

IL-38-Specific Antibodies**Cross-Reference to Related Applications**

[0001] This application claims the priority benefit of U.S. provisional application 63/167,105, filed March 28, 2021 and U.S. provisional application 63/236,454, filed August 24, 2021, the contents of each of which are hereby incorporated by reference in its entirety.

Submission of Sequence Listing on ASCII Text file

[0002] The content of the following submission on ASCII text file is incorporated herein by reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name: 224192000340SEQLIST.TXT, date recorded: March 25, 2022, size: 61,255 bytes).

Field of the invention

[0003] The present invention relates to antibodies that bind to IL38.

Background

[0004] The human adaptive immune system responds through both cellular (T cell) and humoral (B cell) processes. The humoral response results in selection and clonal amplification of B cells that express surface bound immunoglobulin (Ig) molecules capable of binding to antigens. The processes of somatic hypermutation and class switching take place concordant with the clonal amplification. Together these processes lead to secreted antibodies that have been affinity matured against a target antigen and contain a constant domain belonging to one of the five general classes, also called isotypes, (M, D, A, G, or E). Each class of antibody (IgM, IgD, IgA, IgG, and IgE) interact in distinct ways with the cellular immune system. Hallmarks of antibodies that have been affinity matured against a target antigen can include: 1) nucleotide, and subsequent amino acid, changes relative to the germline gene, 2) high binding affinity for the target antigen, 3) binding selectivity for the target antigen as compared to other proteins.

[0005] It is well understood that oncology patients can mount an immune response against tumor antigens. Those antigens can result either from genetic changes within the tumor that lead to mutated proteins or aberrant presentation, of otherwise normal, proteins to the immune system. Aberrant presentation may occur through processes that include, but are not limited to, ectopic expression of neonatal proteins, over-expression of proteins to a high level, alternative splicing, mis-localization of intracellular proteins to the cell surface, or lysis of cells. Post-translational

modifications, such as aberrant glycosylation of proteins, which may occur because of changes in the expression of enzymes, such as, but not limited to, glycosyltransferases, can also result in generation of non-self antigens that are recognized by the humoral immune system.

[0006] Antibodies, which bind selectively to disease-related proteins, including proteins related to cancer, have proven successful at modulating the functions of their target proteins in ways that lead to therapeutic efficacy. The ability of the human immune system to mount antibody responses against mutated, or otherwise aberrant, proteins suggests that patients' immune responses may include antibodies that are capable of recognizing, and modulating the function of, critical tumor-drivers.

[0007] The tumor microenvironment is essential for tumor cells to evade detection and elimination by the immune system. This is achieved through several mechanisms including the recruitment of suppressive immune cells, expression of immune checkpoint molecules like PD-1, and the presence of immunosuppressive cytokines. Therefore, some immune-oncology therapies aim to target key molecules that are responsible for the immunosuppressive barriers within the tumor microenvironment. Successful immune-oncology therapies can lead to the infiltration of cytotoxic T cells and NK cells as well as an upregulation of Th1 cytokines and the induction of a successful anti-tumor response.

[0008] The IL-1 family of cytokines, of which IL-38 is a member, elicit downstream signaling events by binding to, and forming complexes with, membrane bound receptors. An example of an IL-1 receptor complex is represented in Fig. 1. As with other cytokines, such as Tumor Necrosis Factor Alpha (TNFa), binding of antibodies to one, or more, epitopes on the cytokines can be predicted to interfere with the ability of the cytokine to form complexes with their cognate receptors (Hu et al., JBC, 2013). Residues of IL-1, represented in spheres in Fig. 1, are predicted to form an interaction face between IL-1 and the receptors Interleukin-1 Receptor 2 (IL-1R2) and Interleukin-1 Receptor Accessory Protein (IL-1RAP). Residues within this predicted receptor binding region of IL-1 may comprise epitopes to which antibodies can bind. Binding of antibodies to those epitopes would be predicted to block binding of IL-1 to its cognate receptors and antagonize, or inhibit, the cytokine's biological function. The homology between IL-1 and IL-38 suggests that a corresponding epitope, or epitopes, exist on IL-38. Binding of antibodies to that epitope, or epitopes, would be predicted to antagonize, or inhibit, the function of IL-38.

[0009] The IL-1 family of cytokines plays an important role in regulating inflammation during tumor development as well as during treatment (Baker et al., 2019). While some IL-1 family members promote inflammation, others can suppress it. IL-38 is an antagonist that blocks signaling

through IL-1 family receptors, including IL-36R and IL1RALP1, and serves as an immune checkpoint by dampening inflammation (Veerdonk et al., 2017). In particular, IL-38 is shown to reduce the production of inflammatory cytokines, which can play key roles in an effective anti-tumor response. In addition, IL-38 secreted from apoptotic tumor cells can suppress macrophage and T cell responses *in vitro* (Mora et al., 2016). Similarly, IL-38 expression has been shown to correlate with poor prognosis in patients with lung adenocarcinoma as well as correlate with PDL1 expression (Takada et al., 2017). However, conflicting evidence has been reported in non small cell lung cancer, where lower expression of IL-38 correlates with poor prognosis (Wang et al., 2018). IL-38 may serve as an immunosuppressive cytokine within the tumor microenvironment and thus blocking IL-38 signaling may trigger an effective anti-tumor response (Fig. 2).

Summary of the Invention

[0010] In some aspects the present invention relates to discovery of the role of the IL-38 cytokine in various cancers. Accordingly, in some embodiments, provided herein are antibodies that specifically bind to and inhibit IL-38 that are effective for treatment of cancer. In some embodiments, the antibody that specifically binds to IL-38 comprises the CDR sequences of M3. In some embodiments, the antibody that specifically binds to IL-38 comprises a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO:23, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO:24, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 25, a VL CDR1 comprising the amino acid sequence set forth in SEQ ID NO:58, a VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO:59, and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO:60. In some embodiments, the antibody that specifically binds to IL-38 comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:98 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:101. In some embodiments, the cancer is selected from the group consisting of head and neck squamous cell carcinoma (HNSC), esophagus cancer (ESCA), squamous cell cancer of the lung (LUSC), cancer of the cervix (CESC), bladder cancer (BLCA), skin cutaneous melanoma (SKCM), prostate adenocarcinoma (PRAD), gastroesophageal carcinoma, cervical squamous cell carcinoma, cervical squamous cell carcinoma, skin squamous cell carcinoma, basal cell carcinoma, skin cutaneous melanoma, lung adenocarcinoma, endocervical adenocarcinoma, bladder urothelial carcinoma and prostate adenocarcinoma and lung adenocarcinoma (LUAD).

[0011] This invention relates to antibodies specific for human interleukin-38 (IL-38), including isolated antibodies, or antigen-binding fragments thereof, which contain at least 3, at least 4, at

least 5, at least 6, at least 7, or at least 8 optionally contiguous amino acids of at least one complementary-determining region (CDR) contained within the variable heavy chain (VH) amino acid sequences of: SEQ ID NO: 22, SEQ ID NO: 27, SEQ ID NO: 32, SEQ ID NO: 37, SEQ ID NO: 42, SEQ ID NO: 47, SEQ ID NO: 52, SEQ ID NO: 2, or SEQ ID NO: 7; and/or contained within the variable light chain (VL) amino acid sequences of: SEQ ID NO: 57, SEQ ID NO: 62, SEQ ID NO: 67, SEQ ID NO: 72, SEQ ID NO: 77, SEQ ID NO: 82, SEQ ID NO: 4, or SEQ ID NO: 9.

[0012] More particularly, the CDRs of an antibody of the invention, or antigen-binding fragment may contain at least 3, at least 4, at least 5, at least 6, at least 7, or at least 8 optionally contiguous amino acids of: the VH CDR1 amino acid sequences of SEQ ID NOS: 23, 28, 33, 38, 43, 48, 53, or 15; the VH CDR2 amino acid sequences of SEQ ID NOS: 24, 29, 34, 39, 44, 49, 54, or 16; the VH CDR3 amino acid sequences of SEQ ID NOS: 25, 30, 35, 40, 45, 50, 55, or 17; the VL CDR1 amino acid sequences of SEQ ID NOS: 58, 63, 68, 73, 78, 83, or 18; the VL CDR2 amino acid sequences of SEQ ID NOS: 59, 64, 69, 74, 79, 84, or 19; and/or the VL CDR3 amino acid sequences of SEQ ID NOS: 60, 65, 70, 75, 80, 85, or 20.

[0013] Antibodies of the invention, or antigen-binding fragments thereof, partially or fully blocks, inhibits, or neutralizes a biological activity of IL 38. Accordingly, methods of the invention include methods of inhibiting tumor growth or metastasis in an individual afflicted by tumor growth and/or metastasis by administering a therapeutically effective amount of a composition comprising the antibody or an antigen-binding fragment thereof, to the individual for treatment in which the antibody of the invention, or an antigen-binding fragment thereof, partially or fully blocks, inhibits, or neutralizes a biological activity of IL-38 that promotes or sustains tumor growth and/or metastasis.

[0014] The disclosure of PCT Application No. PCT/US2020/044260 is incorporated herein in its entirety.

Brief Description of the Figures

[0015] Fig. 1 is a representation of a co-crystal structure of an Interleukin-1 (IL-1) receptor complex (RCSB PDB file number 3O4O) visualized using PyMol. The co-crystal comprises IL-1Beta, Interleukin-1 receptor type 2 (IL-1R2), and Interleukin-1 receptor accessory protein (IL-1RAP). IL-1R2 and IL-1RAP are depicted in black and light gray cartoons, respectively. IL-1Beta is depicted in medium gray ribbon. Residues of IL-1Beta depicted in spheres are those predicted to be within 4 Angstroms of either IL-1R2 or IL-1RAP.

- [0016] Fig. 2 is a cartoon depicting how blocking IL-38 function may elicit an inflammatory response that could lead to an anti-tumor response.
- [0017] Fig. 3. is a graph showing binding of PR087-29B5 hybridoma-produced antibodies in a LICOR-based screen.
- [0018] Fig. 4 is a graph showing selective, dose-dependent binding of PR087-29B5 to recombinant human IL-38 in a dot blot format.
- [0019] Fig. 5 is a graph showing binding of IMM20130 to various cell lines by flow cytometry
- [0020] Fig 6. is a graph showing IL-38 concentration in conditioned media of cancer cell lines cultured under apoptotic conditions for varying timepoints.
- [0021] Fig. 7 is a set of graphs showing RNA expression analyses based on data from the TCGA database comparing IL-38 expression levels with immune cell lineage-specific markers in prostate adenocarcinoma (PRAD), colorectal adenocarcinoma (COAD), lung adenocarcinoma (LUAD), skin cutaneous melanoma (SKCM), uterine corpus endometrial carcinoma (UCEC), head & neck squamous cell carcinoma (HNSC) and pancreatic adenocarcinoma (PAAD).
- [0022] Fig. 8 is a graph of IL-6 and TNF α expression in conditioned media from LPS-stimulated THP-1 macrophages.
- [0023] Fig. 9 is a graph of mRNA expression of markers that were downregulated in LPS-stimulated macrophages when treated with IL-38.
- [0024] Fig. 10 is a graph of the phosphorylation status of Jnk and STAT3 in THP-1 macrophages stimulated with LPS for indicated timepoints.
- [0025] Fig. 11 is a graph showing IL-6 production of LPS-stimulated THP-1 cells treated with various combinations of IL-38, IMM20130, and an isotype control.
- [0026] Fig. 12 is a graph showing IL-6 production of LPS-stimulated THP-1 cells treated with various combinations of IL-38 and anti-IL-38 polyclonal antibodies.
- [0027] Fig. 13 is a graph showing binding of monoclonal hybridoma supernatants to plate-bound recombinant IL-38.
- [0028] Fig. 14 is a graph showing percent rescue of monoclonal hybridoma supernatants, defined by their ability to restore IL-6 production in IL-38-treated, LPS-stimulated cells.
- [0029] Fig. 15 is a graph showing binding kinetics for different concentrations of a selected anti-human IL-38 antibody
- [0030] Fig. 16A is a graph showing a dose response of lead candidate antibodies tested for their ability to restore IL-6 production in IL-38-treated, LPS-stimulated THP-1 cells.

- [0031] Fig. 16B is a graph showing a dose response of lead candidate antibodies tested for their ability to restore GM-CSF production in IL-38-treated, LPS-stimulated THP-1 cells.
- [0032] Fig. 17 is a graph of the plasma levels of lead candidate antibodies over time in C57BL/6 mice dosed 10 mg/kg both i.v. and i.p.
- [0033] Fig. 18 is a growth curve of B16.F10 tumors implanted in C57BL/6 mice treated with CD1-M3, paclitaxel, or a combination.
- [0034] Fig. 19 is a graph characterizing tumor-infiltrating myeloid and lymphocyte populations by flow cytometry in B16.F10 tumors treated with CD1-M3.
- [0035] Fig. 20 is a growth curve of MMTV-PyMT tumors implanted in FVB mice treated with CD1-M3 and a graph showing IL-6 levels within these tumors.
- [0036] Fig. 21 is a growth curve of A549 tumors implanted in *scid* mice treated with CD1-M3, CD1-M8, CD1-M26, and NZB-M8.
- [0037] Fig. 22 is a graph characterizing the tumor infiltrating myeloid populations by flow cytometry in A549 tumors treated with CD1-M3, CD1-M8, CD1-M26, and NZB-M8.
- [0038] Fig. 23 is a set of graphs showing RNA expression analyses based on data from the TCGA database examining IL-38 expression levels in head & neck squamous cell carcinoma (HNSC), esophagus cancer (ESCA), squamous cell cancer of the lung (LUSC), cancer of the cervix (CESC), bladder cancer (BLCA), skin cutaneous melanoma (SKCM), prostate adenocarcinoma (PRAD), and lung adenocarcinoma (LUAD).
- [0039] Fig. 24 depicts identification and analysis of IMM20130 binding and specificity. FIG. 24A depicts overview of IMM20130 isolation from primary patient B cells using the Immunome platform. FIG. 24B depicts binding of IMM20130 to recombinant human IL-38 protein using Bio-Layer Interferometry (BLI) analysis. FIG. 24C depicts specificity of IMM20130 assessed by protein microarray. Binding signal and S-Scores shown for the top 200 proteins based on binding signal. FIG. 24D depicts cell-based binding of IMM20130 to cancer and cell lines by flow cytometry.
- [0040] Fig. 25 depicts IL-38 expressed in multiple tumor types and is associated with lower immune cell infiltration. Tumor and normal tissue RNAseq data was used to investigate IL-38 expression in multiple cancers. Samples were normalized for IL-38 expression and subdivided based on high (outside of box) and low (inside of box) expression. Thresholds were set according to a value of 10-15% of normal tissues. Numbers in each plot represent frequency of IL-38 positive samples in all tumor samples.

[0041] Fig. 26 depicts immune cell co-expression signature analysis (IMMSig) calculated on each of the segregated tumor types to determine the difference of immune cell tumor infiltration between IL38 positive and negative tumor samples.

[0042] Fig. 27 depicts IMM20324 binding human IL-38 and suppresses primary human immune cell function *in vitro* by neutralizing receptor binding. FIG. 27A depicts intracellular binding of IMM20324 to HEK293 cells overexpressing mouse or human IL-38. FIG. 27B depicts ELISA-based binding analysis of IMM20324 to plate-bound recombinant full-length human IL38. FIG. 27C depicts dose-dependent inhibition of IL38 binding to IL-36R and IL1RAPL1 receptors by IMM20324. FIG. 27D depicts that IMM20324 reverses IL-38-mediated suppression of IL-6 secretion in THP-1 cells *in vitro*. IMM20324 reverses IL-38-mediated suppression of GM-CSF secretion in THP-1 cells *in vitro*.

[0043] Fig. 28 depicts favorable PK of IMM20324 in naive mice *in vivo*. FIG. 28A depicts *in vivo* single dose pharmacokinetic analysis of IMM20324 following administration of antibody by intravenous (IV) and intraperitoneal (IP) routes in naive C57BL/6 female mice. Dose shown is 10mg/kg. FIG. 28B depicts analysis of serum IMM20324 concentrations following repeated administration of antibody by IP injection. Groups were dosed once weekly (QW) or twice weekly (BIW) with the labelled dose in mg/kg (mpk).

[0044] Fig. 29 depicts that IMM20324 is well tolerated in mice. FIG. 29A depicts that average body weight of animals from each treatment group were monitored throughout the duration of the study. FIG. 29B depicts that average spleen weights of animals comprising each treatment group were assessed upon study termination. FIG. 29C depicts assessment of circulating cytokine levels in serum following administration of IMM20324. Blood was sampled following study termination.

[0045] Fig. 30 depicts IMM20324 inhibiting growth of B16.F10 tumors *in vivo*. Mice were randomized at day 0 based on tumor volume and treated with 10 mg/Kg or 50 mg/Kg dose of IMM20324 or isotype control intraperitoneally every three days for 6 total doses. Tumor volumes were measured every 3 days. Tumor growth curves were fitted with exponential model. Fig. 30A depicts growth curves of individual animal from the indicated group over the duration of the study. Fig. 30B depicts interim tumor volumes of individual animal on day 10, * $p < 0.05$. Fig. 30C depicts calculated average tumor growth rate over the duration of study. Fig. 30D calculated days when tumor volume reaches 1500 mm³ based on fitting of tumor growth curve.

[0046] Fig. 31A-D depicts IMM20324 inhibiting tumor growth and elicits immunological memory against EMT6 tumors *in vivo*. Mice were randomized at day 0 based on tumor volume and treated with three different doses of IMM20324 or a vehicle control twice per week for 6 total doses, Fig. 31A depicts individual growth curves for each mouse in the indicated group up to Day 44. Fig. 31B

depicts individual tumor volumes at day 15. In Fig. 31C EMT6 cells were implanted into the opposite flank of IMM20324-treated mice following full regression of the primary tumor. No additional antibody was administered during secondary challenge. Secondary EMT6 tumor growth curves compared to untreated age-matched mice. Fig. 31D depicts survival curves of rechallenged mice showing % survival per group. * $p < 0.05$.

[0047] Figure 32A-C show that IMM20324 treatments dose dependently inhibit B16.F10 tumor growth in vivo. C57BL/6 mice were inoculated with B16.F10 melanoma cells in right flank. At day 0, mice were randomized based on tumor volume and treated with 3 mg/Kg, 10 mg/Kg or 30 mg/Kg dose of IMM20324 or 30 mg/Kg isotype control or 10 mg/Kg anti-PD-L1 intraperitoneally every three days for 4 total doses. Tumor volumes were measured every 3 days. Fig. 32A shows that IMM20324 achieved dose dependent exposures after single dose in B16.F10 tumor bearing mice. Fig 32B depicts the terminal tumor volumes on day 11 in different treatment groups. Fig. 32C depicts the concentration of IMM20324 in serum 24 hours post 4th dose (ng/ml).

[0048] FIG. 33 shows concentrations of IMM20324 in terminal serum are negatively correlated with CXCR3 ligand chemokines: CXCL9/MIG (Fig. 33A) and CXCL10/IP10 (Fig. 33B) in tumors.

[0049] FIG. 34 shows concentrations of IMM20324 in terminal serum are negatively correlated with CC family chemokines: CCL3/MIP1 α (Fig. 34 A) and CCL4/MIP1 β (Fig. 34 B) in tumors.

[0050] FIG. 35 shows concentrations of IMM20324 in terminal serum are negatively correlated with CCL11/Eotaxin in tumors.

[0051] FIG. 36 shows cytoplasmic and nuclear expression analysis of IL-38 in primary human head and neck cancer samples. Paraffin-embedded, formalin-fixed tumor sections were stained with IMM20130 anti-IL38 mouse antibody and detected with a polyclonal anti-mouse secondary antibody. Staining was noted only in cytoplasmic or nuclear compartments. Labelled sections were scored for staining intensity and localization by a board-certified pathologist. Samples (x-axis) are ordered based on stage.

[0052] FIG. 37 shows cytoplasmic and nuclear expression of IL-38 in primary human head and neck cancer samples was compared based on the stage of disease. Paraffin-embedded, formalin-fixed tumor sections were stained with IMM20130 anti-IL38 mouse antibody detected with a polyclonal anti-mouse secondary antibody. The frequency of samples showing staining in cytoplasmic or nuclear compartments was plotted for each cancer stage.

[0053] FIG. 38 shows cytoplasmic and nuclear expression analysis of IL-38 in primary cervical squamous cancer samples. Paraffin-embedded, formalin-fixed tumor sections were stained with IMM20130 anti-IL38 mouse antibody and detected with a polyclonal anti-mouse secondary

antibody. Staining was noted only in cytoplasmic or nuclear compartments. Labelled sections were scored for staining intensity and localization by a board-certified pathologist. Samples (x-axis) are ordered based on stage.

[0054] FIG. 39 shows cytoplasmic and nuclear expression of IL-38 in primary human cervical squamous cancer samples was compared based on the stage of disease. Paraffin-embedded, formalin-fixed tumor sections were stained with IMM20130 anti-IL38 mouse antibody detected with a polyclonal anti-mouse secondary antibody. Staining in cytoplasmic or nuclear compartments was identify positive versus negative samples.

[0055] FIG. 40 shows cytoplasmic and nuclear expression analysis of IL-38 in primary lung cancer samples. Paraffin-embedded, formalin-fixed tumor sections were stained with IMM20130 anti-IL38 mouse antibody and detected with a polyclonal anti-mouse secondary antibody. Staining was noted only in cytoplasmic or nuclear compartments. Labelled sections were scored for staining intensity and localization by a board-certified pathologist. Samples (x-axis) are ordered based on stage.

[0056] FIG. 41 shows cytoplasmic and nuclear expression of IL-38 in primary human lung cancer samples was compared based on the stage of disease. Paraffin-embedded, formalin-fixed tumor sections were stained with IMM20130 anti-IL38 mouse antibody detected with a polyclonal anti-mouse secondary antibody. Staining in cytoplasmic or nuclear compartments was identify positive versus negative samples.

[0057] FIG. 42 shows cytoplasmic and nuclear expression analysis of IL-38 in primary lung cancer samples by immunohistochemistry. Paraffin-embedded, formalin-fixed tumor sections were stained with IMM20130 anti-IL38 mouse antibody detected with a polyclonal anti-mouse secondary antibody. Staining in cytoplasmic or nuclear compartments was identify positive versus negative samples. Lung tumors are sorted according to pathological tumor type.

[0058] FIG. 43 shows relative staining intensity for IL-38 in different lung cancer subsets. Tumors sections were stained with IMM20130 anti-IL38 mouse antibody and detected with a polyclonal anti-mouse secondary antibody. Pathology scoring was performed using a 0 (no expression) to 6 (strong expression) scale for intensity. Data shows the mean intensity score for each subset +/- SEM.

[0059] FIG. 44 shows relative staining intensity for IL-38 in melanoma. Melanoma sections were stained with a commercial mouse anti-human IL-38 antibody (H127c; Thermo Scientific) or IMM20130 and detected with a polyclonal anti-mouse secondary antibody. Pathology scoring was performed using a 0 (negative) to 4 (strong expression) scale for intensity. Samples are ordered by stage.

[0060] FIG. 45 shows relative staining intensity for IL-38 in primary human melanoma samples. Tumor sections were stained with IMM20130 anti-IL38 mouse antibody and detected with a polyclonal anti-mouse secondary antibody. Pathology scoring was performed using a 0 (negative) to 4 (strong expression) scale for intensity. Data shows the mean intensity score for each subset +/- SEM.

[0061] FIG. 46A-D show deep dive analysis of IL-38 expression in Head and neck squamous carcinoma. FIG. 46A-C shows that IL-38 expression (Y-axis, Log2 (TPM+1)) is negatively associated with estimated infiltration (X-axis) of CD8+ T cells (FIG. 46A), macrophages (FIG. 46B) and NK cells (FIG. 46C). FIG. 46D shows IL-38 expression is significantly higher in HPV negative vs HPV positive populations.

Detailed Description

[0062] The invention described herein relates to antibodies that specifically bind to Interleukin-38 (IL 38). Accordingly, the invention includes compositions of antibodies specific for IL-38, methods for using antibodies specific for IL-38, and methods for preparing and formulating antibodies specific for IL-38. Methods of using antibodies of the invention may include methods of treating individuals in need thereof. Accordingly, methods of using antibodies of the invention in methods of treatment may include methods of administering antibody compositions of the invention. Methods of the invention may also include using the antibodies in *in vivo* and *in vitro* diagnostic methods.

Diagnostic methods of the invention may be included as a step in a multi-step method of treatment.

[0063] An antibody according to the invention may be an intact immunoglobulin, or a variant of an immunoglobulin, or a portion of an immunoglobulin. A naturally occurring immunoglobulin has two heavy (H) chains and two light (L) chains, each of which, contains a constant region and a variable region, and are interconnected by disulfide bonds. There are two types of light chains, which are termed lambda ("λ") and kappa ("κ"). There are five main heavy chain classes, also known as isotypes, which determine functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE. In addition to its variable domain, an IgA, IgD, or IgG heavy chain has three constant domains (CH1, CH2, CH3). IgM and IgE heavy chains have four constant domains (CH1, CH2, CH3, CH4).

[0064] Light and heavy chain variable regions contain "framework" regions interrupted by three hypervariable regions, called complementarity-determining regions ("CDRs"). The CDRs are primarily responsible for binding to an epitope of an antigen. The sequences of the framework regions of different light or heavy chains are relatively conserved within a species, and serve to position and align the CDRs in three-dimensional space. The three CDRs of each chain are typically referred to as CDR1, CDR2, and CDR3, numbered sequentially starting from the N-terminus, and are often identified by the chain in which the particular CDR is located. Accordingly, heavy chain CDRs are designated H-CDR1, H-CDR2, and H-CDR3; likewise, light chain CDRs are designated L-CDR1, L-CDR2, and L-CDR3. An antigen-binding fragment that is composed of one constant and one variable domain of each of the heavy and the light chain is referred to as an Fab fragment. An F(ab)₂ fragment contains two Fab fragments, and can be generated by cleaving an immunoglobulin molecule below its hinge region.

[0065] The amino acid sequence of an antibody of the invention can also contain a variant of one or more of the amino acid sequences of: SEQ. ID. NOS. 2, 4, 7, 10, 12, 14-20, 22-25, 27-30, 32-35, 37-40, 42-45, 47-50, 52-55, 57-60, 62-65, 67-70, 72-75, 77-80, 82-85, 87-90, and 92-95; or one or more of the amino acid sequences encoded by SEQ. ID. NOS. 1, 3, 5, 6, 8, 9, 11, 13, 21, 26, 31, 36, 41, 46,

51, 56, 61, 66, 71, 76, 81, 86 and 91. Antibody variants typically contain amino acid sequence modifications, and can be made for any reason, including, for example, to improve specificity, affinity, or stability (*i.e.*, half-life). Examples of antibody variants of the invention include, but are not limited to, fragments of antibodies, amino acid substitutions, amino acid deletions, chimeric antibodies, and any combination of the foregoing.

[0066] A variant antibody of the invention that contains one or more amino acid substitutions generally contain no more than 15, no more than 12, no more than 10, no more than 9, no more than 8, no more than 7, no more than 6, no more than 5, no more than 4, no more than 3, no more than 2 or no more than 1 conservative amino acid substitution relative to an amino acid sequence of SEQ. ID. NOS. 2, 4, 7, 10, 12, 14-20, 22-25, 27-30, 32-35, 37-40, 42-45, 47-50, 52-55, 57-60, 62-65, 67-70, 72-75, 77-80, 82-85, 87-90, and 92-95; or one or more of the amino acid sequences encoded by SEQ. ID. NOS. 1, 3, 5, 6, 8, 9, 11, 13, 21, 26, 31, 36, 41, 46, 51, 56, 61, 66, 71, 76, 81, 86, and 91; and/or no more than 5, no more than 4, no more than 3, or no more than 2 non-conservative amino acid substitutions, or no more than 1 non-conservative amino acid substitution, relative to an amino acid sequence of: SEQ. ID. NOS. 2, 4, 7, 10, 12, 14-20, 22-25, 27-30, 32-35, 37-40, 42-45, 47-50, 52-55, 57-60, 62-65, 67-70, 72-75, 77-80, 82-85, 87-90, and 92-95; or one or more of the amino acid sequences encoded by SEQ. ID. NOS. 1, 3, 5, 6, 8, 9, 11, 13, 21, 26, 31, 36, 41, 46, 51, 56, 61, 66, 71, 76, 81, 86, and 91.

[0067] A conservative amino acid substitution is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan). Variant antibodies of the invention may include amino acid substitutions with amino acid analogs as well as amino acids, as described herein. An antibody of the invention can contain one or more amino acid sequences that share at least 85% sequence identity with the amino acid sequence of one or more of the amino acid sequences of SEQ. ID. NOS. 2, 4, 7, 10, 12, 14-20, 22-25, 27-30, 32-35, 37-40, 42-45, 47-50, 52-55, 57-60, 62-65, 67-70, 72-75, 77-80, 82-85, 87-90, and 92-95; or one or more of the amino acid sequences encoded by SEQ. ID. NOS. 1, 3, 5, 6, 8, 9, 11, 13, 21, 26, 31, 36, 41, 46, 51, 56, 61, 66, 71, 76, 81, 86, and 91. Accordingly, an antibody of the invention can have an amino acid sequence that shares at least 85%, 86%, 87%, 88%, 89% 90%

91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the amino acid sequence of one or more of the amino acid sequences of SEQ. ID. NOS. 2, 4, 7, 10, 12, 14-20, 22-25, 27-30, 32-35, 37-40, 42-45, 47-50, 52-55, 57-60, 62-65, 67-70, 72-75, 77-80, 82-85, 87-90, and 92-95; or one or more of the amino acid sequences encoded by SEQ. ID. NOS. 1, 3, 5, 6, 8, 9, 11, 13, 21, 26, 31, 36, 41, 46, 51, 56, 61, 66, 71, 76, 81, 86, and 91. As used herein, the term "sequence identity" refers to the similarity between two, or more, amino acid sequences. Sequence identity is typically measured in terms of percentage identity (or similarity or homology) between amino acid sequences; the higher the percentage, the more similar to each other are the compared sequences.

[0068] The amino acid sequence of the V_H framework region of some antibodies of the invention may contain a sequence, or fragment thereof, of SEQ ID NOS: 22, 27, 32, 37, 42, 47, 52, 87, 2, or 7.. Similarly, the amino acid sequences of the V_L framework region of the same, or different, antibodies may contain a sequence, or antigen-binding fragment thereof, of SEQ ID NOS: 57, 62, 67, 72, 77, 82, 92, 4, or 10.

[0069] An antibody of the invention contains at least 1, at least 2, at least 3, at least 4, at least five, or at least 6 CDRs. The amino acid sequences of CDRs in antibodies of the invention can be determined by known methods, and definitions of CDRs, in the art, including, for example: the ImMunoGeneTics database ("IMGT") numbering system, (Lefranc, M.-P. et al., *Nucleic Acids Research*, 27, 209-212 (1999)); the CDR definition(s) described by North, B. et al. (A new clustering of antibody CDR loop conformations, *J Mol Biol* (2011)); CDR definition(s) Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991); CDR definition(s) Chothia et al., *J. Mol. Biol.* 196:901-917 (1987); and CDR definition(s) MacCallum et al., *J. Mol. Biol.* 262:732-745 (1996).

[0070] One or more of the CDRs of some antibodies, or antigen-binding fragments, of the invention contain at least 3, at least 4, at least 5, at least 6, at least 7, or at least 8, at least 9, at least 10, at least 11, or at least 12 contiguous amino acids of: the V_H CDR1 are selected from the amino acid sequences of SEQ ID NOS: 23, 28, 33, 38, 43, 48, 53, 88, or 15; the V_H CDR2 are selected from the amino acid sequences of SEQ ID NOS: 24, 29, 34, 39, 44, 49, 54, 89, or 16; the V_H CDR3 are selected from the amino acid sequences of SEQ ID NOS: 25, 30, 35, 40, 45, 50, 55, 90, or 17; the V_L CDR1 are selected from the amino acid sequences of SEQ ID NOS: 58, 63, 68, 73, 78, 83, 93, or 18; the V_L CDR2 are selected from the amino acid sequences of SEQ ID NOS: 59, 64, 69, 74, 79, 84, 94, or 19; and the V_L CDR3 are selected from the amino acid sequences of SEQ ID NOS: 60, 65, 70, 75, 80, 85, 95, or 20.

[0071] In some embodiments, an antibody of the invention contains at least one, at least two, at least three, at least four, at least five, or at least six of: a VH CDR1 of SEQ ID NO:23, a VH CDR2 of SEQ ID NO: 24, a VH CDR3 of SEQ ID NO: 25, a VL CDR1 of SEQ ID NO: 58, a VL CDR2 of SEQ ID NO:59, and a VL CDR3 of SEQ ID NO: 60.

[0072] In other embodiments, an antibody of the invention contains at least one, at least two, at least three, at least four, at least five, or at least six of: a VH CDR1 of SEQ ID NO:28, a VH CDR2 of SEQ ID NO: 29, a VH CDR3 of SEQ ID NO: 30, a VL CDR1 of SEQ ID NO: 58, a VL CDR2 of SEQ ID NO:59, and a VL CDR3 of SEQ ID NO: 60.

[0073] In other embodiments, an antibody of the invention contains at least four, at least five, or at least six of: a VH CDR1 of SEQ ID NO: 33, a VH CDR2 of SEQ ID NO: 34, a VH CDR3 of SEQ ID NO: 35, a VL CDR1 of SEQ ID NO: 63, a VL CDR2 of SEQ ID NO: 64, and a VL CDR3 of SEQ ID NO: 65.

[0074] In other embodiments, an antibody of the invention contains at least four, at least five, or at least six of: a VH CDR1 of SEQ ID NO: 38, a VH CDR2 of SEQ ID NO: 39, a VH CDR3 of SEQ ID NO: 40, a VL CDR1 of SEQ ID NO: 68, a VL CDR2 of SEQ ID NO: 69, and a VL CDR3 of SEQ ID NO: 70.

[0075] In other embodiments, an antibody of the invention contains at least four, at least five, or at least six of: a VH CDR1 of SEQ ID NO: 43, a VH CDR2 of SEQ ID NO: 44, a VH CDR3 of SEQ ID NO: 45, a VL CDR1 of SEQ ID NO: 73, a VL CDR2 of SEQ ID NO: 74, and a VL CDR3 of SEQ ID NO: 75.

[0076] In other embodiments, an antibody of the invention contains at least four, at least five, or at least six of: a VH CDR1 of SEQ ID NO: 48, a VH CDR2 of SEQ ID NO: 49, a VH CDR3 of SEQ ID NO: 50, a VL CDR1 of SEQ ID NO: 78, a VL CDR2 of SEQ ID NO: 79, and a VL CDR3 of SEQ ID NO: 80.

[0077] In other embodiments, an antibody of the invention contains at least four, at least five, or at least six of: a VH CDR1 of SEQ ID NO: 53, a VH CDR2 of SEQ ID NO: 54, a VH CDR3 of SEQ ID NO: 55, a VL CDR1 of SEQ ID NO: 83, a VL CDR2 of SEQ ID NO: 84, and a VL CDR3 of SEQ ID NO: 85.

[0078] In other embodiments, an antibody of the invention contains at least four, at least five, or at least six of: a VH CDR1 of SEQ ID NO: 15, a VH CDR2 of SEQ ID NO: 16, a VH CDR3 of SEQ ID NO: 17, a VL CDR1 of SEQ ID NO: 18, a VL CDR2 of SEQ ID NO: 19, and a VL CDR3 of SEQ ID NO: 20

[0079] In other embodiments, an antibody of the invention contains at least four, at least five, or at least six of: a VH CDR1 of SEQ ID NO: 88, a VH CDR2 of SEQ ID NO: 89, a VH CDR3 of SEQ ID NO: 90, a VL CDR1 of SEQ ID NO: 93, a VL CDR2 of SEQ ID NO: 94, and a VL CDR3 of SEQ ID NO: 95.

[0080] Antibodies according to the invention are monoclonal antibodies, meaning an antibody is produced by a single clonal B-lymphocyte population, a clonal hybridoma cell population, or a clonal population of cells into which the genes of a single antibody, or portions thereof, have been transfected. Monoclonal antibodies are produced by methods known to those of skill in the art, for

instance by making hybrid antibody-forming cells from a fusion of myeloma cells with immune lymphocyte cells.

[0081] Monoclonal antibodies according to the invention are also typically humanized monoclonal antibodies. More specifically, a "human" antibody, also called a "fully human" antibody, according to the invention, is an antibody that includes human framework regions and CDRs from a human immunoglobulin. For example, the framework and the CDRs of an antibody are from the same originating human heavy chain, or human light chain amino acid sequence, or both. Alternatively, the framework regions may originate from one human antibody, and be engineered to include CDRs from a different human antibody. "Humanizing substitutions" are amino acid substitutions in which the amino acid residue present at a particular position in the VH or VL domain of an antibody, such as an IL-38 antibody, is replaced with an amino acid residue which occurs at an equivalent position in a reference human VH or VL domain. The reference human VH or VL domain may be a VH or VL domain encoded by the human germline. Humanizing substitutions may be made in the framework regions and/or the CDRs of the antibodies, defined herein. A "humanized variant" is a variant antibody of the invention, which contains one or more "humanizing substitutions" relative to a reference antibody, wherein a portion of the reference antibody (e.g. the VH domain and/or the VL domain or parts thereof containing at least one CDR) has an amino acid derived from a non-human species, and the "humanizing substitutions" occur within the amino acid sequence derived from a non-human species

[0082] An antibody according to the invention may also be an "antigen-binding fragment". An antigen-binding fragment refers to a polypeptide fragment of an immunoglobulin or antibody that binds antigen or competes with intact antibody (i.e., with the intact antibody from which they were derived) for antigen binding (i.e., specific binding to IL-38). As used herein, the term "fragment" of an antibody molecule includes antigen-binding fragments of antibodies, for example, an antibody light chain variable domain (VL), an antibody heavy chain variable domain (VH), a single chain antibody (scFv), a F(ab')₂ fragment, a Fab fragment, an Fd fragment, an Fv fragment, and a single domain antibody fragment (Dab). Fragments can be obtained, e.g., via chemical or enzymatic treatment of an intact or complete antibody or antibody chain or by recombinant means. Examples of immunoglobulin variants that are considered antibodies according to the invention include single-domain antibodies (such as VH domain antibodies), Fab fragments, Fab' fragments, F(ab')₂ fragments, single chain Fv proteins ("scFv"), and disulfide stabilized Fv proteins ("dsFv"). A VH single-domain antibody is an immunoglobulin fragment consisting of a heavy chain variable domain. An Fab fragment contains a monovalent antigen-binding immunoglobulin fragment, which can be

produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain. Similarly, an Fab' fragment also contains a monovalent antigen-binding immunoglobulin fragment, which can be produced by digestion of whole antibody with the enzyme pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain. Two Fab' fragments are obtained per immunoglobulin molecule. An (Fab')₂ fragment is a dimer of two Fab' fragments, that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction, so Fab' monomers remain held together by two disulfide bonds. An Fv fragment is a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain expressed as two chains. A single chain ("sc") antibody, such as scFv fragment, is a genetically engineered molecule containing the V_L region of a light chain, the V_H region of a heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule. A dimer of a single chain antibody, such as a scFV₂ antibody, is a dimer of a scFV, and may also be known as a "miniantibody". A dsFvs variant also contains a V_L region of an immunoglobulin and a V_H region, but the chains have been mutated to introduce a disulfide bond to stabilize the association of the chains.

[0083] One of skill in the art will realize that conservative variants of the antibodies can be produced. Such conservative variants employed in antibody fragments, such as dsFv fragments or in scFv fragments, will retain critical amino acid residues necessary for correct folding and stabilizing between the V_H and the V_L regions, and will retain the charge characteristics of the residues in order to preserve the low pI and low toxicity of the molecules.

[0084] An antibody according to the invention may also comprise a "tagged" immunoglobulin CH3 domain to facilitate detection of the biologic against a background of endogenous antibodies. More particularly, a tagged CH3 domain is a heterogeneous antibody epitope that has been incorporated into one or more of the AB, EF, or CD structural loops of a human IgG-derived CH3 domain. CH3 tags are preferably incorporated into the structural context of an IgG1 subclass antibody, other human IgG subclasses, including IgG2, IgG3, and IgG4, are also available according to the invention. Epitope-tagged CH3 domains, also referred to as "CH3 scaffolds" can be incorporated into any antibody of the invention having a heavy chain constant region, generally in the form of an immunoglobulin Fc portion. Examples of CH3 scaffold tags, and methods for incorporating them into antibodies are disclosed in International Patent Application No. PCT/US2019/032780. Antibodies used to detect epitope tagged CH3 scaffolds, and antibodies of the invention, that comprise epitope tagged CH3 scaffolds, are generally referred to herein as "detector antibodies".

[0085] Therapeutic and diagnostic effectiveness of an antibody according to the invention correlates with its binding affinity for its target antigen. Binding affinity may be calculated by a modification of the Scatchard method described by Frankel et al., *Mol. Immunol.*, 16:101-106, 1979. Alternatively, binding affinity may be measured by the dissociation rate of an antibody from its antigen. Various methods can be used to measure binding affinity, including, for example, surface plasmon resonance (SPR), competition radioimmunoassay, ELISA, and flow cytometry. An antibody that "specifically binds" an antigen is an antibody that binds the antigen with high affinity and does not significantly bind other unrelated antigens.

[0086] High affinity binding of an antibody to its antigen is mediated by the binding interaction of one or more of the antibody's CDRs to an epitope, also known as an antigenic determinant, of the antigen target. Epitopes are particular chemical groups or peptide sequences on a molecule that are antigenic, meaning they are capable of eliciting a specific immune response. An epitope that is specifically bound by an antibody according to the invention may be formed by a linear sequence of amino acids contained within IL-38. Such an epitope is called a "linear epitope", and it may remain functional with respect to the specific binding of an antibody according to the invention to a denatured form of IL-38. Alternatively, the specific binding of an antibody according to the invention may depend on a particular three-dimensional structure of the IL-38 target, such that the contributing residues of an epitope are not necessarily in a linear sequence. In other words, an epitope of an antibody according to the invention may be a "conformational epitope".

[0087] IL-38 may be present on the surface of various carcinoma cells, including carcinoma cells of epithelial origin or secreted into the extracellular environment by carcinoma cells, normal epithelial cells, or by cells of the immune system. Therefore, antibodies described herein can be included in compositions, which are useful for methods of diagnosing or treating various diseases where IL-38 acts to modulate disease progression. Those include, but are not limited to, cancer, including but not limited to cancers such as esophagus, bladder, cervix, prostate, breast, renal, colorectal, pancreatic, melanoma, uterine, head & neck and lung (either squamous or adenocarcinoma).

[0088] Therapeutic and diagnostic uses of antibodies according to the invention may include uses of immunoconjugates. As described herein, an immunoconjugate is a chimeric molecule, which comprises an effector molecule linked to an antibody according to the invention. As referred to herein, an effector molecule is the portion of an immunoconjugate that is intended to have a desired effect on a cell to which the immunoconjugate is targeted, or an effector molecule may serve to increase the half-life or bioavailability of an antibody according to the invention. General examples of effector molecules include therapeutic agents, (such as toxins and chemotherapeutic drugs),

diagnostic agents, (such as detectable markers), and half-life and bioavailability-enhancing molecules, (such as lipids or polyethylene glycol).

[0089] Effector molecules can be conjugated to antibodies according to the invention using any number of means known to those of skill in the art, including covalent and noncovalent attachment means. The procedure for attaching an effector molecule to an antibody may vary according to the chemical structure of the effector. Polypeptides typically contain a variety of functional groups, such as a carboxylic acid (COOH) group, a free amine ($-NH_2$), and a sulfhydryl (SH) group, which are available for reaction with a suitable functional group on an antibody to result in the binding of the effector molecule. Alternatively, an antibody according to the invention can be derivatized to expose, or attach, additional reactive functional groups. Derivatization may involve attachment of any of a number of known linker molecules, which serve to join an antibody to an effector molecule.

[0090] A linker molecule is capable of forming covalent bonds to the antibody and effector molecule. Suitable linkers include, but are not limited to, straight or branched-chain carbon linkers, heterocyclic carbon linkers, or peptide linkers. If an effector molecule is a polypeptide, a linker may be joined to the constituent amino acids of the polypeptide through their side groups, such as through a disulfide linkage to cysteine, or to the alpha carbon amino and carboxyl groups of the terminal amino acids. Recombinant technology may be used to make two or more polypeptides, including linker peptides, into one contiguous polypeptide molecule.

[0091] An effector molecule may also be contained within a directly attached, or linked encapsulation system, that shields the effector molecule from direct exposure to the circulatory system. Means of preparing liposomes attached to antibodies are well known to those of skill in the art (see, for example, U.S. Pat. No. 4,957,735; and Connor et al., Pharm Ther 28:341-365, 1985).

[0092] The effector molecules of immunoconjugates according to the invention are generally useful for the treatment of cancer, and diseases characterized by abnormal cell growth, generally. Accordingly, the effector molecules of immunoconjugates according to the invention can be chemotherapeutic agents, including: small molecule drugs; nucleic acids, such as antisense nucleic acids, derivatized oligonucleotides for covalent cross-linking with single or duplex DNA, and triplex forming oligonucleotides; proteins; peptides; amino acids, and amino acid derivatives; glycoproteins; radioisotopes; lipids; carbohydrates; recombinant viruses; and toxins, such as, but not limited to, abrin, ricin, Pseudomonas exotoxin ("PE", such as PE35, PE37, PE38, and PE40), diphtheria toxin ("DT"), botulinum toxin, saporin, restrictocin, gelonin, bouganin, and modified toxins thereof.

[0093] In some circumstances, it is desirable to free the effector molecule from the antibody when the immunoconjugate has reached its target site. Therefore, in these circumstances,

immunoconjugates will comprise linkages that are cleavable in the vicinity of the target site. Cleavage of a linker to release the effector molecule from an antibody according to the invention may be prompted by enzymatic activity or conditions to which the immunoconjugate is subjected either inside the target cell or in the vicinity of the target site. Alternatively, after specifically binding its target antigen, an antibody according to the invention can be internalized by the cell expressing the target antigen.

[0094] Therapeutic antibodies according to the invention, including therapeutic immunoconjugates, can be used in methods for preventing, treating, or ameliorating a disease in a subject. In certain embodiments of the invention, antibodies according to the invention can be used for preventing, treating, or ameliorating cancer in a subject. For example, antibodies according to the invention can be used to prevent, treat or ameliorate, cancer, including but not limited to, cancers of the esophagus, bladder, cervix, prostate, breast, kidney, colon, pancreatic, melanoma, uterine, head & neck and lung (either squamous or adenocarcinoma).

[0095] "Preventing" a disease refers to inhibiting the full development of a disease. "Treating" refers to a therapeutic intervention that ameliorates a sign or symptom of a disease or pathological condition after it has begun to develop. "Ameliorating" refers to the reduction in the number or severity of signs or symptoms of a disease. With respect to the use of antibodies according to the invention to prevent, treat or ameliorate cancer, signs or symptoms of the disease may correlate to tumor burden or the number or size of metastases.

[0096] A method for preventing, treating, or ameliorating cancer may require the administration of a composition, comprising an effective amount of an antibody according to the invention, to a subject to inhibit tumor growth or metastasis, comprising selecting a subject with a cancer characterized by tumor cells that expresses the antigen target of the antibody, or otherwise present cell membrane-associated target antigens of an antibody according to the invention. For example, an antibody according to the invention can contact a tumor cell *via* binding to its target antigen, to modulate, inhibit, or neutralize the target antigen's function. An antibody according to the invention may also deliver cytotoxic therapy upon binding to its target antigen on the surface of a tumor cell.

[0097] An antibody according to the invention can bind a target antigen in a fluid, such as, but not limited to, blood or a blood derivative, like plasma and serum, or a fluid within a tumor microenvironment. As is the case with a cell membrane-attached target antigen, binding of an antibody according to the invention to a secreted target antigen can result in the modulation, inhibition, or neutralization of the target antigen's biological function. Thus, the binding of an

antibody according to the invention to its target antigen in solution, can, for example, modulate, inhibit, or neutralize its target antigen's activity, or the membrane-bound vesicle's activity, *in vivo*.

[0098] There are also uses of antibodies according to the invention, in which, an antibody binds a target antigen that is associated with an extracellular matrix ("ECM") or ECM protein. For example, an antibody according to the invention may bind to a target antigen that is, itself associated with an ECM that migrating or differentiating endothelial cells are attached, or would be expected to encounter, to modulate, inhibit, or neutralize its target. The presence of ECM-associated target antigen may correlate with various disease states, including diseases associated with the presence of ECM-associated target antigen in the tumor microenvironment.

[0099] As stated above, antibodies disclosed herein can be administered to slow or inhibit the growth of primary tumors or inhibit the metastasis of various types of tumors. For example, antibodies according to the invention can be administered to slow or inhibit the growth or metastasis of cancers, including but not limited to, esophagus, bladder, cervix, prostate, breast, renal, colorectal, pancreatic, melanoma, uterine, head & neck and lung (either squamous or adenocarcinoma). In these applications, a therapeutically effective amount of an antibody is administered to a subject in an amount sufficient to inhibit growth, replication or metastasis of cancer cells, or to inhibit a sign or a symptom of the cancer. Suitable subjects may include those diagnosed with a cancer, in which the tumor cells express a target antigen of an antibody according to the invention. A therapeutically effective amount of an antibody according to the invention will depend upon the severity of the cancer, and the general state of the patient's health. A therapeutically effective amount of the antibody is that which provides either subjective relief of a symptom(s) or an objectively identifiable improvement as noted by a clinician or other qualified professional.

[0100] Antibodies according to the invention, that are administered to subjects in need thereof, are formulated into compositions. More particularly, the antibodies can be formulated for systemic administration, or local administration, such as intra-tumor administration. For example, an antibody according to the invention may be formulated for parenteral administration, such as intravenous administration. The compositions can be prepared in unit dosage forms for administration to a subject. The amount and timing of administration are at the discretion of the treating clinician to achieve the desired outcome.

[0101] Administration of antibodies according to the invention can also be accompanied by administration of other anti-cancer agents or therapeutic treatments, such as surgical resection of a tumor. Any suitable anti-cancer agent can be administered in combination with the antibodies

disclosed herein. Exemplary anti-cancer agents include, but are not limited to, chemotherapeutic agents, such as, for example, mitotic inhibitors, alkylating agents, anti-metabolites, intercalating antibiotics, growth factor inhibitors, cell cycle inhibitors, enzymes, topoisomerase inhibitors, anti-survival agents, biological response modifiers, immune modulators, anti-hormones (e.g. anti-androgens) and anti-angiogenesis agents. Other anti-cancer treatments include radiation therapy and other antibodies that specifically target cancer cells.

[0102] The compositions for administration can include a solution of the antibody dissolved in a pharmaceutically acceptable carrier, such as an aqueous carrier. In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, or glycerol as a vehicle. For solid compositions, such as powder, pill, tablet, or capsule forms, conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate. The foregoing carrier solutions are sterile and generally free of undesirable matter, and may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, and toxicity adjusting agents such as sodium acetate, sodium chloride, potassium chloride, calcium chloride, and sodium lactate. The concentration of antibody in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the subject's needs.

[0103] Options for administering an antibody according to the invention include, but are not limited to, administration by slow infusion, or administration via an intravenous push or bolus. Other options for antibody administration may be optimized for intraocular administration. Prior to being administered, an antibody composition according to the invention may be provided in lyophilized form, and rehydrated in a sterile solution to a desired concentration before administration. The antibody solution may then be added to an infusion bag containing 0.9% sodium chloride, USP, and in some cases administered at a dosage of from 0.5 to 15 mg/kg of body weight. In one example of administration of an antibody composition according to the invention, a

higher loading dose is administered, with subsequent, maintenance doses being administered at a lower level. For example, an initial loading dose of 4 mg/kg may be infused over a period of some 90 minutes, followed by weekly maintenance doses for 4-8 weeks of 2 mg/kg infused over a 30 minute period if the previous dose was well tolerated.

[0104] Antibody compositions according to the invention may also be controlled release formulations. Controlled release parenteral formulations, for example, can be made as implants, or oily injections. Particulate systems, including microspheres, microparticles, microcapsules, nanocapsules, nanospheres, and nanoparticles, may also be used to deliver antibody compositions according to the invention. Microcapsules, as referred to herein, contain an antibody according to the invention as a central core component. In microspheres, an antibody according to the invention is dispersed throughout the particle. Particles, microspheres, and microcapsules smaller than about 1 μm are generally referred to as nanoparticles, nanospheres, and nanocapsules, respectively.

[0105] As described above, antibodies according to the invention may also be useful for diagnosing or monitoring the presence of a pathologic condition, such as, but not limited to cancer of the esophagus, bladder, cervix, prostate, breast, renal, colorectal, pancreatic, melanoma, uterine, head & neck and lung (either squamous or adenocarcinoma). More particularly, methods of the invention are useful for detecting expression of the antigen target of an antibody according to the invention. Detection may be in vitro or in vivo. Any tissue sample may be used for in vitro diagnostic detection, including, but not limited to, tissue from biopsies, autopsies and pathology specimens. Biological samples include sections of tissues, for example, frozen sections taken for histological purposes. Biological samples further include body fluids, such as blood, serum, plasma, sputum, spinal fluid or urine.

[0106] A method determines if a subject has a disease by contacting a sample from the subject with an antibody according to the invention; and detecting binding of the antibody to its target antigen present in the sample. An increase in binding of the antibody to its target antigen in the sample, as compared to binding of the antibody in a control sample identifies the subject as having a disease associated with IL-38 expression, such as, cancer or any other type of disease that expresses IL-38. In general, a control sample is a sample from a subject without disease.

[0107] Diagnostic methods differ in their sensitivity and specificity. The "sensitivity" of a diagnostic assay is the percentage of diseased individuals who test positive (percent of true positives). The "specificity" of a diagnostic assay is one minus the false positive rate, where the false

positive rate is defined as the proportion of those without the disease who test positive. While a particular diagnostic method may not provide a definitive diagnosis of a condition, it suffices if the method provides a positive indication that aids in diagnosis. "Prognostic" is the probability of development (e.g., severity) of a pathologic condition, such as pancreatic cancer or metastasis.

[0108] Antibodies of the invention can be linked to a detectable label to form immunoconjugates that are useful as diagnostic agents. A detectable label, as referred to herein, is a compound or composition that is conjugated directly or indirectly to an antibody according to the invention, for the purpose of facilitating detection of a molecule that correlates to presence of a disease, such as, for example, a tumor cell antigen that is the antigen target of an antibody according to the invention. Detectable labels useful for such purposes are well known in the art, and include: radioactive isotopes, such as ^{35}S , ^{11}C , ^{13}N , ^{15}O , ^{18}F , ^{19}F , technetium-99m ($^{99\text{m}}\text{Tc}$), ^{124}I , ^{131}I , ^{89}Zr , ^3H , ^{14}C , ^{15}N , ^{90}Y , ^{111}In and ^{125}I ; fluorophores; chemiluminescent agents; enzymatic labels, such as horseradish peroxidase, beta-galactosidase, luciferase, alkaline phosphatase; biotinyl groups; predetermined polypeptide epitopes recognized by a secondary reporter, such as a leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags; and magnetic agents, such as gadolinium chelates. A labeled antibody according to the invention may also be referred to as a "labeled antibody", or more specifically a "radiolabeled antibody". For some antibodies according to the invention, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

[0109] A diagnostic method comprising a step of using an antibody according to the invention may, in certain applications, be an immunoassay. While the details of immunoassays may vary with the particular format employed, a method of detecting the antigen target of an antibody according to the invention in a biological sample generally includes the steps of contacting the biological sample with the antibody which specifically reacts with the antigen, under immunologically reactive conditions to form an immune complex. The presence of the resulting immune complex can be detected directly or indirectly. In other words, an antibody according to the invention can function as a primary antibody (1° Ab) in a diagnostic method, and a labeled antibody, specific for the antibody according to the invention, functions as the 2° Ab . In the case of indirect detection of an immune complex, the use of an antibody according to the invention for a diagnostic method will also include the use of a labelled secondary antibody (2° Ab) to detect binding of the primary antibody – the antibody according to the invention – to its target antigen. Suitable detectable labels for a secondary antibody include the labels, described above, for directly labeled antibodies according to

the invention. A 2° Ab, used in a diagnostic method according to the invention, may also be a "detector antibody", as defined, above, for use in conjunction with an antibody according to the invention that contains a CH3 epitope tag, as described in International Patent Application No. PCT/US2019/032780.

[0110] Antibodies according to the invention can also be used for fluorescence activated cell sorting (FACS). A FACS analysis of a cell population employs a plurality of color channels, low angle and obtuse light-scattering detection channels, and impedance channels, among other more sophisticated levels of detection, to separate or sort cells (see U.S. Pat. No. 5,061,620).

[0111] Reagents used in a diagnostic application of an antibody according to the invention, as described above, may be provided in a kit for detecting the antigen target of an antibody according to the invention a biological sample, such as a blood sample or tissue sample. Such a kit can be used to confirm a cancer diagnosis in a subject. For example, a diagnostic kit comprising an antibody according to the invention can be used to perform a histological examination for tumor cells in a tissue sample obtained from a biopsy. In a more particular example, a kit may include antibodies according to the invention that can be used to detect lung cancer cells, either adenocarcinoma or squamous, in tissue or cells obtained by performing a lung biopsy. In an alternative, particular example, a kit may include antibodies according to the invention that can be used to detect pancreatic cancer cells in a tissue biopsy. In an alternative, particular example, a kit may include antibodies according to the invention that can be used to detect esophagus cancer cells in a tissue biopsy. In an alternative, particular example, a kit may include antibodies according to the invention that can be used to detect cervix cancer cells in a tissue biopsy. In an alternative, particular example, a kit may include antibodies according to the invention that can be used to detect bladder cancer cells in a tissue biopsy. In an alternative, particular example, a kit may include antibodies according to the invention that can be used to detect melanoma cancer cells in a tissue biopsy. In an alternative, particular example, a kit may include antibodies according to the invention that can be used to detect prostate cancer cells in a tissue biopsy. In an alternative, particular example, a kit may include antibodies according to the invention that can be used to detect head & neck cancer cells in a tissue biopsy. Kits for detecting an antigen target of an antibody according to the invention will typically comprise an antibody according to the invention in the form of a monoclonal antibody, or an antigen-binding fragment thereof, such as an scFv fragment, a VH domain, or a Fab. The antibody may be unlabeled or labeled by a detectable marker, such as a fluorescent, radioactive, or an enzymatic label, as described above. A kit also generally includes instructional materials disclosing

means of use of an antibody according to the invention. The instructional materials may be written, in an electronic form, such as a portable hard drive, and the materials also be visual, such as video files. Instructional materials may also refer to a website or link to an application software program, such as a mobile device or computer "app", that provides instructions. A kit may also include additional components to facilitate the particular application for which the kit is designed. For example, a kit may also contain a means of detecting a label (such as enzyme substrates for enzymatic labels, filter sets to detect fluorescent labels, appropriate secondary labels such as a secondary antibody, or the like). Buffers and other reagents, which are routinely in methods of using an antibody according to the invention for diagnostic purposes.

[0112] Antibodies according to the invention can be produced by various recombinant expression systems. In other words, the antibodies can be produced by the expression of nucleic acid sequences encoding their amino acid sequences in living cells in culture. An "isolated" antibody according to the invention is one which has been substantially separated or purified away from other biological components environment, such as a cell, proteins and organelles. For example, an antibody may be isolated if it is purified to: i) greater than 95%, 96%, 97%, 98%, or 99% by weight of protein as determined by the Lowry method, and alternatively, more than 99% by weight; ii) a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator; iii) homogeneity by SDS-PAGE, under reducing or nonreducing conditions, using Coomassie blue or silver stain. Isolated antibody may also be an antibody according to the invention that is in situ within recombinant cells, since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

[0113] A variety of host-expression vector systems may be utilized to express an antibody according to the invention, by transforming or transfecting the cells with an appropriate nucleotide coding sequences for an antibody according to the invention. Examples of host-expression cells include, but are not limited to: Bacteria, such as *E.coli* and *B. Subtilis*, which may be transfected with antibody coding sequences contained within recombinant bacteriophage DNA, plasmid DNA, or cosmid DNA expression vectors; Yeast, such as *Saccharomyces* and *Pichia*, transformed with recombinant yeast expression vectors containing antibody coding sequences; Insect cell systems, infected with recombinant virus expression vectors, such as baculovirus, containing antibody coding sequences; Plant cell systems infected with recombinant virus expression vectors, such as cauliflower mosaic virus ("CaMV"), or tobacco mosaic virus ("TMV"), containing antibody coding

sequences; and Mammalian cell systems, such as, but not limited to COS, Chinese hamster ovary ("CHO") cells, ExpiCHO, baby hamster kidney ("BHK") cells, HEK293, Expi293, 3T3, NSO cells, harboring recombinant expression constructs containing promoters derived from the genome of mammalian cell, such as the metallothionein promoter or elongation factor I alpha promoter, or from mammalian viruses, such as the adenovirus late promoter, and the vaccinia virus 7.5K promoter. For example, mammalian cells such as Human Embryonic Kidney 293 (HEK293) or a derivative thereof, such as Expi293, in conjunction with a dual promoter vector that incorporates mouse and rat elongation factor 1 alpha promoters to express the heavy and light chain fragments, respectively, is an effective expression system for antibodies according to the invention, which can be advantageously selected, depending upon the use intended for the antibody molecule being expressed. Alternatively, a two-vector system that expresses the heavy and light chain fragments from separate plasmids under control of the cytomegalovirus (CMV) enhancer and promoter sequences, is an effective expression system for the antibodies, in conjunction with CHO cells, HEK cells, or their derivatives

[0114] When a large quantity of an antibody according to the invention is to be produced for the generation of a pharmaceutical composition of the antibody, vectors which direct the expression of high levels of readily purified fusion protein products may be desirable. Such vectors include, but are not limited to: a pUR278 vector (Ruther *et al.* *EMBO J.* 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with a lac Z coding region so that a fusion protein is produced; a pIN vector (Inouye & Inouye, *Nucleic Acids Res.* 13:3101-3109 (1985))

[0115] A host expression cell system may also be chosen which modulates the expression of inserted sequence(s) coding for an antibody according to the invention, or modifies and processes the gene product as desired. For example, modifications, including the glycosylation and processing, such as cleavage of protein products, may be important for the function of the protein. Indeed, different host cells have characteristic and specific mechanisms for the posttranslational processing and modification of proteins and gene products. To this end, eukaryotic host cells, which possess appropriate cellular machinery for proper processing of a primary transcript, as well as the glycosylation and phosphorylation of a gene product according to the invention may be used.

[0116] The vector used to produce an antibody according to the invention comprises a nucleic acid molecule encoding at least a portion of that particular antibody. For example, such a nucleic

acid sequence can comprise a DNA sequence corresponding to any polynucleotide sequences comprising VH and VL domains therein, including codon optimized sequences, or a portion thereof. Thus, a first nucleic acid encoding at least a portion of an antibody according to the invention, that is operably linked with a second nucleic acid sequence that is placed in a functional relationship with the first nucleic acid sequence, such as a promoter, is a nucleic acid according to the invention. An operable linkage exists if a linked promoter sequence affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, may also join two, or more, protein-coding regions, in the same reading frame.

[0117] When a nucleic acid comprising a DNA sequence according to the invention is substantially separated or purified away from other biological components in the environment, such as a cell, other chromosomal and extra-chromosomal DNA and RNA, proteins and organelles, it may be considered to be an "isolated nucleic acid" according to the invention. For example, a nucleic acid, which has been purified by standard purification methods, is an isolated nucleic acid.

[0118] Nucleic acids according to the invention also include degenerate variants of nucleotides encoding an antibody according to the invention. More particularly, a "degenerate variant" refers to a polynucleotide, which encodes an antibody according to the invention, but is degenerate as a result of the genetic code. All degenerate nucleotide sequences are included, according to the invention, as long as the amino acid sequence of the encoded antibody specifically binds the antigen target of an antibody according to the invention.

EMBODIMENTS

1. An isolated Interleukin-38 (IL-38)-binding antibody, or antigen-binding fragment thereof, comprising at least 3, at least 4, at least 5, at least 6, at least 7, or at least 8 optionally contiguous amino acids of at least one complementary-determining region (CDR) contained within the variable heavy chain (VH) amino acid sequences of: SEQ ID NO: 22, SEQ ID NO: 27, SEQ ID NO: 32, SEQ ID NO: 37, SEQ ID NO: 42, SEQ ID NO: 47, SEQ ID NO: 52, SEQ ID NO: 2, SEQ ID NO: 87 or SEQ ID NO: 7; and/or contained within the variable light chain (VL) amino acid sequences of: SEQ ID NO: 57, SEQ ID NO: 62, SEQ ID NO: 67, SEQ ID NO: 72, SEQ ID NO: 77, SEQ ID NO: 82, SEQ ID NO: 92, SEQ ID NO: 4, or SEQ ID NO: 9.
2. The isolated antibody, or antigen-binding fragment thereof of embodiment 1, wherein the CDRs are defined by the *North* method or by the *Kabat* method.

3. An antibody, or antigen-binding fragment thereof, comprising:
at least 3, at least 4, at least 5, at least 6, at least 7, or at least 8 amino acids of:
the VH CDR1 are selected from the amino acid sequences of SEQ ID NOS: 23, 28, 33, 38, 43, 48, 53, 88, or 15;
the VH CDR2 are selected from the amino acid sequences of SEQ ID NOS: 24, 29, 34, 39, 44, 49, 54, 89 or 16;
the VH CDR3 are selected from the amino acid sequences of SEQ ID NOS: 25, 30, 35, 40, 45, 50, 55, 90, or 17;
the VL CDR1 are selected from the amino acid sequences of SEQ ID NOS: 58, 63, 68, 73, 78, 83, 93, or 18;
the VL CDR2 are selected from the amino acid sequences of SEQ ID NOS: 59, 64, 69, 74, 79, 84, 94, or 19; and
the VL CDR3 are selected from the amino acid sequences of SEQ ID NOS: 60, 65, 70, 75, 80, 85, 95, or 20.
4. The antibody, or antigen-binding fragment thereof, of embodiment 3, comprising at least one CDR selected from: a VH CDR1 of SEQ ID NO: 33, a VH CDR2 of SEQ ID NO: 34, a VH CDR3 of SEQ ID NO: 35, a VL CDR1 of SEQ ID NO: 63, a VL CDR2 of SEQ ID NO: 64, and a VL CDR3 of SEQ ID NO: 65.
5. The antibody, or antigen-binding fragment thereof, of embodiment 3, comprising at least one CDR selected from: a VH CDR1 of SEQ ID NO: 38, a VH CDR2 of SEQ ID NO: 39, a VH CDR3 of SEQ ID NO: 40, a VL CDR1 of SEQ ID NO: 68, a VL CDR2 of SEQ ID NO: 69, and a VL CDR3 of SEQ ID NO: 70.
[00100]
6. The antibody, or antigen-binding fragment thereof, of embodiment 3, comprising at least one CDR selected from: a VH CDR1 of SEQ ID NO: 43, a VH CDR2 of SEQ ID NO: 44, a VH CDR3 of SEQ ID NO: 45, a VL CDR1 of SEQ ID NO: 73, a VL CDR2 of SEQ ID NO: 74, and a VL CDR3 of SEQ ID NO: 75.
7. The antibody, or antigen-binding fragment thereof, of embodiment 3, comprising at least one CDR selected from: a VH CDR1 of SEQ ID NO: 48, a VH CDR2 of SEQ ID NO: 49, a VH CDR3 of SEQ ID NO: 50, a VL CDR1 of SEQ ID NO: 78, a VL CDR2 of SEQ ID NO: 79, and a VL CDR3 of SEQ ID NO: 80.

8. The antibody, or antigen-binding fragment thereof, of embodiment 3, comprising at least one CDR selected from: a VH CDR1 of SEQ ID NO: 53, a VH CDR2 of SEQ ID NO: 54, a VH CDR3 of SEQ ID NO: 55, a VL CDR1 of SEQ ID NO: 83, a VL CDR2 of SEQ ID NO: 84, and a VL CDR3 of SEQ ID NO: 85.
9. The antibody, or antigen-binding fragment thereof, of embodiment 3, comprising at least one CDR selected from: SEQ ID NO: 15, a VH CDR2 of SEQ ID NO: 16, a VH CDR3 of SEQ ID NO: 17, a VL CDR1 of SEQ ID NO: 18, a VL CDR2 of SEQ ID NO: 19, and a VL CDR3 of SEQ ID NO: 20
10. The antibody, or antigen-binding fragment thereof, of embodiment 3, comprising at least one CDR selected from: SEQ ID NO: 88, a VH CDR2 of SEQ ID NO: 89, a VH CDR3 of SEQ ID NO: 90, a VL CDR1 of SEQ ID NO: 93, a VL CDR2 of SEQ ID NO: 94, and a VL CDR3 of SEQ ID NO: 95
11. The antibody, or antigen-binding fragment of any one of embodiments 1-10, wherein the IL-38 is a component of a multi-protein complex.
12. The antibody, or antigen-binding fragment of any one of embodiments 1-11, wherein the antibody, or antigen-binding fragment partially or fully blocks, inhibits, or neutralizes a biological activity of IL-38.
13. The antibody or antigen-binding fragment of any one of embodiments 1-12, wherein the IL-38 is present in a body fluid.
14. The antibody or antigen-binding fragment of embodiment 13, wherein the body fluid is blood or a blood derivative.
15. The antibody or antigen-binding fragment of embodiment 14, wherein the blood derivative is plasma or serum.
16. The antibody or antigen-binding fragment of any one of embodiments 1-15, wherein the IL-38 is associated with an extracellular matrix ("ECM"), or ECM protein.
17. The antibody or antigen-binding fragment of embodiment 16, wherein the IL-38 is present in a tumor micro-environment.

18. The antigen-binding fragment of any one of embodiments 1-17, wherein the antigen-binding fragment is an isolated variable heavy (VH) single domain monoclonal antibody.
19. The antigen-binding fragment of any one of embodiments 1-17, wherein the antigen-binding fragment is a single chain (sc)Fv-Fc fragment.
20. The antigen-binding fragment of any one of embodiments 1-17, wherein the isolated antigen-binding fragment comprises an Fv, scFv, Fab, F(ab')₂, or Fab' fragment, diabody, or any fragment whose half-life may have been increased.
21. The antibody or antigen-binding fragment of any one of the embodiments 1-20, wherein the antibody or antigen-binding fragment comprises a CH3 scaffold, comprising at least one modification of the wild-type amino acid sequence of the CH3 domain derived from an immunoglobulin Fc region.
22. The antibody or antigen-binding fragment of any one of embodiments 1-21, wherein the antibody or antigen-binding fragment is monoclonal.
23. The antibody or antigen-binding fragment of any one of embodiments 1-22, wherein the antibody or antigen-binding fragment is human, humanized, or bi-specific.
24. A method of inhibiting tumor growth or metastasis in a subject, comprising administering to the subject a therapeutically effective amount of a composition comprising the antibody or an antigen-binding fragment of any of embodiments 1-23, wherein the antibody or an antigen-binding fragment partially or fully blocks, inhibits, or neutralizes a biological activity of IL-38.
- 1A. A method of treating cancer in an individual comprising administering to the individual an antibody that binds to IL-38, wherein the antibody comprises
a VH CDR1, a VH CDR2 and a VH CDR3 of a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:22 and a VL CDR1, VL CDR2 and a VL CDR3 of a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:57;

a VH CDR1, a VH CDR2 and a VH CDR3 of a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:98 and a VL CDR1, VL CDR2 and a VL CDR3 of a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:101;

a VH CDR1, a VH CDR2 and a VH CDR3 of a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:7 and a VL CDR1, VL CDR2 and a VL CDR3 of a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:10;

a VH CDR1, a VH CDR2 and a VH CDR3 of a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:87 and a VL CDR1, VL CDR2 and a VL CDR3 of a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:92;

a VH CDR1, a VH CDR2 and a VH CDR3 of a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:27 and a VL CDR1, VL CDR2 and a VL CDR3 of a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:57;

a VH CDR1, a VH CDR2 and a VH CDR3 of a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:37 and a VL CDR1, VL CDR2 and a VL CDR3 of a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:67;

a VH CDR1, a VH CDR2 and a VH CDR3 of a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:42 and a VL CDR1, VL CDR2 and a VL CDR3 of a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:72;

a VH CDR1, a VH CDR2 and a VH CDR3 of a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:47 and a VL CDR1, VL CDR2 and a VL CDR3 of a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:77;

a VH CDR1, a VH CDR2 and a VH CDR3 of a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:32 and a VL CDR1, VL CDR2 and a VL CDR3 of a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:62;

a VH CDR1, a VH CDR2 and a VH CDR3 of a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:52 and a VL CDR1, VL CDR2 and a VL CDR3 of a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:82;

a VH CDR1, a VH CDR2 and a VH CDR3 of a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:2 and a VL CDR1, VL CDR2 and a VL CDR3 of a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:4;

2A. The method of embodiment 1A, wherein the antibody comprises

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO:23, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO:24, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 25, a VL CDR1 comprising the amino acid sequence set forth in SEQ ID NO:58, a VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO:59, and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO:60;

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO:15, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO:16, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO:17, a VL CDR1 comprising the amino acid sequence set forth in SEQ ID NO:18, a VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO:19 and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO:20;

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO:88, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO:89, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 90, a VL CDR1 comprising the amino acid sequence set forth in SEQ ID NO:93, VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO:94, and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO:95

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 33, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 34, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 35, a VL CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 63, a VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 64, and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 65;

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 48, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 49, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 50, a VL CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 78, a VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 79, and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 80;

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 38, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 39, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 40, a VL CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 68, a VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 69, and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 70;

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 43, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 44, a VH CDR3 of SEQ ID NO: 45, a VL CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 73, a VL CDR2 comprising the

amino acid sequence set forth in SEQ ID NO: 74, and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 75; or

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 28, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 29, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 30, a VL CDR1 of SEQ ID NO: 58, a VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 59, and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 60;

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 53, a VH CDR2 of SEQ ID NO: 54, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 55, a VL CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 83, a VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 84, and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 85.

3A. The method of embodiment 1A or 2A, wherein the antibody that specifically binds to IL-38 comprises

a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:22 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:57;

a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:98 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:101;

a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:7 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:10; and

a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:87 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:92.

4A. The method of any one of embodiments 1A-3A, wherein the antibody that specifically binds to IL-38 comprises

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO:23, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO:24, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 25, a VL CDR1 comprising the amino acid sequence set forth in SEQ ID NO:58, a VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO:59, and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO:60;

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO:15, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO:16, a VH CDR3 comprising the amino

acid sequence set forth in SEQ ID NO:17, a VL CDR1 comprising the amino acid sequence set forth in SEQ ID NO:18, a VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO:19 and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO:20;

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO:88, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO:89, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 90, a VL CDR1 comprising the amino acid sequence set forth in SEQ ID NO:93, VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO:94, and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO:95

5A. The method of embodiment 4A, wherein the antibody that specifically binds to IL-38 comprises

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO:23, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO:24, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 25, a VL CDR1 comprising the amino acid sequence set forth in SEQ ID NO:58, a VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO:59, and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO:60.

6A. The method of embodiment 5A, wherein the antibody that specifically binds to IL-38 comprises

a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:98 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:101.

7A. The method of any one of embodiments 1A, 2A, 4A, or 5A, wherein the CDRs are defined by the North method or the Kabat method.

8A. The method of any one of embodiments 1A-7A, wherein the antibody that specifically binds to IL-38 is a humanized antibody.

9A. The method of any one of embodiment 1A-4A, wherein the antibody that specifically binds to IL-38 is a human antibody.

10A. The method of any one of embodiments 1A-9A, wherein the cancer expresses a high level of IL-38.

- 11A. The method of any one of embodiments 1A-10A, wherein the antibody that specifically binds to IL-38 partially or fully blocks, inhibits, or neutralizes a biological activity of IL-38.
- 12A. The method of any one of embodiments 1A-11A, wherein the individual is human.
- 13A. The method of any one of embodiments 1A-12A, wherein the level of one or more proinflammatory cytokines is increased.
- 14A. The method of any one of claims 1A-13A, wherein the level of one or more of IL-6, CCL4, CCL3, CXCL1, CXCL10, or GM-CSF is increased.
- 15A. The method of any one of embodiments 1A-14A, wherein the cancer is a stage I, stage II, stage III, or stage IV cancer.
- 16A. The method of any one of embodiments 1A-15A, wherein the antibody that specifically binds to IL-38 is administered at a dose of about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 10 mg/kg, about 15 mg/kg, about 25 mg/kg, 30 mg/kg, about 15 mg/kg, or about 50 mg/kg.
- 17A. The method of any one of embodiments 1A-16A, wherein the antibody that specifically binds to IL-38 is a bispecific antibody.
- 18A. The method of any one of embodiments 1A-17A, wherein the antibody that specifically binds to IL-38 is an isolated variable heavy (VH) single domain monoclonal antibody, a (sc)Fv-Fc, a Fv, a scFv, a Fab, a F(ab')₂, a Fab' fragment, a bispecific antibody, or a diabody.
- 19A. The method of any one of embodiments 1A-18A, wherein the cancer is a squamous cell carcinoma or an adenocarcinoma.
- 20A. The method of any one of embodiments 1A-19A, wherein the cancer is selected from the group consisting of gastroesophageal cancer, bladder cancer, cervical cancer, prostate cancer, breast cancer, renal cancer, colorectal cancer, pancreatic cancer, melanoma, uterine cancer, head and neck cancer, lung cancer and skin cancer.

21A. The method of any one of embodiments 1A-20A, wherein the cancer is selected from the group consisting of head and neck squamous cell carcinoma (HNSC), esophagus cancer (ESCA), squamous cell cancer of the lung (LUSC), cancer of the cervix (CESC), bladder cancer (BLCA), skin cutaneous melanoma (SKCM), prostate adenocarcinoma (PRAD), gastroesophageal carcinoma, cervical squamous cell carcinoma, cervical squamous cell carcinoma, skin squamous cell carcinoma, basal cell carcinoma, skin cutaneous melanoma, lung adenocarcinoma, endocervical adenocarcinoma, bladder urothelial carcinoma and prostate adenocarcinoma and lung adenocarcinoma (LUAD).

22A. The method of embodiment 21A, wherein the cancer is squamous cell cancer of the lung (LUSC).

23A. The method of embodiment 21A, wherein the cancer is gastroesophageal carcinoma.

24A. The method of embodiment 21A, wherein the cancer is head and neck squamous cell carcinoma.

25A. An isolated antibody that specifically binds to IL-38 comprising a VH CDR1, a VH CDR2 and a VH CDR3 of a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:87 and a VL CDR1, VL CDR2 and a VL CDR3 of a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:92.

26A. The isolated antibody that specifically binds to IL-38 of embodiment 25A, wherein the antibody comprises a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO:88, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO:89, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 90, a VL CDR1 comprising the amino acid sequence set forth in SEQ ID NO:93, VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO:94, and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO:95.

27A. An isolated antibody that specifically binds to IL-38 comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:98 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:101; or

a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:87 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:92

28A. The antibody that specifically binds to IL-38 of embodiment 27A comprising
a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:98 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:101.

29A. The antibody that specifically binds to IL-38 of embodiment 26A, wherein the CDRs are defined by the North method or the Kabat method.

30A. The antibody that specifically binds to IL-38 of any one of embodiments 25A-29A, wherein the antibody that specifically binds to IL-38 is a humanized antibody.

31A. The antibody that specifically binds to IL-38 of any one of embodiments 25A-30A, wherein the antibody that specifically binds to IL-38 partially or fully blocks, inhibits, or neutralizes a biological activity of IL-38.

32A. The antibody that specifically binds to IL-38 of any one of embodiments 25A-31A, wherein the antibody that specifically binds to IL-38 is an isolated variable heavy (VH) single domain monoclonal antibody, a (sc)Fv-Fc, a Fv, a scFv, a Fab, a F(ab')₂, a Fab' fragment, a bispecific antibody, or a diabody.

33A. A composition comprising the antibody of any one of embodiments 25A-32A.

34A. Nucleic acid(s) encoding the antibody of any one of embodiments 25A-32A.

35A. A vector comprising the nucleic acid(s) of embodiment 34A.

36A. A host cell comprising the nucleic acid of embodiment 34A or the vector of embodiment 35A.

37A. The host cell of embodiment 36A, wherein the host cell is a CHO cell.

Examples

[0119] The following Examples describe the isolation and characterization of IMM20130, an antibody which binds to an epitope on IL-38; an evaluation of the immunosuppressive effects of IL-38; the generation of additional anti-IL-38 antibodies; and their characterization both *in vitro* and *in vivo*. IL-38, can, in certain contexts, be soluble, or associated with a cell membrane, including at a cell surface, including in context of a multi-protein complex.

[0120] **Example 1. Isolation of a human hybridoma producing an antibody that binds the surface of intact human cancer cells.** PR087-29B5 hybridoma cells were generated from the fusion of human B cells isolated from the lymph node of a head and neck cancer patient, with the B56T fusion partner. Fusion of human B cells with B56T was carried out by electrofusion essentially as described in USPTO#EP2242836 "Method of making hybrid cells that express useful antibodies". Post-fusion, hybridomas were plated and allowed to grow for approximately two weeks. Conditioned medias from IgG/A-positive hybridomas were then collected and screened for the ability of the antibodies to bind to the surface of cancer cell lines. Binding of PR087-29B5 -produced Abs to pools of live, intact cancer cell lines was detected using fluorophore-labelled anti-human IgG secondary Abs and a LI-COR Odyssey™ Sa imaging system configured for 96-well plates. Prior to screening, the cancer cells were mixed in equal proportions and the pools were aliquoted into 96-well plates and allowed to attach for 24 hours. Hybridoma supernatants were incubated with cells and binding to the cancer cell lines was assessed relative to a positive control comprising a mixture of anti-basigin, anti-EGFR, and anti-ERBB2 (BCH) antibodies in equal ratios. The BCH positive control was incubated with cells at 66.6, 22.2, and 7.4 ng/mL of each antibody. An anti-Integrin (ITGA3) antibody (20 ng/mL) was also used as a positive control. Secondary antibody alone was used as a negative control. The combination of controls provides a range of absolute signal intensities across both the cell line pools and the detection range of the LI-COR instrument. The BCH (7.4 ng/mL) positive control exhibited a signal approximately 160% that of the background, where the background was defined as the average of the signal found in the four wells of secondary alone controls. The signals across those four wells had a standard deviation of 8.5%. PR087-29B5 did not exhibit a signal above the level of background, but rather presented as a low level punctate staining pattern that was selected for subsequent follow-up (Fig. 3).

[0121] **Example 2. The PR087-29B5 hybridoma produces an IgG comprising IGHV1/IGLV2 variable domains.** Nucleotide sequences encoding the variable heavy chain (V_H) and variable light chain (V_L) domains of PR087-29B5 were obtained by RT-PCR amplification of RNA isolated from cells

of the PR087-29B5 hybridoma line and subjecting the resulting antibody cDNA to sequencing reactions. SEQ ID NO: 1 corresponds to the nucleotide sequence of the V_H and SEQ ID NO: 3 corresponds to the nucleotide sequence of the V_L of PR087-29B5 isolated from the hybridoma. SEQ ID NO: 2 and SEQ ID NO: 4 correspond to the corresponding amino acid sequences of the V_H and V_L of PR087-29B5 isolated from the hybridoma. IGHV1-18 and IGKV3-20 gene assignments were predicted based upon homology to known germline gene sequences and were used to generate the 5' ends of the V_H and V_L to create full-length coding sequences represented by SEQ ID NO: 5 and SEQ ID NO: 8, which encode the amino acid sequences SEQ ID NO: 7 and SEQ ID NO: 10, respectively. A two plasmid system was used to facilitate recombinant expression of antibodies that contain the variable domains of PR087-29B5 with IgG1 heavy chain and kappa light chain constant domains. Codon optimization was carried out on SEQ ID NO: 5 and a nucleotide fragment corresponding to SEQ ID NO: 6, which encodes the amino acid sequence corresponding to SEQ ID NO: 7, was synthesized to facilitate expression of antibodies comprising the V_H domain of PR087-29B5. Codon optimization was also carried out on SEQ ID NO: 8 and a nucleotide fragment corresponding to SEQ ID NO: 9, which encodes SEQ ID NO: 10, was synthesized to facilitate expression of antibodies comprising the V_L domain of PR087-29B5. Vectors expressing either the heavy chain or light chain of PR087-29B5 were generated by synthesis and cloning of V_H and V_L domains into a two vector system that encode for a full-length IgG1 antibody comprised of amino acid sequences corresponding to SEQ ID NO: 12 and SEQ ID NO: 14. Antibodies containing the PR087-29B5 V_H and V_L domains, were expressed recombinantly by transient transfection into mammalian cell lines, such as Chinese Hamster Ovary (CHO) and human embryonic kidney (HEK), using standard conditions. Recombinant antibodies, referred to as IMM20130, were purified from conditioned media by affinity chromatography using techniques well known to one of ordinary skill in the art.

[0122] **Example 3. IMM20130 Ab binds an epitope on IL-38.** To identify the target antigen bound by IMM20130, the antibody was screened against CDI HuProt arrays where target proteins were spotted in their native conformation. More specifically, IMM20130 was incubated (1 microgram / mL) overnight at 4° C against the native CDI HuProt arrays. Slides were washed and IMM20130 binding was detected with an Alexa-647 conjugated anti-H+L secondary antibody. Non-specific hits that were bound by the secondary antibody were eliminated from any analysis. Selective binding to target proteins was analyzed by a combination of Z-score, to determine reproducibility of binding to replicates on each slide, and S-score, to determine difference in selectivity versus possible targets. An S-score >3 between the top and second-ranked hits is considered as highly specific for the top hit.

[0123] IMM20130 bound selectively to IL1F10/IL-38 found on the native array. IL-38 represented the top hit on the CDI array with a Z-score of 119.635 and an S-score of 51.643. See Table 1.

Table 1. IMM20130 binding to human proteome in protein microarray format

CDI Native Array				
Rank	Protein	Z Score	S Score	F635
1	IL1F10/IL38	119.635	51.643	18395.5
2	IL1F10/IL38	67.992	32.891	10473.5
3	STIP1	35.101	8.615	5428
4	DNAJC7	26.486	6.004	4106.5
5	STIP1	20.482	8.866	3185.5
6	TTC1	11.616	0.251	1825.5
7	ZNF41	11.365	1.829	1787
8	PPIA	9.536	0.029	1506.5
9	HIST1H1B	9.507	0.049	1502
10	FAM104B	9.458	0.094	1494.5

[0124] Binding of IMM20130 to recombinant IL-38 was confirmed by dot-blot analysis. Increasing doses of recombinant human IL-38 (NovusBio, catalog number NBP2-22645) was spotted onto nitrocellulose and incubated with IMM20130. As shown in Fig. 4, IMM20130 interacted with IL-38 in a dose-dependent manner. A commercial anti-IL-38 antibody served as the positive control for the assay and an anti-Dengue antibody of the same isotype served as a negative control. The recombinant protein STIP1, a lower level hit in the original CDI array, was used as a non-specific control.

[0125] Binding of IMM20130 to IL-38 (NovusBio, catalog number NBP2-22645) was quantified by surface plasmon resonance (SPR). Briefly, IMM20130 was diluted in SPR running buffer (10 mM HEPES, pH7.4, 150 mM NaCl, 0.0005% Tween-20, 0.2% bovine serum albumin) to final concentrations of 150 nM and 25 nM and captured at four different surface densities on an anti-human Fc coated CM5 sensor chip. Surface densities ranged from 600 to 3200 RU. IL-38 (NovusBio, catalog number NBP2-22645) was diluted in SPR running buffer to a concentration of 600 nM and a 3-fold dilution series was run across the four different IMM20130 density surfaces. Data were

collected at 25 °C. Data from all four surfaces were fitted to a 1:1 interaction model yielding the rate constants depicted in Table 2.

Table 2. SPR Quantification of IMM20130 binding to human IL-38

SPR analysis		
k_a	k_d	K_D
4.98(8)e3	6.80(1)e-5	1.36(2)e-8

[0126] IMM20130 also specifically bound to the surface of several endogenously-expressing cell lines (Fig. 5). Live cells were stained with IMM20130 or isotype control and a fluorochrome-conjugated anti-human secondary antibody. Propidium iodide was used to exclude dead cells. Data is expressed as fold change of MFI over isotype control. Since IL-38 can be secreted under apoptotic conditions (Mora et al, 2016), several IMM20130-binding cancer cell lines were tested for their ability to secrete IL-38. Cancer cell lines were treated with 20 ng/mL TNF α and 10 μ g/mL cycloheximide for the indicated time period. For 0 h and 4 h timepoints, cells were cultured in plain RPMI for 16 hours post-treatment. For the 16 h timepoint, cells were cultured with TNF α and cycloheximide for the full 16 hours in plain RPMI. The concentration of IL-38 in the supernatants was determined by direct ELISA using a protocol adapted from *Mora et al 2016*. In brief, 100 μ L of supernatant was added to a high binding 96 well plate (Corning) and compared to a standard curve of seven two-fold dilutions of recombinant IL-38 (Adipogen, catalog number AG-40A-0191-C050) in plain RPMI. Plates were incubated overnight at 4°C. Wells were blocked with PBS 2% BSA, washed 3 times with PBS 0.05% Tween, and incubated with a rat anti-human IL-38 antibody (R&D Systems) for 2 hours at room temperature. After 3 washes, wells were incubated with a biotinylated anti-rat secondary antibody (Invitrogen) for 2 hours at room temperature. Following 3 washes, streptavidin-HRP (R&D Systems) in PBS 2% BSA was added for 20 minutes. After 3 washes, 100 μ L of OPD substrate diluted in phospho-citrate/sodium perborate buffer was added per well, developed for 5-30 minutes, and absorbance was measure at 450nm. Indeed, multiple cancer cell lines secreted IL-38 under apoptosis-inducing conditions (Fig. 6), identifying tumor cells as a potential source of IL-38.

[0127] IL-38 RNA expression across various tumor types was assessed using the TCGA database (Fig. 23). Multiple cancers, including head & neck squamous cell carcinoma (HNSC), esophagus

cancer (ESCA), squamous cell cancer of the lung (LUSC), cancer of the cervix (CESC), bladder cancer (BLCA), skin cutaneous melanoma (SKCM), prostate adenocarcinoma (PRAD), and lung adenocarcinoma (LUAD) were shown to have a subset of tumors that overexpress IL-38 as compared to normal tissues from the same organ. Based upon the 90th percentile of IL-38 expression in normal tissues, tumor samples from the same organ were divided into two separate groups: High and low IL-38 expressing tumors. The level of expression differed between organs and the percentage of tumors from each organ that were classified as high IL-38 expressing tumors. The Y-axis in Fig. 7 represents an enrichment score, with higher numbers correlating with higher IL-38 RNA expression. The values differ for each tissue type assessed. The percentages in Fig. 7 represent the percentage of tumors from each anatomical location that were classified as high IL-38 expressing tumors. In the case of lung cancer, squamous and adenocarcinoma sub-types were analyzed separately and showed similar trends

[0128] **Example 4. IL-38 is a pro-tumorigenic, immunosuppressive cytokine that inhibits inflammatory responses.** After assessing the expression of IL-38 in various cancer cell lines with IMM20130, the TCGA database was used to assess the effect of IL-38 on the tumor microenvironment. Gene expression analysis was performed using TCGA Firehouse Legacy datasets from the indications specified above. The number of samples in each dataset is indicated along with the R-squared values for each analysis. RNA_Seq_v2_mRNA_median_Zscore data was used for data analysis. In multiple cancer types, expression of IL-38 correlated with reduced expression of genes associated with immune cell types essential for an effective anti-tumor response, including T cells and myeloid cells (Fig. 7), suggesting that IL-38 can play an important role in suppressing immune cell infiltration into the tumor microenvironment. Similar results were observed for head and neck cancer, esophagus cancer, cervical cancer, melanoma, and lung squamous carcinoma.

[0129] To identify how IL-38 could suppress the immune system, an *in vitro* model was established using the THP-1 monocyte cell line (ATCC, catalog number TIB-202). THP-1 monocytes were differentiated to macrophages by culturing with 100 nM PMA for 72 hours. After differentiation, PMA was removed, macrophages were washed with PBS, and cultured in plain RPMI with or without 1 µg/mL recombinant full length human IL-38 (Adipogen, catalog number AG-40A-0191-C050) for 24 hours. To stimulate macrophages and induce production of inflammatory cytokines, 10 ng/mL LPS was added for an additional 24 hours. Supernatants were harvested and cytokine expression was measured using CBA Human Inflammatory Cytokines Kit (BD Biosciences, catalog number 551811) per manufacturer's instructions. Treatment of THP-1 macrophages with IL-

38 resulted in a reduction in several inflammatory cytokines, namely IL-6 and TNF α (Fig. 8). In order to have a more complete understanding of the effect of IL-38 on macrophage inflammatory responses, THP-1 cells were assayed for RNA expression of important inflammatory markers using the Nanostring PanCancer IO 360 Gene Expression Panel. THP-1 cells were differentiated and stimulated as described for Fig. 8. After LPS stimulation, cells were harvested and RNA was isolated using RNeasy kit (Qiagen). Nanostrings PanCancer IO 360 Gene Expression Panel was used to assess gene expression using the nCounter Platform (Nanostring Technologies). nSolver software was used for data analysis. The LPS-stimulated sample was used to normalize gene expression to 1. Several important inflammatory markers were reduced in IL-38-treated cells, including pro-inflammatory M1 macrophage markers (CD80, IL-6) and chemokines important for immune cell recruitment (CXCL10, CXCL13) (Fig. 9).

[0130] To determine how IL-38 suppresses inflammatory responses in THP-1 cells, phosphorylation of key signaling proteins was measured. Using the *in vitro* system described in Fig. 8, differentiated THP-1 macrophages pretreated with IL-38 were stimulated with 10 ng/mL LPS for various timepoints. Following stimulation, cells were lysed in 1% Triton-containing lysis buffer (Cell Signaling) with phosphatase and protease inhibitors. 20 μ g per lane was loaded onto a 4% to 12% polyacrylamide gel (Invitrogen) and transferred onto a nitrocellulose membrane. The membrane was probed overnight with rabbit anti-human antibodies recognizing p-STAT3 and GAPDH and a mouse anti-human p-Jnk antibody (Cell Signaling). Then, the membranes were incubated with fluorescent anti-rabbit and anti-mouse secondary antibodies (LI-COR Biosciences) for 1 hour. Blots were scanned using LI-COR imaging system and quantification performed in Image Studio software (LI-COR Biosciences). Phosphorylation of Jnk was impaired in IL-38-treated THP-1 macrophages (Fig. 10). Conversely, phosphorylation of STAT3 was increased in IL-38-treated THP-1 macrophages prior to stimulation with LPS.

[0131] **Example 5. Generation of anti-IL-38 antibodies that block IL-38 function.** The *in vitro* system established in Fig. 8 was used to test the ability of IMM20130 to block the function of IL-38. THP-1 monocytes were differentiated into macrophages with 100 nM PMA for 72 hours. After differentiation, 1 μ g/mL IL-38 (Adipogen, catalog number AG-40A-0191-C050) and 10 μ g/mL of the indicated antibody were incubated at room temperature for 1 hour in plain RPMI. Macrophages were washed with PBS and cultured with the indicated IL-38/antibody-containing media for 24 hours. Subsequently, cells were stimulated with 10 ng/mL LPS for 24 hours, supernatants were harvested and IL-6 production was measured using the Human IL-6 DuoSet ELISA kit (R&D Systems).

Inhibition of IL-38 should result in the restoration of IL-6 production in IL-38-treated, LPS-stimulated THP-1 cells to levels comparable to LPS-stimulated cells. However, IMM20130 was unable to restore IL-6 production of these cells (Fig. 11). As controls, two polyclonal antibodies raised against different portions of the IL-38 protein (Lifespan Biosciences, catalog numbers LS-C135753 and LS-C201139) were also tested for their ability to restore IL-6 production in this system. One polyclonal antibody raised against a portion of the C-terminus of IL-38 successfully restored IL-6 production of IL-38-treated, LPS-stimulated THP-1 macrophages (Fig. 12), indicating that IMM20130 binds to an epitope of IL-38 that does not block IL-38 function.

[0132] Although IMM20130 did not block the function of IL-38, it did identify IL-38 as an important modulator of the inflammatory response and a promising cancer target. Therefore, an antibody generation campaign was initiated to isolate anti-IL-38 antibodies that also block IL-38 function. NZB/w and CD-1 mice were immunized with full length recombinant IL-38. At Day 21 post immunization, mouse sera were assayed for the presence of anti-IL-38 antibodies by the Direct ELISA described in Example 2 using an HRP-conjugated anti-mouse secondary antibody (Jackson ImmunoResearch Laboratories, catalog number 115-035-071). Spleens from animals with the highest anti-IL-38 sera titer were fused with a myeloma cell line to generate a polyclonal hybridoma library. After polyclonal supernatants were confirmed to have anti-IL-38 antibodies by ELISA, individual hybridomas were single cell sorted and cultured in 96 well plates to generate antibody-containing monoclonal supernatants. A direct IL-38 ELISA was performed with the indicated controls on monoclonal supernatants from NZB/w and CD-1 mice-derived hybridomas as described in Fig. 6. Multiple monoclonal supernatants contained anti-IL-38 antibodies (Fig. 13). The selected IL-38 binding monoclonal supernatants were also tested for the ability to block IL-38 function in the *in vitro* system described in Fig. 11. IL-6 production was measured using two preparations of each monoclonal supernatant. Blocking efficiency was determined by normalizing IL-6 production of LPS-stimulated THP-1 cells to 100% and IL-6 production of IL-38-treated, LPS-stimulated THP-1 cells to 0%. Therefore, the percent rescue of each monoclonal supernatant was calculated as the IL-6 production of [(sample containing monoclonal sup, LPS, and IL-38) – (LPS, IL-38 Control)] / [(LPS alone control) – (LPS, IL-38 control)]. High blocking efficiency was observed with multiple monoclonal supernatants (Fig. 14) and these were selected for further development.

[0133] Antibody-containing solutions, including mouse hybridoma supernatants and purified antibodies, were tested on Octet Qke instrument using ForteBio's anti-mouse Fc (AMC) biosensors and soluble recombinant human (Adipogen, catalog number 40A-0191-C050) or mouse (Lifespan

Biosciences, catalog number LS-G3934) IL-38 proteins that were serially diluted in the ForteBio's kinetics buffer. Antibody was loaded to AMC probes, blocked in the kinetics buffer for 1 min (baseline) and dipped into an appropriate IL-38 solution. IL-38 association to antibodies of interest was measured for 180 seconds at 28°C. Biosensors were subsequently dipped into kinetics buffer-containing wells and the protein dissociation was measured for 600s. Raw traces were analyzed as shown in Table 3. KD, Kon and Kdis values were identified using the 1:1 built-in model of the ForteBio's Data Analysis 9 software. Binding kinetics of a selected anti-human IL-38 antibody are demonstrated in Fig. 15.

Table 3. Binding characteristics of lead candidate anti-IL-38 antibodies.

Antibody	human IL-38			mouse IL-38		
	Kon	Koff	KD	Kon	Koff	KD
CD1-M3	2.64E+04	1.85E-05	7.21E-10	9.25E+03		
CD1-M8	2.80E+04					
CD1-M26	3.50E+04	2.31E-04	6.60E-09			
CD1-M27	1.66E+04					

[0134] To confirm specificity to IL-38, several lead candidate antibodies (CD1-M3, CD1-M8, NZB-M8) were analyzed in a High-Spec Cross-reactivity Assay using native HuProt arrays (CDI Laboratories). 1 μ L of antibody was incubated overnight in a cold room, washed, and probed with anti-human secondary antibodies. Fluorescence intensity (F635) as an indicator of binding was measured for each spot. CDI software quantified the specificity of the antibody to each spot based on a Z-score. Z-score is defined as $[F635 - (\text{Average } F635 \text{ on array})] / (\text{Standard deviation of } F635 \text{ on array})$. S-score is defined as the difference between the Z-score of a given protein and the Z-score of the next highest protein. For CD1-M3 and CD1-M8, bound selectively to IL-38 on the native array with Z-scores of 139.533 and 142.421, respectively (Table 4).

Table 4. Binding of lead candidate antibodies to human proteome in protein microarray format.

CD1-M3				
Rank	Protein	Z-Score	S-Score	F635
1	IL1F10/IL38	139.533	90.338	53998.5
2	IL1F10/IL38	49.195	26.187	19058.5
3	U2AF2	23.008	3.434	8930
4	SUV39H1	19.574	8.976	7602
5	Hnrnp1	10.598	5.005	4130
6	HNRNPH1	5.593	0.518	2194.5
7	DBN1	5.075	0.201	1994

CD1-M8				
Rank	Protein	Z-Score	S-Score	F635
1	IL1F10/IL38	142.421	91.494	45625.5
2	IL1F10/IL38	50.927	40.838	16334
3	DBN1	10.089	4.376	3260
4	ESYT2_Frag	5.713	2.152	1859
5	CROCCP2	3.561	0.747	1170
6	CROCCP2	2.814	1.068	931
7	NDOR1	1.746	0.155	589

NZB-M8				
Rank	Protein	Z-Score	S-Score	F635
1	IFNG	56.061	3.471	7518.5
2	GORASP1	52.58	4.166	7056.5
3	COL5A2	48.414	1.349	6502
4	NEDD4L	47.065	9.039	6322.5
5	TTC5	38.026	3.689	5119.5
6	LBHD1	34.337	0.297	4628.5
7	IL1F10/IL38	34.04	0.383	4589
8	IL1F10/IL38	33.657	1.056	4538

[0135] NZB-M8, however, identified IFN γ as the top hit and IL-38 as the 7th hit, suggesting low fidelity to IL-38. Since IL-38 is an IL-1 family member, antibody binding was also compared with other IL-1 family members. Despite homology between IL-38 and other IL-1 family members, no cross-reactivity was identified (Table 5).

[0136] CD1-M3, CD1-M8, and CD1-M26 were isolated from monoclonal antibody supernatants and PBS-purified. These antibodies were tested in the established *in vitro* system described in Fig. 11 using half log dilutions of each antibody. IL-6 and GM-CSF production was assessed using Human IL-6 and Human GM-CSF DuoSet ELISA Kits (R&D Systems) per the manufacturer's instructions. All lead antibodies were capable of restoring IL-6 and GM-CSF production of IL-38-treated, LPS-stimulated THP-1 macrophages (Figs. 16A-B).

Table 5. Binding of lead candidate antibodies to IL-1 family members in protein microarray format.

IL-1 Family Member	CD1-M3 (Z-Score)	CD1-M8 (Z-score)	NZB-M8 (Z-score)
IL-38	139.533	142.421	34.04
IL-1 α	-0.013	-0.014	-0.147
IL-1 β	-0.016	-0.017	-0.143
IL-1Ra	-0.013	0.003	-0.166
IL-18	-0.016	-0.016	-0.132
IL-36Ra	-0.017	-0.019	-0.034
IL-36a	-0.008	0.012	0.033
IL-37	-0.013	-0.016	-0.113
IL-36b	-0.016	-0.01	-0.087
IL-36g	-0.017	-0.008	-0.143
IL-33	-0.02	-0.014	-0.17

[0137] **Example 6. Evaluating the effect of anti-IL-38 antibodies in *in vivo* tumor models.**

After CD1-M3, CD1-M8, and CD1-M26 were confirmed to bind and block IL-38 function *in vitro*, they were assessed for their ability to persist in mouse plasma in a pharmacokinetic study. 6-7 week old C57BL/6 mice were dosed at 10mg/kg i.p. and i.v. at 0 h (n = 9 per group). Each mouse was retro-orbitally bled for two timepoints and terminally bled at a final timepoint. Plasma was isolated using K2 EDTA tubes and assayed for antibody via direct IL-38 ELISA. 100 μ L of IL-38 in PBS (50 ng/mL for CD1-M3, M8 ; 600 ng/mL for CD1-M26) was added per well of a 96 well high binding plate and plates were incubated overnight at 4°C. After 3 washes with PBS 0.05% Tween, plates were blocked with PBS 2% BSA for 1 h at room temperature. After 3 washes, 100 μ L mouse plasma diluted in PBS 2% BSA was added each well. To generate a standard curve, CD1-M3, M8, M26 antibodies were spiked into untreated mouse plasma diluted with PBS 2% BSA starting at 500ng/mL. Plates were incubated for 2 h at room temperature and washed 3 times. 100 μ L of HRP-conjugated anti-mouse antibody diluted 1:2000 in PBS 2% BSA was added per well and incubated for 2 h at room temperature. After 3 washes, 100 μ L of OPD substrate diluted in phospho-citrate/sodium perborate buffer was added per well, developed for 5-30 minutes, and absorbance was measure at 450nm. After a 10 mg/kg dose, all antibodies reached 100,000 ng/mL plasma concentration soon after dosing, which slowly decreased over time (Fig. 17). i.v. and i.p. administration of CD1-M3, CD1-M8, and CD1-M26 resulted in similar plasma levels throughout the one week study.

[0138] Of the selected lead candidate antibodies, CD1-M3 was the only antibody capable of binding human and mouse IL-38. Therefore, this antibody was tested in several syngeneic tumor models, where blocking IL-38 in the tumor microenvironment could be evaluated in an immune competent mouse. In the first study, 2×10^5 B16.F10 cells were implanted into the flank of 6-8 week

old C57BL/6 female mice. When mean tumor size reached 85 mm³, mice were randomized into indicated groups (n = 10). Mice were treated with CD1-M3 and/or paclitaxel as indicated and tumor volumes were taken 3 times per week with a caliper. Treatment with anti-CD1-M3 resulted in a small reduction in tumor volume compared with vehicle control (Fig. 18). CD1-M3 treatment also reduced tumor volume when combined with the chemotherapeutic agent paclitaxel.

[0139] Due to the reported role of IL-38 in suppressing inflammatory immune responses, an additional study was performed to evaluate the effect that CD1-M3 would have on the tumor infiltrating myeloid and lymphocyte populations. 2x10⁵ B16.F10 cells were implanted into the flank for 7-8 week old C57BL/6 female mice. When mean tumor size reached 104mm³, mice were randomized into Vehicle and CD1-M3-treated groups and dosed at 10mg/mL IP QWx2. On Day 9, 24 hours after the 2nd dose, mice were euthanized and tumors were dissociated into single cell suspensions. Lymphocyte and myeloid populations were assessed using two flow cytometry panels. T cell panel includes Zombie NIR Viability dye (Biolegend) and fluorochrome-conjugated antibodies recognizing CD3, CD4, CD8, CD45, CD25, PD-1, CD69, FoxP3, CD49b/CD335, TCRgd. Myeloid panel includes Zombie NIR Viability dye (Biolegend) and fluorochrome-conjugated antibodies recognizing CD45, CD11b, CD11c, CD24, Ly-6C, Ly-6G, F4/80, MHCII, and CD206. Cell number was calculated using Precision Count beads (BioLegend) and normalized based on tumor size. All populations were gated on single, live CD45+ cells. Populations are defined as follows: Treg – CD4⁺CD25⁺FoxP3⁺; NK cell – CD3⁻CD49b⁺CD335⁺; NKT cell – CD3⁺CD49b⁺CD335⁺; G-MDSC – CD11b⁺Ly6G⁺; M-MDSC – CD11b⁺Ly6C⁺; Macrophage – CD11b⁺F4/80⁺ (exclude MDSC); M1 – CD206⁻MHCII⁺ Macrophages; M2 – CD206⁺ Macrophages; Dendritic cells – CD24⁺F4/80⁻CD11c⁺MHCII⁺. Percent change was calculated as [(cells/gram of CD1-M3 sample) – (cells/gram of Vehicle control group)] / (cells/gram of Vehicle control group) x 100%. Notably, CD1-M3 treatment resulted in an increase in the tumor infiltrating T cells, including CD4, CD8, and $\gamma\delta$ T cells (Fig. 19, top). The B cell population was also increased compared with vehicle control. CD1-M3 did not affect the expression of the activation marker CD69 on intratumoral CD8 T cells, however, it did decrease the amount of CD8 T cells expressing PD-1 (Fig. 19, bottom).

[0140] The second syngenic model evaluated was the MMTV-PyMT orthotopic mouse model. 10⁶ MMTV-PyMT cells were implanted orthotopically into the mammary fat pad of female FVB mice. When mean tumor size reached 150mm³, mice were randomized into groups (n = 10) and dosed as indicated. Tumor volumes were measured with a caliper every 2-3 days and study was concluded on Day 9, 24 hours after the 2nd dose. CD1-M3 treatment again resulted in a small reduction in tumor

volume compared with the vehicle control (Fig. 20, top). Since CD1-M3 can restore IL-6 production of IL-38-treated macrophages *in vitro*, intratumor cytokine levels were evaluated in this study. Flash frozen tumors were homogenized in lysis buffer containing 0.5% NP-40 using a bead beating homogenizer (Omni International). Protein concentration was measured by Pierce BCA Protein Assay Kit (ThermoFisher) and normalized. Cytokine concentration was measured on a Luminex-based platform. Similar to *in vitro* data showing restoration of IL-6 production with CD1-M3, intratumoral IL-6 was increased in CD1-M3-treated mice.

[0141] To evaluate additional lead candidate antibodies that only bind human IL-38, a xenograft model was also evaluated in immune-deficient *scid* mice. 5×10^6 A549 cells, which have been previously shown to secrete IL-38 under apoptotic conditions, were implanted into 7-8 week old female *scid* mice. When mean tumor size reached 134 mm^3 , mice were randomized into groups and dosed as indicated. Tumor volumes were measured with a caliper every 2-3 days and study was concluded on Day 9, 24 hours after the 2nd dose. No major reduction in tumor volume was observed after treatment the lead candidate antibodies (Fig. 21). This may be due to the lack of T and B cells in this model, which were increased in B16.F10 tumors (Fig. 19). The myeloid compartment, which is still present in *scid* mice, was evaluated in these mice by harvesting the tumors from Fig. 21 and dissociating them into single cell suspensions and flow cytometry was performed as described in Fig. 19. Overall, CD1-M3 slightly increased multiple myeloid populations, while CD1-M8, CD1-M26, and NZB-M8 largely resulted in a slight decrease in these populations (Fig. 22).

[0142] **Example 7. Identification of IMM20130 from the B cell repertoire of a head and neck cancer patient.** IMM20130 was isolated from a human B cell hybridoma generated by fusion of memory B cells from the lymph node of a treatment-naïve head and neck cancer patient (schematic shown in Figure 24a). This antibody was a class-switched IgG composed of hIGHV1-18 paired with a hIGKV3-20 light chain. It contained moderate levels of somatic hypermutation, with 93% and 98% homology to the germline heavy and light chain sequences, respectively. Bio-Layer Interferometry (BLI) analysis demonstrate binding of IMM20310 to human full-length IL-38 with an affinity of 13.6nM (Figure 24b). Analysis of binding promiscuity using a native human protein demonstrated exquisite specificity and selectivity to human IL-38 protein (Figure 24c). Binding to human cancer cell lines was performed by flow cytometry and identified multiple IL-38-expressing lines, including A549, which have been previously shown to secrete IL-38 under apoptotic conditions. In functional experiments, however, IMM20130 was unable to reverse IL-38-mediated suppression of IL-6 production by LPS-stimulated THP-1 cells, indicating that it did not block binding of IL-38 to its

cognate receptor. Together, although IMM20130 could not be used therapeutically to block IL-38 function, these data demonstrate targeting of IL-38 by the immune system in cancer and identify IL-38 as a potential target of interest.

[0143] *LPS Stimulation of THP-1 macrophages.* THP-1 monocytes were differentiated to macrophages by culturing with 100 nM PMA for 72 hours. After differentiation, PMA was removed by washing with PBS. THP-1 macrophages were then cultured in plain RPMI with or without 1 µg/mL recombinant full length human IL-38 (Adipogen) for 24 hours. Where indicated, IMM antibodies or commercial polyclonal anti-IL-38 antibodies (Lifespan Biosciences) were pre-incubated with human IL-38 for 1 hour at room temperature prior to addition to the THP-1 macrophages. To induce production of inflammatory cytokines, 10 ng/mL LPS was added for an additional 24 hours. Supernatants were harvested and cytokine expression was measured using the Human IL-6 DuoSet ELISA (R&D Systems) per manufacturer's instructions.

[0144] *Affinity of anti-IL-38 antibodies to IL-38 measured by biolayer interference (BLI).* Initial screening and binding experiments were performed using a biolayer interferometry approach (BLI) on Octet Qke instrument (ForteBio). Antibody was loaded on anti-human Fc probe at 5 mg/mL concentration and probed against serial dilutions of a soluble IL-38 human protein in 0.5% of Ig-free BSA in PBS. Baseline and Baseline2 steps were measured for 60 s, Loading for 120 s, Association for 90-120 s and Dissociation for 240-360 s. Regeneration was performed using 10 mM glycine pH 1.7 buffer. Experimental data were analyzed using a 1:1 (antibody to analyte) interaction model using ForteBio Data Analysis software V9.0.0.14. Data fitting was performed using the closest of fit based on the chi-square value, which describes the deviation between the experimental and fitted curves. The fitting algorithm seeks to minimize chi square.

[0145] *Flow cytometry.* Flow cytometry was performed to assess binding to tumor cell lines. Briefly, adherent cells were harvested using non-enzymatic detachment buffer and incubated at 37°C for 10-30 minutes. Following release, cells were washed in complete media and stained with fixable live/dead dye. Primary antibody binding was performed at 4°C followed using IMM20324. Detection was performed by staining with a polyclonal AF647 goat anti-mouse IgG2a secondary antibody. Samples were analyzed on an Attune NxT flow cytometer (Life Technologies) and analyzed using FlowJo X (BD BioSciences). Binding curves were generated in Prism V9 using the sigmoidal, 4PL, log function.

[0146] **Example 8. IL-38 is overexpressed in squamous cell carcinomas and correlates with poor immune infiltration.** To confirm the relevance of IL-38 to other tumor types we mined RNAseq data from The Cancer Genome Atlas (TCGA). Tumors from 6 different cancer types were separated into IL-38 positive and negative tumors and compared with normal tissue. In all tumors analyzed IL-38 expression was significantly higher than normal adjacent tissue. When comparing positive and negative head and neck tumors, a significant fraction (23%) of squamous cell carcinomas expressed IL-38 (Figure 25), supporting the relevance of IL-38 in the indication from which the antibody was derived. Interestingly, IL-38 transcripts were also detectable in multiple additional tumor types, particularly esophageal carcinoma, squamous cell carcinoma, cervical squamous cell carcinoma, where prevalence was >40% for each, as well as skin cutaneous melanoma, lung adenocarcinoma (Figure 25). Positivity was also observed in other cancer types, including endocervical adenocarcinoma, bladder urothelial carcinoma and prostate adenocarcinoma. Interestingly, squamous cell carcinomas exhibited the highest IL-38 expression and highest frequency of IL-38 positive patient samples.

[0147] To confirm expression of IL-38 mRNA transcripts across different cancer types in Tempus dataset (Beaubier N, Bontrager M, Huether R, Igartua C, Lau D, Tell R, Bobe AM, Bush S, Chang AL, Hoskinson DC, Khan AA. Integrated genomic profiling expands clinical options for patients with cancer. *Nature Biotechnology*. 2019 Nov;37(11):1351-60), RNA sequencing data from real world database were mined using Tempus platform. Among more than 60 cancer types, the top 3 cancers with high frequency of IL-38 mRNA expression are Gastroesophageal squamous carcinoma, Head and neck squamous carcinoma and Lung squamous carcinoma (Table 6). Interestingly, IL-38 mRNA expression is significantly higher in HPV negative populations (Fig. 46D), whom have poor prognosis and are more difficult to treat than HPV positive population. These results confirm the findings from TCGA analysis that IL-38 is overexpressed in squamous carcinoma.

[0148] **Table 6** depicts that IL-38 mRNA expression analysis based on Tempus database and frequency of IL-38 mRNA expression in tumor samples.

		IL-38 mRNA			
[00101]	Cancer type	Mean expression	Stdev	Sample size	% positive
		[Log2(TPM+1)]			

Gastroesophageal squamous carcinoma	0.774	0.885	88	86.4
Head and neck squamous carcinoma	0.646	0.940	467	63.8
Lung squamous carcinoma	0.260	0.497	753	47.7

TPM: transcript per million. Stdev: standard deviation.

[0149] Since IL-38 is associated with the suppression of inflammation, we also correlated immune cell lineage-specific gene expression to IL-38 expression. IL-38 expression was negatively correlated with IMMSig enrichment scores of T cells, B cells, NK cells, monocytes, and DCs in multiple tumor types, suggesting that IL-38 expression is negatively correlated with immune infiltration (Figure 26). These data suggest that targeting IL-38 in human tumors may enhance immune infiltration, inflammatory cytokine expression and induce productive anti-tumor immunity.

[0150] To confirm IL-38 expression is negatively associated with infiltration of immune cells in tumors in Tempus dataset, enrichment scores of different immune cell populations in each tumor sample were generated using proprietary algorithm by Tempus (Reiman et al. Pacific Symposium on Biocomputing (2019)). Pearson correlation of IL-38 expression levels with enrichment scores were calculated and depicted in Table 7. In Head and neck squamous carcinoma, IL-38 expression is negatively associated with total immune cells, CD8+ T cells (FIG. 46A), macrophages (FIG. 46B), and NK cells (FIG. 46C). In Lung squamous carcinoma, IL-38 expression is negatively associated with total immune cells, CD8+ T cells and macrophages. Similar trends were also observed in Gastroesophageal squamous carcinoma. These results confirm the findings from TCGA analysis: high IL-38 mRNA expression is associated with less infiltration of immune cell populations, confirming our hypothesis that IL-38 is an inhibitory cytokine and blocks migration of immune cells into tumors.

[0151] **Table 7.** IL-38 expression is negatively associated with immune cell infiltration in selected cancers.

Cancer type, cell population	Pearson Correlation	P value
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Gastroesophageal squamous carcinoma (n=62)		
Total immune cells	-0.152	0.238
CD4+ T cells	-0.178	0.167
CD8+ T cells	-0.176	0.172
Macrophage	-0.159	0.216
NK	-0.015	0.907
Head and neck squamous carcinoma (n=396)		
Total immune cells	-0.199	<0.001
CD4+ T cells	-0.189	0.078
CD8+ T cells	-0.243	<0.001
Macrophage	-0.217	<0.001
NK	-0.147	0.003
Lung squamous carcinoma (n=594)		
Total immune cells	-0.124	0.002
CD4+ T cells	-0.035	0.397
CD8+ T cells	-0.149	<0.001
Macrophage	-0.186	<0.001
NK	0.002	0.961

[0152] Taken together, the discovery of IMM20130 in head and neck cancer patients, confirmation of IL-38 expression in multiple tumor types, and IL-38's immune modulatory functions suggest that IL-38 could be a key immune checkpoint shaping tumor microenvironment and anti-tumor response. We hypothesize that an antibody against IL-38 could inhibit tumor growth by promoting innate immunity.

[0153] *TCGA analysis.* RNAseq data from multiple tumor types were download from The Cancer Genome Atlas (TCGA) portal. IL-38 expression in individual samples is depicted in scatter plots. The

IL-38 expression thresholds in different tumor types are chosen based on expression level of IL-38 in 90th percentile normal samples. Samples with an IL-38 expression level above the threshold are considered as IL-38 positive samples. Conversely, samples with an IL-38 expression level below the threshold are considered as IL-38 negative samples. Enrichment of IMMsig scores for each immune cell population are calculated for IL-38 positive and IL-38 negative samples.

[0154] *Tempus analysis. Whole genome RNA sequencing data from more than 60 different types of cancers are mined.* IL-38 positive samples are defined as more than one copies of IL-38 mRNA are detected in the sequencing reads.

[0155] **Example 9. Discovery of IMM20324.** To identify neutralizing anti-IL-38 antibodies with therapeutic potential, mouse hybridomas were generated by immunization of CD-1 mice with recombinant human IL-38. Among multiple IL-38 binders, IMM20324 was selected due to its high affinity to human IL-38, in vitro neutralization potency, and cross-reactivity with cynomolgus and murine IL-38. IMM20324 binds to human and cynomolgus IL-38 with high affinity and mouse IL-38 with slightly lower affinity (Figure 27a). Similar to IMM20130, IMM20324 exhibited strong specificity for IL-38 in a native human protein array with no binding to other IL-1 family members or other non-related proteins. IMM20324 binds to plate-bound recombinant IL-38 in a dose-dependent manner with an EC50 value of 4.3 ng/mL (Figure 27b). IMM20324 also neutralizes binding of recombinant human IL-38 to plate-bound IL1RAPL1-Fc or human IL-36R-Fc in a dose-dependent manner with IC50 values of 190 ng/mL and 220 ng/mL, respectively (Figure 27c). The ability of IMM20324 to block the function of IL-38 in a cell-based assay was assessed using LPS-stimulated THP-1 cells (Figure 27d). IL-38 inhibits the production of IL-6 following LPS treatment in this assay. IMM20324 addition significantly increases IL-6 production, albeit not completely. Together, these data demonstrate that IMM20324 binds to IL-38, prevents IL-38 binding its putative receptors and inhibits function in a human monocytic cell line.

[0156] *Affinity of anti-IL-38 antibodies to IL-38 measured by surface plasmon resonance (SPR).* Samples were diluted in running buffer (HBS-EP + 1 mg/mL BSA). Antibodies were loaded to Protein A capture sensor chip (Cytiva). IL-38 protein (NovoPro) was diluted in 5 serial three-fold dilutions from 270 nM to 0.33 nM. Experiment was performed using 30 mL/min flow rate at 25°C on a Biacore 8K instrument running Biacore Insight Evaluation software V3.0.12.15655. Association step was measured for 300 s, dissociation for 1500 s. Regeneration was performed using 10 mM glycine pH 1.7 buffer. Analysis was performed assuming 1:1 (antibody to antibody) binding with double

reference subtraction (i.e. subtracting the reference response first and then subtracting the zero-concentration sensogram to compensate for drift and small differences between the reference and the active channel). Data was processed using Langmuir (1:1) binding analysis. The model describes a 1:1 interaction and is commonly used for initial evaluation. Data fitting was performed using the closest of fit based on the chi-square value, which describes the deviation between the experimental and fitted curves. The fitting algorithm seeks to minimize chi square.

[0157] *Antibody binding to plate-bound recombinant human IL-38.* Recombinant human IL-38 (Full-length, Adipogen) was diluted to a final concentration of 0.1 µg/ml in PBS, dispensed into high binding plates (Corning) and incubated overnight at 4°C. The plate was then washed three times with wash buffer (PBS+ 0.05% Tween) and blocked with blocking buffer (PBS + 2% BSA) at room temperature (RT) for 1h. Anti-IL-38 antibodies were incubated for 2h at RT followed by three washes with wash buffer. The HRP-conjugated rabbit anti-mouse secondary antibody (Southern Biotech) was incubated at RT for 2h followed by three washes with wash buffer. OPD substrate (Sigma) was incubated for 10-15 min in dark at RT prior to the addition of stop solution (2N sulfuric acid, R&D systems). The amount of hu-IL-38 binding to sample/standard was determined by measuring absorbance at 450 nm using an EnSpire plate reader (model number: 2300-0000, PerkinElmer).

[0158] *Neutralization of human IL-38 binding to IL1RAPL1 or IL-36R.* Human IL1RAPL1-Fc (Sino Biological, #10177-H02H) and human IL-36R-Fc (R&D Systems) were diluted in coating Buffer (50 mM Carbonate-Bicarbonate Buffer, pH 9.4) at final concentration of 0.5 µg/mL and coated on MSD high bind plates (MSD) for approximately two hours at RT. Recombinant human IL-38 (Adipogen) was diluted in assay Diluent (1% BSA, 0.1% Tween 20, in 1x PBS). For antibody testing, anti-IL-38 antibodies were pre-incubated with IL-38 for approximately one hour at RT. IL-38 or antibody-IL-38 mixture was transferred to pre-coated MSD plates and incubated for approximately two hours at RT. After washing, the MSD plate was incubated with biotinylated mouse anti-human IL-38 (clone H127C, Thermo Fisher) at final concentration of 100 ng/mL and Sulfo-Tag-Streptavidin (MSD) at final concentration of 100 ng/mL for 1 hour at RT. Binding of IL-38 to plate-bound IL1RAPL1-Fc or human IL-36R-Fc was detected using the Meso Sector S 600 plate reader. Data are plotted using GraphPad Prism version 9.0.1. Dose response curves were fitted with "[Inhibitor] vs. response -- Variable slope (four parameters)" method.

[0159] **Example 10. IMM20324 demonstrates a favorable PK profile and is well tolerated in C57BL/6 mice.** Since IMM20324 binds to mouse IL-38, we used syngeneic mouse models to assess

pharmacokinetics and anti-tumor activity. After a single 10 mg/kg dose of IMM20324, plasma concentrations remained above 10 mg/mL after one week when administered i.p. and i.v. (Figure 28a; Table 8).

Table 8. Pharmacokinetics of IMM20324 following dosing by IV and IP administration routes.

<i>Parameter</i>	<i>Units</i>	<i>IV Dosing</i>	<i>IP Dosing</i>
t _{1/2}	h	91.0429	146.566
T _{max}	h	0.083	24
C _{max}	ng/ml	192288	80131
AUC 0-t	ng/ml*h	9534732	9601759
Cl _{obs}	(mg/kg)/(ng/ml)/h	7.4E-07	5.7E-07
V _{ss_obs}	(mg/kg)/(ng/ml)	9.8E-05	0.00012

Serum concentrations of IMM20324 were further increased when multiple doses were administered once or twice per week for 3 weeks (Figure 28b). Importantly, plasma levels of IMM20324 correlated with the dosing schedule, as groups that received higher and more frequent doses of IMM20324 had the highest plasma levels. IMM20324 was also well tolerated, as all mice gained weight normally throughout the study (Figure 29a). On day 21, mice were sacrificed, and gross analysis of the organs revealed no obvious abnormalities and no significant enlargement of the spleen (Figure 29b). To determine whether any systemic upregulation of inflammatory cytokines was occurring in these mice, a Luminex-based multiplex analysis of the plasma was performed. Overall, there was no significant increase in inflammatory cytokines in any group, however, some individual mice did exhibit a small increase in several cytokines and chemokines after IMM20324 treatment (Figure 29c). Together, these data demonstrate that IMM20324 shows good systemic stability and has limited toxicities following administration.

[0160] *Pharmacokinetic and tolerability analysis of IMM20324.* For single dose pharmacokinetic analysis, 6-7-week-old C57BL/6 female mice were given a 10 mg/kg dose of IMM20324 either i.p. or i.v. At each timepoint, blood from three mice was collected retro-orbitally or by cardiac puncture in K2-EDTA tubes and plasma was isolated. For multi-dose tolerability study, 5-6-week-old C57BL/6 female mice were randomly assigned to groups and dosed as indicated and body weight was measured twice per week. On Day 21, blood was collected in K2-EDTA tubes and plasma was isolated. Spleens from all mice were removed and weighed.

[0161] Plasma concentration of IMM20324 was determined by a direct IL-38 ELISA. 25 ng/mL human IL-38 (Adipogen, #AG-40A-0191-C050) was coated to each well of a high binding 96 well plate (Corning, #3369) overnight 4°C. Wells were blocked with PBS + 2% BSA at room temperature for 1 hour then washed 3 times with PBS + 0.05% Tween. Plasma samples were diluted in PBS, added to each well for 2 hours at room temperature, and then wells were washed 3 times with PBS + 0.05% Tween. Anti-mouse IgG-HRP was added to each well for 2 hours at room temperature, and then wells were washed 3 times with PBS + 0.05% Tween. OPD substrate (Sigma, #P8287) diluted in phospho-citrate/sodium perborate buffer was added per well, developed for 5-30 minutes, and absorbance was measure at 450 nm.

[0162] **Example 11. IMM20324 demonstrates anti-tumor therapeutic efficacy as a single agent in B16.F10 syngeneic mouse model.** Antibodies targeting the PD-1/PD-L1 axis are most successful in tumors, which have high immune cell infiltration. The B16.F10 melanoma syngeneic mouse model is considered a rapidly growing and immunologically cold tumor. Previous reports showed that anti-PD-1/PD-L1 treatments demonstrated limited efficacy in this model. To test whether anti-IL-38 could induce an anti-tumor response in these immunologically cold tumors semi-therapeutically, IMM20324 was administered i.p. for every three days starting when tumor volume reached 75 mm³. Significantly reduced tumor size was observed throughout the duration of the study after IMM20324 treatment at both 10 mg/kg and 50 mg/kg doses (Figure 30a; Table 9).

Table 9. Linear mixed effects modeling of IMM20324 treatment in the B16.F10 syngeneic model.

Predictors	Estimates	CI	Volume		df
			p		
(Intercept)	-418.67	-774.01 – -63.33	0.021		186.93
IMM20324 (10 mg/kg)	-48.24	-93.35 – -3.14	0.036		248.38
IMM20324 (50 mg/kg)	-12.90	-58.27 – 32.46	0.576		247.38

At Day 10, 15 of 15 mice in the 10 mg/kg group and 14 of 15 mice in the 50 mg/kg IMM20324-treated group had a tumor volume below the mean of that observed in the isotype control group (Figure 30b). Ultimately IMM20324 treatment led to delayed time when tumors reached 1500 mm³ in volume during dosing period or throughout the duration of the study (Figure 30c). In conclusion, IMM20324 treatment is efficacious in immunologically cold, B16.F10 melanoma syngeneic tumor.

[0163] **PK/PD analysis.** C57BL/6 mice were inoculated with B16.F10 melanoma cells in right flank. At day 0, mice were randomized based on tumor volume and treated with 3 mg/Kg, 10 mg/Kg or 30 mg/Kg dose of IMM20324 or 30 mg/Kg isotype control or 10 mg/Kg anti-PD-L1 intraperitoneally every three days for 4 total doses. Tumor volumes were measured every 3 days. The half-life of

IMM20324 in tumor bearing mice is 190~222 hours, which supports every three days dosing (FIG. 32A). After antibody treatments for every three days (4 times total doses) at depicted doses, IMM20324 showed dose dependent inhibition of tumor growth. The effects of IMM20324 at 30 mg/Kg dose is comparable to positive control, anti-mPD-L1 at 10 mg/Kg (FIG. 32B). Higher IMM20324 concentrations in terminal serum 24 hr post 4th doses were observed in mice in higher dose groups. There is a trend of negative association of terminal tumor volumes and terminal exposures of IMM20324 in serum (FIG. 32C). Chemokines expressed in tumors can recruit different immune cells and initiate anti-tumor immune responses. Higher chemokine concentrations are associated with smaller terminal tumor volumes (Data not shown). Total protein is extracted from snap frozen B16.F10 tumors after IMM20324 treatment. Chemokines in tumor extract were measured by Mouse Cytokine/Chemokine 31-plex Discovery Assay Array. IMM20324 treatment showed exposure dependent increase of chemokines in tumors. Concentrations of IMM20324 in terminal serum (24 hr after 4th dose) are negatively correlated with CXCR3 ligands: CXCL9/MIG or CXCL10/IP10 (FIG.33) and CCRs ligands: CCL3/MIP1 α , CCL4/MIP1 β (FIG.34) and CCL11/Eotaxin (FIG.35). These data demonstrated that blockade of IL-38 in vivo with IMM20324 results in increase of chemokine production and potentially recruitment of immune cells into tumors. These findings are also in line with findings from TCGA and Tempus analysis that IL-38 negatively regulates immune infiltration in tumors.

[0164] *B16.F10 syngeneic tumor model.* 2.5×10^5 B16.F10 cells were implanted into the flank for 6-week-old C57BL/6 female mice. When average tumor volume reached 50-100 mm³ approximately 8 days later, mice were randomly assigned to indicated treatment groups and first dose of CD1-M3 was administered. For the duration of the study, tumors were measured with a digital caliper twice per week and tumor volume was calculated by (longest diameter) x (shortest diameter)²/2. Mice were sacrificed when tumor volume reached 3000 mm³, in accordance with the IACUC protocol.

[0165] *Analysis of tumor growth rate and time-tumor-volume.* Tumor growth curves for each animal were fitted using the exponential model of growth (tumor volume = $a \cdot \exp(b \cdot \text{days})$), which provided the highest R² and lowest AIC value. The time to reach a predicted tumor volume was then calculated by intercept the tumor volume with fitted tumor growth curve. Tumor growth rate in percentages were calculated using a difference in time with lag and difference in tumor volume with lag using equations below:

$$\text{Difference in Days} = \text{Timepoint} - \text{lag}(\text{Timepoint})$$

$$\text{Difference in Growth} = \text{Volume} - \text{lag}(\text{Volume})$$

$$\text{Tumor growth rate \%} = (\text{Difference in Growth} / \text{Difference in Days}) / \text{lag}(\text{Volume}) * 100$$

Finally, the tumor volume comparison between treatment arms was analyzed using linear mixed effects model using tumor volume as the outcome and treatment groups as the fixed variable absorbing the variance of time. As this was a nested experimental design, individual animals were controlled for as a random effect. Results allow the comparison of each treatment arm compared to control with and without absorbing the variance of time. This LME analysis was completed with all the time points.

[0166] Analysis of cytokine and chemokines. Multiplexing analysis was performed using the Luminex™ 200 system (Luminex, Austin, TX, USA) by Eve Technologies Corp. (Calgary, Alberta). Thirty-two markers were simultaneously measured in the samples using Eve Technologies' Mouse Cytokine 32-Plex Discovery Assay® (MilliporeSigma, Burlington, Massachusetts, USA) according to the manufacturer's protocol. The 32-plex consisted of Eotaxin, G-CSF, GM-CSF, IFN γ , IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17, IP-10, KC, LIF, LIX, MCP-1, M-CSF, MIG, MIP-1 α , MIP-1 β , MIP-2, RANTES, TNF α , and VEGF. Assay sensitivities of these markers range from 0.3 – 30.6 pg/mL for the 32-plex. Individual analyte sensitivity values are available in the MilliporeSigma MILLIPLEX® MAP protocol.

[0167] **Example 12. IMM20324 induces long-term anti-tumor immunity in a subset of EMT6 tumor-bearing mice and protects against tumor re-challenge.** IMM20324 was also tested for the ability to inhibit tumor growth of the EMT6 breast cancer syngeneic mouse model. At Day 15, half of IMM20324 treated mice showed decreased tumor growth (Figure 31a; Table 10) and several IMM20324 treated mice had no detectable tumor after all doses were administered (Figure 31b).

Table 10. Linear mixed effects modeling of IMM20324 treatment in the EMT6 syngeneic model.

Predictors	Estimates	CI	Volume	
			p	df
(Intercept)	-92.16	-361.66 – 177.33	0.498	80.75
Anti-CTLA4 (10 mg/kg)	-87.14	-96.92 – -77.37	<0.001	674.87
IMM20324 (10 mg/kg)	-31.57	-42.57 – -20.56	<0.001	680.48
IMM20324 (15 mg/kg)	-55.58	-66.67 – -44.49	<0.001	682.05
IMM20324 (25 mg/kg)	-56.44	-66.85 – -46.04	<0.001	679.34

To determine whether IMM20324 treatment induced a protective anti-tumor memory response, cured mice were then re-challenged with EMT6 tumor cells in the opposite flank without additional IMM20324 dosing. All cured mice tested did not exhibit appreciable tumor growth several weeks after inoculation compared to naïve age-matched controls (Figure 31c) leading to a significant increase in survival (Figure 31d).

[0168] Taken together, the above results indicate that blocking the immunosuppressive function of IL-38 with IMM20324 can restore inflammatory cytokine production. Furthermore, IMM20324 treatment induces significant tumor regression in immunologically cold tumors such as B16.F10, that is mediated by adaptive immune cells.

[0169] *EMT6 syngeneic tumor model.* 5×10^5 EMT6 cells were implanted into the flank for 6-8-week-old BALB/c female mice. When average tumor volume reached 50-100 mm³ approximately 8 days later, mice were randomly assigned to indicated treatment groups and first dose of CD1-M3 was administered. For the duration of the study, tumors were measured with a digital caliper twice per week and tumor volume was calculated by (longest diameter) x (shortest diameter)² x 0.52. Mice were sacrificed when tumor volume reached 3000 mm³, in accordance with the IACUC protocol.

[0170] Analysis of tumor growth rate and time-tumor-volume was as described above.

[0171] **Example 13. IL-38 protein expression in tumor samples detected by immunohistochemistry (IHC).** To evaluate IL-38 protein expression in primary cancer samples, tumor microarrays comprising tumors from multiple cancer indications were assessed for IL-38 expression using IL-38-specific antibodies using immunohistochemistry. Cancer types were selected based on RNA expression data and arrays were composed of tumors from multiple subtypes of each indication and those at distinct stages. Following antigen retrieval and blocking, staining was performed using IMM20130 anti-IL38 mouse antibody, which were detected with a horseradish peroxidase (HRP) labeled secondary antibody. Images were scored for positivity by a board-certified pathologist using a 0 (no expression) to 6 (strong expression) scale. Expression was also noted as either cytoplasmic or nuclear. Staining was not observed in other compartments.

[0172] Analysis of head and neck squamous carcinoma (HNSCC) samples demonstrated expression in multiple samples, consistent with previous RNA expression analyses. Cytoplasmic expression was generally weak to moderate in intensity, while nuclear staining was more intense and was present on higher frequency of samples (Figure 36). Although nuclear staining was lower in

stage I samples compared to later stages of this disease, this group was limited in terms of the number of samples it contained (Figure 37). No differences in cytoplasmic expression were observed in specific stages of disease. Cervical squamous carcinoma (CSCC) showed a similar pattern of staining with expression noted in a significant number of samples, mainly within the nuclear compartment of tumor cells (Figure 38). Expression in the nucleus was of higher intensity compared to that observed within the cytoplasm. The intensity of nuclear staining was universally high, except in stage IIIa samples where it was lower (Figure 39). Cytoplasmic staining was more variable with highest expression at stages Ia and IIb. Analysis of lung cancer tumor tissues showed a high frequency of positive tissues, with results again demonstrating that nuclear staining was of a higher intensity compared to cytoplasmic expression (Figure 40). Nuclear expression was consistently high throughout distinct stages. In contrast, cytoplasmic expression was not observed in stage I samples but was equivalent in tumors from a later stages of disease (Figure 41). Analysis of expression based on subtypes of lung cancer demonstrated cytoplasmic staining that was evident in a small number of most subtypes including adenocarcinoma and squamous cell carcinoma (Figure 42). In contrast, nuclear expression, although present in all subtypes of lung cancer, was highest in intensity in small cell undifferentiated carcinoma and squamous carcinoma Figure 43.

[0173] Tumor microarrays composed of human melanoma samples were also assessed for IL-38 expression using both IMM20130 and H127c, a commercially available mouse anti-human IL-38 antibody (Thermo Scientific). Scoring was performed using a scale between 0 (no expression) and 5 (strong expression). Localization was not scored in this study. The two antibodies showed some differences in the intensity in individual samples. A higher percentage of positive samples was observed with IMM20130 and these were at higher levels of intensity, indicating that this antibody was more sensitive than H127c. This data provided confirmation of the accuracy of IMM20130 for the quantification of IL-38 expression.

[0174] Overall, the IHC data showed good correlation with RNA expression data, and supported the conclusion that IL-38 expression is a feature of tumor cells in multiple cancer indications.

What is claimed:

1. A method of treating cancer in an individual comprising administering to the individual an antibody that binds to IL-38, wherein the antibody comprises

a VH CDR1, a VH CDR2 and a VH CDR3 of a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:22 and a VL CDR1, VL CDR2 and a VL CDR3 of a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:57;

a VH CDR1, a VH CDR2 and a VH CDR3 of a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:98 and a VL CDR1, VL CDR2 and a VL CDR3 of a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:101;

a VH CDR1, a VH CDR2 and a VH CDR3 of a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:7 and a VL CDR1, VL CDR2 and a VL CDR3 of a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:10;

a VH CDR1, a VH CDR2 and a VH CDR3 of a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:87 and a VL CDR1, VL CDR2 and a VL CDR3 of a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:92;

a VH CDR1, a VH CDR2 and a VH CDR3 of a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:27 and a VL CDR1, VL CDR2 and a VL CDR3 of a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:57;

a VH CDR1, a VH CDR2 and a VH CDR3 of a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:37 and a VL CDR1, VL CDR2 and a VL CDR3 of a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:67;

a VH CDR1, a VH CDR2 and a VH CDR3 of a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:42 and a VL CDR1, VL CDR2 and a VL CDR3 of a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:72;

a VH CDR1, a VH CDR2 and a VH CDR3 of a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:47 and a VL CDR1, VL CDR2 and a VL CDR3 of a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:77;

a VH CDR1, a VH CDR2 and a VH CDR3 of a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:32 and a VL CDR1, VL CDR2 and a VL CDR3 of a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:62;

a VH CDR1, a VH CDR2 and a VH CDR3 of a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:52 and a VL CDR1, VL CDR2 and a VL CDR3 of a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:82;

a VH CDR1, a VH CDR2 and a VH CDR3 of a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:2 and a VL CDR1, VL CDR2 and a VL CDR3 of a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:4;

2. The method of claim 1, wherein the antibody comprises

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO:23, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO:24, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 25, a VL CDR1 comprising the amino acid sequence set forth in SEQ ID NO:58, a VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO:59, and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO:60;

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO:15, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO:16, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO:17, a VL CDR1 comprising the amino acid sequence set forth in SEQ ID NO:18, a VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO:19 and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO:20;

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO:88, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO:89, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 90, a VL CDR1 comprising the amino acid sequence set forth in SEQ ID NO:93, VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO:94, and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO:95

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 33, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 34, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 35, a VL CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 63, a VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 64, and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 65;

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 48, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 49, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 50, a VL CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 78, a VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 79, and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 80;

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 38, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 39, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 40, a VL CDR1 comprising the amino acid sequence set forth in

SEQ ID NO: 68, a VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 69, and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 70;

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 43, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 44, a VH CDR3 of SEQ ID NO: 45, a VL CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 73, a VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 74, and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 75; or

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 28, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 29, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 30, a VL CDR1 of SEQ ID NO: 58, a VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 59, and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 60;

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 53, a VH CDR2 of SEQ ID NO: 54, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 55, a VL CDR1 comprising the amino acid sequence set forth in SEQ ID NO: 83, a VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO: 84, and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 85.

3. The method of claim 1 or 2, wherein the antibody that specifically binds to IL-38 comprises

a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:22 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:57;

a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:98 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:101;

a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:7 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:10; and

a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:87 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:92.

4. The method of any one of claims 1-3, wherein the antibody that specifically binds to IL-38 comprises

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO:23, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO:24, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 25, a VL CDR1 comprising the amino acid sequence set forth in

SEQ ID NO:58, a VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO:59, and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO:60;

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO:15, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO:16, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO:17, a VL CDR1 comprising the amino acid sequence set forth in SEQ ID NO:18, a VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO:19 and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO:20;

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO:88, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO:89, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 90, a VL CDR1 comprising the amino acid sequence set forth in SEQ ID NO:93, VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO:94, and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO:95

5. The method of claim 4, wherein the antibody that specifically binds to IL-38 comprises

a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO:23, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO:24, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 25, a VL CDR1 comprising the amino acid sequence set forth in SEQ ID NO:58, a VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO:59, and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO:60.

6. The method of claim 5, wherein the antibody that specifically binds to IL-38 comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:98 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:101.

7. The method of any one of claims 1, 2, 4, or 5, wherein the CDRs are defined by the North method or the Kabat method.

8. The method of any one of claims 1-7, wherein the antibody that specifically binds to IL-38 is a humanized antibody.

9. The method of any one of claims 1-4, wherein the antibody that specifically binds to IL-38 is a human antibody.

10. The method of any one of claims 1-9, wherein the cancer expresses a high level of IL-38.
11. The method of any one of claims 1-10, wherein the antibody that specifically binds to IL-38 partially or fully blocks, inhibits, or neutralizes a biological activity of IL-38.
12. The method of any one of claims 1-11, wherein the individual is human.
13. The method of any one of claims 1-12, wherein the level of one or more proinflammatory cytokines is increased.
14. The method of any one of claims 1-13, wherein the level of one or more of IL-6, CCL4, CCL3, CXCL1, CXCL10, or GM-CSF is increased.
15. The method of any one of claims 1-14, wherein the cancer is a stage I, stage II, stage III, or stage IV cancer.
16. The method of any one of claims 1-15, wherein the antibody that specifically binds to IL-38 is administered at a dose of about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 10 mg/kg, about 15 mg/kg, about 25 mg/kg, 30 mg/kg, about 15 mg/kg, or about 50 mg/kg.
17. The method of any one of claims 1-16, wherein the antibody that specifically binds to IL-38 is a bispecific antibody.
18. The method of any one of claims 1-17, wherein the antibody that specifically binds to IL-38 is an isolated variable heavy (VH) single domain monoclonal antibody, a (sc)Fv-Fc, a Fv, a scFv, a Fab, a F(ab')₂, a Fab' fragment, a bispecific antibody, or a diabody.
19. The method of any one of claims 1-18, wherein the cancer is a squamous cell carcinoma or an adenocarcinoma.
20. The method of any one of claims 1-19, wherein the cancer is selected from the group consisting of gastroesophageal cancer, bladder cancer, cervical cancer, prostate cancer, breast

cancer, renal cancer, colorectal cancer, pancreatic cancer, melanoma, uterine cancer, head and neck cancer, lung cancer and skin cancer.

21. The method of any one of claims 1-20, wherein the cancer is selected from the group consisting of head and neck squamous cell carcinoma (HNSC), esophagus cancer (ESCA), squamous cell cancer of the lung (LUSC), cancer of the cervix (CESC), bladder cancer (BLCA), skin cutaneous melanoma (SKCM), prostate adenocarcinoma (PRAD), gastroesophageal carcinoma, cervical squamous cell carcinoma, cervical squamous cell carcinoma, skin squamous cell carcinoma, basal cell carcinoma, skin cutaneous melanoma, lung adenocarcinoma, endocervical adenocarcinoma, bladder urothelial carcinoma and prostate adenocarcinoma and lung adenocarcinoma (LUAD).

22. The method of claim 21, wherein the cancer is squamous cell cancer of the lung (LUSC).

23. The method of claim 21, wherein the cancer is gastroesophageal carcinoma.

24. The method of claim 21, wherein the cancer is head and neck squamous cell carcinoma.

25. An isolated antibody that specifically binds to IL-38 comprising a VH CDR1, a VH CDR2 and a VH CDR3 of a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:87 and a VL CDR1, VL CDR2 and a VL CDR3 of a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:92.

26. The isolated antibody that specifically binds to IL-38 of claim 25, wherein the antibody comprises a VH CDR1 comprising the amino acid sequence set forth in SEQ ID NO:88, a VH CDR2 comprising the amino acid sequence set forth in SEQ ID NO:89, a VH CDR3 comprising the amino acid sequence set forth in SEQ ID NO: 90, a VL CDR1 comprising the amino acid sequence set forth in SEQ ID NO:93, VL CDR2 comprising the amino acid sequence set forth in SEQ ID NO:94, and a VL CDR3 comprising the amino acid sequence set forth in SEQ ID NO:95.

27. An isolated antibody that specifically binds to IL-38 comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:98 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:101; or

a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:87 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:92

28. The antibody that specifically binds to IL-38 of claim 27 comprising
a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:98 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO:101.
29. The antibody that specifically binds to IL-38 of claim 26, wherein the CDRs are defined by the North method or the Kabat method.
30. The antibody that specifically binds to IL-38 of any one of claims 25-29, wherein the antibody that specifically binds to IL-38 is a humanized antibody.
31. The antibody that specifically binds to IL-38 of any one of claims 25-30, wherein the antibody that specifically binds to IL-38 partially or fully blocks, inhibits, or neutralizes a biological activity of IL-38.
32. The antibody that specifically binds to IL-38 of any one of claims 25-31, wherein the antibody that specifically binds to IL-38 is an isolated variable heavy (VH) single domain monoclonal antibody, a (sc)Fv-Fc, a Fv, a scFv, a Fab, a F(ab')₂, a Fab' fragment, a bispecific antibody, or a diabody.
33. A composition comprising the antibody of any one of claims 25-32.
34. Nucleic acid(s) encoding the antibody of any one of claims 25-32.
35. A vector comprising the nucleic acid(s) of claim 34.
36. A host cell comprising the nucleic acid of claim 34 or the vector of claim 35.
37. The host cell of claim 36, wherein the host cell is a CHO cell.