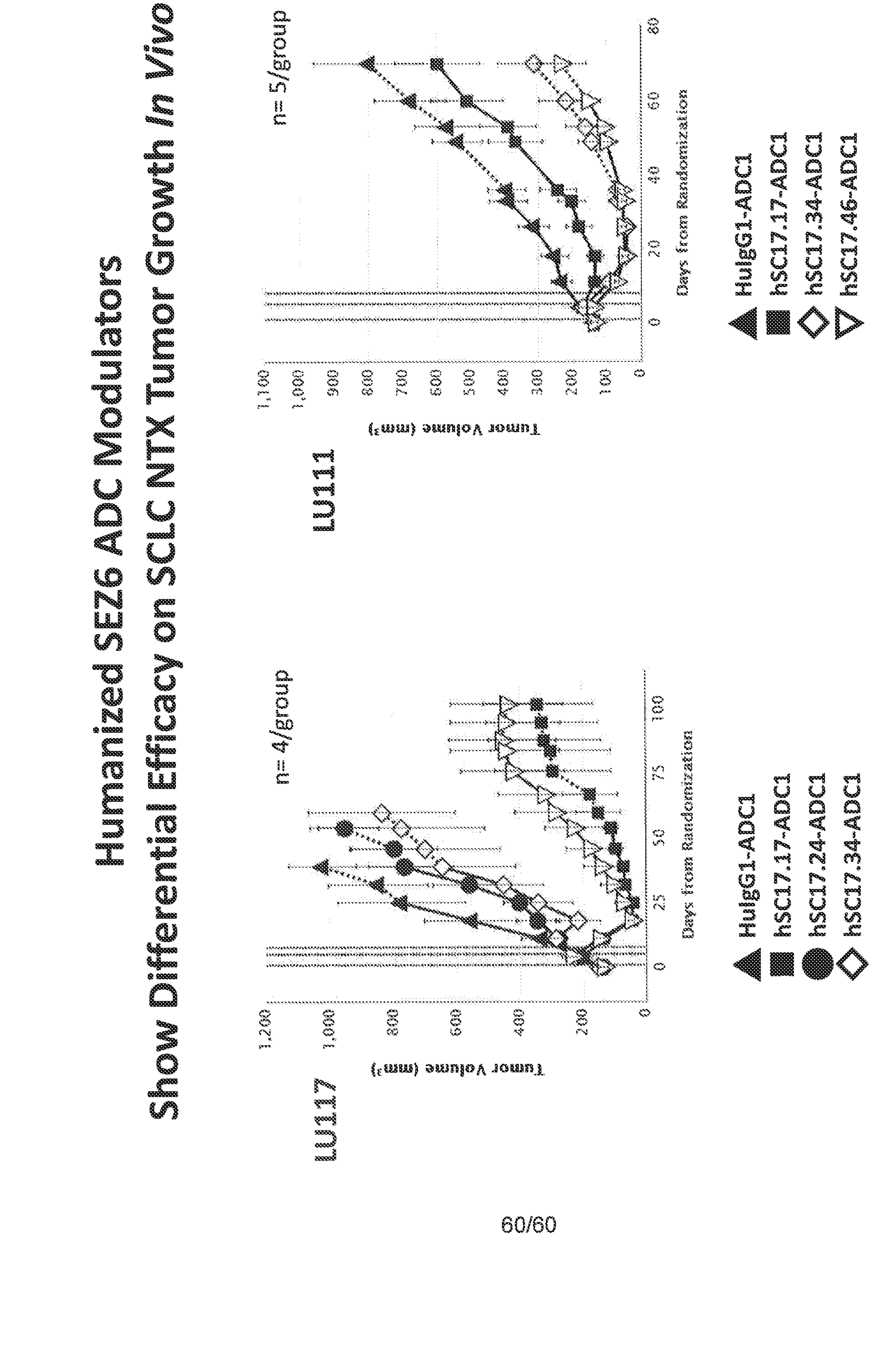


FIG. 18A


Humanized SEZ6 ADC Modulators
Differential Efficacy on SCLC NTX Tumor Growth In Vivo
n= 5/group
LU111
Tumor Volume (mm³)
1,100
1,000
900
800
700
600
500
400
300
200
100
0
0
20
40
60
80
Days from Randomization
HuIgG1-ADC1
hSC17.17-ADC1
hSC17.34-ADC1
hSC17.46-ADC1
18B
n= 4/group
LU117
Tumor Volume (mm³)
1,200
1,000
800
600
400
200
0
0
25
50
75
100
Days from Randomization
HuIgG1-ADC1
hSC17.17-ADC1
hSC17.24-ADC1
hSC17.34-ADC1
hSC17.46-ADC1
Shown
FIG.