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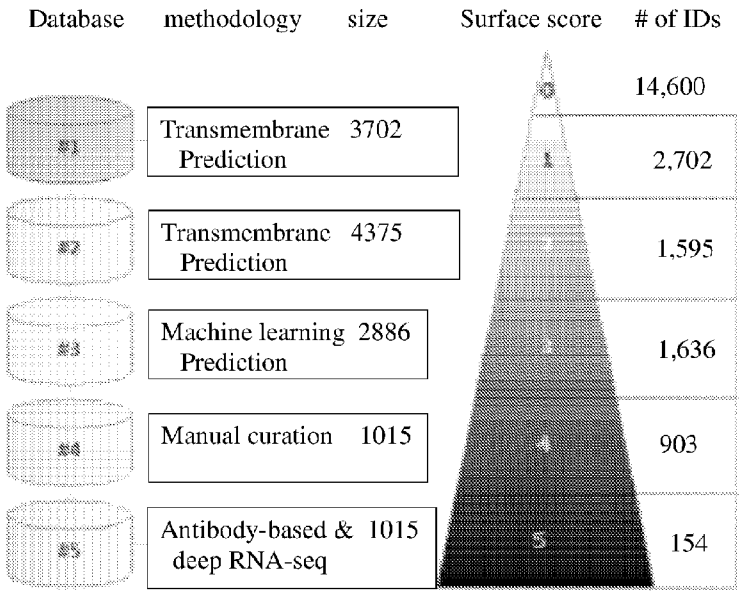
Fig. 1B



(57) Abstract: Surface proteins predominantly associated with multiple myeloma are identified as potential targets for developing anti-multiple myeloma therapeutics. In accordance with one embodiment antibodies are generated that specifically bind to epitopes of the identified protein that are associated with multiple myeloma cells. These antibodies can then be used to target the delivery of cytotoxic agents to multiple myeloma cells in a patient or used to prepare CAR T-cells for the treatment of multiple myeloma patients.

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IMMUNOTHERAPEUTIC TARGETS IN MULTIPLE MYELOMA AND METHODS FOR THEIR IDENTIFICATION

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application No. 63/000,694 filed on March 27, 2020, the disclosure of which is expressly incorporated herein.

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

Incorporated by reference in its entirety is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: 1,490 kilobytes ASCII (text) file named "335022_ST25," created on March 22, 2021.

BACKGROUND OF THE DISCLOSURE

Multiple myeloma (MM), also known as plasma cell myeloma, is a cancer of plasma cells, a type of white blood cell that normally produces antibodies. Globally, multiple myeloma affected 488,000 people and resulted in 101,100 deaths in 2015. In the United States, it develops in 6.5 per 100,000 people per year and 0.7% of people are affected at some point in their lives. Without treatment, typical survival is seven months. With current treatments, survival is usually 4–5 years.

Targeting tumor antigens with immunotherapy is rapidly emerging as a promising approach for cancer treatment. This is based on successes of antibody-mediated checkpoint blockade and engineered T cells. In MM, monoclonal antibodies, antibody-drug conjugates, bi-specific antibody constructs, and Chimeric Antigen Receptor (CAR) T-cell therapy targeting BCMA (B-cell maturation antigen) are significantly improving survival in patients with MM. Data from >20 clinical trials involving anti-BCMA CAR T cells have demonstrated that patients with relapsed and/or refractory MM can achieve objective responses.

While tremendous progress in the treatment of Multiple Myeloma (MM) has been made over the past 25 years, myeloma remains an incurable disease, with a particularly poor prognosis for patients with refractory relapsed MM (RRMM) or high-risk cytogenetics. The remarkable success of CD19-Chimeric Antigen Receptor

(CAR) T cells in patients with lymphoid malignancies has prompted the development of CAR T cells for treating MM. BCMA (aka TNFRSF17) is the first surface target utilized to generate CAR T cells for patients with RRMM who have undergone at least three prior treatments, including treatment with a proteasome inhibitor and an immunomodulatory agent. Targeting BCMA with antibody conjugates and bispecific T-cell engagers (BiTE; a unique artificial bispecific monoclonal antibody that has two linked, single-chain variable fragments and having a 1 + 1 antigen-binding valency) has been demonstrated to have efficacy in treating MM. Both BCMA targeting immune-therapies were granted breakthrough status for patients with RRMM by FDA. All these trials demonstrate impressive results with the ability of anti-BCMA CAR T cells to induce deep responses in highly pretreated RRMM, however, despite this, remissions are not sustained and the majority of patients eventually relapse. One of the mechanisms of resistance lies in the antigen loss or downregulation with the emergence of low BCMA or BCMA-negative subclones. Identifying alternative targets is crucial to provide therapeutic options to patients who failed BCMA CAR therapy and/or design combinatorial strategies limiting the risk of antigen escape. One of the most important determinants of the success of CAR T-cell therapy is the choice of the target antigen; an ideal target should be highly expressed on all tumor cells, on cancer stem cells, in most patients, absent in normal counterparts and most organs of the whole body. Given that, studying the myeloma surface proteome is critical to identify additional immunotherapeutic targets and understand the role of an altered surfaceome in the disease biology. In fact, surface proteins may mediate regulatory mechanisms underpinning myeloma manipulation of the bone marrow microenvironment.

To this purpose, one aspect of the present disclosure is directed to the use of high-quality Mass-Spectrometry methodologies and generated integrative bioinformatics tools to unbiasedly and accurately map the cell surface of MM cell lines and primary MM patient samples bearing distinct genetic backgrounds. These helped overcome the challenge of studying surface proteins that present with low abundance, high hydrophobicity and heavy post-translational modifications compared to intracellular proteins. In accordance with the present disclosure methods are provided for identifying additional targets that can be used to design alternative CAR T-cell therapeutics beyond BCMA.

SUMMARY

The present disclosure is directed to the identification of candidate cell surface antigens that can be utilized as immuno-therapeutic targets for developing novel drugs including antibodies and CAR T cells for diagnosing and treating patients with Multiple Myeloma. The antibodies of the present invention can also be used to identify disease markers for diagnostic purposes like flow cytometry. In accordance with one embodiment the identified targets disclosed herein have the potential to serve as marker for identifying and treating those Multiple Myeloma (MM) patients that display surface targets significantly associated with features of poor prognosis (i.e. associated with high-risk MM).

In accordance with one embodiment a method is provided for identifying target cell surface antigens that are specific for multiple myeloma cells and can be used as immuno-therapeutic targets. In one embodiment the method comprises performing surface-specific proteomic analyses of multiple myeloma cell lines bearing distinct genetic abnormalities using biotin labeling followed by Mass-Spectrometry analysis (MS). In a further embodiment RNA-seq datasets of MM patients are investigated to identify surface proteins whose gene expression is elevated in MM patients relative to normal tissues. In one embodiment, a method is provided for identifying target cell surface antigens that are specific for multiple myeloma cells wherein the method comprises Mass-Spectrometry analysis of surface labeled proteins and analysis of RNA-seq datasets of MM patients to identify proteins common to both analysis as target cell surface antigens.

In accordance with one embodiment cell surface polypeptides having the sequence of SEQ ID NO: 1 through SEQ ID NO: 155 have been identified as being associated with MM cells. Accordingly, the peptides of SEQ ID NO: 1-155 represent targets for immuno-based therapeutic strategies for treating MM. In accordance with one embodiment an antibody that specifically binds to a polypeptide selected from the group consisting of SEQ ID NO: 1-155 is provided. In accordance with one embodiment the antibody is a monoclonal antibody. Also encompassed by the present invention are antibody fragments wherein the fragment retains the ability to bind to the same epitope as the whole antibody.

In accordance with one embodiment cell surface polypeptides IL12RB1, SLC5A3, CCR1, ANKH, IL27RA, S1PR4, TLR1, KCNA3, PSEN2, BCMA, IL2RG, LILRB4, IL6R, ITGB7, LRP10, FCRL3, IFNGR1, SLAMF6, SLCO3A1, LILRB1,

PLXNA3, SLC17A5, CD28, LAX1, NEMP1, TMEM154, SEMA4A, C10orf54, ITM2C, LY9, SLAMF7, ITGA4, LRRC8A, LRRC8D, CD320, KCNN4, PLXNC1, CD37, SELPLG, DAGLB, ABCC4, ADAM9, CD4, CD180, CD48, CD40, MCUR1, ABCC5, IL6ST, LRP8, SLC5A6, SLC7A6, HLA-F, ICAM2, LEPROT, ITGAL, TMEM63A, CMTM7, IL10RB, NDC1, PTPRCAP, ANTXR2, ABCA7, FCGR2B, ACVR1B, STS, ABHD12, TNFRSF10A, HVCN1, SLC39A10, EMP3, ABCC1, SLC26A6, SLC6A6, CCR10, SLC30A1, SLC231A1, ADCY3, IFNAR1, PTPRJ, CLDND1, SLC30A5, SLC6A9, ADAM15, IGF2R, INSR, NOTCH2, CD53, SLC12A9, SLC15A4, CEMIP2, ADAM17, MPZL1 and TACI have been identified as being associated with MM cells. Accordingly, these peptides represent targets for immuno-based therapeutic strategies for treating MM. In accordance with one embodiment an antibody that specifically binds to one of these polypeptides is provided. In accordance with one embodiment the antibody is a monoclonal antibody. Also encompassed by the present invention are antibody fragments wherein the fragment retains the ability to bind to the same epitope as the whole antibody.

In accordance with one embodiment cell surface polypeptides IL12RB1, CCR1, LILRB4, FCRL3, IFNGR1, SLAMF6, LAX1, SEMA4A, ITGA4, LRRC8D and CD320 have been identified as being associated with MM cells. Accordingly, these peptides represent targets for immuno-based therapeutic strategies for treating MM. In accordance with one embodiment an antibody that specifically binds to one of these polypeptides is provided. In accordance with one embodiment the antibody is a monoclonal antibody. Also encompassed by the present invention are antibody fragments wherein the fragment retains the ability to bind to the same epitope as the whole antibody.

In accordance with one embodiment an antibody or antibody fragment is provided that binds to a polypeptide selected from the group consisting of SEQ ID NO: 1-115. In accordance with one embodiment the antibody or antibody fragment binds to a polypeptide selected from the group consisting of SEQ ID NO: 1-10. In accordance with one embodiment the antibody or antibody fragment binds to a polypeptide selected from the group consisting of SEQ ID NO: 11-20. In accordance with one embodiment the antibody or antibody fragment binds to a polypeptide selected from the group consisting of SEQ ID NO: 21-30. In accordance with one embodiment the antibody or antibody fragment binds to a polypeptide selected from the group consisting of SEQ ID NO: 31-40. In accordance with one embodiment the

antibody or antibody fragment binds to a polypeptide selected from the group consisting of SEQ ID NO: 41-50. In accordance with one embodiment the antibody or antibody fragment binds to a polypeptide selected from the group consisting of SEQ ID NO: 51-60. In accordance with one embodiment the antibody or antibody fragment binds to a polypeptide selected from the group consisting of SEQ ID NO: 61-70. In accordance with one embodiment the antibody or antibody fragment binds to a polypeptide selected from the group consisting of SEQ ID NO: 71-80. In accordance with one embodiment the antibody or antibody fragment binds to a polypeptide selected from the group consisting of SEQ ID NO: 81-90. In accordance with one embodiment the antibody or antibody fragment binds to a polypeptide selected from the group consisting of SEQ ID NO: 91-100. In accordance with one embodiment the antibody or antibody fragment binds to a polypeptide selected from the group consisting of SEQ ID NO: 101-110. In accordance with one embodiment the antibody or antibody fragment binds to a polypeptide selected from the group consisting of SEQ ID NO: 111-120. In accordance with one embodiment the antibody or antibody fragment binds to a polypeptide selected from the group consisting of SEQ ID NO: 121-130. In accordance with one embodiment the antibody or antibody fragment binds to a polypeptide selected from the group consisting of SEQ ID NO: 131-140. In accordance with one embodiment the antibody or antibody fragment binds to a polypeptide selected from the group consisting of SEQ ID NO: 141-147.

In accordance with one embodiment a monoclonal antibody is provided that specifically binds to a polypeptide selected from the group consisting of SEQ ID NO: 168-208. In accordance with one embodiment a monoclonal antibody is provided wherein the antibody specifically binds to a polypeptide having as least 90, 95 or 99% sequence identity to a polypeptide selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 37, SEQ ID NO: 42, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 79, SEQ ID NO: 112 and SEQ ID NO: 104.

In accordance with one embodiment a monoclonal antibody is provided wherein the antibody specifically binds to a polypeptide having as least 90, 95 or 99% sequence identity to a polypeptide selected from the group consisting of CCR1 (SEQ ID NO: 60), CD320 (SEQ ID NO: 56), FCRL3(SEQ ID NO: 57), IFNGR1 (SEQ ID NO: 79), IL12RB1 (SEQ ID NO: 19), ITGA4 (SEQ ID NO: 42), LILRB4 (SEQ ID

NO: 20), LRRC8D (SEQ ID NO: 112), SEMA4A (SEQ ID NO: 104), SLAMF6 (SEQ ID NO: 37) and TNFRSF17 (SEQ ID NO: 167).

In accordance with one embodiment a monoclonal antibody is provided wherein the antibody specifically binds to a polypeptide having as least 90, 95 or 99% sequence identity to a polypeptide selected from the group consisting of IL12RB1, CCR1(SEQ ID NO: 60), LILRB4 (SEQ ID NO: 20), FCRL3 (SEQ ID NO: 57), IFNGR1 (SEQ ID NO: 79), SLAMF6 (SEQ ID NO: 37), SEMA4A (SEQ ID NO: 104), ITGA4 (SEQ ID NO: 42), LRRC8D (SEQ ID NO: 112) and CD320 (SEQ ID NO: 56).

In accordance with one embodiment a monoclonal antibody is provided wherein the antibody specifically binds to a polypeptide having as least 90, 95 or 99% sequence identity to a polypeptide selected from the group consisting of SEQ ID NO: 1-155 or 168-208, optionally wherein the polypeptide selected from the group consisting of SEQ ID NO: 168-208.

In accordance with one embodiment any of the antibodies disclosed herein optionally further comprises a detectable marker and/or a cytotoxic agent linked to the antibody.

In accordance with one embodiment a composition is provided comprising 2, 3, 4, 5, 6, 7, 8, 9 or more of any of the antibodies disclosed herein wherein the plurality of antibodies each binds a different epitope displayed by the polypeptides of SEQ ID NO: 1 through SEQ ID NO: 155.

In accordance with one embodiment any of the antibodies disclosed herein is linked to a solid support. In one embodiment any of the antibodies disclosed herein is covalently linked to a detectable label. In one embodiment any of the antibodies disclosed herein is covalently linked a cytotoxic agent.

In accordance with one embodiment a chimeric antigen receptor (CAR) is provided comprising

an antibody, or antigen binding fragment thereof, that binds one or more epitopes of a polypeptide selected from the group consisting of SEQ ID NO: 1-155,

a transmembrane domain; and

a T -cell antigen receptor chain, wherein the transmembrane domain links the an antibody, or antigen binding fragment thereof to the T -cell antigen receptor chain.

In accordance with one embodiment a chimeric antigen receptor (CAR) is provided comprising

an antibody single-chain variable fragment that specifically binds to one or more epitopes of a polypeptide selected from the group consisting of SEQ ID NO: 1-155, a transmembrane domain; and a T -cell antigen receptor chain, wherein the transmembrane domain links the antibody single-chain variable fragment to the T -cell antigen receptor chain. In one embodiment the T -cell antigen receptor chain of the chimeric antigen receptor comprises a CD3ζ chain (zeta-chain). In one embodiment the chimeric antigen receptor specifically binds to a polypeptide selected from the group consisting of SEQ ID NO: 1-25, or a polypeptide selected from the group consisting of SEQ ID NO: 25-50, or polypeptide selected from the group consisting of SEQ ID NO: 50-75, or a polypeptide selected from the group consisting of SEQ ID NO: 75-100, or a polypeptide selected from the group consisting of SEQ ID NO: 100-125. In accordance with one embodiment the chimeric antigen receptor further comprises a hinge region located between the antibody single-chain variable fragment and the transmembrane domain.

In accordance with one embodiment a modified T-cell (CAR T-cell) is provided wherein the T-cell has been transformed to express a chimeric antigen receptor of the present disclosure that specifically binds to a polypeptide selected from the group consisting of SEQ ID NO 1-155. In one embodiment the T -cell antigen receptor chain of the chimeric antigen receptor is a CD3ζ chain (zeta-chain).

In one embodiment a pharmaceutical composition is provided comprising any of the antibodies, chimeric antigen receptors or CAR T-cells as disclosed herein, optionally including a pharmaceutically acceptable carrier. The pharmaceutical composition can be formulated using standard techniques for any suitable route of administration including intravenously or intraperitoneally.

In accordance with one embodiment a method for treating multiple myeloma is provided. In one embodiment the method comprises administering a therapeutic amount of any of the antibodies, chimeric antigen receptors or CAR T-cells of the present disclosure to a patient in need of such treatment. In one embodiment the compositions is administered as a pharmaceutical composition comprising any of the antibodies, chimeric antigen receptors or CAR T-cells as disclosed herein and a pharmaceutically acceptable carrier.

In accordance with one embodiment a method of treating MM comprises administering CAR T-cells that express chimeric antigen receptors that specifically bind to a polypeptide selected from the group consisting of SEQ ID NO: 1-155. In

one embodiment the treatment is conducted in conjunction with other known therapeutic treatments known to the skilled practitioner, including the co-administration of CAR T-cells directed to BCMA or other known surface candidate targets in MM including the antigens SLAMF7, CD38, GPRC5D, ITGB7, CD229, CD56, TACI, CD19 and CD70.

BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A-1D represent the steps for identifying target MM genes, Fig. 1A is a schematic representation of the biotinylation of the surface proteins of 7 different MM cell lines followed by Spectrometry analysis. Each cell line bears unique combinations of chromosomal rearrangements and p53 mutations as shown. This analysis led to the identification of 5,454 uniprot IDs corresponding to 4761 proteins Fig. 1B is a schematic representation of the integrated database used to generated for cell surface molecule annotation. For each repository we indicated the methodology used for cell surface molecule annotation and relative size. This also served as a scoring system with 0 denoting a protein not at the cell surface location and 5 a protein detected in all five repositories. The number of IDs per score is also indicated. 16,462 transcripts derived from 904 patients with multiple myeloma were annotated for cell surface molecules. Fig. 1C presents a Venn Diagram showing the overlap between cell surface molecules with a score equal or higher than 3 as detected by Mass-Spectrometer analysis in cell lines and RNA-seq in primary patient samples. As shown in Fig. 1D expression levels of the cell surface molecules was analyzed and by excluding molecules with an expression below 1 SD from the average patient gene expression 326 surface proteins were selected for further analyses.

Figs. 2A-2C: Enrichment analysis of candidate targets. 326 surface proteins selected in Fig. 1D were analyzed by STRING. A significant number of edges was identified (490) that is higher than expected (109) with an average local functional and/or physical local clustering coefficient of 0.326 and a PPI enrichment p value <1.0e-16. In yellow the largest cluster. The largest cluster (227/326 proteins) involves proteins with a functional enrichment related to immune pathways (Fig. 2A). The other two clusters involve transporters and adhesion molecules Fig. 2B). Additional enrichment analysis of the largest cluster by the KEGG collection is shown in Fig. 2C. Further enrichment analysis of the largest cluster by the Reactome collection is shown.

Fig. 3 provides a Venn Diagram overlapping the targets with a potential biological relevance (227 molecules involved in immune-related pathways) and therapeutic relevance (94 molecules with minimal expression in normal tissues). 67 common targets are common. 24 out of those 67 targets presented the most favorable expression profile in primary patients and were used for validation in patient samples including: CCR1, CD28, CD320, FCRL3, IFNGR1, IL12RB1. IL27RA. IL2RG, IL6R, ITGA4, KCNN4, LAX1, LILRB1, LILRB4, LRRC8A, LRRC8D, PLXNA3, PLXNC1, S1PR4, SELPLG, SEMA4A, SLAMF6, TLR1 and BCMA.

DETAILED DESCRIPTION

DEFINITIONS

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs.

The term "about" as used herein means greater or lesser than the value or range of values stated by 10 percent, but is not intended to designate any value or range of values to only this broader definition. Each value or range of values preceded by the term "about" is also intended to encompass the embodiment of the stated absolute value or range of values.

As used herein the terms "native" or "natural" define a condition found in nature. A "native DNA sequence" is a DNA sequence present in nature that was produced by natural means but not generated by genetic engineering (e.g., using molecular biology/transformation techniques)

As used herein the term "solid support" relates to a solvent insoluble substrate that is capable of forming linkages (preferably covalent bonds) with soluble molecules. The support can be either biological in nature, such as, without limitation, a cell or bacteriophage particle, or synthetic, such as, without limitation, an acrylamide derivative, glass, plastic, agarose, cellulose, nylon, silica, or magnetized particles. The support can be in particulate form or a monolythic strip or sheet. The surface of such supports may be solid or porous and of any convenient shape.

The term "linked" or like terms refers to the connection between two groups. The linkage may comprise a covalent, ionic, or hydrogen bond or other interaction that binds two compounds or substances to one another.

As used herein, the term “purified” and like terms relate to an enrichment of a molecule or compound relative to other components normally associated with the molecule or compound in a native environment. The term “purified” does not necessarily indicate that complete purity of the particular molecule has been achieved during the process. A “highly purified” compound as used herein refers to a compound that is greater than 90% pure.

"Therapeutic agent," "pharmaceutical agent" or "drug" refers to any therapeutic or prophylactic agent which may be used in the treatment (including the prevention, diagnosis, alleviation, or cure) of a malady, affliction, disease or injury in a patient.

As used herein, the term “pharmaceutically acceptable carrier” includes any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions such as an oil/water or water/oil emulsion, and various types of wetting agents. The term also encompasses any of the agents approved by a regulatory agency of the US Federal government or listed in the US Pharmacopeia for use in animals, including humans.

As used herein, the term "treating" includes alleviating the symptoms associated with a specific disorder or condition and/or preventing or eliminating said symptoms. For example, treating cancer includes preventing or slowing the growth and/or division of cancer cells as well as killing cancer cells.

As used herein, the term "antibody" refers to a polyclonal or monoclonal antibody or a binding fragment thereof such as Fab, $F(ab')_2$ and Fv fragments. Antibodies as disclosed herein include, but are not limited to, monoclonal, multispecific, human or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, anti-idiotypic (anti-Id)antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), intracellularly-made antibodies (i.e., intrabodies), and epitope-binding fragments of any of the above. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule

As used herein, the term "biologically active fragments" of the antibodies described herein encompasses natural or synthetic portions of the respective full-length antibody that retain the capability of specific binding to the target epitope.

As used herein, the term "parenteral" includes administration subcutaneously, intravenously or intramuscularly.

As used herein the characterization of expression level such as “high expression” is based on the parameters established in Perna et al., Cancer Cell 32, 506–519. October 9, 2017, the teachings of which are expressely incorporated herein. By merging 3 proteomic databases a cut-off for low medium and high expression was established and was used in the context of the present invention.

EMBODIMENTS

The present disclosure is based on applicant's analysis of the expression of cell surface candidate targets in MM. Biotinylation of the surface proteins of 7 different MM cell lines followed by Spectrometry analysis led to the identification of 5,454 uniprot IDs corresponding to 4761 proteins. As detailed in Fig. 1B an integrated database was used to generate cell surface molecule annotation. A combination of cell surface molecule annotation and exclusion based on expression levels (see Fig. 1C and 1D) identified 326 surface proteins for further analysis by STRING.

A heatmap reveals the protein annotation of 94 selected targets in several normal tissues and organs of the whole body. These molecules were selected by merging the Human Protein Atlas, Human Protein Map and Proteomics database as previously reported (Perna F et al., Cancer Cell 2017). Molecules with high expression in any normal tissue except hematopoietic tissues were excluded. Molecules with available annotation in less than 2 out of the 3 proteomic databases were excluded. The 94 targets include the cell surface polypeptides IL12RB1, SLC5A3, CCR1, ANKH, IL27RA, S1PR4, TLR1, KCNA3, PSEN2, BCMA, IL2RG, LILRB4, IL6R, ITGB7, LRP10, FCRL3, IFNGR1, SLAMF6, SLCO3A1, LILRB1, PLXNA3, SLC17A5, CD28, LAX1, NEMP1, TMEM154, SEMA4A, C10orf54, ITM2C, LY9, SLAMF7, ITGA4, LRRC8A, LRRC8D, CD320, KCNN4, PLXNC1, CD37, SELPLG, DAGLB, ABCC4, ADAM9, CD4, CD180, CD48, CD40, MCUR1, ABCC5, IL6ST, LRP8, SLC5A6, SLC7A6, HLA-F, ICAM2, LEPROT, ITGAL, TMEM63A, CMTM7, IL10RB, NDC1, PTPRCAP, ANTXR2, ABCA7, FCGR2B, ACVR1B, STS, ABHD12, TNFRSF10A, HVCN1, SLC39A10, EMP3, ABCC1, SLC26A6, SLC6A6, CCR10, SLC30A1, SLC231A1, ADCY3, IFNAR1, PTPRJ, CLDND1, SLC30A5, SLC6A9, ADAM15, IGF2R, INSR, NOTCH2, CD53, SLC12A9, SLC15A4, CEMIP2, ADAM17, MPZL1 and TACI.

A Venn Diagram overlapping the targets with a potential biological relevance (227 molecules involved in immune-related pathways) and therapeutic relevance (94 molecules with minimal expression in normal tissues) revealed 67 common targets (see Fig. 3). 24 out of those 67 targets presented the most favorable expression profile in primary patients and were used for validation in patient samples including: CCR1, CD28, CD320, FCRL3, IFNGR1, IL12RB1. IL27RA. IL2RG, IL6R, ITGA4, KCNN4, LAX1, LILRB1, LILRB4, LRRC8A, LRRC8D, PLXNA3, PLXNC1, S1PR4, SELPLG, SEMA4A, SLAMF6, TLR1 and BCMA.

Further analysis as described in Example 2 has further identified the following 12 genes that encode products that in some aspects are targets for the generation of immunotherapeutics: CCR1 (SEQ ID NO: 156), CD320 (SEQ ID NO: 157), FCRL3(SEQ ID NO: 158), IFNGR1 (SEQ ID NO: 159), IL12RB1 (SEQ ID NO: 160), ITGA4 (SEQ ID NO: 161), LAX1 (SEQ ID NO: 162), LILRB4 (SEQ ID NO: 163), LRRC8D (SEQ ID NO: 164), SEMA4A (SEQ ID NO: 165), SLAMF6 (SEQ ID NO: 166), and TNFRSF17 (SEQ ID NO: 167).

Western Blot analysis identified the expression of 11 selected targets in in 25 MM patients relative to normal cord blood CD34+ cells revealing these proteins to be of particular interest as targets. BCMA was used as a control. VCP was used as loading control. Specifically, the identified proteins include IL12RB1, CCR1, LILRB4, FCRL3, IFNGR1, SLAMF6, LAX1, SEMA4A, ITGA4, LRRC8D and CD320 by western blot analysis.

In accordance with at least one embodiment Mass-Spectrometer analysis reveals 5,454 Uniprot ID and MMRF Compass revealed 16,462 transcripts. Common targets of this analysis revealed a total of 401 proteins with surface score greater than or equal to (=>) 3. Overlap with high expressors (average expression is greater than (>) median) in primary patient samples (MMRF) was determined and the average expression of each gene was calculated; low expressing genes (1 SD below the mean) were removed and genes having expression over the cutoff were selected identifying 326 surface proteins.

Annotation in normal tissues from at least 2 out of 3 databases (HPA, HPM and PDB) and exclusion of proteins with high expression in any tissue except hematopoietic tissues identified 94 out of the 326 targets. Analysis of immune-related + therapeutic value reduced this number to 67 targets. Analysis in high-risk cytogenetics excluded targets with decreased expression in standard risk vs high-risk

and validation in primary MM samples produce 11 targets identified as CCR1 (SEQ ID NO: 156), CD320 (SEQ ID NO: 157), FCRL3 (SEQ ID NO: 158), IFNGR1 (SEQ ID NO: 159), IL12RB1 (SEQ ID NO: 160), ITGA4 (SEQ ID NO: 161), LAX1 (SEQ ID NO: 162), LILRB4 (SEQ ID NO: 163), LRRC8D (SEQ ID NO: 164), SEMA4A (SEQ ID NO: 165), and SLAMF6 (SEQ ID NO: 166).

In accordance with at least one embodiment an antibody is provided that specifically binds to a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO: 1-155. In some embodiments an antibody is provided that specifically binds to a polypeptide comprising a sequence selected from the group consisting of (SEQ ID NO: 156), (SEQ ID NO: 157), (SEQ ID NO: 158), (SEQ ID NO: 159), (SEQ ID NO: 160), (SEQ ID NO: 161), (SEQ ID NO: 162), (SEQ ID NO: 163), (SEQ ID NO: 164), (SEQ ID NO: 165), and (SEQ ID NO: 166). Such antibodies can be linked to detectable markers for diagnostic purposes. Alternatively, the antibodies can be linked to cytotoxic agents and the conjugated antibodies can be administered to patients for targeted delivery of cytotoxins to MM cells.

In at least one embodiment antibodies generated against the peptides disclosed in Table 1 (SEQ ID NO: 1-155), or a polypeptide comprising a sequence selected from the group consisting of (SEQ ID NO: 156), (SEQ ID NO: 157), (SEQ ID NO: 158), (SEQ ID NO: 159), (SEQ ID NO: 160), (SEQ ID NO: 161), (SEQ ID NO: 162), (SEQ ID NO: 163), (SEQ ID NO: 164), (SEQ ID NO: 165), and (SEQ ID NO: 166) can be used to generate an antibody single-chain variable fragment which can then be used to prepare a chimeric antigen receptor (CAR). The antibody single-chain variable fragment is a chimeric protein made up of the light (VL) and heavy (VH) chains of immunoglobins, connected with a short linker peptide. In accordance with the present disclosure the VL and VH regions are selected in advance for their binding ability to a target antigen selected from the polypeptides of SEQ ID NO: 1-155, or a polypeptide comprising a sequence selected from the group consisting of (SEQ ID NO: 156), (SEQ ID NO: 157), (SEQ ID NO: 158), (SEQ ID NO: 159), (SEQ ID NO: 160), (SEQ ID NO: 161), (SEQ ID NO: 162), (SEQ ID NO: 163), (SEQ ID NO: 164), (SEQ ID NO: 165), and (SEQ ID NO: 166). In some embodiments the linker between the VL and VH regions consists of hydrophilic residues with stretches of glycine and serine in it for flexibility as well as stretches of glutamate and lysine for added solubility.

In some aspects, the antibody single-chain variable fragment can then be covalently linked to an intracellular immune cell signaling domain typically through a transmembrane domain to create a chimeric antigen receptor (CAR). In some aspects, the immune cell signaling domain can be a T-cell, NK cell, macrophage, and/or a myeloid cell. For example, in some aspects, the antibody single-chain variable fragment can then be covalently linked to an intracellular T-cell signaling domain typically through a transmembrane domain to create a chimeric antigen receptor (CAR). Such CARs when expressed in immune cells such as T-cells, NK cells, macrophages, and/or a myeloid cells can provide the immune cells a new ability to target a specific protein. Accordingly, CAR T-cells, NK cells, macrophages, and/or myeloid cells comprising CARs that specifically bind to polypeptides selected from the group consisting of SEQ ID NO: 1-155, or a polypeptide comprising a sequence selected from the group consisting of (SEQ ID NO: 156), (SEQ ID NO: 157), (SEQ ID NO: 158), (SEQ ID NO: 159), (SEQ ID NO: 160), (SEQ ID NO: 161), (SEQ ID NO: 162), (SEQ ID NO: 163), (SEQ ID NO: 164), (SEQ ID NO: 165), and (SEQ ID NO: 166) can be used to treat MM patients. In particular, in some embodiments CAR T-cells are prepared by isolating the patient's own cells and transforming T-cells with nucleic acid sequences encoding CAR having specificity to a polypeptide selected from the group consisting of SEQ ID NO: 1-155. In particular, in some embodiments the CAR T-cells are prepared by providing allogeneic T-cells and transforming T-cells with nucleic acid sequences encoding CAR having specificity to a polypeptide selected from the group consisting of SEQ ID NO: 1-155, 156-167, and 168-208, and optionally selected from the group consisting of (SEQ ID NO: 156), (SEQ ID NO: 157), (SEQ ID NO: 158), (SEQ ID NO: 159), (SEQ ID NO: 160), (SEQ ID NO: 161), (SEQ ID NO: 162), (SEQ ID NO: 163), (SEQ ID NO: 164), (SEQ ID NO: 165), and (SEQ ID NO: 166). It is understood that for any of the above cell types, either autologous or allogeneic approaches may be utilized. In accordance with embodiments, the method of treating a patient with MM comprises administering 1, 2, 3, 4, 5 or more CAR T-cells, CAR NK cells, CAR macrophages, and/or CAR myeloid cells to a MM patient in need to therapy, wherein each of the CAR T-cells targets a different antigen present on a polypeptide selected from the group consisting of SEQ ID NO: 1-155, or a polypeptide comprising a sequence selected from the group consisting of (SEQ ID NO: 156), (SEQ ID NO: 157), (SEQ ID NO: 158), (SEQ ID

NO: 159), (SEQ ID NO: 160), (SEQ ID NO: 161), (SEQ ID NO: 162), (SEQ ID NO: 163), (SEQ ID NO: 164), (SEQ ID NO: 165), and (SEQ ID NO: 166).

In accordance with at least one embodiment an antibody is provided that specifically binds to a gene product of CCR1 (SEQ ID NO: 156), CD320 (SEQ ID NO: 157), FCRL3(SEQ ID NO: 158), IFNGR1 (SEQ ID NO: 159), IL12RB1 (SEQ ID NO: 160), ITGA4 (SEQ ID NO: 161), LAX1 (SEQ ID NO: 162), LILRB4 (SEQ ID NO: 163), LRRC8D (SEQ ID NO: 164), SEMA4A (SEQ ID NO: 165), SLAMF6 (SEQ ID NO: 166), and TNFRSF17 (SEQ ID NO: 167). In one embodiment an antibody is provided that specifically binds to a polypeptide comprising a sequence selected from the group consisting of SEQ ID NO: 168-208. Such antibodies can be linked to detectable markers for diagnostic purposes. Alternatively, the antibodies can be linked to cytotoxic agents and the conjugated antibodies can be administered to patients for targeted delivery of cytotoxins to MM cells.

In some embodiments antibodies generated against the peptides disclosed in Table 1 (SEQ ID NO: 168-208) can be used to generate an antibody single-chain variable fragment which can then be used to prepare a chimeric antigen receptor (CAR). The antibody single-chain variable fragment is a chimeric protein made up of the light (VL) and heavy (VH) chains of immunoglobins, connected with a short linker peptide. In accordance with the present disclosure the VL and VH regions are selected in advance for their binding ability to a target antigen selected from the polypeptides of SEQ ID NO: 168-208. In one embodiment the linker between the VL and VH regions consists of hydrophilic residues with stretches of glycine and serine in it for flexibility as well as stretches of glutamate and lysine for added solubility.

The antibody single-chain variable fragment can then be covalently linked to an intracellular T-cell signaling domain typically through a transmembrane domain to create a chimeric antigen receptor (CAR). Such CARs when expressed in T-cells can provide T cells a new ability to target a specific protein. Accordingly, CAR T-cells comprising CARs that specifically bind to polypeptides selected from the group consisting of SEQ ID NO: 168-208 can be used to treat MM patients. In particular, in some embodiments the CAR T-cells are prepared by isolating the patient's own cells and transforming T-cells with nucleic acid sequences encoding CAR having specificity to a polypeptide selected from the group consisting of SEQ ID NO: 168-208. In accordance with one embodiment the method of treating a patient with MM comprises administering 1, 2, 3, 4, 5 or more CAR T-cells to a MM patient in need to

therapy, wherein each of the CAR T-cells targets a different antigen present on a polypeptide selected from the group consisting of SEQ ID NO: 168-208.

In accordance with one embodiment the transmembrane domain of the CARs of the present disclosure comprises a hydrophobic alpha helix that spans the cell membrane. It anchors the CAR to the plasma membrane, bridging the extracellular antigen recognition domains (i.e., antibody single-chain variable fragment) with the intracellular signaling region. In one embodiment the CAR further comprises a hinge region located between the antigen recognition domains and the transmembrane domain. The ideal hinge enhances the flexibility of the scFv receptor head, reducing the spatial constraints between the CAR and its target antigen. This promotes antigen binding and synapse formation between the CAR-T cells and target cells. In one embodiment the hinge sequences is based on membrane-proximal regions from other immune molecules including IgG, CD8, and CD28.

The intracellular T-cell signaling domain of the CAR when expressed a cell will remain inside the cell. After an antigen is bound to the external antigen recognition domain, CAR receptors cluster together and transmit an activation signal. Then the internal cytoplasmic end of the receptor perpetuates signaling inside the T cell. Normal T cell activation relies on the phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) present in the cytoplasmic domain of CD3-zeta. To mimic this process, in one embodiment the CARS of the present disclosure comprise the CD3-zeta's cytoplasmic domain as the main CAR endodomain component.

T cells also require co-stimulatory molecules in addition to CD3 signaling in order to persist after activation. In a further embodiment, the endodomains of CAR receptors also include one or more chimeric domains from co-stimulatory proteins known to those skilled in the art. Signaling domains from a wide variety of co-stimulatory molecules have been successfully tested, including CD28, CD27, CD134 (OX40), and CD137. In a further embodiment co-stimulatory domains, like CD28 or 4-1BB, CD28-41BB or CD28-OX40, and cytokines, such is IL-2, IL-5, IL-12 can be added to the endodomains of CAR receptors to augment T cell activity.

In accordance with embodiment 1, a method for identifying target multiple myeloma associated surface antigens is provided wherein said method comprises

identifying a plurality of genes that express cell-surface proteins in a first multiple myeloma sample and a second multiple myeloma sample;

selecting nucleic acids from said first multiple myeloma sample that have expression levels higher than a control gene unrelated to hematopoietic cells, and identifying the proteins corresponding to the detected elevated expressed nucleic acids to designate a first pool of selected proteins;

conducting mass spec analysis on proteins isolated from said second myeloma sample to identify proteins that are present in higher concentration in said second multiple myeloma relative to normal tissues, wherein such proteins represent a second pool of selected proteins;

excluding proteins with high expression in brain, spinal cord, gut, liver and kidney from said first and second pools to produce a modified first and second pool of proteins; and

identifying proteins common to said first and second modified pool of proteins as target multiple myeloma associated surface antigens.

In accordance with embodiment 2, the method of embodiment 1 is provided wherein said first multiple myeloma sample and a second multiple myeloma sample are taken from the same tissue source.

In accordance with embodiment 3, the method of embodiment 1 is provided wherein said first multiple myeloma sample is a nucleic acid pool of expressed genes from MM patients and the second multiple myeloma sample represents proteins expressed in MM cell lines.

In accordance with embodiment 4, the method of any one of embodiments 1-3 is provided wherein the target multiple myeloma associated surface antigen has an expression level in a normal tissue sample that is more than about one standard deviation below the normal peak of the protein expression level distribution of the normal tissue sample.

In accordance with embodiment 5, the method of any one of embodiments 1-4 wherein mRNA is measured to determine the expression level of the nucleic acids used to identify proteins for the first pool of selected proteins.

In accordance with embodiment 6, the method of any one of embodiments 1-5 is provided wherein proteins are identified as cell surface proteins based on the use of an integrative computational tool assigning a score relative to five published databases disclosed in Example 2.

In accordance with embodiment 7, the method of any one of embodiments 1-5 is provided wherein said first pool of proteins is identified by

analyzing an RNA-seq dataset from Multiple Myeloma patients to identify surface targets based on the use of an integrative computational tool assigning a score relative to five published databases disclosed in Example 2;

removing low expressing genes (1 SD below the mean); and

excluding proteins with high expression in any normal tissue except hematopoietic tissues; wherein the remaining proteins constitute said first pool of proteins.

In accordance with embodiment 8, a method for identifying target multiple myeloma associated surface antigens is provided wherein said method comprises

analyzing an RNA-seq dataset from Multiple Myeloma patients to identify surface targets based on the use of an integrative computational tool assigning a score relative to five published databases disclosed in Example 2;

removing low expressing genes (1 SD below the mean); and

excluding proteins with high expression in any normal tissue except hematopoietic tissues; wherein the remaining proteins constitute target multiple myeloma associated surface antigens.

In accordance with embodiment 9, a monoclonal antibody that specifically binds to a polypeptide selected from the group consisting of SEQ ID NO: 1-155 or 168-208 is provided or a monoclonal antibody that specifically binds to a polypeptide having at least 90, 95, or 99% sequence identity with a polypeptide selected from the group consisting of SEQ ID NO: 1-155 or 168-208.

In accordance with embodiment 10, the monoclonal antibody of embodiment 9 is provided wherein the antibody specifically binds to a polypeptide selected from the group consisting of SEQ ID NO: 168-208.

In accordance with embodiment 11, the monoclonal antibody of embodiment 9 is provided wherein the antibody specifically binds to a polypeptide selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 37, SEQ ID NO: 42, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 79, SEQ ID NO: 112 and SEQ ID NO: 104.

In accordance with embodiment 12, the monoclonal antibody of any one of embodiments 9-11 is provided wherein the antibody specifically binds to a polypeptide having at least 90% sequence identity to a sequence selected from the

group consisting of CCR1 (SEQ ID NO: 60), CD320 (SEQ ID NO: 56), FCRL3(SEQ ID NO: 57), IFNGR1 (SEQ ID NO: 79), IL12RB1 (SEQ ID NO: 19), ITGA4 (SEQ ID NO: 42), LILRB4 (SEQ ID NO: 20), LRRC8D (SEQ ID NO: 112), SEMA4A (SEQ ID NO: 104), and SLAMF6 (SEQ ID NO: 37).

In accordance with embodiment 13, the monoclonal antibody of any one of embodiments 9-12 is provided wherein the antibody specifically binds to a polypeptide having at least 90% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 1-155 or 168-208.

In accordance with embodiment 14, the monoclonal antibody of any one of embodiments 9-12 is provided wherein the antibody specifically binds to a polypeptide having at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 1-155 or 168-208.

In accordance with embodiment 15, the monoclonal antibody of any one of embodiments 9-14 is provided wherein said antibody further comprises a detectable label covalently linked to the antibody.

In accordance with embodiment 16, the monoclonal antibody of any one of embodiments 9-14 is provided wherein said antibody further comprises a cytotoxic agent linked to the antibody.

In accordance with embodiment 17, a chimeric antigen receptor (CAR) is provided comprising an antibody of any one of embodiments 9-15, or antigen binding fragment thereof;

a transmembrane domain; and

an immune cell antigen receptor chain, wherein the transmembrane domain links the an antibody, or antigen binding fragment thereof to the immune cell antigen receptor chain.

In accordance with embodiment 18, a chimeric antigen receptor of embodiment 17 is provided wherein the antibody or antigen binding fragment thereof, specifically binds to a polypeptide selected from the group consisting of SEQ ID NO: 168-208.

In accordance with embodiment 19, a chimeric antigen receptor of embodiment 17 is provided wherein the antibody or antigen binding fragment thereof, specifically binds to a polypeptide having at least 95% sequence identity to a sequence selected from the group consisting of CCR1 (SEQ ID NO: 60), CD320 (SEQ ID NO: 56), FCRL3(SEQ ID NO: 57), IFNGR1 (SEQ ID NO: 79), IL12RB1

(SEQ ID NO: 19), ITGA4 (SEQ ID NO: 42), LILRB4 (SEQ ID NO: 20), LRRC8D (SEQ ID NO: 112), SEMA4A (SEQ ID NO: 104) and SLAMF6 (SEQ ID NO: 37).

In accordance with embodiment 20, a chimeric antigen receptor of embodiment 17 is provided wherein the antibody, or antigen binding fragment thereof, binds one or more epitopes of a polypeptide having at least 90% homology to a sequence selected from the group consisting of SEQ ID NO: 1-155 or 168-208.

In accordance with embodiment 21, a chimeric antigen receptor of embodiment 17 is provided wherein the antibody, or antigen binding fragment thereof, binds one or more epitopes of a polypeptide having at least 95% homology to a sequence selected from the group consisting of SEQ ID NO: 1-155 or 168-208.

In accordance with embodiment 22, a chimeric antigen receptor of embodiment 17 is provided wherein the antibody or antigen binding fragment thereof, specifically binds to a polypeptide selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 37, SEQ ID NO: 42, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 79, SEQ ID NO: 112 and SEQ ID NO: 104.

In accordance with embodiment 23, a chimeric antigen receptor of any one of embodiments 17-22 is provided wherein said antibody or antigen binding fragment comprises an antibody single-chain variable fragment.

In accordance with embodiment 24, a chimeric antigen receptor of embodiment 23 is provided further comprising a hinge region located between the antibody single-chain variable fragment and the transmembrane domain.

In accordance with embodiment 25, a T-cell modified to express the chimeric antigen receptor of any one of embodiments 16-23 is provided.

In accordance with embodiment 26, the T-cell of embodiment 25 is provided wherein the T-cell is a tumor infiltrating leukocyte.

In accordance with embodiment 27, an NK cell modified to express the chimeric antigen receptor of any one of embodiments 17-24 is provided.

In accordance with embodiment 28, a myeloid cell modified to express the chimeric antigen receptor of any one of embodiments 17-24 is provided.

In accordance with embodiment 29, a macrophage modified to express the chimeric antigen receptor of any one of embodiments 17-24 is provided.

In accordance with embodiment 30, T-cell of embodiment 25 or 26 is provided wherein the T -cell antigen receptor chain is the CD3ζ chain (zeta-chain).

In accordance with embodiment 31 a pharmaceutical composition comprising the antibody of any one of embodiments 9-16, chimeric antigen receptor of any one of embodiments 17-24, the T-cell of embodiments 25 or 26, the NK cell of embodiment 27, the myeloid cell of embodiment 28 or the macrophage of embodiment 29.

In accordance with embodiment 32 a method for treating multiple myeloma is provided wherein said method comprises administering the pharmaceutical composition of embodiment 31 to a patient in need of such treatment.

In accordance with embodiment 33 an isolated immunoresponsive cell is provided wherein the cell comprises an antigen recognizing receptor that binds to an antigen present on a polypeptide selected from the group consisting of CCR1 (SEQ ID NO: 60), CD320 (SEQ ID NO: 56), FCRL3(SEQ ID NO: 57), IFNGR1 (SEQ ID NO: 79), IL12RB1 (SEQ ID NO: 19), ITGA4 (SEQ ID NO: 42), LILRB4 (SEQ ID NO: 20), LRRC8D (SEQ ID NO: 112), SEMA4A (SEQ ID NO: 104), and SLAMF6 (SEQ ID NO: 37) or a polypeptide having 90, 95 or 99% sequence identity to any of said proteins.

In accordance with embodiment 34 an isolated immunoresponsive cell of embodiment 33 is provided wherein the antigen is present on a polypeptide selected from the group consisting of IL12RB1 (SEQ ID NO: 19), LILRB4 (SEQ ID NO: 20), SLAMF6 (SEQ ID NO: 37), CCR1 (SEQ ID NO: 60), and CD320 (SEQ ID NO: 56).

In accordance with embodiment 35 an isolated immunoresponsive cell of embodiment 33 or 34 is provided wherein said antigen recognizing receptor is a T cell receptor (TCR), or a chimeric antigen receptor (CAR).

In accordance with embodiment 36 an isolated immunoresponsive cell of embodiment 35 is provided wherein said antigen recognizing receptor is a CAR.

In accordance with embodiment 37 an isolated immunoresponsive cell of embodiment 36 is provided wherein the intracellular signaling domain of said CAR is the CD3C-chain, CD97, CD1 la-CD 18, CD2, ICOS, CD27, CD 154, CD8, OX40, 4-IBB, CD28 signaling domain, or combinations thereof.

In accordance with embodiment 38 an isolated immunoresponsive cell of any one of embodiments 33-37 is provided wherein the cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, a human embryonic stem cell, and a pluripotent stem cell from which lymphoid cells may be differentiated.

In accordance with embodiment 39 an isolated immunoresponsive cell is provided comprising:

(a) a first antigen recognizing receptor that binds to a first antigen, and

(b) a second antigen recognizing receptor that binds to a second antigen, wherein each of the first antigen and the second antigen is selected from the group consisting of CCR1 (SEQ ID NO: 60), CD320 (SEQ ID NO: 56), FCRL3(SEQ ID NO: 57), IFNGR1 (SEQ ID NO: 79), IL12RB1 (SEQ ID NO: 19), ITGA4 (SEQ ID NO: 42), LILRB4 (SEQ ID NO: 20), LRRC8D (SEQ ID NO: 112), SEMA4A (SEQ ID NO: 104), and SLAMF6 (SEQ ID NO: 37), and the first antigen and the second antigen are different.

In accordance with embodiment 40 an isolated immunoresponsive cell of embodiment 37 is provided, wherein each of said antigen recognizing receptor is a T cell receptor (TCR) or a chimeric antigen receptor (CAR), optionally wherein the said antigen recognizing receptor is a CAR.

In accordance with embodiment 41 a method of reducing tumor burden in a subject is provided, comprising administering to the subject an effective amount of the immunoresponsive cell of any one of embodiments 33-40.

EXAMPLE 1

Surface biotinylation and MS analysis

As multiple myeloma is characterized by significant molecular heterogeneity, 7 known and distinct MM cell lines were selected for additional analysis. The 7 cell lines are: OPM-2 bearing p53 mutation and t(4;14), NCI-H929 with t(4;14) and 8q+, JJN3 with t(14;16) and t(8;14), KMS11 with p53 null and t(4;14), t(14;16) and t(8;14), U266 p53 mut and t(11;14), AMO-1 with p53 WT and t(12;14) and RPMI-8226 with p53 mut, t(16;22), t(8;22) and KRas mut as disclosed in Table 1.

Table 1. MM cell lines used for Mass-Spectrum analysis

	Cell Lines	Molecular Genetics	BCMA expression level (flow cytometry)	TP53 status
1	OPM-2	t(4;14)	+++	Mut
2	NCI-H929	t(4;14) + 8q+	+++	WT
3	JJN3	t(14;16) + t(8;14)	++ (60%)	+/-
4	KMS11	t(4;14) + t(14;16) + t(8;14)	+++	Null
5	U266	t(11;14)	+++	Mut
6	AMO-1	t(12;14)	+++	WT
7	RPMI-8226	t(16;22) + t(8;22)	+++	Mut

Surface-specific proteomic analyses of these seven multiple myeloma cell lines bearing distinct genetic abnormalities was conducted using biotin labeling followed by Mass-Spectrometry analysis (MS). Cell surface proteins were labeled with biotin on live cells; biotin-tagged proteins were then captured on avidin beads, digested with trypsin and analyzed by Mass-Spectrometry and data independent annotation (DIA).

More particularly, labeling with biotin was conducted using procedures to ensure that only surface proteins were exposed to the biotinylation reagents. Pierce® Cell Surface Protein Biotinylation and isolation Kit (Thermo Fisher, A44390) was used for labeling and isolation of surface proteins. Briefly, the cells were incubated with the membrane-impermeable Sulfo-NHS-SS-biotin reagent for 10 min at RT, after the incubation excess labeling reagent was removed and the cells were washed several times to remove any unbound labeling reagent before lysis. Biotinylated proteins were captured on Neutravidin resin, and any non-specific bound unbiotinylated proteins were removed by repetitive washing of the resins. Prior to MS analysis, the bound biotinylated proteins on the Neutravidin resins were released from the resin by trypsin cleavage overnight. The digested peptides were desalted and purified using StageTip, desalted peptides were analyzed by LC-MS/MS using 170 mins LC gradient and DIA method on Orbitrap Fusion. Samples specific library (SSL) was generated using equal amount of digested proteins from each sample. MS Raw data were searched with SSL using Spectronaut software. Protein expression quantification was done using Label Free Quantification (LFQ). This generated an unbiased pool of 5,454 Uniprot IDs corresponding to 4,761 proteins (Fig 1A). Table 2 155 proteins identified by Mass spec analysis.

Table 2:

Protein Name	Gene Name	Protein Sequence
Anthrax toxin receptor 2 (Capillary morphogenesis gene 2 protein) (CMG-2)	ANTXR2 CMG2	SEQ ID NO: 1
C-type lectin domain family 1 member A (C-type lectin-like receptor 1) (CLEC-1)	CLEC1A CLEC1 UNQ569/PRO1131	SEQ ID NO: 2
T-cell-specific surface glycoprotein CD28 (TP44) (CD antigen CD28)	CD28	SEQ ID NO: 3
Complement receptor type 2 (Cr2)	CR2 C3DR	SEQ ID NO: 4

(Complement C3d receptor) (Epstein-Barr virus receptor) (EBV receptor) (CD antigen CD21)		
T-cell surface glycoprotein CD8 beta chain (CD antigen CD8b)	CD8B CD8B1	SEQ ID NO: 5
Y+L amino acid transporter 2 (Cationic amino acid transporter, y+ system) (Solute carrier family 7 member 6) (y(+)L-type amino acid transporter 2) (Y+LAT2) (y+LAT-2)	SLC7A6 KIAA0245	SEQ ID NO: 6
Solute carrier family 52, riboflavin transporter, member 2 (Porcine endogenous retrovirus A receptor 1) (PERV-A receptor 1) (Protein GPR172A) (Riboflavin transporter 3) (hRFT3)	SLC52A2 GPR172A PAR1 RFT3	SEQ ID NO: 7
Tumor necrosis factor receptor superfamily member 5 (B-cell surface antigen CD40) (Bp50) (CD40L receptor) (CDw40) (CD antigen CD40)	CD40 TNFRSF5	SEQ ID NO: 8
Sodium-coupled neutral amino acid transporter 1 (Amino acid transporter A1) (N-system amino acid transporter 2) (Solute carrier family 38 member 1) (System A amino acid transporter 1) (System N amino acid transporter 1)	SLC38A1 ATA1 NAT2 SAT1 SNAT1	SEQ ID NO: 9
T-cell surface glycoprotein CD4 (T-cell surface antigen T4/Leu-3) (CD antigen CD4)	CD4	SEQ ID NO: 10
Leukocyte antigen CD37 (Tetraspanin-26) (Tspan-26) (CD antigen CD37)	CD37 TSPAN26	SEQ ID NO: 11
CD180 antigen (Lymphocyte antigen 64) (Radioprotective 105 kDa protein) (CD antigen CD180)	CD180 LY64 RP105	SEQ ID NO: 12
Adhesion G protein-coupled receptor E2 (EGF-like module receptor 2) (EGF-like module-containing mucin-like hormone receptor-like 2) (CD antigen CD312)	ADGRE2 EMR2	SEQ ID NO: 13
Myeloid cell surface antigen CD33 (Sialic acid-binding Ig-like lectin 3) (Siglec-3) (gp67) (CD antigen CD33)	CD33 SIGLEC3	SEQ ID NO: 14
Phospholipid-transporting ATPase ABCA7 (EC 7.6.2.1) (ABCA-SSN) (ATP-binding cassette sub-family A member 7) (Autoantigen SS-N) (Macrophage ABC transporter)	ABCA7	SEQ ID NO: 15
Sodium-dependent multivitamin transporter (Na(+)-dependent multivitamin transporter) (Solute carrier family 5	SLC5A6 SMVT	SEQ ID NO: 16

member 6)		
Leukosialin (GPL115) (Galactoglycoprotein) (GALGP) (Leukocyte sialoglycoprotein) (Sialophorin) (CD antigen CD43) [Cleaved into: CD43 cytoplasmic tail (CD43-ct) (CD43ct)]	SPN CD43	SEQ ID NO: 17
Integrin alpha-L (CD11 antigen-like family member A) (Leukocyte adhesion glycoprotein LFA-1 alpha chain) (LFA-1A) (Leukocyte function-associated molecule 1 alpha chain) (CD antigen CD11a)	ITGAL CD11A	SEQ ID NO: 18
Interleukin-12 receptor subunit beta-1 (IL-12 receptor subunit beta-1) (IL-12R subunit beta-1) (IL-12R-beta-1) (IL-12RB1) (IL-12 receptor beta component) (CD antigen CD212)	IL12RB1 IL12R IL12RB	SEQ ID NO: 19
Leukocyte immunoglobulin-like receptor subfamily B member 1 (LIR-1) (Leukocyte immunoglobulin-like receptor 1) (CD85 antigen-like family member J) (Immunoglobulin-like transcript 2) (ILT-2) (Monocyte/macrophage immunoglobulin-like receptor 7) (MIR-7) (CD antigen CD85j)	LILRB1 ILT2 LIR1 MIR7	SEQ ID NO: 20
T-lymphocyte surface antigen Ly-9 (Cell surface molecule Ly-9) (Lymphocyte antigen 9) (SLAM family member 3) (SLAMF3) (Signaling lymphocytic activation molecule 3) (CD antigen CD229)	LY9 CDABP0070	SEQ ID NO: 21
Disintegrin and metalloproteinase domain-containing protein 28 (ADAM 28) (EC 3.4.24.-) (Epididymal metalloproteinase-like, disintegrin-like, and cysteine-rich protein II) (eMDC II) (Metalloproteinase-like, disintegrin-like, and cysteine-rich protein L) (MDC-L)	ADAM28 ADAM23 MDCL	SEQ ID NO: 22
Epithelial membrane protein 3 (EMP-3) (Hematopoietic neural membrane protein 1) (HNMP-1) (Protein YMP)	EMP3 YMP	SEQ ID NO: 23
Low-density lipoprotein receptor-related protein 10 (LRP-10)	LRP10 MSTP087 SP220 UNQ389/PRO724	SEQ ID NO: 24
Receptor-type tyrosine-protein phosphatase C (EC 3.1.3.48) (Leukocyte common antigen) (L-CA) (T200) (CD antigen CD45)	PTPRC CD45	SEQ ID NO: 25
Cytokine receptor common subunit gamma (Interleukin-2 receptor subunit gamma) (IL-	IL2RG	SEQ ID NO: 26

2 receptor subunit gamma) (IL-2R subunit gamma) (IL-2RG) (gammaC) (p64) (CD antigen CD132)		
Tumor necrosis factor receptor superfamily member 17 (B-cell maturation protein) (CD antigen CD269)	TNFRSF17 BCM BCMA	SEQ ID NO: 27
Tumor necrosis factor receptor superfamily member 10A (Death receptor 4) (TNF-related apoptosis-inducing ligand receptor 1) (TRAIL receptor 1) (TRAIL-R1) (CD antigen CD261)	TNFRSF10A APO2 DR4 TRAILR1	SEQ ID NO: 28
SLAM family member 7 (CD2 subset 1) (CD2-like receptor-activating cytotoxic cells) (CRACC) (Membrane protein FOAP-12) (Novel Ly9) (Protein 19A) (CD antigen CD319)	SLAMF7 CS1 UNQ576/PRO1138	SEQ ID NO: 29
Tumor necrosis factor receptor superfamily member 1B (Tumor necrosis factor receptor 2) (TNF-R2) (Tumor necrosis factor receptor type II) (TNF-RII) (TNFR-II) (p75) (p80 TNF-alpha receptor) (CD antigen CD120b) (Etanercept) [Cleaved into: Tumor necrosis factor receptor superfamily member 1b, membrane form; Tumor necrosis factor-binding protein 2 (TBP-2) (TBPII)]	TNFRSF1B TNFBR TNFR2	SEQ ID NO: 30
Leukocyte-associated immunoglobulin-like receptor 1 (LAIR-1) (hLAIR1) (CD antigen CD305)	LAIR1 CD305	SEQ ID NO: 31
Toll-like receptor 1 (EC 3.2.2.6) (Toll/interleukin-1 receptor-like protein) (TIL) (CD antigen CD281)	TLR1 KIAA0012	SEQ ID NO: 32
Toll-like receptor 7	TLR7 UNQ248/PRO285	SEQ ID NO: 33
Tumor necrosis factor receptor superfamily member 18 (Activation-inducible TNFR family receptor) (Glucocorticoid-induced TNFR-related protein) (CD antigen CD357)	TNFRSF18 AITR GITR UNQ319/PRO364	SEQ ID NO: 34
Tumor necrosis factor receptor superfamily member 8 (CD30L receptor) (Ki-1 antigen) (Lymphocyte activation antigen CD30) (CD antigen CD30)	TNFRSF8 CD30 D1S166E	SEQ ID NO: 35
Interferon alpha/beta receptor 2 (IFN-R-2) (IFN-alpha binding protein) (IFN-alpha/beta receptor 2) (Interferon alpha binding protein) (Type I interferon receptor 2)	IFNAR2 IFNABR IFNARB	SEQ ID NO: 36
SLAM family member 6 (Activating NK	SLAMF6 KALI	SEQ ID NO: 37

receptor) (NK-T-B-antigen) (NTB-A) (CD antigen CD352)	UNQ6123/PRO20080	
Tumor necrosis factor receptor superfamily member 10D (Decoy receptor 2) (DcR2) (TNF-related apoptosis-inducing ligand receptor 4) (TRAIL receptor 4) (TRAIL-R4) (TRAIL receptor with a truncated death domain) (CD antigen CD264)	TNFRSF10D DCR2 TRAILR4 TRUNDD UNQ251/PRO288	SEQ ID NO: 38
P-selectin glycoprotein ligand 1 (PSGL-1) (Selectin P ligand) (CD antigen CD162)	SELPLG	SEQ ID NO: 39
T-cell surface protein tactile (Cell surface antigen CD96) (T cell-activated increased late expression protein) (CD antigen CD96)	CD96	SEQ ID NO: 40
Porimin (Keratinocytes-associated transmembrane protein 3) (KCT-3) (Pro-oncosis receptor inducing membrane injury) (Transmembrane protein 123)	TMEM123 KCT3 PSEC0111 UNQ641/PRO1271	SEQ ID NO: 41
Integrin alpha-4 (CD49 antigen-like family member D) (Integrin alpha-IV) (VLA-4 subunit alpha) (CD antigen CD49d)	ITGA4 CD49D	SEQ ID NO: 42
G-protein coupled receptor 15 (Brother of Bonzo) (BoB)	GPR15	SEQ ID NO: 43
Multidrug resistance-associated protein 1 (EC 7.6.2.2) (ATP-binding cassette sub-family C member 1) (Glutathione-S-conjugate-translocating ATPase ABCC1) (EC 7.6.2.3) (Leukotriene C(4) transporter) (LTC4 transporter)	ABCC1 MRP MRP1	SEQ ID NO: 44
CX3C chemokine receptor 1 (C-X3-C CKR-1) (CX3CR1) (Beta chemokine receptor-like 1) (CMK-BRL-1) (CMK-BRL1) (Fractalkine receptor) (G-protein coupled receptor 13) (V28)	CX3CR1 CMKBRL1 GPR13	SEQ ID NO: 45
B-lymphocyte antigen CD20 (B-lymphocyte surface antigen B1) (Bp35) (Leukocyte surface antigen Leu-16) (Membrane-spanning 4-domains subfamily A member 1) (CD antigen CD20)	MS4A1 CD20	SEQ ID NO: 46
HLA class II histocompatibility antigen, DM beta chain (MHC class II antigen DMB) (Really interesting new gene 7 protein)	HLA-DMB DMB RING7	SEQ ID NO: 47
Low affinity immunoglobulin gamma Fc region receptor II-b (IgG Fc receptor II-b) (CDw32) (Fc-gamma RII-b) (Fc-gamma-RIIb) (FcRII-b) (CD antigen CD32)	FCGR2B CD32 FCG2 IGFR2	SEQ ID NO: 48
HLA class I histocompatibility antigen, alpha chain F (CDA12) (HLA F antigen)	HLA-F HLA-5.4 HLAF	SEQ ID NO: 49

(Leukocyte antigen F) (MHC class I antigen F)		
Disintegrin and metalloproteinase domain-containing protein 9 (ADAM 9) (EC 3.4.24.-) (Cellular disintegrin-related protein) (Meltrin-gamma) (Metalloprotease/disintegrin/cysteine-rich protein 9) (Myeloma cell metalloproteinase)	ADAM9 KIAA0021 MCMP MDC9 MLTNG	SEQ ID NO: 50
IgG receptor FcRn large subunit p51 (FcRn) (IgG Fc fragment receptor transporter alpha chain) (Neonatal Fc receptor)	FCGRT FCRN	SEQ ID NO: 51
Interleukin-6 receptor subunit beta (IL-6 receptor subunit beta) (IL-6R subunit beta) (IL-6R-beta) (IL-6RB) (CDw130) (Interleukin-6 signal transducer) (Membrane glycoprotein 130) (gp130) (Oncostatin-M receptor subunit alpha) (CD antigen CD130)	IL6ST	SEQ ID NO: 52
Lymphocyte function-associated antigen 3 (Ag3) (Surface glycoprotein LFA-3) (CD antigen CD58)	CD58 LFA3	SEQ ID NO: 53
C-C chemokine receptor type 10 (C-C CKR-10) (CC-CKR-10) (CCR-10) (G-protein coupled receptor 2)	CCR10 GPR2	SEQ ID NO: 54
C-type lectin domain family 7 member A (Beta-glucan receptor) (C-type lectin superfamily member 12) (Dendritic cell-associated C-type lectin 1) (DC-associated C-type lectin 1) (Dectin-1) (CD antigen CD369)	CLEC7A BGR CLECSF12 DECTIN1 UNQ539/PRO1082	SEQ ID NO: 55
CD320 antigen (8D6 antigen) (FDC-signaling molecule 8D6) (FDC-SM-8D6) (Transcobalamin receptor) (TCblR) (CD antigen CD320)	CD320 8D6A UNQ198/PRO224	SEQ ID NO: 56
Fc receptor-like protein 3 (FcR-like protein 3) (FcRL3) (Fc receptor homolog 3) (FcRH3) (IFGP family protein 3) (hIFGP3) (Immune receptor translocation-associated protein 3) (SH2 domain-containing phosphatase anchor protein 2) (CD antigen CD307c)	FCRL3 FCRH3 IFGP3 IRTA3 SPAP2	SEQ ID NO: 57
Plexin-C1 (Virus-encoded semaphorin protein receptor) (CD antigen CD232)	PLXNC1 VESPR	SEQ ID NO: 58
Interleukin-17 receptor A (IL-17 receptor A) (IL-17RA) (CDw217) (CD antigen CD217)	IL17RA IL17R	SEQ ID NO: 59
C-C chemokine receptor type 1 (C-C CKR-1) (CC-CKR-1) (CCR-1) (CCR1) (HM145) (LD78 receptor) (Macrophage inflammatory	CCR1 CMKBR1 CMKR1 SCYAR1	SEQ ID NO: 60

protein 1-alpha receptor) (MIP-1alpha-R) (RANTES-R) (CD antigen CD191)		
T-lymphocyte activation antigen CD80 (Activation B7-1 antigen) (BB1) (CTLA-4 counter-receptor B7.1) (B7) (CD antigen CD80)	CD80 CD28LG CD28LG1 LAB7	SEQ ID NO: 61
Proteinase-activated receptor 4 (PAR-4) (Coagulation factor II receptor-like 3) (Thrombin receptor-like 3)	F2RL3 PAR4	SEQ ID NO: 62
Interleukin-6 receptor subunit alpha (IL-6 receptor subunit alpha) (IL-6R subunit alpha) (IL-6R-alpha) (IL-6RA) (IL-6R 1) (Membrane glycoprotein 80) (gp80) (CD antigen CD126)	IL6R	SEQ ID NO: 63
Hepatitis A virus cellular receptor 2 (HAVcr-2) (T-cell immunoglobulin and mucin domain-containing protein 3) (TIMD-3) (T-cell immunoglobulin mucin receptor 3) (TIM-3) (T-cell membrane protein 3) (CD antigen CD366)	HAVCR2 TIM3 TIMD3	SEQ ID NO: 64
Interleukin-27 receptor subunit alpha (IL-27 receptor subunit alpha) (IL-27R subunit alpha) (IL-27R-alpha) (IL-27RA) (Cytokine receptor WSX-1) (Cytokine receptor-like 1) (Type I T-cell cytokine receptor) (TCCR) (ZcytoR1)	IL27RA CRL1 TCCR WSX1 UNQ296/PRO336	SEQ ID NO: 65
Interleukin-13 receptor subunit alpha-2 (IL-13 receptor subunit alpha-2) (IL-13R subunit alpha-2) (IL-13R-alpha-2) (IL-13RA2) (Interleukin-13-binding protein) (CD antigen CD213a2)	IL13RA2 IL13R	SEQ ID NO: 66
Integrin beta-7 (Gut homing receptor beta subunit)	ITGB7	SEQ ID NO: 67
Fc receptor-like protein 2 (FcR-like protein 2) (FcRL2) (Fc receptor homolog 2) (FcRH2) (IFGP family protein 4) (Immunoglobulin receptor translocation-associated protein 4) (SH2 domain-containing phosphatase anchor protein 1) (CD antigen CD307b)	FCRL2 FCRH2 IFGP4 IRTA4 SPAP1 UNQ9236/PRO31998	SEQ ID NO: 68
L-selectin (CD62 antigen-like family member L) (Leukocyte adhesion molecule 1) (LAM-1) (Leukocyte surface antigen Leu-8) (Leukocyte-endothelial cell adhesion molecule 1) (LECAM1) (Lymph node homing receptor) (TQ1) (gp90-MEL) (CD antigen CD62L)	SELL LNHR LYAM1	SEQ ID NO: 69
Intercellular adhesion molecule 2 (ICAM-2)	ICAM2	SEQ ID NO: 70

(CD antigen CD102)		
CD82 antigen (C33 antigen) (IA4) (Inducible membrane protein R2) (Metastasis suppressor Kangai-1) (Suppressor of tumorigenicity 6 protein) (Tetraspanin-27) (Tspan-27) (CD antigen CD82)	CD82 KAI1 SAR2 ST6 TSPAN27	SEQ ID NO: 71
CD83 antigen (hCD83) (B-cell activation protein) (Cell surface protein HB15) (CD antigen CD83)	CD83	SEQ ID NO: 72
Activin receptor type-1B (EC 2.7.11.30) (Activin receptor type IB) (ACTR-IB) (Activin receptor-like kinase 4) (ALK-4) (Serine/threonine-protein kinase receptor R2) (SKR2)	ACVR1B ACVRLK4 ALK4	SEQ ID NO: 73
Lymphocyte antigen 75 (Ly-75) (C-type lectin domain family 13 member B) (DEC-205) (gp200-MR6) (CD antigen CD205)	LY75 CD205 CLEC13B	SEQ ID NO: 74
Junctional adhesion molecule B (JAM-B) (Junctional adhesion molecule 2) (JAM-2) (Vascular endothelial junction-associated molecule) (VE-JAM) (CD antigen CD322)	JAM2 C21orf43 VEJAM UNQ219/PRO245	SEQ ID NO: 75
Low-density lipoprotein receptor-related protein 8 (LRP-8) (Apolipoprotein E receptor 2)	LRP8 APOER2	SEQ ID NO: 76
Cytokine receptor common subunit beta (CDw131) (GM-CSF/IL-3/IL-5 receptor common beta subunit) (CD antigen CD131)	CSF2RB IL3RB IL5RB	SEQ ID NO: 77
Interleukin-10 receptor subunit beta (IL-10 receptor subunit beta) (IL-10R subunit beta) (IL-10RB) (Cytokine receptor class-II member 4) (Cytokine receptor family 2 member 4) (CRF2-4) (Interleukin-10 receptor subunit 2) (IL-10R subunit 2) (IL-10R2) (CD antigen CDw210b)	IL10RB CRFB4 D21S58 D21S66	SEQ ID NO: 78
Interferon gamma receptor 1 (IFN-gamma receptor 1) (IFN-gamma-R1) (CDw119) (Interferon gamma receptor alpha-chain) (IFN-gamma-R-alpha) (CD antigen CD119)	IFNGR1	SEQ ID NO: 79
Adhesion G-protein coupled receptor G6 (Developmentally regulated G-protein-coupled receptor) (G-protein coupled receptor 126) (Vascular inducible G protein-coupled receptor) [Cleaved into: ADGRG6 N-terminal fragment (ADGRG6-NTF); ADGRG6 C-terminal fragment (ADGRG6-CTF)]	ADGRG6 DREG GPR126 VIGR	SEQ ID NO: 80
Adenosine receptor A2a	ADORA2A ADORA2	SEQ ID NO: 81

Progressive ankylosis protein homolog (ANK)	ANKH KIAA1581 UNQ241/PRO274	SEQ ID NO: 82
Cell adhesion molecule-related/down-regulated by oncogenes	CDON CDO	SEQ ID NO: 83
CSC1-like protein 1 (Transmembrane protein 63A)	TMEM63A KIAA0489 KIAA0792	SEQ ID NO: 84
Toll-like receptor 9 (CD antigen CD289)	TLR9 UNQ5798/PRO19605	SEQ ID NO: 85
Sodium- and chloride-dependent taurine transporter (Solute carrier family 6 member 6)	SLC6A6	SEQ ID NO: 86
CD48 antigen (B-lymphocyte activation marker BLAST-1) (BCM1 surface antigen) (Leukocyte antigen MEM-102) (SLAM family member 2) (SLAMF2) (Signaling lymphocytic activation molecule 2) (TCT.1) (CD antigen CD48)	CD48 BCM1 BLAST1	SEQ ID NO: 87
ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase 1 (EC 3.2.2.6) (2'-phospho-ADP-ribosyl cyclase) (2'-phospho-ADP-ribosyl cyclase/2'-phospho-cyclic-ADP-ribose transferase) (EC 2.4.99.20) (2'-phospho-cyclic-ADP-ribose transferase) (ADP-ribosyl cyclase 1) (ADPRC 1) (Cyclic ADP-ribose hydrolase 1) (cADPr hydrolase 1) (T10) (CD antigen CD38)	CD38	SEQ ID NO: 88
Muscarinic acetylcholine receptor M3	CHRM3	SEQ ID NO: 89
Macrophage colony-stimulating factor 1 (CSF-1) (M-CSF) (MCSF) (Lanimostim) [Cleaved into: Processed macrophage colony-stimulating factor 1]	CSF1	SEQ ID NO: 90
Multidrug resistance-associated protein 4 (ATP-binding cassette sub-family C member 4) (MRP/cMOAT-related ABC transporter) (Multi-specific organic anion transporter B) (MOAT-B)	ABCC4 MRP4	SEQ ID NO: 91
Potassium voltage-gated channel subfamily A member 3 (HGK5) (HLK3) (HPCN3) (Voltage-gated K(+) channel HuKIII) (Voltage-gated potassium channel subunit Kv1.3)	KCNA3 HGK5	SEQ ID NO: 92
Sialic acid-binding Ig-like lectin 7 (Siglec-7) (Adhesion inhibitory receptor molecule 1) (AIRM-1) (CDw328) (D-siglec) (QA79 membrane protein) (p75) (CD antigen CD328)	SIGLEC7 AIRM1	SEQ ID NO: 93
Sphingosine 1-phosphate receptor 4 (S1P	S1PR4 EDG6	SEQ ID NO: 94

receptor 4) (S1P4) (Endothelial differentiation G-protein coupled receptor 6) (Sphingosine 1-phosphate receptor Edg-6) (S1P receptor Edg-6)		
Transmembrane protein 106B	TMEM106B	SEQ ID NO: 95
Proton-coupled folate transporter (G21) (Heme carrier protein 1) (PCFT/HCP1) (Solute carrier family 46 member 1)	SLC46A1 HCP1 PCFT	SEQ ID NO: 96
Allergin-1 (Allergy inhibitory receptor 1) (Mast cell antigen 32) (MCA-32) (Mast cell immunoglobulin-like receptor 1)	MILR1 C17orf60 MCA32	SEQ ID NO: 97
Steryl-sulfatase (EC 3.1.6.2) (Arylsulfatase C) (ASC) (Estrone sulfatase) (Steroid sulfatase) (Steryl-sulfate sulfohydrolase)	STS ARSC1	SEQ ID NO: 98
Tumor necrosis factor receptor superfamily member 6 (Apo-1 antigen) (Apoptosis-mediating surface antigen FAS) (FASLG receptor) (CD antigen CD95)	FAS APT1 FAS1 TNFRSF6	SEQ ID NO: 99
Presenilin-2 (PS-2) (EC 3.4.23.-) (AD3LP) (AD5) (E5-1) (STM-2) [Cleaved into: Presenilin-2 NTF subunit; Presenilin-2 CTF subunit]	PSEN2 AD4 PS2 PSNL2 STM2	SEQ ID NO: 100
Solute carrier organic anion transporter family member 4A1 (OATP4A1) (Colon organic anion transporter) (Organic anion transporter polypeptide-related protein 1) (OATP-RP1) (OATPRP1) (POAT) (Organic anion-transporting polypeptide E) (OATP-E) (Sodium-independent organic anion transporter E) (Solute carrier family 21 member 12)	SLCO4A1 OATP1 OATP4A1 OATPE SLC21A12	SEQ ID NO: 101
Solute carrier organic anion transporter family member 3A1 (OATP3A1) (Organic anion transporter polypeptide-related protein 3) (OATP-RP3) (OATPRP3) (Organic anion-transporting polypeptide D) (OATP-D) (PGE1 transporter) (Sodium-independent organic anion transporter D) (Solute carrier family 21 member 11)	SLCO3A1 OATP3A1 OATPD SLC21A11	SEQ ID NO: 102
Transmembrane protein 154	TMEM154	SEQ ID NO: 103
Semaphorin-4A (Semaphorin-B) (Sema B)	SEMA4A SEMAB SEMB UNQ783/PRO1317	SEQ ID NO: 104
Multidrug resistance-associated protein 5 (ATP-binding cassette sub-family C member 5) (Multi-specific organic anion transporter C) (MOAT-C) (SMRP) (pABC11)	ABCC5 MRP5	SEQ ID NO: 105

Transmembrane protein 132E	TMEM132E	SEQ ID NO: 106
Netrin receptor UNC5C (Protein unc-5 homolog 3) (Protein unc-5 homolog C)	UNC5C UNC5H3	SEQ ID NO: 107
Scavenger receptor class B member 1 (SRB1) (CD36 and LIMPII analogous 1) (CLA-1) (CD36 antigen-like 1) (Collagen type I receptor, thrombospondin receptor-like 1) (SR-BI) (CD antigen CD36)	SCARB1 CD36L1 CLA1	SEQ ID NO: 108
Zinc transporter ZIP6 (Estrogen-regulated protein LIV-1) (Solute carrier family 39 member 6) (Zrt- and Irt-like protein 6) (ZIP-6)	SLC39A6 LIV1 ZIP6	SEQ ID NO: 109
Inactive tyrosine-protein kinase transmembrane receptor ROR1 (Neurotrophic tyrosine kinase, receptor-related 1)	ROR1 NTRKR1	SEQ ID NO: 110
Solute carrier family 26 member 6 (Anion exchange transporter) (Pendrin-like protein 1) (Pendrin-L1)	SLC26A6	SEQ ID NO: 111
SLIT and NTRK-like protein 5 (Leucine-rich repeat-containing protein 11)	SLITRK5 KIAA0918 LRRC11	SEQ ID NO: 112
Cell surface glycoprotein CD200 receptor 1 (CD200 cell surface glycoprotein receptor) (Cell surface glycoprotein OX2 receptor 1)	CD200R1 CD200R CRTR2 MOX2R OX2R UNQ2522/PRO6015	SEQ ID NO: 113
Discoidin, CUB and LCCL domain-containing protein 1	DCBLD1	SEQ ID NO: 114
CD109 antigen (150 kDa TGF-beta-1-binding protein) (C3 and PZP-like alpha-2-macroglobulin domain-containing protein 7) (Platelet-specific Gov antigen) (p180) (r150) (CD antigen CD109)	CD109 CPAMD7	SEQ ID NO: 115
C3a anaphylatoxin chemotactic receptor (C3AR) (C3a-R)	C3AR1 AZ3B C3R1 HNFAG09	SEQ ID NO: 116
CD70 antigen (CD27 ligand) (CD27-L) (Tumor necrosis factor ligand superfamily member 7) (CD antigen CD70)	CD70 CD27L CD27LG TNFSF7	SEQ ID NO: 117
Large neutral amino acids transporter small subunit 4 (L-type amino acid transporter 4) (Solute carrier family 43 member 2)	SLC43A2 LAT4 PP7664	SEQ ID NO: 118
HLA class II histocompatibility antigen, DR beta 5 chain (DR beta-5) (DR2-beta-2) (Dw2) (MHC class II antigen DRB5)	HLA-DRB5	SEQ ID NO: 119
Ephrin type-A receptor 5 (EC 2.7.10.1) (Brain-specific kinase) (EPH homology kinase 1) (EHK-1) (EPH-like kinase 7) (EK7) (hEK7)	EPHA5 BSK EHK1 HEK7 TYRO4	SEQ ID NO: 120
Monocarboxylate transporter 2 (MCT 2)	SLC16A7 MCT2	SEQ ID NO: 121

(Solute carrier family 16 member 7)		
Cysteine-rich motor neuron 1 protein (CRIM-1) (Cysteine-rich repeat-containing protein S52) [Cleaved into: Processed cysteine-rich motor neuron 1 protein]	CRIM1 S52 UNQ1886/PRO4330	SEQ ID NO: 122
Nuclear envelope integral membrane protein 1	NEMP1 KIAA0286 TMEM194 TMEM194A	SEQ ID NO: 123
HLA class II histocompatibility antigen, DR beta 4 chain (MHC class II antigen DRB4)	HLA-DRB4	SEQ ID NO: 124
Cationic amino acid transporter 2 (CAT-2) (CAT2) (Low affinity cationic amino acid transporter 2) (Solute carrier family 7 member 2)	SLC7A2 ATRC2 CAT2	SEQ ID NO: 125
Integral membrane protein 2C (Cerebral protein 14) (Transmembrane protein BRI3) [Cleaved into: CT-BRI3]	ITM2C BRI3 hucep-14 NPD018 PSEC0047	SEQ ID NO: 126
Ephrin type-A receptor 3 (EC 2.7.10.1) (EPH-like kinase 4) (EK4) (hEK4) (HEK) (Human embryo kinase) (Tyrosine-protein kinase TYRO4) (Tyrosine-protein kinase receptor ETK1) (Eph-like tyrosine kinase 1)	EPHA3 ETK ETK1 HEK TYRO4	SEQ ID NO: 127
Lysosome-associated membrane glycoprotein 5 (Brain and dendritic cell-associated LAMP) (Brain-associated LAMP-like protein) (BAD-LAMP) (Lysosome-associated membrane protein 5) (LAMP-5)	LAMP5 C20orf103	SEQ ID NO: 128
Metal transporter CNNM2 (Ancient conserved domain-containing protein 2) (Cyclin-M2)	CNNM2 ACDP2	SEQ ID NO: 129
CD177 antigen (Human neutrophil alloantigen 2a) (HNA-2a) (NB1 glycoprotein) (NB1 GP) (Polycythemia rubra vera protein 1) (PRV-1) (CD antigen CD177)	CD177 NB1 PRV1 UNQ595/PRO1181	SEQ ID NO: 130
Zinc transporter ZIP10 (Solute carrier family 39 member 10) (Zrt- and Irt-like protein 10) (ZIP-10)	SLC39A10 KIAA1265 ZIP10	SEQ ID NO: 131
Plexin-A3 (Plexin-4) (Semaphorin receptor SEX)	PLXNA3 PLXN4 SEX	SEQ ID NO: 132
Phospholipid phosphatase 2 (EC 3.1.3.-) (EC 3.1.3.4) (Lipid phosphate phosphohydrolase 2) (PAP2-gamma) (PAP2-G) (Phosphatidate phosphohydrolase type 2c) (Phosphatidic acid phosphatase 2c) (PAP-2c) (PAP2c)	PLPP2 LPP2 PPAP2C	SEQ ID NO: 133
Serine incorporator 3 (Tumor differentially expressed protein 1)	SERINC3 DIFF33 TDE1 SBBI99	SEQ ID NO: 134

Sodium/myo-inositol cotransporter (Na(+)/myo-inositol cotransporter) (Sodium/myo-inositol transporter 1) (SMIT1) (Solute carrier family 5 member 3)	SLC5A3	SEQ ID NO: 135
Teneurin-3 (Ten-3) (Protein Odd Oz/ten-m homolog 3) (Tenascin-M3) (Ten-m3) (Teneurin transmembrane protein 3)	TENM3 KIAA1455 ODZ3 TNM3	SEQ ID NO: 136
Sodium-coupled neutral amino acid transporter 5 (Solute carrier family 38 member 5) (System N transporter 2)	SLC38A5 JM24 SN2 SNAT5 PP7194	SEQ ID NO: 137
Solute carrier family 23 member 2 (Na(+)/L-ascorbic acid transporter 2) (Nucleobase transporter-like 1 protein) (Sodium-dependent vitamin C transporter 2) (hSVCT2) (Yolk sac permease-like molecule 2)	SLC23A2 KIAA0238 NBTL1 SLC23A1 SVCT2 YSPL2	SEQ ID NO: 138
Sialin (H(+)/nitrate cotransporter) (H(+)/sialic acid cotransporter) (AST) (Membrane glycoprotein HP59) (Solute carrier family 17 member 5) (Vesicular H(+)/Aspartate-glutamate cotransporter)	SLC17A5	SEQ ID NO: 139
Urokinase plasminogen activator surface receptor (U-PAR) (uPAR) (Monocyte activation antigen Mo3) (CD antigen CD87)	PLAUR MO3 UPAR	SEQ ID NO: 140
Sn1-specific diacylglycerol lipase beta (DGL-beta) (EC 3.1.1.-) (KCCR13L)	DAGLB	SEQ ID NO: 141
Embigin	EMB	SEQ ID NO: 142
Protein HEG homolog 1	HEG1 KIAA1237	SEQ ID NO: 143
Plexin-A4	PLXNA4 KIAA1550 PLXNA4A PLXNA4B UNQ2820/PRO34003	SEQ ID NO: 144
Protein ELFN1 (Extracellular leucine-rich repeat and fibronectin type-III domain-containing protein 1) (Protein phosphatase 1 regulatory subunit 28)	ELFN1 PPP1R28	SEQ ID NO: 145
Adhesion G-protein coupled receptor G5 (G-protein coupled receptor 114) (G-protein coupled receptor PGR27)	ADGRG5 GPR114 PGR27 UNQ2524/PRO6017	SEQ ID NO: 146
Leucine-rich repeat and fibronectin type III domain-containing protein 1 (Synaptic adhesion-like molecule 2)	LRFN1 KIAA1484 SALM2	SEQ ID NO: 147
CD160 antigen (Natural killer cell receptor BY55) (CD antigen CD160) [Cleaved into: CD160 antigen, soluble form]	CD160 BY55	SEQ ID NO: 148
Battenin (Batten disease protein) (Protein CLN3)	CLN3 BTS	SEQ ID NO: 149
Transmembrane protein 178B	TMEM178B	SEQ ID NO: 150
RGM domain family member B (DRG11-	RGMB	SEQ ID NO: 151

responsive axonal guidance and outgrowth of neurite) (DRAGON)		
Reversion-inducing cysteine-rich protein with Kazal motifs (hRECK) (Suppressor of tumorigenicity 15 protein)	RECK ST15	SEQ ID NO: 152
Natural cytotoxicity triggering receptor 3 ligand 1 (B7 homolog 6) (B7-H6)	NCR3LG1 B7H6	SEQ ID NO: 153
Testisin (EC 3.4.21.-) (Eosinophil serine protease 1) (ESP-1) (Serine protease 21)	PRSS21 ESP1 TEST1 UNQ266/PRO303	SEQ ID NO: 154
UL16-binding protein 3 (ALCAN-gamma) (NKG2D ligand 3) (N2DL-3) (NKG2DL3) (Retinoic acid early transcript 1N)	ULBP3 N2DL3 RAET1N	SEQ ID NO: 155

Example 2

Additional MM Surface Protein Analysis

The unbiased pool of 5,454 Uniprot IDs corresponding to 4,761 proteins identified in the surface-specific proteomic analyses of seven multiple myeloma cell lines as described in Example 1 was subjected to further analysis.

Given the lack of bioinformatics tools enabling accurate detection of proteins at the cell surface location, we developed an integrated scoring database for surface protein annotation. To this purpose we merged five published databases using different methodologies in identifying molecules that localized to the plasma membrane: #1 (Díaz-Ramos et al., Immunol Lett 2011, 134, 183-187, doi:10.1016/j.imlet.2010.09.016) was manually curated from the literature; #2 (Baush-Fluck et al. Proc Natl Acad Sci U S A 2018, 115, E10988-E10997, doi:10.1073/pnas.1808790115.) was computationally compiled using a random forest classifier trained on domain-specific protein features and tested on a set of α-helical transmembrane proteins; #3 (Town et al. Proc Natl Acad Sci U S A 2016, 113, 3603-3608, doi:10.1073/pnas.1521251113) was computationally compiled using GO and Uniprot annotation followed by transmembrane domain prediction and signal peptide prediction; #4 (da Cunha et al. Proc Natl Acad Sci U S A 2009, 106, 16752-16757, doi:10.1073/pnas.0907939106) was computationally compiled by transmembrane domain prediction; #5 (Thule et al.) was experimentally determined by antibody-based immunofluorescence microscopy. In our integrative computational database, each uniprot ID is scored based on the number of databases it was identified in, with 0

denoting the protein was not found in any and 5 denoting the protein was found in all five (Fig. 1B). Based on this integrative computational tool cutoff 1 includes 1229 uniprot IDs, cutoff 2: 699 uniprot IDs, cutoff 3: 448 uniprot IDs, cutoff 4: 260 uniprot IDs and cutoff 5: 63 uniprot IDs. For further analyses we selected proteins with a surface score equal or higher than three representing a high-level of confidence for cell surface location.

We integrated the CoMMpass RNA-seq dataset providing gene expression data from 904 MM patients. This integrative approach served to identify surface targets that are relevant to the patient population. We applied our surface scoring system to 16,462 transcripts from MM patients and, identified 402 targets with a surface score higher than three and detected by MS (Fig. 1C). We have further selected 326 top surface proteins overlapping with high expressors in patients by calculating 1SD from the mean gene expression in patients. We calculated the average expression of each gene and removed low expressing genes (1 SD below the mean) and selected the genes that have expression over the cutoff (326 transcripts kept) (Fig 1D). Of note, patient gene expression was unimodal except for 7 transcripts presenting a non-unimodal expression (NCAM1, TPBG, ROB1, LAMP5, APP, PTRCAP, PLXAN2).

This group of 326 surface proteins includes known MM -associated surface proteins, some of which are targeted in clinical and pre-clinical studies such as BCMA, SLAMF7, TACI, LY9, CD38. GPCR5D and FCRL5, also currently investigated in clinical trials for patients with MM did not result in this list because they were identified by transcriptomic analysis in patients, but we did not detect their protein expression by MS analysis.

The MM surfaceome mainly consists in immune-related proteins

In order to gain insights into the function of these candidate surface proteins we performed functional enrichment analyses and, found that they can be divided into three main functional clusters: 1) proteins involved in immune-mediated pathways 2) transporters 3) proteins mediating the adhesion to the stroma. The surface proteins related to immune-mediated pathways (top cluster) involve 227 out of 326 molecules and thus, might mediate the interplay between immune cells and myeloma cells.

By further digging into this group of surface proteins with the Kegg and Reactome collections we found that cytokine-dependent mechanisms and NK-mediated cytotoxicity represent enriched mechanisms. These findings are consistent

with previous studies in the immunobiology of MM, however a detailed profile of the surface proteins mediating these mechanisms had remained unknown or only partially known so-far.

Normal tissue annotation identifies targetable antigens

In order to identify therapeutically relevant targets, we used a pipeline we previously established for leukemia. This combines three public proteomic databases for human proteome annotation (Human Protein Atlas, Human Protein Map and Proteomics DataBase) and allowed the annotation of each candidate protein in a panel of 42 normal tissues and organs. Given that, we excluded proteins with high expression in any normal tissue except hematopoietic tissues and with an available annotation in less than two out of the three databases. Through this, we identified 94 out of 326 targets with minimal expression in normal tissues. This list includes BCMA, SLAMF7, ITGB7, TACI and Ly9. We also ranked each one of the 94 targets based on their expression in normal tissues and thus, predicted on-target off-tumor toxicity.

By overlapping the list of immune-related proteins (227) with that of proteins with a favorable profile in normal tissues we obtained 67 targets with both functional and therapeutic relevance (Fig. 3). Such latter list still includes BCMA, CD229, SLAMF7, ITGB7, TACI that are targeted in current pre-clinical trials for immunotherapy of MM.

Validation in primary patient samples identifies 11 targets

Based on the expression in normal tissues we chose 24 targets for further validation in primary MM patient samples. Eleven of these targets include CCR1 (SEQ ID NO: 60), CD320 (SEQ ID NO: 56), FCRL3(SEQ ID NO: 57), IFNGR1 (SEQ ID NO: 79), IL12RB1 (SEQ ID NO: 19), ITGA4 (SEQ ID NO: 42), LILRB4 (SEQ ID NO: 20), LRRC8D (SEQ ID NO: 112), SEMA4A (SEQ ID NO: 104), and SLAMF6 (SEQ ID NO: 37) and BCMA, and in some aspects, these targets can be integrated into modified or synthesized chimeric antigen receptors, antibodies or antibody binding fragments thereof, and immune cells including T-Cells containing or expressing the foregoing.

Claims:

1. A method for identifying target multiple myeloma associated surface antigens, said method comprising:

identifying a plurality of genes that express cell-surface proteins in a first multiple myeloma sample and a second multiple myeloma sample;

selecting nucleic acids from said first multiple myeloma sample that have expression levels higher than a control gene unrelated to hematopoietic cells, and identifying the proteins corresponding to the detected elevated expressed nucleic acids to designate a first pool of selected proteins;

conducting mass spec analysis on proteins isolated from said second myeloma sample to identify proteins that are present in higher concentration in said second multiple myeloma relative to normal tissues, wherein such proteins represent a second pool of selected proteins;

excluding proteins with high expression in brain, spinal cord, gut, liver and kidney from said first and second pools to produce a modified first and second pool of proteins; and

identifying proteins common to said first and second modified pool of proteins as target multiple myeloma associated surface antigens.

2. The method of claim 1 wherein said first multiple myeloma sample and said second multiple myeloma sample are taken from the same tissue source.

3. The method of claim 1 wherein said first multiple myeloma sample is a nucleic acid pool of expressed genes from MM patients and said second multiple myeloma sample represents proteins expressed in MM cell lines.

4. The method of claim 1, wherein the target multiple myeloma associated surface antigen has an expression level in a normal tissue sample that is more than about one standard deviation below the normal peak of the protein expression level distribution of the normal tissue sample.

5. The method of claim 1, wherein mRNA is measured to determine the expression level of the nucleic acids used to identify proteins for the first pool of selected proteins.

6. A monoclonal antibody that specifically binds to a polypeptide having at least 90% sequence identity to a polypeptide selected from the group consisting of SEQ ID NO: 1-155 or 168-208.

7. A monoclonal antibody that specifically binds to a polypeptide selected from the group consisting of SEQ ID NO: 168-208.

8. A monoclonal antibody in accordance with claim 6 wherein the antibody specifically binds to a polypeptide selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 37, SEQ ID NO: 42, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 79, SEQ ID NO: 112 and SEQ ID NO: 104.

9. The monoclonal antibody of any one of claims 6-8, wherein the antibody specifically binds to a polypeptide having at least 90% sequence identity to a sequence selected from the group consisting of CCR1 (SEQ ID NO: 60), CD320 (SEQ ID NO: 56), FCRL3(SEQ ID NO: 57), IFNGR1 (SEQ ID NO: 79), IL12RB1 (SEQ ID NO: 19), ITGA4 (SEQ ID NO: 42), LILRB4 (SEQ ID NO: 20), LRRC8D (SEQ ID NO: 112), SEMA4A (SEQ ID NO: 104), and SLAMF6 (SEQ ID NO: 37).

10. The monoclonal antibody of any one of claims 6-8, wherein the antibody specifically binds to a polypeptide having at least 90% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 1-155 or 168-208.

11. The monoclonal antibody of any one of claims 6-8, wherein the antibody specifically binds to a polypeptide having at least 95% sequence identity to a sequence selected from the group consisting of SEQ ID NO: 1-155 or 168-208.

12. The monoclonal antibody of any one of claims 6-11 further comprising a detectable label covalently linked to the antibody.

13. The monoclonal antibody of any one of claims 6-11 further comprising a cytotoxic agent linked to the antibody.

14. A chimeric antigen receptor (CAR) comprising

an antibody, or antigen binding fragment thereof, that binds one or more epitopes of a polypeptide selected from the group consisting of SEQ ID NO: 1-155 or 168-208,

a transmembrane domain; and

an immune cell antigen receptor chain, wherein the transmembrane domain links the an antibody, or antigen binding fragment thereof to the immune cell antigen receptor chain.

15. The chimeric antigen receptor of claim 14 wherein the antibody or antigen binding fragment thereof, specifically binds to a polypeptide selected from the group consisting of SEQ ID NO: 168-208.

16. The chimeric antigen receptor of claim 14 wherein the antibody or antigen binding fragment thereof, specifically binds to a polypeptide having at least 95% sequence identity to a sequence selected from the group consisting of CCR1 (SEQ ID NO: 60), CD320 (SEQ ID NO: 56), FCRL3(SEQ ID NO: 57), IFNGR1 (SEQ ID NO: 79), IL12RB1 (SEQ ID NO: 19), ITGA4 (SEQ ID NO: 42), LILRB4 (SEQ ID NO: 20), LRRC8D (SEQ ID NO: 112), SEMA4A (SEQ ID NO: 104) and SLAMF6 (SEQ ID NO: 37).

17. The chimeric antigen receptor of claim 14, wherein the antibody, or antigen binding fragment thereof, binds one or more epitopes of a polypeptide having at least 90% homology to a sequence selected from the group consisting of SEQ ID NO: 1-155 or 168-208.

18. The chimeric antigen receptor of claim 17, wherein the antibody, or antigen binding fragment thereof, binds one or more epitopes of a polypeptide having at least 95% homology to a sequence selected from the group consisting of SEQ ID NO: 1-155 or 168-208.

19. The chimeric antigen receptor of claim 15 wherein the antibody or antigen binding fragment thereof, specifically binds to a polypeptide selected from the group consisting of SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 37, SEQ ID NO: 42, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 79, SEQ ID NO: 112 and SEQ ID NO: 104.

20. The chimeric antigen receptor of any one of claims 15-19 wherein said antibody or antigen binding fragment comprises an antibody single-chain variable fragment.

21. The chimeric antigen receptor of claim 20 further comprising a hinge region located between the antibody single-chain variable fragment and the transmembrane domain.

22. A T-cell modified to express the chimeric antigen receptor of claim 20 or 21.

23. The T-cell of claim 22 wherein the T-cell is a tumor infiltrating leukocyte.

24. An NK cell modified to express the chimeric antigen receptor of claim 15-21.

25. A myeloid cell modified to express the chimeric antigen receptor of claim 15-21.

26. A macrophage modified to express the chimeric antigen receptor of claim 15-21.

27. The T-cell of claim 22 wherein the T -cell antigen receptor chain is the CD3ζ chain (zeta-chain).

28. A pharmaceutical composition comprising the antibody of any one of claims 6-12, chimeric antigen receptor of any one of claims 15-21, the T-cell of

claims 22 or 23, the NK cell of claim 24, the myeloid cell of claim 2325 or the macrophage of claim 26.

29. A method for treating multiple myeloma, said method comprising administering the pharmaceutical composition of claim 24 to a patient in need of such treatment.

30. An isolated immunoresponsive cell comprising an antigen recognizing receptor that binds to an antigen selected from the group consisting of CCR1 (SEQ ID NO: 60), CD320 (SEQ ID NO: 56), FCRL3(SEQ ID NO: 57), IFNGR1 (SEQ ID NO: 79), IL12RB1 (SEQ ID NO: 19), ITGA4 (SEQ ID NO: 42), LILRB4 (SEQ ID NO: 20), LRRC8D (SEQ ID NO: 112), SEMA4A (SEQ ID NO: 104), and SLAMF6 (SEQ ID NO: 37).

31. The isolated immunoresponsive cell of claim 30, wherein the antigen is selected from the group consisting of IL12RB1 (SEQ ID NO: 19), LILRB4 (SEQ ID NO: 20), SLAMF6 (SEQ ID NO: 37), CCR1 (SEQ ID NO: 60), and CD320 (SEQ ID NO: 56).

32. The isolated immunoresponsive cell of claim 30 or 31, wherein said antigen recognizing receptor is a T cell receptor (TCR), or a chimeric antigen receptor (CAR).

33. The isolated immunoresponsive cell of claim 32, wherein said antigen recognizing receptor is a CAR.

34. The isolated immunoresponsive cell of claim 33, wherein the intracellular signaling domain of said CAR is the CD3C-chain, CD97, CD1 la-CD 18, CD2, ICOS, CD27, CD 154, CD8, OX40, 4- IBB, CD28 signaling domain, or combinations thereof.

35. The isolated immunoresponsive cell of any one of claims 30-34, wherein the cell is selected from the group consisting of a T cell, a Natural Killer (NK) cell, a cytotoxic T lymphocyte (CTL), a regulatory T cell, a Natural Killer T (NKT) cell, a

human embryonic stem cell, and a pluripotent stem cell from which lymphoid cells may be differentiated.

36. An isolated immunoresponsive cell comprising:
(a) a first antigen recognizing receptor that binds to a first antigen, and
(b) a second antigen recognizing receptor that binds to a second antigen, wherein each of the first antigen and the second antigen is selected from the group consisting of CCR1 (SEQ ID NO: 60), CD320 (SEQ ID NO: 56), FCRL3(SEQ ID NO: 57), IFNGR1 (SEQ ID NO: 79), IL12RB1 (SEQ ID NO: 19), ITGA4 (SEQ ID NO: 42), LILRB4 (SEQ ID NO: 20), LRRC8D (SEQ ID NO: 112), SEMA4A (SEQ ID NO: 104), and SLAMF6 (SEQ ID NO: 37), and the first antigen and the second antigen are different.

37. The isolated immunoresponsive cell of claim 36, wherein each of said antigen recognizing receptor is a T cell receptor (TCR) or a chimeric antigen receptor (CAR).

38. The isolated immunoresponsive cell of claim 36, wherein each of said antigen recognizing receptor is a CAR.

39. A method of reducing tumor burden in a subject, comprising administering to the subject an effective amount of the immunoresponsive cell of any one of claims 30-38.

Fig. 1A

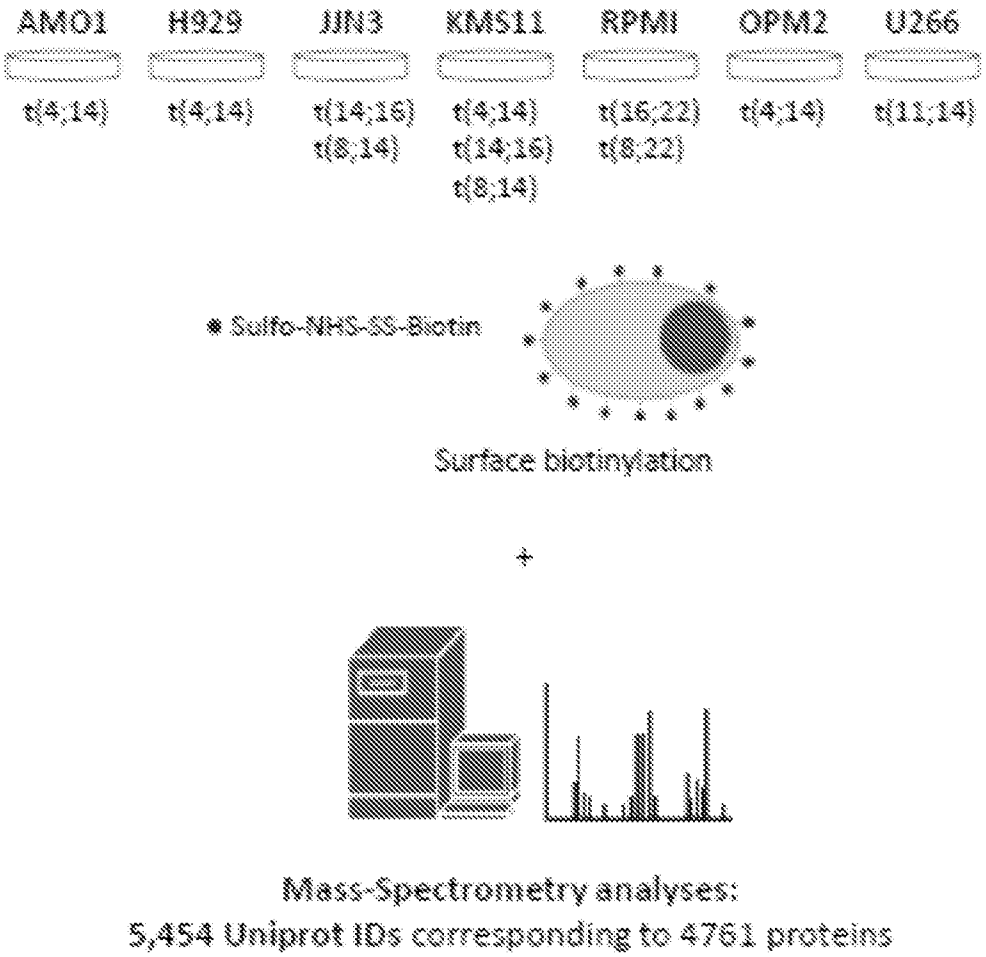

AMO1
H929
JJN3
KMS11
RPMI
OPM2
U266
t(4;14)
t(4;14)
t(14;16)
t(8;14)
t(4;14)
t(14;16)
t(8;14)
t(16;22)
t(8;22)
t(4;14)
t(11;14)
Sulfo-NHS-SS-Biotin
Surface biotinylation
+
Mass-Spectrometry analyses:
5,454 Uniprot IDs corresponding to 4761 proteins

Fig. 1B

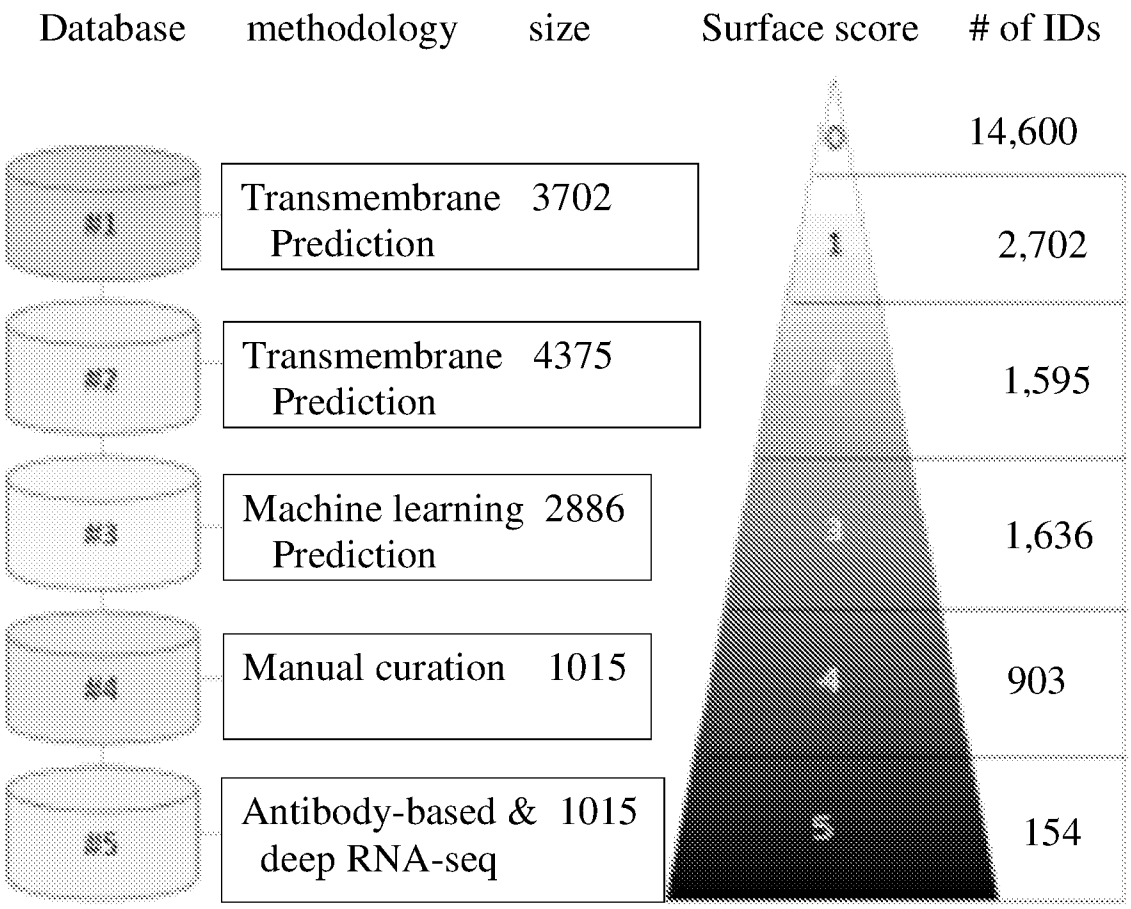

Database
methodology
size
Surface score
# of IDs
S1
Transmembrane Prediction
3702
S2
Transmembrane Prediction
4375
S3
Machine learning Prediction
2886
S4
Manual curation
1015
S5
Antibody-based & deep RNA-seq
1015
0
14,600
1
2,702
2
1,595
3
1,636
4
903
5
154

Fig. 1C

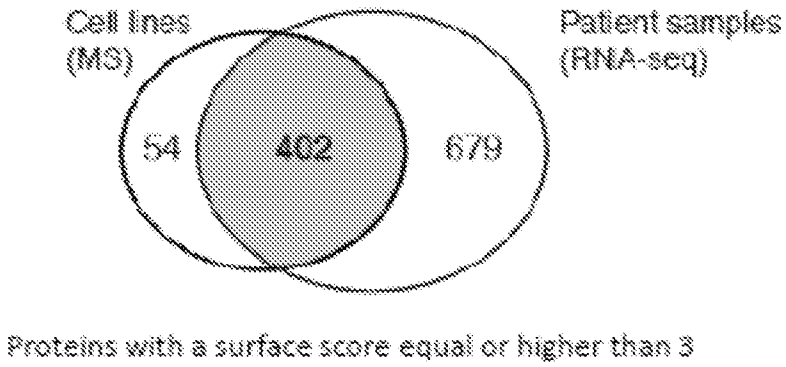

Cell lines
(MS)
Patient samples
(RNA-seq)
54
402
679
Proteins with a surface score equal or higher than 3

Fig. 1D

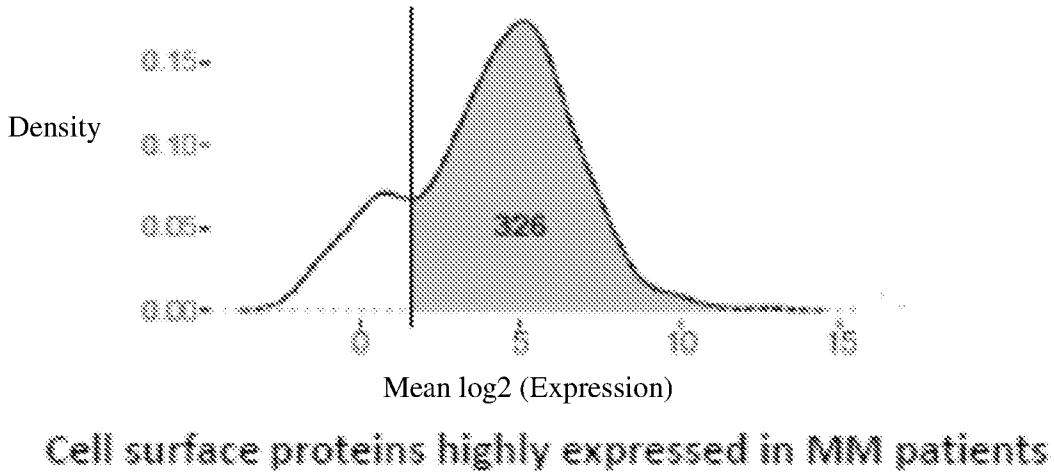

Density
0.15
0.10
0.05
0.00
326
0
5
10
15
Mean log2 (Expression)
Cell surface proteins highly expressed in MM patients

Fig. 2A

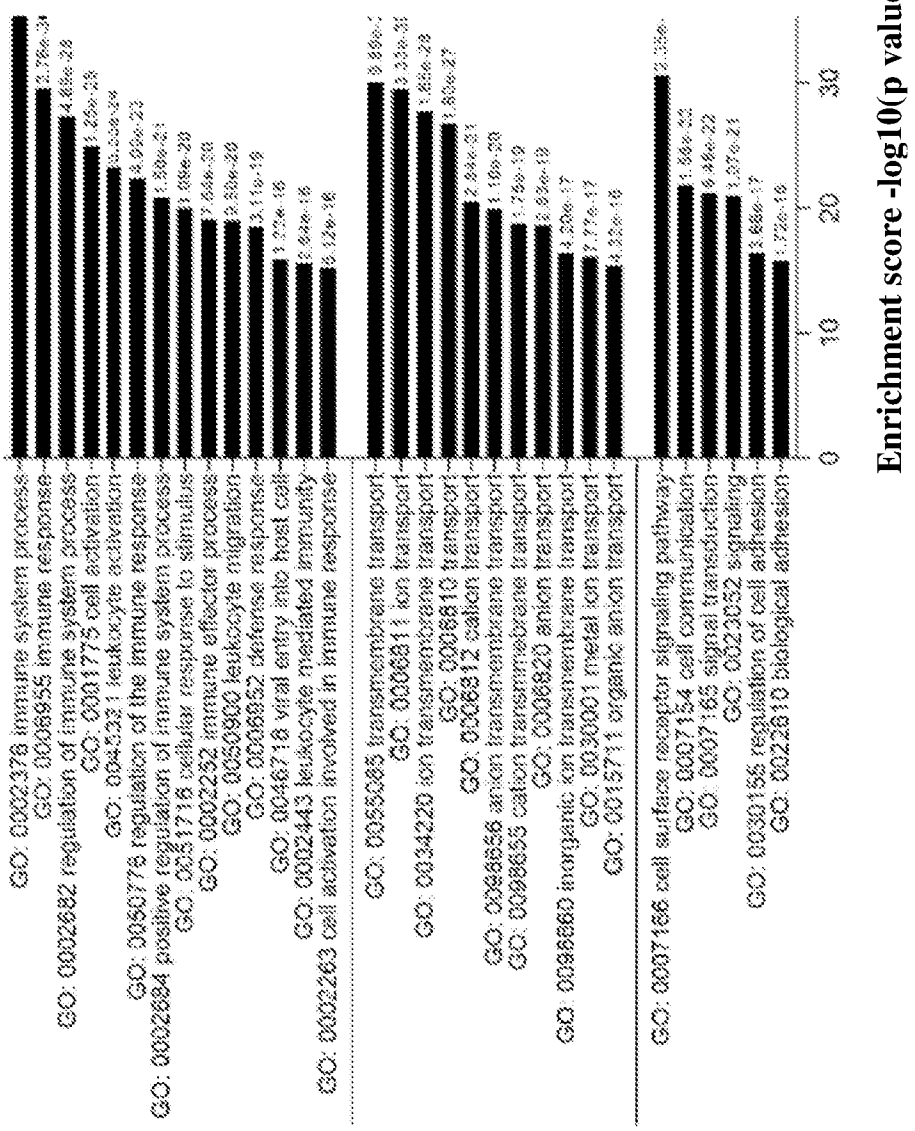

Biological Process (Gene Ontology)
GO: 0002376 immune system process
GO: 0006955 immune response
GO: 0002682 regulation of immune system process
GO: 0001775 cell activation
GO: 0045321 leukocyte activation
GO: 0050776 regulation of the immune response
GO: 0002684 positive regulation of immune system process
GO: 0051716 cellular response to stimulus
GO: 0002252 immune effector process
GO: 0050900 leukocyte migration
GO: 0006952 defense response
GO: 0046718 viral entry into host cell
GO: 0002443 leukocyte mediated immunity
GO: 0002263 cell activation involved in immune response
GO: 0055085 transmembrane transport
GO: 0006811 ion transport
GO: 0034220 ion transmembrane transport
GO: 0006810 transport
GO: 0006812 cation transport
GO: 0098656 anion transmembrane transport
GO: 0098655 cation transmembrane transport
GO: 0006820 anion transport
GO: 0098660 inorganic ion transmembrane transport
GO: 0030001 metal ion transport
GO: 0015711 organic anion transport
GO: 0007186 cell surface receptor signaling pathway
GO: 0007154 cell communication
GO: 0007165 signal transduction
GO: 0023052 signaling
GO: 0030155 regulation of cell adhesion
GO: 0022610 biological adhesion
0
10
20
30
Enrichment score -log10(p value)

Fig. 2B

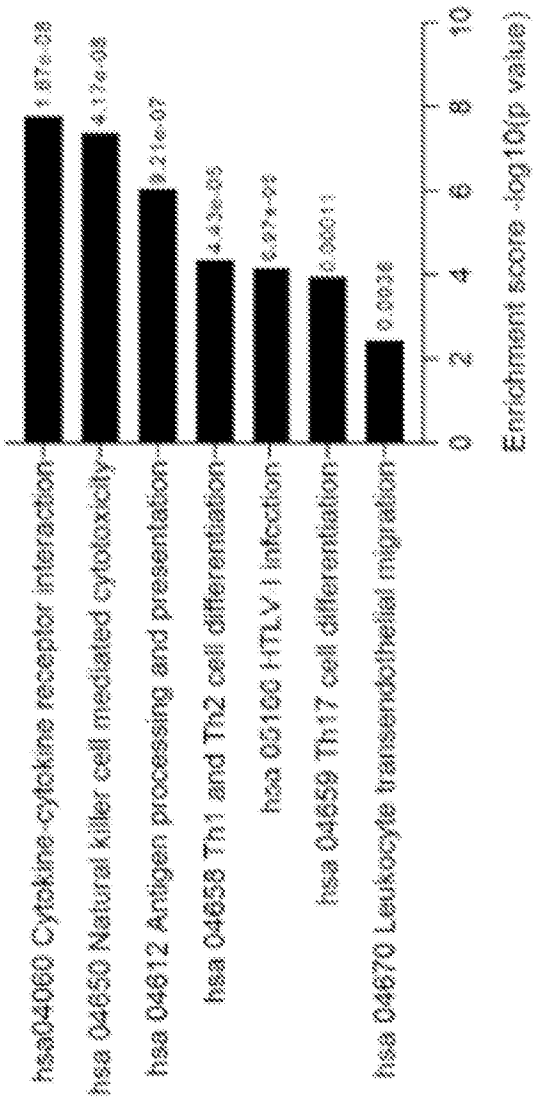

hsa04060 Cytokine-cytokine receptor interaction
hsa 04650 Natural killer cell mediated cytotoxicity
hsa 04612 Antigen processing and presentation
hsa 04658 Th1 and Th2 cell differentiation
hsa 05166 HTLV I infection
hsa 04659 Th17 cell differentiation
hsa 04670 Leukocyte transendothelial migration
0 2 4 6 8 10
Enrichment score -log10(p value)

Fig. 2C

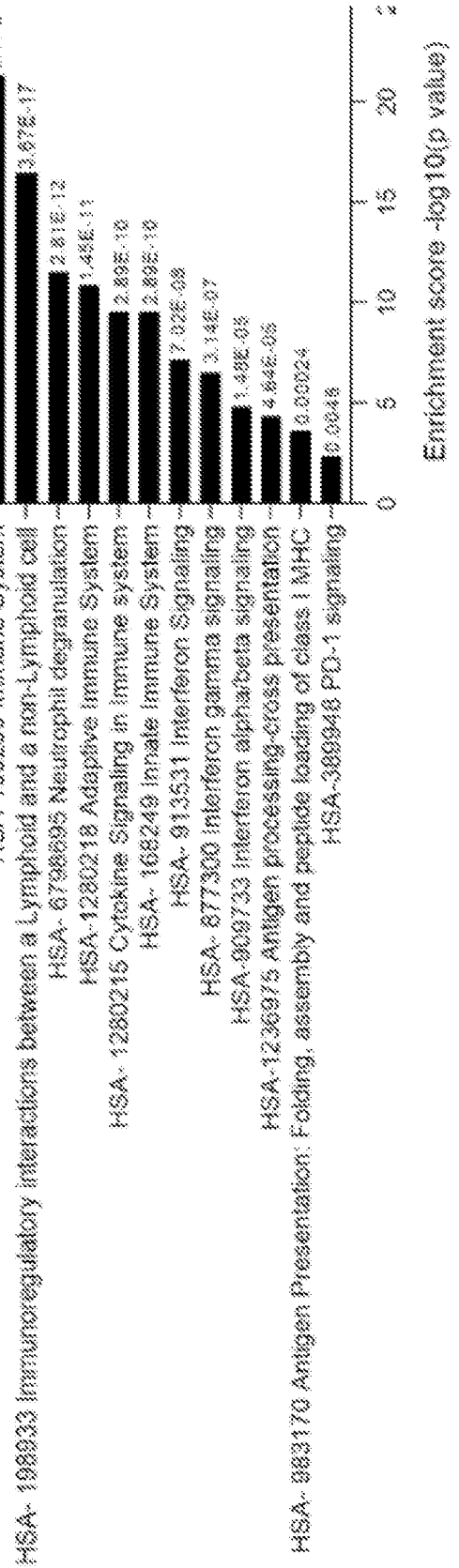
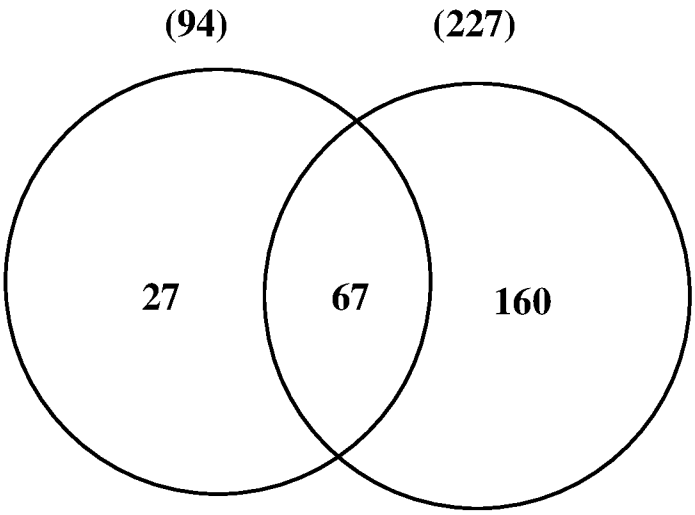

HSA-168256 Immune System
HSA-198933 Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell
HSA-6798695 Neutrophil degranulation
HSA-1280218 Adaptive Immune System
HSA-1280215 Cytokine Signaling in Immune system
HSA-168249 Innate Immune System
HSA-913531 Interferon Signaling
HSA-877300 Interferon gamma signaling
HSA-909733 Interferon alpha/beta signaling
HSA-1236975 Antigen processing-cross presentation
HSA-983170 Antigen Presentation: Folding, assembly and peptide loading of class I MHC
HSA-389948 PD-1 signaling
8.17E-
3.67E-17
2.81E-12
1.45E-11
2.89E-10
2.89E-10
7.02E-08
3.14E-07
1.48E-05
4.64E-05
0.00024
0.0048
0
5
10
15
20
Enrichment score -log10(p value)

Fig. 3



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/024431

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61P 35/00; C07K 16/28; G01N 33/566; G01N 33/574; G01N 33/68; G06F 19/12 (2021.01)
CPC - A61K 35/17; A61K 35/19; A61P 35/00; G01N 33/566; G01N 33/57492; G06F 19/30; G06K 9/00496 (2021.05)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
see Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
see Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
see Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6,046,012 A (CHIZZONITE et al) 04 April 2000 (04.04.2000) entire document	6, 8-11
X	US 6,635,468 B2 (ASHKENAZI et al) 21 October 2003 (21.10.2003) entire document	7
X	WO 2019/094360 A1 (THE BOARD OF REGENTS OF THE UNIVERSITY OF TEXAS SYSTEM) 16 May 2019 (16.05.2019) entire document	14-21, 30-34
X	US 2018/0201901 A1 (CELLECTIS) 19 July 2018 (19.07.2018) entire document	36-38
X -- Y	US 2017/0074883 A1 (LEUKEMIA THERAPEUTICS, LLC) 16 March 2017 (16.03.2017) entire document	1 --- 2-5
Y	US 2010/0143912 A1 (WELLS et al) 10 June 2010 (10.06.2010) entire document	2-5
A	WO 2019/099440 A1 (ARCELLX, INC.) 23 May 2019 (23.05.2019) entire document	1-11, 14-21, 30-34, 36-38
A	WO 2020/033585 A1 (THE BROAD INSTITUTE, INC. et al) 13 February 2020 (13.02.2020) entire document	1-11, 14-21, 30-34, 36-38
A	US 2015/0301058 A1 (CARIS SCIENCE, INC.) 22 October 2015 (22.10.2015) entire document	1-11, 14-21, 30-34, 36-38
A	US 2017/0234874 A1 (CLEARBRIDGE BIOPHOTONICS PTE LTD.) 17 August 2017 (17.08.2017) entire document	1-11, 14-21, 30-34, 36-38

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 June 2021	JUL 15 2021
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Harry Kim Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/024431

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2020/0041513 A1 (HORIBA ABX SAS et al) 06 February 2020 (06.02.2020) entire document	1-11, 14-21, 30-34, 36-38
A	US 2019/0194327 A1 (JOUNCE THERAPEUTICS, INC.) 27 June 2019 (27.06.2019) entire document	1-11, 14-21, 30-34, 36-38
A	US 2018/0348227 A1 (MEMORIAL SLOAN-KETTERING CANCER CENTER) 06 December 2018 (06.12.2018) entire document	1-11, 14-21, 30-34, 36-38

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/024431

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 1-155 and 168-208 were searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2021/024431

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 12, 13, 22-29, 35, 39
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.