

COMPOSITIONS AND METHODS RELATED TO CELL SYSTEMS FOR PENETRATING SOLID TUMORS

RELATED APPLICATIONS

5 This application claims priority to U.S. Serial No. 62/429,275 filed December 2, 2016,
the contents of which are incorporated herein by reference in their entirety.

BACKGROUND

10 Red blood cells have been considered for use as drug delivery systems, e.g., to degrade
toxic metabolites or inactivate xenobiotics, and in other biomedical applications.

SEQUENCE LISTING

15 The instant application contains a Sequence Listing which has been submitted
electronically in ASCII format and is hereby incorporated by reference in its entirety. Said
ASCII copy, created on December 1, 2017, is named R2081-7018WO_SL.txt and is 220,057
bytes in size.

SUMMARY OF THE INVENTION

20 The invention includes cell systems for treating cancer, e.g., by killing cancer cells and/or
by stimulating an immune response to cancer. One challenge in treatment of solid tumors is that
therapeutic agents, especially large agents such as cell therapies, sometimes fail to penetrate the
tumor mass. This disclosure shows, among other things, that erythroid cells comprising a
binding agent can be delivered to the vasculature, and then exit the vasculature and accumulate
in solid tumors. The disclosure also shows that erythroid cells comprising an anti-cancer agent
25 (e.g., antibody) can treat cancers that are resistant to the antibody alone. Thus, the disclosure
provides, e.g., compositions and methods related to cell systems for penetrating solid tumors.

30 In some embodiments, the cell systems involve erythroid cells that express one or more
exogenous polypeptides with targeting function, cancer cell killing function, immune checkpoint
inhibition, and/or costimulation.

The disclosure provides, in some aspects, a method of treating a subject having a cancer (e.g., a cancer described herein), comprising administering to the subject a preparation comprising a plurality of erythroid cells (e.g., erythroid cells described herein), each erythroid cell comprising an anti-cancer agent (e.g., an anti-cancer agent described herein). The anti-

5 cancer agent may be an agent that binds a tumor moiety (e.g., a polypeptide, e.g., an antibody, that binds a cell surface tumor moiety) and/or an agent that has an anti-tumor effect, e.g., an immunostimulatory cytokine, a tumor starvation enzyme (e.g. asparaginase, methionine gamma lyase, or serine dehydrogenase), a cytotoxic small molecule, radionuclide, chemotherapeutic, toxin, small molecule, protein therapeutic, cancer vaccine, checkpoint modulator, pro-apoptotic agent, complement-dependent cytotoxicity (CDC) stimulator, or a fragment or variant thereof.

10 In embodiments, the immunostimulatory cytokine is a Type 1 cytokine or a Type 2 cytokine, or a fragment or variant thereof. In some embodiments, one agent can have both a tumor binding activity and an anti-tumor effect. In some embodiments, each erythroid cell of the plurality has a tumor binding agent and a different agent that has an anti-tumor effect, and the agents may be
15 separate or linked, e.g., expressed as separate agents (e.g., separate polypeptides) or as linked sequences, e.g., fusions).

The present disclosure provides, in some aspects, a method of treating a subject having a resistant, e.g., a refractory or relapsed cancer, comprising:

20 administering (e.g., to the subject's bloodstream) to the subject a preparation comprising a plurality of cells, e.g., erythroid cells (e.g., enucleated erythroid cells), each cell of the plurality comprising, on its surface, an exogenous polypeptide comprising a binding agent, e.g., an antibody, against a tumor cell antigen, in an amount sufficient to treat the cancer,

thereby treating the cancer.

25 The present disclosure provides, in some aspects, a method of delivering an agent, e.g., a binding agent, to a cell of a resistant, e.g., a refractory or relapsed, cancer in a subject, comprising:

administering (e.g., to the subject's bloodstream) to the subject a preparation comprising
30 a plurality of cells, e.g., erythroid cells (e.g., enucleated erythroid cells), each cell of the plurality

comprising, on its surface, an exogenous polypeptide comprising a binding agent, e.g., an antibody, against a tumor cell antigen,
thereby delivering the agent to the cell of the resistant cancer.

5 The present disclosure also provides, in some aspects, a method of treating a subject having a treatment naïve resistant cancer, comprising:

 administering (e.g., to the subject's bloodstream) to the subject a preparation comprising a plurality of cells, e.g., erythroid cells (e.g., enucleated erythroid cells), each cell of the plurality comprising, on its surface, an exogenous polypeptide comprising a binding agent, e.g., an
10 antibody, against a tumor cell antigen, in an amount sufficient to treat the cancer,
 thereby treating the cancer.

 The present disclosure also provides, in some aspects, a method of delivering an agent, e.g., a binding agent, to a cell of a treatment naïve resistant cancer in a subject, comprising:

15 administering (e.g., to the subject's bloodstream) to the subject a preparation comprising a plurality of cells, e.g., erythroid cells (e.g., enucleated erythroid cells), each cell of the plurality comprising, on its surface, an exogenous polypeptide comprising a binding agent, e.g., an antibody, against a tumor cell antigen,
 thereby delivering the agent to the cell of the treatment naïve resistant cancer.

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 The present disclosure also provides, in some aspects, a method of treating a subject having a cancer, wherein the cancer comprises an oncogenic mutation, e.g., a translocation that places a cell cycle gene under control of a constitutive promoter, comprising:

 administering (e.g., to the subject's bloodstream) to the subject a preparation comprising
25 a plurality of cells, e.g., erythroid cells (e.g., enucleated erythroid cells), each cell of the plurality comprising, on its surface, an exogenous polypeptide comprising a binding agent, e.g., an antibody, against a tumor cell antigen, in an amount sufficient to treat the cancer,
 thereby treating the cancer.

30 The present disclosure also provides, in some aspects, a method of delivering an agent, e.g., a binding agent, to a cell of a cancer in a subject, wherein the cancer comprises an

oncogenic mutation, e.g., a translocation that places a cell cycle gene under control of a constitutive promoter, comprising:

administering (e.g., to the subject's bloodstream) to the subject a preparation comprising a plurality of cells, e.g., erythroid cells (e.g., enucleated erythroid cells), each cell of the plurality comprising, on its surface, an exogenous polypeptide comprising a binding agent, e.g., an antibody, against a tumor cell antigen, thereby delivering the agent to the cell of the cancer.

The present disclosure also provides, in some aspects, a method of treating a subject having a B cell cancer, or of delivering an agent to a cancerous B cell in a subject, wherein the subject has developed resistance to an antibody selected from Table 1, e.g., an anti-CD20 antibody, comprising:

administering to the subject a preparation of cells, e.g., erythroid cells (e.g., enucleated erythroid cells) comprising a fusion protein comprising a transmembrane domain and a binding domain that binds a tumor antigen (e.g., wherein the binding domain is an anti-CD20 antibody domain), wherein:

a) the number of fusion proteins on the outer surface of the engineered erythroid cell is greater than 10^4 , e.g., greater than 2×10^4 , 3×10^4 , 4×10^4 , 5×10^4 , 6×10^4 , 7×10^4 , 8×10^4 , 9×10^4 or greater than 10^5 , e.g., greater than 2×10^5 , 3×10^5 , 4×10^5 , 5×10^5 , 6×10^5 , 7×10^5 , 8×10^5 , 9×10^5 , 1×10^6 , 2×10^6 , 5×10^6 , or 1×10^7 (and optionally up to 1×10^7 or 1×10^8), or about 1×10^4 - 3×10^4 , 1×10^4 - 5×10^4 , 1×10^4 - 7×10^4 , 1×10^4 - 9×10^4 , or

b) the number of fusion proteins on the outer surface of the engineered erythroid cell or the affinity of the binding domain for the tumor antigen is sufficient to induce:

i) clustering of a tumor antigen, e.g., an antigen described herein, e.g., an antigen of Table 1, e.g., CD20 molecules on the surface of target cancer cells, e.g., B cells,

ii) hyper-clustering of a tumor antigen, e.g., an antigen described herein, e.g., an antigen of Table 1, e.g., CD20, on the surface of target cancer cells, e.g., B cells,

iii) cross-linking of a tumor antigen, e.g., an antigen described herein, e.g., an antigen of Table 1, e.g., CD20, on the surface of target cancer cells, e.g., B cells,

iv) hyper cross-linking of a tumor antigen, e.g., an antigen described herein, e.g., an antigen of Table 1, e.g., CD20, on the surface of target cancer cells, e.g., B cells,

v) apoptosis, e.g., caspase-independent apoptosis and/or caspase-dependent apoptosis, of target cancer cells, e.g., B cells,

vi) activation of Src family tyrosine kinase, clustering of Fas molecule, inhibition or downregulation of BCL2, and/or inhibition or downregulation of BCLx1, in target cancer cells, e.g., B cells, or a combination thereof, thereby treating the subject or delivering the agent to the cancerous B cell.

The present disclosure also provides, in some aspects, a cell, e.g., an erythroid cell (e.g., enucleated erythroid cell) comprising a fusion protein comprising a transmembrane domain and a binding domain that binds a tumor antigen (e.g., wherein the binding domain is an anti-CD20 antibody domain or an anti-PD-L1 antibody domain) wherein:

a) the number of fusion proteins on the outer surface of the erythroid cell is greater than 10^4 , e.g., greater than 2×10^4 , 3×10^4 , 4×10^4 , 5×10^4 , 6×10^4 , 7×10^4 , 8×10^4 , 9×10^4 or greater than 10^5 , e.g., greater than 2×10^5 , 3×10^5 , 4×10^5 , 5×10^5 , 6×10^5 , 7×10^5 , 8×10^5 , 9×10^5 , 1×10^6 , 2×10^6 , 5×10^6 , or 1×10^7 (and optionally up to 1×10^7 or 1×10^8), or about 1×10^4 - 3×10^4 , 1×10^4 - 5×10^4 , 1×10^4 - 7×10^4 , 1×10^4 - 9×10^4 , or

b) the number of fusion proteins on the outer surface of the erythroid cell or the affinity of the binding domain for the tumor antigen is sufficient to induce:

i) clustering of a tumor antigen, e.g., an antigen described herein, e.g., an antigen of Table 1, e.g., CD20 molecules on the surface of target cancer cells, e.g., B cells,

ii) hyper-clustering of a tumor antigen, e.g., an antigen described herein, e.g., an antigen of Table 1, e.g., CD20, on the surface of target cancer cells, e.g., B cells,

iii) cross-linking of a tumor antigen, e.g., an antigen described herein, e.g., an antigen of Table 1, e.g., CD20, on the surface of target cancer cells, e.g., B cells,

iv) hyper cross-linking of a tumor antigen, e.g., an antigen described herein, e.g., an antigen of Table 1, e.g., CD20, on the surface of target cancer cells, e.g., B cells,

v) apoptosis, e.g., caspase-independent apoptosis and/or caspase-dependent apoptosis, of target cancer cells, e.g., B cells,

vi) activation of Src family tyrosine kinase, clustering of Fas molecule, inhibition or downregulation of BCL2, and/or inhibition or downregulation of BCLx1, in target cancer cells, e.g., B cells, or a combination thereof.

The present disclosure also provides, in certain aspects, a method of treating a vascularized solid tumor in a subject comprising:

5 administering to the subject's bloodstream a preparation comprising a plurality of cells, e.g., erythroid cells (e.g., enucleated erythroid cells), each cell of the plurality comprising, on its surface, an exogenous polypeptide comprising a binding agent, e.g., an antibody, against a tumor cell antigen, in an amount sufficient to treat the vascularized solid tumor thereby treating the vascularized solid tumor.

10 The present disclosure also provides, in some aspects, a method of delivering an agent, e.g., a binding agent to a tumor cell of a vascularized solid tumor in a subject comprising:

administering to the subject's bloodstream a preparation comprising a plurality of cells, e.g., erythroid cells (e.g., enucleated erythroid cells), each cell of the plurality comprising, on its surface, an exogenous polypeptide comprising a binding agent, e.g., an antibody, against a tumor
15 cell antigen, in an amount sufficient to deliver the binding agent to a tumor cell of the vascularized solid tumor,
thereby delivering the agent, e.g., binding agent to the tumor cell of the vascularized solid tumor.

20 The present disclosure also provides, in some aspects, a method of enriching an anti-cancer agent, e.g., an anti-cancer antibody, at a solid tumor in a subject, or treating a solid tumor in the subject, comprising:

administering to the subject's bloodstream a preparation comprising a plurality of cells, e.g., erythroid cells (e.g., enucleated erythroid cells), each cell of the plurality comprising the
25 anti-cancer agent, in an amount sufficient to treat the solid tumor,

wherein optionally the anti-cancer agent comprises an exogenous polypeptide comprising a binding agent, e.g., an antibody, against a tumor cell antigen,
thereby enriching an anti-cancer agent or treating the solid tumor.

The present disclosure also provides, in some aspects, a method of enriching an anti-cancer agent, e.g., an anti-cancer antibody, at a solid tumor in a subject, or treating a solid tumor in the subject, comprising:

administering to the subject's bloodstream a preparation comprising a plurality of cells, e.g., erythroid cells, each cell of the plurality comprising the anti-cancer agent, in an amount sufficient to treat the solid tumor,

wherein the anti-cancer agent comprises an exogenous polypeptide comprising a binding agent, e.g., an antibody, against a tumor cell antigen,

thereby enriching an anti-cancer agent or treating the solid tumor.

The disclosure further provides, in certain aspects, a method of delivering an erythroid cell to an extravascular site in a subject, comprising:

administering to the subject's bloodstream an effective amount of a preparation comprising a plurality of cells, e.g., erythroid cells (e.g., enucleated erythroid cells), each cell of the plurality comprising, on its surface, an exogenous polypeptide comprising a binding agent, e.g., an antibody, against an antigen present at the extravascular site, e.g., a tumor antigen, thereby delivering the erythroid cell to the extravascular site.

The disclosure further provides, in certain aspects, a method of delivering an agent, e.g., a binding agent, to an extravascular site in a subject, comprising:

administering to the subject's bloodstream an effective amount of a preparation comprising a plurality of cells, e.g., erythroid cells (e.g., enucleated erythroid cells), each cell of the plurality comprising, on its surface, an exogenous polypeptide comprising a binding agent, e.g., an antibody, against an antigen present at the extravascular site, e.g., a tumor antigen, thereby delivering the agent, e.g., binding agent, to the extravascular site.

The present disclosure also provides, in some aspects, a method of treating a non-vascularized, e.g., a prevascularized, solid tumor in a subject comprising:

administering to the subject's bloodstream a preparation comprising a plurality of cells, e.g., erythroid cells (e.g., enucleated erythroid cells), each cell of the plurality comprising, on its

surface, an exogenous polypeptide comprising a binding agent, e.g., an antibody, against a tumor cell antigen, in an amount sufficient to treat the non-vascularized solid tumor thereby treating a prevascularized solid tumor.

5 The present disclosure also provides, in some aspects, a method of delivering an agent, e.g., a binding agent to a tumor cell of a non-vascularized, e.g., prevascularized, solid tumor in a subject comprising:

 administering to the subject's bloodstream a preparation comprising a plurality of cells, e.g., erythroid cells (e.g., enucleated erythroid cells), each cell of the plurality comprising, on its
10 surface, an exogenous polypeptide comprising a binding agent, e.g., an antibody, against a tumor cell antigen, in an amount sufficient to deliver the binding agent to a tumor cell of the non-vascularized solid tumor,
 thereby delivering the agent to the tumor cell of the non-vascularized solid tumor.

15 The present disclosure also provides, in some aspects, a method of treating a subject having a cancer, comprising:

 administering (e.g., to the subject's bloodstream) to the subject a preparation comprising a plurality of cells, e.g., erythroid cells (e.g., enucleated erythroid cells), each cell of the plurality comprising, on its surface, an exogenous polypeptide comprising a binding agent, e.g., an
20 antibody, against a tumor cell antigen,

 wherein the density of binding agents on the surface of the erythroid cell is sufficient that, upon binding of the binding agents with tumor cell antigen, a tumor antigen accumulates in a lipid raft, the distribution of the antitumor cell antigen is sufficiently perturbed to alter signalling in the tumor cell, the density of tumor antigens on the surface of the cancer cell is significantly
25 altered, an anti-apoptotic pathway (e.g., BCL2 and/or BCLxl pathway) is inhibited, an apoptotic pathway of the cancer cell is induced, a necrotic pathway of the cancer cell is induced, or the membrane properties of the cancer cell are significantly altered, or a combination thereof
 thereby treating the cancer.

The present disclosure also provides, in some aspects, a cell, e.g., an erythroid cell (e.g., enucleated erythroid cell) comprising an exogenous polypeptide comprising a binding agent, e.g., an antibody, against a tumor cell antigen,

wherein the density of binding agents on the surface of the erythroid cell is sufficient that,
5 upon binding of the binding agents with tumor cell antigen, a tumor antigen accumulates in a lipid raft, the distribution of the antitumor cell antigen is sufficiently perturbed to alter signalling in the tumor cell, the density of tumor antigens on the surface of the cancer cell is significantly altered, an anti-apoptotic pathway (e.g., BCL2 and/or BCLxl pathway) is inhibited, an apoptotic pathway of the cancer cell is induced, a necrotic pathway of the cancer cell is induced, or the
10 membrane properties of the cancer cell are significantly altered, or a combination thereof

The present disclosure also provides, in some aspects, a method of treating a tumor, e.g., a vascularized tumor, in a subject comprising:

administering (e.g., to the subject's bloodstream) to the subject an erythroid cell (e.g.,
15 enucleated erythroid cell) comprising, e.g., on its surface,

a moiety that interferes with the ability of an immune checkpoint-ligand (e.g., PD-L1) to functionally engage an immune checkpoint molecule (e.g., PD1), e.g., an immune checkpoint molecule expressed on a tumor infiltrating lymphocyte,

wherein if the moiety is expressed as a fusion protein:

- 20 i) at least 50, 60, 70, 80, 90, 95, or 99% of the fusion proteins on the surface of the erythroid cell have an identical sequence,
ii) at least 50, 60, 70, 80, 90, 95, or 99% of the fusion protein have the same transmembrane region,
iii) the fusion protein does not include a full length endogenous membrane protein, e.g.,
25 comprises a segment of a full length endogenous membrane protein, which segment lacks at least 1, 2, 3, 4, 5, 10, 20, 50, 100, 200, or 500 amino acids of the full length endogenous membrane protein;
iv) at least 50, 60, 70, 80, 90, 95, or 99% of the fusion proteins do not differ from one another by more than 1, 2, 3, 4, 5, 10, 20, or 50 amino acids,
30 v) the moiety lacks a sortase transfer signature (i.e., a sequence that can be created by a sortase reaction) such as LPXTG,

- vi) the moiety is present on less than 1, 2, 3, 4, or 5 sequence-distinct fusion polypeptides;
- vii) the moiety is present on a single fusion polypeptide;
- viii) the fusion protein does not contain Gly-Gly at the junction of an endogenous

5 transmembrane protein and the moiety; or

- ix) the fusion protein does not contain Gly-Gly, or does not contain Gly-Gly in an extracellular region, does not contain Gly-Gly in an extracellular region that is within 1, 2, 3, 4, 5, 10, 20, 50, or 100 amino acids of a transmembrane segment; or a combination thereof, thereby treating the tumor.

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The present disclosure also provides, in some aspects, an erythroid cell comprising, e.g., on its surface,

a moiety that interferes with the ability of an immune checkpoint-ligand (e.g., PD-L1) to functionally engage an immune checkpoint molecule (e.g., PD1), e.g., an immune checkpoint

15 molecule expressed on a tumor infiltrating lymphocyte,

wherein if the moiety is expressed as a fusion protein:

- i) at least 50, 60, 70, 80, 90, 95, or 99% of the fusion proteins on the surface of the erythroid cell have an identical sequence,

- ii) at least 50, 60, 70, 80, 90, 95, or 99% of the fusion protein have the same

20 transmembrane region,

- iii) the fusion protein does not include a full length endogenous membrane protein, e.g., comprises a segment of a full length endogenous membrane protein, which segment lacks at least 1, 2, 3, 4, 5, 10, 20, 50, 100, 200, or 500 amino acids of the full length endogenous membrane protein;

25 iv) at least 50, 60, 70, 80, 90, 95, or 99 % of the fusion proteins do not differ from one another by more than 1, 2, 3, 4, 5, 10, 20, or 50 amino acids,

- v) the moiety lacks a sortase transfer signature,

- vi) the moiety is present on less than 1, 2, 3, 4, or 5 sequence-distinct fusion polypeptides;

30 vii) the moiety is present on a single fusion polypeptide;

viii) the fusion protein does not contain Gly-Gly at the junction of an endogenous transmembrane protein and the moiety; or

ix) the fusion protein does not contain Gly-Gly, or does not contain Gly-Gly in an extracellular region, does not contain Gly-Gly in an extracellular region that is within 1, 2, 3, 4,
5 5, 10, 20, 50, or 100 amino acids of a transmembrane segment, or a combination thereof.

The present disclosure also provides, in some aspects, a method of treating a tumor, e.g., a vascularized tumor, in a subject comprising:

administering (e.g., to the subject's bloodstream) to the subject an erythroid cell (e.g.,
10 enucleated erythroid cell) comprising, e.g., on its surface, a stimulatory molecule, e.g., a costimulatory molecule, e.g., 4-1BBL or a fragment thereof, wherein

the level of the stimulatory molecule, e.g., costimulatory molecule or the affinity of the stimulatory molecule, e.g., costimulatory molecule for a binding partner on an immune cell (e.g., T cell) is sufficient to: induce immune cell proliferation, increase secretion of a cytokine (e.g.,
15 IL2 or IFN-gamma), or reduce activation-induced cell death in an immune cell, e.g., tumor infiltrating lymphocyte and/or T cell,

wherein if the moiety is expressed as a fusion protein:

i) at least 50, 60, 70, 80, 90, 95, or 99% of the fusion proteins on the surface of the erythroid cell have an identical sequence,

20 ii) at least 50, 60, 70, 80, 90, 95, or 99% of the fusion protein have the same transmembrane region,

iii) the fusion protein does not include a full length endogenous membrane protein, e.g., comprises a segment of a full length endogenous membrane protein, which segment lacks at least 1, 2, 3, 4, 5, 10, 20, 50, 100, 200, or 500 amino acids of the full length
25 endogenous membrane protein;

iv) at least 50, 60, 70, 80, 90, 95, or 99% of the fusion proteins do not differ from one another by more than 1, 2, 3, 4, 5, 10, 20, or 50 amino acids,

v) the moiety lacks a sortase transfer signature,

vi) the moiety is present on less than 1, 2, 3, 4, or 5 sequence-distinct fusion
30 polypeptides;

vii) the moiety is present on a single fusion polypeptide;

viii) the fusion protein does not contain Gly-Gly at the junction of an endogenous transmembrane protein and the moiety; or

ix) the fusion protein does not contain Gly-Gly, or the fusion protein does not contain Gly-Gly, or does not contain Gly-Gly in an extracellular region, does not contain Gly-Gly in an extracellular region that is within 1, 2, 3, 4, 5, 10, 20, 50, or 100 amino acids of a transmembrane segment; or a combination thereof,

thereby treating the tumor.

The present disclosure also provides, in some aspects, an erythroid cell comprising, e.g., on its surface, a stimulatory molecule, e.g., a costimulatory molecule, e.g., 4-1BBL or a fragment thereof, wherein

the level of the stimulatory molecule, e.g., costimulatory molecule or the affinity of the stimulatory molecule, e.g., costimulatory molecule for a binding partner on an immune cell (e.g., T cell) is sufficient to: induce immune cell proliferation, increase secretion of a cytokine (e.g., IL2 or IFN-gamma), or reduce activation-induced cell death in an immune cell, e.g., tumor infiltrating lymphocyte and/or T cell,

wherein if the moiety is expressed as a fusion protein:

i) at least 50, 60, 70, 80, 90, 95, or 99% of the fusion proteins on the surface of the erythroid cell have an identical sequence,

ii) at least 50, 60, 70, 80, 90, 95, or 99% of the fusion protein have the same transmembrane region,

iii) the fusion protein does not include a full length endogenous membrane protein, e.g., comprises a segment of a full length endogenous membrane protein, which segment lacks at least 1, 2, 3, 4, 5, 10, 20, 50, 100, 200, or 500 amino acids of the full length endogenous membrane protein;

iv) at least 50, 60, 70, 80, 90, 95, or 99 % of the fusion proteins do not differ from one another by more than 1, 2, 3, 4, 5, 10, 20, or 50 amino acids,

v) the moiety lacks a sortase transfer signature,

vi) the moiety is present on less than 1, 2, 3, 4, or 5 sequence distinct fusion polypeptides;

vii) the moiety is present on a single fusion polypeptide;

viii) the fusion protein does not contain Gly-Gly at the junction of an endogenous transmembrane protein and the moiety;

ix) the fusion protein does not contain Gly-Gly, or the fusion protein does not contain Gly-Gly, or does not contain Gly-Gly in an extracellular region, does not contain Gly-Gly in an extracellular region that is within 1, 2, 3, 4, 5, 10, 20, 50, or 100 amino acids of a transmembrane segment; or a combination thereof.

The present disclosure also provides, in some aspects, a method of stimulating an immune effector cell, e.g., a T cell, in a subject, comprising:

administering (e.g., to the subject's bloodstream) to the subject a preparation comprising a plurality of cells, e.g., erythroid cells (e.g., enucleated erythroid cells), each cell of the plurality comprising, on its surface, an exogenous polypeptide comprising a costimulatory molecule (e.g., 4-1BB-L, OX40-L, GITR-L, or ICOS-L), in an amount sufficient to stimulate the immune effector cell, thereby stimulating the immune effector cell.

The present disclosure also provides, in some aspects, a method of detecting a cancer, e.g. a solid tumor, comprising:

administering (e.g., to the subject's bloodstream) to the subject a preparation comprising a plurality of cells, e.g., erythroid cells (e.g., enucleated erythroid cells), each cell of the plurality comprising, on its surface, an exogenous polypeptide comprising a binding agent, e.g., an antibody, against a tumor cell antigen,

wherein each cell in the plurality also comprises (e.g., on the cell surface on inside the cell) a label detectable by in vivo imaging, e.g., a radionuclide, fluorophore, bioluminescent agent, or MRI contrast agent; and

detecting the label, e.g., by MRI or radiological detection, thereby detecting the cancer.

The present disclosure also provides, in some aspects, an erythroid cell (e.g., enucleated erythroid cell) comprising:

on its surface, an exogenous polypeptide comprising a binding agent, e.g., an antibody, against a tumor cell antigen, and

a label detectable by in vivo imaging, e.g., a radionuclide, fluorophore, bioluminescent agent, or MRI contrast agent, (e.g., on the cell surface or inside the cell).

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In some aspects, the present disclosure provides a method of delivering, presenting, or expressing an anti-cancer agent comprising providing an erythroid cell described herein.

10 In some aspects, the present disclosure provides a method of producing an erythroid cell (e.g., enucleated erythroid cell) described herein, providing contacting an erythroid cell precursor with one or more nucleic acids encoding the exogenous polypeptides and placing the cell in conditions that allow enucleation to occur.

15 In some aspects, the present disclosure provides a preparation, e.g., pharmaceutical preparation, comprising a plurality of erythroid cells (e.g., enucleated erythroid cells) described herein, e.g., at least 10^8 , 10^9 , 10^{10} , 10^{11} , or 10^{12} cells.

The following embodiments can apply to any of the aspects herein, e.g., any of the compositions and methods herein above.

20 In some embodiments, the agent is a therapeutic agent (e.g., an anti-cancer agent) or a diagnostic agent (e.g., a label detectable by in vivo imaging). In embodiments, the agent promotes T cell activation, stimulation, or proliferation. In embodiments, the agent inhibits cancer cell growth or survival. In embodiments, the agent has two or more properties of agents described herein.

25 In some embodiments, the cell, e.g., erythroid cell, is autologous. In some embodiments, the cell is allogeneic.

In some embodiments, the cell system is administered in an amount and for a time effective to result in one of (or more, e.g., 2 or more, 3 or more, 4 or more of): (a) reduced tumor size, (b) reduced rate of tumor growth, (c) increased tumor cell death (d) reduced tumor
30 progression, (e) reduced number of metastases, (f) reduced rate of metastasis, (g) decreased

tumor recurrence (h) increased survival of subject, (i) increased progression free survival of subject.

In embodiments, the tumor is non-metastatic. In embodiments, the tumor is metastatic. In embodiments, the tumor is vascularized and non-metastatic. In embodiments, the tumor comprises one or more tumor blood vessels. In embodiments, the tumor (e.g., vascularized tumor) secretes VEGF or bFGF. In embodiments, the tumor (e.g., non-vascularized tumor) does not produce VEGF or bFGF. In embodiments, the tumor (e.g., non-vascularized tumor) produces an anti-VEGF enzyme such as PGK. In embodiments, the tumor (e.g., vascularized tumor) does not produce an anti-VEGF enzyme such as PGK. In embodiments, the tumor comprises a necrotic region. In embodiments, the tumor expresses a pro-angiogenic factor.

In embodiments, the cancer expresses one or more tumor antigen herein, e.g., CD19, CD20, CD30, CD33, CD52, EGFR, GD2, HER2/neu, or VEGF, or a combination thereof.

In embodiments, the cancer is a cancer of Table 1 and the cancer antigen is a cancer antigen of Table 1. In embodiments, the cancer is a cancer of Table 3. In embodiments, the cancer lacks a functional caspase apoptosis pathway. In embodiments, the solid tumor is a solid tumor of Table 3. In embodiments, the solid tumor is a primary tumor or a metastatic tumor lesion. In embodiments, the location of the primary tumor is known. In embodiments, the cancer is other than a cancer of unknown primary.

In embodiments, after administration, the erythroid cells are resident or persistent in an extravascular region of the tumor, a non-necrotic region of the tumor, or an interstitial region of the tumor. In embodiments, an erythroid cell that is persistent in a tumor is resident in the tumor for at least 3, 6, or 12 hours or 1, 2, 3, 4, 5, 6, 7, 14, 21, or 28 days (e.g., up to 14 or 28 days).

In embodiments, the amount of binding agent on the surface of the erythroid cell is sufficient, or the binding affinity of the binding agent for its target is sufficient, or the erythroid cells are administered to the subject in an amount sufficient:

a) that the ratio of erythroid cells to tumor cells in the tumor (or in a 1, 2, 5, 10, 20, 50, 100, 200, 500, or 1000 mm³ region of the tumor) is at least about 100:1, 50:1, 20:1, 10:1, 5:1, 2:1, 1:1, 1:2, 1:5, 1:10, 1:20, 1:50, or 1:100 (e.g., up to about 100:1 or 10:1);

b) that the ratio of the erythroid cells to endogenous erythroid cells in the tumor is at least 2:1, 5:1, 10:1, 20:1, 50:1, 100:1, 1,000:1, or 10,000:1 (e.g., up to 100:1, 1,000:1 or 10,000:1);

c) to induce hypercrosslinking of a cancer cell surface protein, e.g., CD20;

d) that the ratio of erythroid cells resident in the tumor to the number of erythroid cells in the subject's bloodstream is greater than 1:1, 2:1, 3:1, 4:1, 5:1 10:1, 20:1, or 100:1 (and optionally up to 10:1 or 100:1), e.g., at least 1 hour or 12 hours or 1 day, 3, days, 5 days, or 7 days after administration of the cells or at the peak of erythroid cell accumulation in the tumor;

5 e) that the amount of binding agent, e.g., antibody, resident in the tumor (or in a 1, 2, 5, 10, 20, 50, 100, 200, 500, or 1000 mm³ region of the tumor) is at least 2, 3, 4, 5, 10, 20, 50, or 100-fold greater (and, optionally up to 10 or 100-fold greater) than the amount of an otherwise similar binding agent, e.g., antibody that is not associated with an erythroid cell, e.g., a free antibody;

10 f) to increase the erythroid cells' persistence in the tumor (e.g., by at least 2, 3, 4, 5, 10, 20, 50, 100, 200, or 500-fold), as compared with a reference, e.g., with a similar erythroid cell that lacks the binding agent;

g) that the concentration of erythroid cells in the tumor or a 1, 2, 5, 10, 20, 50, 100, 200, 500, or 1000 mm³ region of the tumor is enriched compared to an extratumoral compartment, 15 e.g., at least 10, 20, 30, 40, 50, 60, 70, 80, 90, or 95% (and optionally up to 95% or 99%) of the erythroid cells in the subject are found in the tumor and less than 30, 20, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1% of the erythroid cells found in a second compartment, e.g., the liver; e.g., at least 1 hour or 12 hours or 1 day, 3, days, 5 days, or 7 days after administration of the cells or at the peak of erythroid cell accumulation in the tumor;

20 h) that the concentration of erythroid cells in a vasculature-adjacent region of the tumor (e.g., a 1, 2, 5, 10, 20, 50, 100, 200, 500, or 1000 mm³ region) is enriched compared to a vasculature-distant region of the tumor (e.g., a 1, 2, 5, 10, 20, 50, 100, 200, 500, or 1000 mm³ region), e.g., at least 10, 20, 30, 40, 50, 60, 70, 80, 90, or 95% (and optionally up to 95% or 99%) of the erythroid cells in the subject are found in the vasculature-adjacent region and less than 30, 25 20, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1%, or an undetectable amount, of the erythroid cells found in a vasculature-distant region; e.g., at least 1 hour or 12 hours or 1 day, 3, days, 5 days, or 7 days after administration of the cells or at the peak of erythroid cell accumulation in the tumor, wherein optionally the vascular adjacent region is within 0.1, 0.2, 0.5, 1, 2, 3, 4, or 5 cm (e.g., up to 5 cm) of a blood vessel and the vascular-distant region of the tumor is further than 2, 3, 4, 5, 30 or 10 cm of a blood vessel;

j) that the number of erythroid cells in circulation is sufficiently low such that the patient does not experience an infusion reaction, severe mucocutaneous reaction, Hepatitis B virus reactivation, or progressive multifocal leukoencephalopathy, or a combination thereof;

k) that the erythroid cells are present and/or active in the tumor site at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months (and optionally up to 6, 9 or 12 months) after they are administered to the subject; or

l) that cancer cells undergo cell death (e.g., ADCC or apoptosis, e.g., caspase-independent apoptosis) in the presence of the erythroid cells at a higher rate than cancer cells in the presence of the same amount of the same binding agent not comprised by an erythroid cell, or any combination thereof.

In embodiments, the number of erythroid cells comprising a binding agent on their surface in circulation is sufficiently low such that the patient does not experience a side effect that is associated with the free binding agent, e.g., an antibody not associated with an erythroid cell. In embodiments (e.g., wherein the binding agent binds CD20, e.g., wherein the binding agent comprises rituximab or a fragment or variant thereof), the number of erythroid cells in circulation is sufficiently low such that the patient does not experience an infusion reaction, severe mucocutaneous reaction, Hepatitis B virus reactivation, or progressive multifocal leukoencephalopathy, or a combination thereof. In embodiments (e.g., wherein the binding agent binds CD52, e.g., wherein the binding agent comprises alemtuzumab or a fragment or variant thereof), the number of erythroid cells in circulation is sufficiently low such that the patient does not experience a cytopenia, infusion reaction, an infection, or a combination thereof. In embodiments (e.g., wherein the binding agent binds HER2/neu, e.g., wherein the binding agent comprises ado-Trastuzumab or a fragment or variant thereof), the number of erythroid cells in circulation is sufficiently low such that the patient does not experience hepatotoxicity, liver failure, reductions in left ventricular ejection fraction, or embryo-fetal toxicity, or a combination thereof. In embodiments (e.g., wherein the binding agent binds HER2/neu, e.g., wherein the binding agent comprises Trastuzumab or a fragment or variant thereof), the number of erythroid cells in circulation is sufficiently low such that the patient does not experience Cardiomyopathy, Infusion Reaction, Pulmonary Toxicity, or embryo-fetal toxicity, or a combination thereof. In embodiments (e.g., wherein the binding agent binds EGFR, e.g., wherein the binding agent comprises nimotuzumab or a fragment or variant thereof), the number of erythroid cells in

circulation is sufficiently low such that the patient does not experience myalgia, somnolence, disorientation, hematuria and elevated liver function enzymes, or a combination thereof. In embodiments (e.g., wherein the binding agent binds EGFR, e.g., wherein the binding agent comprises cetuximab or a fragment or variant thereof), the number of erythroid cells in

5 circulation is sufficiently low such that the patient does not experience infusion reaction or cardiopulmonary arrest, or a combination thereof. In embodiments (e.g., wherein the binding agent binds VEGF, e.g., wherein the binding agent comprises bevacizumab or a fragment or variant thereof), the number of erythroid cells in circulation is sufficiently low such that the patient does not experience gastrointestinal perforation, surgery and wound healing

10 complications, hemorrhage, or a combination thereof. In embodiments (e.g., wherein the binding agent binds CD33, e.g., wherein the binding agent comprises gemtuzumab or a fragment or variant thereof), the number of erythroid cells in circulation is sufficiently low such that the patient does not experience hypersensitivity reactions including anaphylaxis, infusion reactions, pulmonary events, hepatotoxicity, or a combination thereof. In embodiments (e.g., wherein the

15 binding agent binds CD20, e.g., wherein the binding agent comprises ibritumomab or a fragment or variant thereof), the number of erythroid cells in circulation is sufficiently low such that the patient does not experience serious infusion reactions, cytopenia (e.g., prolonged and/or severe), or severe cutaneous and mucocutaneous reactions, or a combination thereof. In embodiments (e.g., wherein the binding agent binds CD20, e.g., wherein the binding agent comprises

20 Tositumomab or a fragment or variant thereof), the number of erythroid cells in circulation is sufficiently low such that the patient does not experience serious allergic reaction or cytopenia (e.g., prolonged and/or severe), or a combination thereof. In embodiments (e.g., wherein the binding agent binds EGFR, e.g., wherein the binding agent comprises panitumumab or a fragment or variant thereof), the number of erythroid cells in circulation is sufficiently low such

25 that the patient does not experience dermatologic toxicity. In embodiments (e.g., wherein the binding agent binds CD20, e.g., wherein the binding agent comprises ofatumumab or a fragment or variant thereof), the number of erythroid cells in circulation is sufficiently low such that the patient does not experience Hepatitis B Virus reactivation or progressive multifocal leukoencephalopathy, or a combination thereof. In embodiments (e.g., wherein the binding

30 agent binds CTLA-4, e.g., wherein the binding agent comprises ipilimumab or a fragment or variant thereof), the number of erythroid cells in circulation is sufficiently low such that the

patient does not experience immune-mediated adverse reaction (e.g., enterocolitis, hepatitis, dermatitis, neuropathy, or endocrinopathy) or a combination thereof. In embodiments (e.g., wherein the binding agent binds CD30, e.g., wherein the binding agent comprises brentuximab or a fragment or variant thereof), the number of erythroid cells in circulation is sufficiently low
5 such that the patient does not experience progressive multifocal leukoencephalopathy, or a combination thereof. In embodiments (e.g., wherein the binding agent binds Her2, e.g., wherein the binding agent comprises pertuzumab or a fragment or variant thereof), the number of erythroid cells in circulation is sufficiently low such that the patient does not experience left ventricular dysfunction or embryo-fetal toxicity, or a combination thereof. In embodiments (e.g.,
10 wherein the binding agent binds CD20, e.g., wherein the binding agent comprises obinutuzumab or a fragment or variant thereof), the number of erythroid cells in circulation is sufficiently low such that the patient does not experience Hepatitis B Virus reactivation or progressive multifocal leukoencephalopathy, or a combination thereof. In embodiments (e.g., wherein the binding agent binds PD-1, e.g., wherein the binding agent comprises nivolumab or a fragment or variant
15 thereof), the number of erythroid cells in circulation is sufficiently low such that the patient does not experience immune-mediated events (e.g., pneumonitis, colitis, hepatitis, endocrinopathy, nephritis and renal dysfunction, skin adverse reactions, or encephalitis), infusion reaction, complications of allogeneic HSCT, or embryo-fetal toxicity, or a combination thereof. In
20 embodiments (e.g., wherein the binding agent binds PD-1, e.g., wherein the binding agent comprises pembrolizumab or a fragment or variant thereof), the number of erythroid cells in circulation is sufficiently low such that the patient does not experience an immune-mediated adverse reaction (e.g., colitis, hepatitis, hypophysitis, nephritis, hyperthyroidism, hypothyroidism) or embryo-fetal toxicity, or a combination thereof. In embodiments (e.g.,
25 wherein the binding agent binds PDGF-R α , e.g., wherein the binding agent comprises olaratumab or a fragment or variant thereof), the number of erythroid cells in circulation is sufficiently low such that the patient does not experience infusion-related reaction or embryo-fetal toxicity, or a combination thereof. In embodiments (e.g., wherein the binding agent binds PD-L1, e.g., wherein the binding agent comprises atezolizumab or a fragment or variant thereof),
30 the number of erythroid cells in circulation is sufficiently low such that the patient does not experience an immune-related adverse reaction (e.g., pneumonitis, hepatitis, colitis, endocrinopathy, myasthenic syndrome, myasthenia gravis, Guillain-Barré or

meningoencephalitis, pancreatitis), ocular inflammatory toxicity, infection, infusion reaction, or embryo-fetal toxicity, or a combination thereof. In embodiments (e.g., wherein the binding agent binds SLAMF7, e.g., wherein the binding agent comprises elotuzumab or a fragment or variant thereof), the number of erythroid cells in circulation is sufficiently low such that the patient does not experience infusion reaction, infection, second primary malignancy, hHepatotoxicity, or a combination thereof. In embodiments (e.g., wherein the binding agent binds EGFR, e.g., wherein the binding agent comprises necitumumab or a fragment or variant thereof), the number of erythroid cells in circulation is sufficiently low such that the patient does not experience cardiopulmonary arrest, hypomagnesemia, or a combination thereof. In embodiments (e.g., wherein the binding agent binds CD38, e.g., wherein the binding agent comprises daratumumab or a fragment or variant thereof), the number of erythroid cells in circulation is sufficiently low such that the patient does not experience infusion reaction, neutropenia, or thrombocytopenia, or a combination thereof. In embodiments (e.g., wherein the binding agent binds VEGFR2, e.g., wherein the binding agent comprises ramucirumab or a fragment or variant thereof), the number of erythroid cells in circulation is sufficiently low such that the patient does not experience hemorrhage, arterial thromboembolic event, hypertension, infusion related reactions, gastrointestinal perforation, clinical deterioration in patients with cirrhosis, reversible posterior leukoencephalopathy syndrome, or a combination thereof. In embodiments (e.g., wherein the binding agent binds GD2, e.g., wherein the binding agent comprises dinutuximab or a fragment or variant thereof), the number of erythroid cells in circulation is sufficiently low such that the patient does not experience infusion reaction or neuropathy, or a combination thereof. In embodiments (e.g., wherein the binding agent binds CD19 and CD3, e.g., wherein the binding agent comprises blinatumomab or a fragment or variant thereof), the number of erythroid cells in circulation is sufficiently low such that the patient does not experience cytokine release syndrome or neurological toxicity, or a combination thereof. In embodiments (e.g., wherein the binding agent binds IL-6, e.g., wherein the binding agent comprises siltuximab or a fragment or variant thereof), the number of erythroid cells in circulation is sufficiently low such that the patient does not experience infusion related reaction or gastrointestinal perforation, or a combination thereof. In embodiments (e.g., wherein the binding agent binds RANK ligand, e.g., wherein the binding agent comprises denosumab or a fragment or variant thereof), the number of erythroid cells in circulation is sufficiently low such

that the patient does not experience infection, dermatologic reaction, osteonecrosis of the jaw, or suppression of bone turnover, or a combination thereof. In embodiments (e.g., wherein the binding agent binds EpCAM and CD3, e.g., wherein the binding agent comprises catumaxomab or a fragment or variant thereof), the number of erythroid cells in circulation is sufficiently low such that the patient does not experience abdominal pain, pyrexia, fatigue, or nausea/vomiting, or a combination thereof.

In embodiments, at least about 1×10^{10} , 2×10^{10} , 5×10^{10} , 1×10^{11} , 2×10^{11} , 5×10^{11} , (e.g., up to 1×10^{12}), erythroid cells are administered to the subject.

In embodiments, exogenous polypeptide comprising a binding agent further comprises a transmembrane domain. In embodiments, the binding agent is an antibody, antibody fragment, single-chain antibody, scFv, or nanobody. In embodiments, the binding agent comprises an anti-CD20 antibody or an anti-PL-L1 antibody.

In embodiments, the binding agent is other than an anti-CD20 antibody, other than rituximab, or other than an antibody against a cancer stem cell antigen (e.g., CD44, CD47, MET, EpCam, Her2, EGFR, or CD19). In embodiments, the binding agent is other than an antibody against CD133, CD3, CD, CD16, CD19, CD20, CD56, CD44, CD24, or CD133.

In embodiments, the tumor cell antigen is a membrane protein, e.g., a membrane phosphoprotein.

In embodiments, the erythroid cells bind or associate with non-necrotic tumor cells with a greater affinity than an otherwise similar non-genetically engineered erythroid cell, e.g., having a K_d that is lower by at least 2, 5, 10, 20, 50, or 100-fold (and optionally by up to 10-fold or 100-fold).

In embodiments, the binding agent has targeting activity (e.g., causes erythroid cells to accumulate at the tumor at higher levels than otherwise similar erythroid cells that lack the binding agent) and has anti-cancer activity (e.g., causes cancer cell death, or slows tumor growth compared to an untreated cancer). In embodiments, the binding agent has targeting activity and does not have anti-cancer activity.

In embodiments, the anti-cancer agent described herein comprises one or more of an immunostimulatory cytokine, a tumor starvation enzyme (e.g. asparaginase, methionine gamma lyase, or serine dehydrogenase), a cytotoxic small molecule, radionuclide, chemotherapeutic, toxin, small molecule, protein therapeutic, checkpoint modulator, or pro-apoptotic agent, or a

fragment or variant thereof. In embodiments, the immunostimulatory cytokine is a Type 1 cytokine or a Type 2 cytokine, or a fragment or variant thereof. In embodiments, the immunostimulatory cytokine is IL-2 or IL-12, or a fragment or variant thereof.

In embodiments, the erythroid cell further comprises a second agent. In embodiments, the second agent is an anti-cancer agent (e.g., a second anti-cancer agent) and/or a second exogenous polypeptide. In embodiments, the second agent comprises a radionuclide, chemotherapeutic, toxin, small molecule, or protein therapeutic. In embodiments, the second exogenous polypeptide comprises a checkpoint modulator, pro-apoptotic agent, or a tumor starvation enzyme (e.g., asparaginase). In embodiments, the second agent promotes an immune function, e.g., the second agent comprises a proinflammatory cytokine or an inhibitor of an immune checkpoint molecule.

In embodiments, the second agent shows improved safety or reduced side effects when comprised by the erythroid cell, compared to the same amount of an otherwise similar second agent not comprised by an erythroid cell.

In embodiments, the binding agent or second agent increases immune cell activity against cancer cells, recruits immune cells to the cancer, increases immune cell activation, increases immune cell resistance to immune checkpoint inhibition, or a combination thereof. In embodiments, the binding agent or second agent increases apoptosis. In embodiments, the binding agent or second agent modulates ion levels in the cancer cell, e.g., increases or decreases levels of a given ion, e.g., increases calcium levels. In embodiments, the binding agent or second agent modulates calcium channel activity in a tumor cell.

In some embodiments, the resistant cancer is resistant to an antibody therapeutic, e.g., an antibody therapeutic of Table 1. In some embodiments, the resistant cancer is resistant to a small molecule drug. In embodiments, the small molecule drug is a chemotherapeutic agent, e.g., a chemotherapeutic described herein. In embodiments, e.g., embodiments relating to resistant subjects, the subject has failed to respond to treatment with an antibody of Table 1. In embodiments, the subject is naïve to an antibody of Table 1. In embodiments, the antibody is an anti-CD20 antibody, e.g., rituximab. In embodiments, the antibody domain binds a target of Table 1. In embodiments, the antibody domain comprises CDRs or VR from Table 2, e.g., using the Kabat or Chothia definitions.

In embodiments, a method herein (e.g., a method of treating a resistant cancer), comprises administering to the subject a preparation of erythroid cells comprising a fusion protein comprising a transmembrane domain and an antibody domain, wherein the antibody domain binds to a tumor antigen. In embodiments, the cancer has become resistant to an antibody that binds that antigen. In embodiments, the antibody domain comprised by the erythroid cell has the same CDRs as, or differs from the CDRs by no more than 1, 2, 3, 4, or 5 mutations relative to, an antibody therapeutic to which the cancer is resistant, e.g., the patient has relapsed after treatment with that antibody therapeutic.

In embodiments, e.g., embodiments relating to resistant subjects, the patient comprises endogenous antibodies against the anti-cancer antibody therapeutic, e.g., the patient comprises HAMA (human anti-mouse antibodies). In embodiments, after treatment with the erythroid cells, the patient displays a reduced level of anti-drug antibodies, e.g., HAMA, compared to a pre-treatment level of anti-drug antibodies. In embodiments, the patient has an impaired caspase-dependent apoptosis pathway, e.g., has a mutation in a caspase, e.g., an initiator or executioner caspase. In embodiments, the mutation is in Caspase 2, Caspase 8, Caspase 9, Caspase 10, Caspase 3, Caspase 6, or Caspase 7. In embodiments, at least a subset of cancer cells do not have a mutation affecting (e.g., deletion of) a protein bound by the anti-cancer antibody therapeutic, e.g., CD20 or a target of Table 1.

In embodiments, e.g., embodiments involving treating cancers having an oncogenic mutation, the method comprises obtaining knowledge that the cancer has the mutation. In embodiments, the cancer comprises a second oncogenic mutation, e.g., mutation selected from Table 8.

In embodiments, the level of the exogenous polypeptide (e.g., a costimulatory molecule) or the affinity of the exogenous polypeptide for a binding partner on an immune cell (e.g., T cell) is sufficient to induce immune cell proliferation.

In embodiments, the erythroid cells have an osmotic fragility of less than 50% cell lysis at 0.3%, 0.35%, 0.4%, 0.45%, or 0.5% NaCl. In some embodiments, the enucleated erythroid cell has approximately the diameter or volume as a wild-type, untreated erythroid cell, e.g., a reticulocyte. In some embodiments, the erythroid cells comprise greater than 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% or greater than 10% fetal hemoglobin. In some embodiments, the

enucleated erythroid cell has approximately the same phosphatidylserine content on the outer leaflet of its cell membrane as a wild-type, untreated erythroid cell.

In embodiments, the transmembrane domain comprises a transmembrane portion of glycophorin A (GPA), glycophorin B, glycophorin C, glycophorin D, kell, band 3, aquaporin 1, glut 1, kidd antigen protein, or rhesus antigen.

In embodiments, in embodiments involving immune checkpoint modulation, the moiety is an antibody of Table 4 an antibody that binds an antigen of Table 4. In embodiments, the moiety (e.g., moiety that binds to PD-L1) is an anti-PD-L1 antibody, or extracellular fragment of PD1. In embodiments, the moiety binds to an immune checkpoint-ligand (e.g., PD-L1).

In embodiments, a composition herein comprises, or a method comprises administering, a population of erythroid cells that lack the exogenous polypeptide, e.g., admixed together with the plurality of erythroid cells comprising the exogenous polypeptide. In embodiments, the composition comprises, or the method comprises administering, a population of cells that was transfected or transduced with less than 100% efficiency.

In embodiments, e.g., embodiments involving costimulatory molecules, the erythroid cell further comprises a targeting moiety, e.g., an exogenous polypeptide comprising a binding moiety. In embodiments, the binding moiety binds an antigen on an immune effector cell, e.g., a T cell or NK cell. In embodiments, the binding moiety binds an antigen on a tumor cell or present in the tumor microenvironment. In embodiments, the costimulatory molecule is 4-1BB-L, OX40-L, GITR-L, or ICOS-L.

In embodiments, the stimulatory molecule is a molecule that stimulates an immune effector cells, e.g., a T cell. In embodiments, the stimulatory molecule is a primary stimulant or a costimulatory molecule. In embodiments, the erythroid cell comprises a costimulatory molecule and a primary stimulant. In other embodiments, the erythroid comprises a costimulatory molecule and does not comprise a primary stimulant. In embodiments, the erythroid comprises a costimulatory molecule and does not comprise an exogenous primary stimulant.

In embodiments, e.g., embodiments involving resistant subjects, the number of fusion proteins on the outer surface of the engineered erythroid cell or the affinity of the binding domain for the tumor antigen is sufficient to induce binding of the erythroid cells to a target cell, e.g., a B cell.

In embodiments, the administration is systemic or local. In embodiments, the administration is to the bloodstream, e.g., intravenous administration, e.g., intravenous infusion. In embodiments, the administration is to a tumor.

In some embodiments, the erythroid cell comprises on its surface:

- i) a first costimulatory molecule (e.g., a costimulatory molecule described herein, e.g., a costimulatory molecule of Table 5), and
- ii) a second costimulatory molecule (e.g., a costimulatory molecule of Table 5), and
- iii) optionally, a third or more costimulatory molecules (e.g., a costimulatory molecule of Table 5), and
- iv) optionally, a targeting moiety that binds a tumor antigen.

In some embodiments, the erythroid cell comprises on its surface:

- i) a first agent that binds a first immune checkpoint molecule (e.g., an agent that binds an immune checkpoint molecule described herein, e.g., an agent that binds an immune checkpoint molecule of Table 4, e.g., an antibody or other binding agent of Table 4), and
- ii) a second agent that binds a second immune checkpoint molecule (e.g., an agent that binds an immune checkpoint molecule of Table 4, e.g., an antibody or other binding agent of Table 4), and
- iii) optionally, a third or more agents that bind an immune checkpoint molecule (e.g., an immune checkpoint molecule of Table 4), and
- iv) optionally, a targeting moiety that binds a tumor antigen.

In some embodiments, the erythroid cell comprises on its surface:

- i) a costimulatory molecule (e.g., a costimulatory molecule described herein, e.g., a costimulatory molecule of Table 5, e.g., 4-1BBL), and
- ii) an agent that binds an immune checkpoint molecule (e.g., an agent that binds an immune checkpoint molecule described herein, e.g., an agent that binds an immune checkpoint molecule of Table 4, e.g., an antibody or other binding agent of Table 4, e.g., anti-PD-L1), and
- iii) optionally, a targeting moiety that binds a tumor antigen.

In embodiments, e.g., embodiments involving erythroid cells for in vivo imaging, the erythroid cell comprises a label comprising a PET isotope, e.g., ^{11}C , ^{13}N , ^{15}O , ^{18}F , ^{64}Cu , ^{62}Cu , ^{124}I , ^{76}Br , ^{82}Rb , ^{89}Zr and ^{68}Ga . In embodiments, the erythroid cell comprises a label comprising a bioluminescent agent, e.g., luciferase.

5 In some embodiments, the exogenous polypeptide(s) are encoded by one or more exogenous nucleic acid(s) that are not retained by the enucleated erythroid cell.

In embodiments, the erythroid cell promotes T cell proliferation, e.g., proliferation of CD4+ T cells, CD8+ T cells, or both of CD4+ T cells and CD8+ T cells, e.g., by at least about 2, 3, 4, 5, 6, 7, 8, or 10-fold, e.g., compared to a sample lacking erythroid cells, e.g., by a PBMC proliferation assay, e.g., an assay of Example 5. In embodiments, the erythroid cell promotes proliferation of CD8+ T cells more strongly than of CD4+ T cells, e.g., by a factor of at least 2-fold or 3-fold difference in fold increase over the same amount of time, e.g., 5 days. In some
10 embodiments, the ratio of erythroid cells to PMBCs at the beginning of the assay is about 1:1.

In embodiments, the erythroid cell promotes cytokine secretion in a sample of T cells, e.g., secretion of IFN γ , TNF α , or both of IFN γ and TNF α , e.g., by a flow cytometry assay, e.g., an assay of Example 5. In embodiments, the erythroid cell promotes increased cytokine secretion, e.g., an increase of at least 2, 3, 4, or 5 fold compared to an otherwise similar cell sample treated with otherwise similar erythroid cells that lack the exogenous protein. In some
15 embodiments, increased cytokine secretion is due at least in part to increased proliferation of cytokine-secreting cells (e.g., CD4+ T cells, CD8+ T cells, or both of CD4+ T cells and CD8+ T cells).
20

In embodiments, the erythroid cells (e.g., erythroid cells expressing an anti-PD-L1 antibody) are used to treat a cancer that expresses PD-L1. In some embodiments, the cancer cells are exposed to IFN-gamma, e.g., endogenous IFN-gamma, e.g., in an amount sufficient to increase PD-L1 expression in the tumor cells. In some embodiments, the tumor expresses at
25 least 100,000, 125,000, 150,000, 200,000, 250,000, 300,000, 350,000, or 400,000 copies of PD-L1 per cell.

In related aspects, the disclosure provides a method comprising:

(a) acquiring information (e.g., directly or indirectly) about the presence or level of PD-L1 expression on a tumor, e.g., whether the tumor cell has a number of PD-L1 polypeptides per
30

cell that is greater than a reference value, e.g., wherein the reference value is one of 100,000, 125,000, 150,000, 200,000, 250,000, 300,000, 350,000, or 400,000, and

(b) responsive to (a), selecting a treatment or administering a treatment comprising a plurality of enucleated erythroid cells described herein, e.g., an enucleated erythroid cell
5 comprising on its surface an exogenous polypeptide comprising an anti-PD-L1 binding agent, e.g., an anti-PD-L1 antibody.

The cell systems described herein may be used in combination with another (one or more) anti-proliferative, anti-neoplastic or anti-tumor drug or treatment that is not part of the cell system. Such drugs or treatments include chemotherapeutic drugs, e.g., cytotoxic drugs (e.g.,
10 alkylating agents, antimetabolites, anti-tumor antibiotics, topoisomerase inhibitors, mitotic inhibitors, corticosteroids); cancer growth blockers such as tyrosine kinase inhibitors and proteasome inhibitors; T cell therapy (e.g., CAR-T cell therapy) (see, e.g., PMID: 26611350), Natural Killer (NK) cell immunomodulation (see, e.g., PMID: 26697006); and cancer vaccines (PMID: 26579225); other chemical drugs such as L-asparaginase and bortezomib (Velcade®).

15 Hormone therapies (or anti-hormone therapies) may be used, e.g., for hormone-sensitive cancers.

The cell systems described herein may also be used in combination with non-drug therapies for cancer such as surgery, radiotherapy, or cryotherapy. In some cases, treatment methods of the invention may include a cell system described herein in combination with 2 or
20 more other therapies or drugs, e.g., breast cancer may be treated with a combination of a cell system described herein in combination with surgery or radiotherapy and a chemotherapeutic cocktail or biologic (e.g., an anti-HER2 antibody).

The disclosure contemplates all combinations of any one or more of the foregoing aspects and/or embodiments, as well as combinations with any one or more of the embodiments set forth
25 in the detailed description and examples.

Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references (e.g., sequence database reference numbers) mentioned herein are incorporated by reference in their
30 entirety. For example, all GenBank, Unigene, NCBI, and Entrez sequences referred to herein, e.g., in any Table herein, are incorporated by reference. Unless otherwise specified, the

sequence accession numbers specified herein, including in any Table herein, refer to the database entries current as of December 2, 2016. When one gene or protein references a plurality of sequence accession numbers, all of the sequence variants are encompassed.

5 BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graph showing the level of apoptosis in four lymphoma cell lines treated with (from left to right), anti-CD20 antibody alone, anti-CD20 antibody “crosslinked” together with a secondary anti-isotype antibody, control RCT-HA-GPA (at 1:1 or 1:5), or RCT-antiCD20 (at 1:1 or 1:5) antibody alone.

10 Fig. 2 is a graph showing level of apoptosis in CD20+ lymphoma cells treated with RCT-antiCD20 or control RCT-HA-GPA in the presence or absence of a caspase-dependent apoptosis inhibitor (FMK). 1-1 and 1-5 indicate a 1:1 and 1:5 ratio, respectively, of lymphoma cells to RCTs.

Fig. 3 shows tissue sections of CD20+ tumors from mice treated with RCT-antiCD20 or
15 control RCT-HA-GPA. H&E, Hematoxylin and eosin, detects nucleated cells, endogenous red blood cells, and engineered erythroid cells. CD31 staining detects endothelial vasculature. CD20 staining detects Ramos tumor cells. HA detects engineered erythroid cells.

Fig. 4 is a fluorescence image of a xenograft tumor in a mouse treated with control RCT-HA-GPA (top panel) or RCT-antiCD20 (bottom panel).

20 Fig. 5 is a graph showing rescue of IL-2 secretion by RCTs comprising immune checkpoint inhibitors. From left to right, the bars correspond to Jurkat (Jurkat cells alone, baseline), Jurkat/Z138 (Jurkat cells co-cultured with NHL Z138 cells, showing inhibition of IL-2 secretion), Jurkat/Z138/Untransduced RCTs (Jurkat cells co-cultured with NHL Z138 cells and untransduced control erythroid cells, showing IL-2 secretion is not rescued), Jurkat/Z138/atezo-
25 flag RCTs (Jurkat cells co-cultured with NHL Z138 cells and RCT-antiPDL1, showing rescue of IL-2 secretion), Jurkat/Z138/Ipi-flag RCTs (Jurkat cells co-cultured with NHL Z138 cells and RCT-antiCTLA4, showing rescue of IL-2 secretion). The next five bars show low or no IL-2 secretion in the absence of Jurkat cells. The rightmost bar shows roughly baseline levels of IL-2 secretion when Jurkat cells are co-cultured with untransduced control erythroid cells.

Fig. 6 is a graph showing interferon-gamma secretion in a standard antigen recall assay. Leftmost bar, PBMC alone showed baseline interferon-gamma secretion. Next 5 bars, PBMC co-cultured with the indicated numbers of control RCT-HA-GPA cells showed interferon gamma secretion levels similar to baseline. Rightmost 5 bars, PBMC co-cultured with the indicated numbers of RCT-antiPD1 cells showed increased interferon gamma secretion levels.

Fig. 7 is a graph showing NFκB activation as indicated by luciferase activity measured in RLU when Jurkat cells are co-cultured with RCT-41BBL or untransduced control erythroid cells.

Fig. 8 is a graph showing NFκB activation resulting from erythroid cells having the indicated number of copies of 4-1BB-L per cell.

Fig. 9 is a graph showing NFκB activation resulting from the indicated number of 4-1BB-L RCT cells, compared to an anti-4-1BB antibody.

Fig. 10 is a pair of graphs showing, in the left panel, the fold change in CD4+ T cells, and in the right panel, the fold change in CD8+ T cells, induced by 4-1BB-L RCT cells, compared to an anti-4-1BB antibody.

Fig. 11 is a pair of graphs showing, in the left panel, the IFN-gamma secretion, and in the right panel, the TNF-alpha secretion, induced by 4-1BB-L RCT cells, compared to an anti-4-1BB antibody.

Fig. 12 is a time course showing tumor size in mice treated with 41BBL-RCT and an untreated control.

Fig. 13 shows the ratio of erythroid cells in the tumor to erythroid cells in blood vessels for erythroid cells having an anti-PD-L1 antibody or an isotype control antibody.

Fig. 14 is a chart summarizing approximate fold change levels in signalling, proliferation, cytokine levels, and antigen recall activity induced by the indicated RCTs.

Fig. 15 is a graph quantifying the binding of the indicated erythroid cells to the indicated tumor cells.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

As used herein, the term “antibody” refers to a protein or part thereof, e.g., an immunoglobulin chain or fragment thereof, comprising at least one immunoglobulin variable domain sequence. The term “antibody” encompasses antibodies and antibody fragments. In an embodiment, an antibody is a multispecific antibody, e.g., a bispecific antibody. Examples of antibodies include, but are not limited to, Fab, Fab', F(ab')₂, Fv fragments, scFv antibody fragments, disulfide-linked Fvs (sdFv), a Fd fragment consisting of the VH and CH1 domains, linear antibodies, single domain antibodies such as sdAb (either VL or VH), camelid VHH domains, multi-specific antibodies formed from antibody fragments such as a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region, an isolated epitope binding fragment of an antibody, maxibodies, minibodies, nanobodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv.

As used herein, a “combination therapy” or “administered in combination” means that two (or more) different agents or treatments are administered to a subject as part of a treatment regimen for a particular disease or condition. The treatment regimen includes the doses and periodicity of administration of each agent such that the effects of the separate agents on the subject overlap. In some embodiments, the delivery of the two or more agents is simultaneous or concurrent and the agents may be co-formulated. In other embodiments, the two or more agents are not co-formulated and are administered in a sequential manner as part of a prescribed regimen. In some embodiments, administration of two or more agents or treatments in combination is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one agent or treatment delivered alone or in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive (e.g., synergistic). Sequential or substantially simultaneous administration of each therapeutic agent can be effected by any appropriate route including, but not limited to, oral routes, intravenous routes, intramuscular routes, and direct absorption through mucous membrane tissues. The therapeutic agents can be administered by the same route or by different routes. For example, a first therapeutic agent of the combination may be administered

by intravenous injection while a second therapeutic agent of the combination may be administered orally.

The term "complementarity determining region" or "CDR," as used herein, refers to the sequences of amino acids within antibody variable regions which confer antigen specificity and binding affinity. For example, in general, there are three CDRs in each heavy chain variable region (e.g., HCDR1, HCDR2, and HCDR3) and three CDRs in each light chain variable region (LCDR1, LCDR2, and LCDR3). The precise amino acid sequence boundaries of a given CDR can be determined using any of a number of well-known schemes, including those described by Kabat et al. (1991), "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD ("Kabat" numbering scheme), Al-Lazikani et al., (1997) JMB 273,927-948 ("Chothia" numbering scheme), or a combination thereof.

Under the Kabat numbering scheme, in some embodiments, the CDR amino acid residues in the heavy chain variable domain (VH) are numbered 31-35 (HCDR1), 50-65 (HCDR2), and 95-102 (HCDR3); and the CDR amino acid residues in the light chain variable domain (VL) are numbered 24-34 (LCDR1), 50-56 (LCDR2), and 89-97 (LCDR3). Under the Chothia numbering scheme, in some embodiments, the CDR amino acids in the VH are numbered 26-32 (HCDR1), 52-56 (HCDR2), and 95-102 (HCDR3); and the CDR amino acid residues in the VL are numbered 26-32 (LCDR1), 50-52 (LCDR2), and 91-96 (LCDR3). In a combined Kabat and Chothia numbering scheme, in some embodiments, the CDRs correspond to the amino acid residues that are part of a Kabat CDR, a Chothia CDR, or both. For instance, in some embodiments, the CDRs correspond to amino acid residues 26-35 (HCDR1), 50-65 (HCDR2), and 95-102 (HCDR3) in a VH, e.g., a mammalian VH, e.g., a human VH; and amino acid residues 24-34 (LCDR1), 50-56 (LCDR2), and 89-97 (LCDR3) in a VL, e.g., a mammalian VL, e.g., a human VL.

As used herein, "enucleated" refers to a cell that lacks a nucleus, e.g., a cell that lost its nucleus through differentiation into a mature red blood cell.

"Erythroid cells" as used herein, include nucleated red blood cells, red blood cell precursors, and enucleated red blood cells. For example, any of a cord blood stem cell, a CD34+ cell, a hematopoietic stem cell (HSC), a spleen colony forming (CFU-S) cell, a common myeloid

progenitor (CMP) cell, a blastocyte colony-forming cell, a burst forming unit-erythroid (BFU-E), a megakaryocyte-erythroid progenitor (MEP) cell, an erythroid colony-forming unit (CFU-E), a reticulocyte, an erythrocyte, an induced pluripotent stem cell (iPSC), a mesenchymal stem cell (MSC), a polychromatic normoblast, an orthochromatic normoblast, is an erythroid cell. A
5 preparation of erythroid cells can include any of these cells or a combination thereof. In some embodiments, the erythroid cells are immortal or immortalized cells. For example, immortalized erythroblast cells can be generated by retroviral transduction of CD34+ hematopoietic progenitor cells to express Oct4, Sox2, Klf4, cMyc, and suppress TP53 (e.g., as described in Huang et al., Mol Ther 2013, epub ahead of print September 3). In addition, the cells may be intended for
10 autologous use or provide a source for allogeneic transfusion. In some embodiments, erythroid cells are cultured. In an embodiment an erythroid cell is an enucleated red blood cell.

As used herein, the term “exogenous polypeptide” refers to a polypeptide that is not produced by a wild-type cell of that type or is present at a lower level in a wild-type cell than in a cell containing the exogenous polypeptide. In some embodiments, an exogenous polypeptide is
15 a polypeptide encoded by a nucleic acid that was introduced into the cell, which nucleic acid is optionally not retained by the cell.

As used herein, “hyper cross-linking” of a tumor antigen refers to causing that antigen to cluster on the surface of the cell or redistribute into detergent-insoluble cell membrane complexes or signaling-processing centers. This redistribution may be assayed, e.g., as
20 described in Polyak et al., “Identification of a Cytoplasmic Region of CD20 Required for Its Redistribution to a Detergent-Insoluble Membrane Compartment” The Journal of Immunology, October 1, 1998, vol. 161 no. 7 3242-3248. Hyper cross-linking does not require the formation of covalent bonds between tumor antigens.

The term “resident”, as used herein, in reference to an agent being resident in a tumor,
25 refers to the agent being present within the boundaries of the tumor, e.g., between tumor cells or inside a tumor cell.

The term “persistent” as used herein, in reference to an agent being persistent in a tumor, refers to an agent that is continuously resident in the tumor for a prolonged or predetermined time. For instance, if the agent comprises a targeting moiety that binds a tumor antigen, the

agent is persistent in the tumor if the agent comprising the targeting moiety is continuously resident in the tumor for longer than an otherwise similar agent lacking the targeting moiety (e.g., an unmodified erythroid cell). In embodiments, an agent that is persistent in the tumor is resident in the tumor for at least 3, 6, or 12 hours or 1, 2, 3, 4, 5, 6, 7, 14, 21, or 28 days (e.g., up to 14 or 28 days).

A “resistant” tumor, as used herein, refers to a tumor that does not respond to a therapy. A resistant tumor may be a tumor in a subject who was treated with a therapy, and the tumor did not show a response to the therapy, e.g., the patient was refractory to the treatment, e.g., the patient exhibited progressive disease with no period of responsiveness. A resistant tumor may also be a tumor in a subject who was treated with a therapy and showed an initial response (e.g., complete response or partial response), but then the patient ceased to exhibit improvement (e.g., the patient’s response plateaued) or relapsed (e.g., the patient exhibited progressive disease after the period of responsiveness). A resistant tumor may also be a treatment-naïve resistant tumor, e.g., a tumor in a subject who was not treated with the therapy, e.g., wherein the tumor was determined to be resistant to the therapy, e.g., using an in vitro assay of a tumor biopsy, or by tumor sequencing. A resistant tumor may also be a tumor, having a characteristic, e.g., a mutation, or a stage, which indicates it would be resistant to a therapy. In an embodiment, a therapeutic agent delivered by an erythroid cell described herein is more efficacious than the same therapeutic agent delivered freely, that is, not by an erythroid cell described herein, and is useful, e.g., to treat a tumor that is resistant to treatment with that therapeutic agent. In an embodiment, a therapeutic agent delivered by an erythroid cell described herein is administered to treat a tumor that is resistant to treatment with a different therapeutic agent. In an embodiment, treatment with the erythroid cell is initiated prior to resistance. In an embodiment, treatment with the erythroid cell is initiated after resistance.

A “refractory” tumor, as used herein, refers to a tumor that was treated with a therapy but did not show an adequate response, e.g., the patient is classified as having no response (NR) or progressive disease (PD) after the treatment. In an embodiment, a therapeutic agent delivered by an erythroid cell described herein is more efficacious than the same therapeutic agent delivered freely, that is, not by an erythroid cell described herein, and is useful, e.g., to treat a tumor that has is, or has become, refractory to that therapeutic agent. In an embodiment, a therapeutic agent delivered by an erythroid cell described herein is administered to treat a tumor that is, or

has become, refractory to a different therapeutic agent. In an embodiment, treatment with the erythroid cell is initiated prior to the tumor becoming refractory. In an embodiment, treatment with the erythroid cell is initiated after the tumor becomes refractory.

5 A “relapsed” tumor, as used herein, refers to a tumor that went into remission (e.g., after treatment with a therapy, e.g., a complete response or partial response) and then progressed. In an embodiment, a therapeutic agent delivered by an erythroid cell described herein is more efficacious than the same therapeutic agent delivered freely, that is, not by an erythroid cell described herein, and is useful, e.g., to treat a tumor that has relapsed after treatment with that therapeutic agent. In an embodiment, a therapeutic agent delivered by an erythroid cell
10 described herein is administered to treat a tumor that is has relapsed after treatment with a different therapeutic agent. In an embodiment, treatment with the erythroid cell is initiated during remission. In an embodiment, treatment with the erythroid cell is initiated after relapse.

A “tumor” as used herein refers to a plurality of aberrant cells having uncontrolled growth. The terms “tumor” and “cancer” are used interchangeably herein, e.g., both terms
15 encompass solid and liquid, e.g., diffuse or circulating, tumors. In an embodiment the cancer or tumor is premalignant. In an embodiment, the cancer or tumor is malignant. Examples of various cancers are described herein and include but are not limited to, breast cancer, prostate cancer, ovarian cancer, cervical cancer, skin cancer, pancreatic cancer, colorectal cancer, renal cancer, liver cancer, brain cancer, lymphoma, leukemia, lung cancer and the like.

20 The terms “tumor cell” and “cancer cell” are used herein interchangeably to refer to a cell or a tumor or cancer.

“Tumor antigen” which is used herein interchangeably with “cancer antigen”, refers to a moiety (e.g., a peptide or protein) comprised by a tumor cell, e.g., present on the surface of the tumor. In embodiments, the tumor antigen is also present on one or more types of noncancerous
25 cells (e.g., noncancerous cells in the subject having the cancer), e.g., at the same level or at a different level. In an embodiment the tumor antigen is present in higher average numbers on a tumor cell than on a non-tumor cell. In embodiments, an antigen is a polypeptide, or portion thereof, present on the tumor cell. An antigen may be bound by a binding partner, e.g., an antibody or a non-antibody binding partner.

30 As used herein, the term “variant” of a polypeptide refers to a polypeptide having at least one sequence difference compared to that polypeptide, e.g., one or more substitutions, insertions,

or deletions. In some embodiments, the variant has at least 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to that polypeptide. A variant includes a fragment. In some embodiments, a fragment lacks up to 1, 2, 3, 4, 5, 10, 20, or 100 amino acids on the N-terminus, C-terminus, or both (each independently), compared to the full-length polypeptide.

5 As used herein with reference to a tumor, “vascularized” refers to a tumor that has induced angiogenesis and/or comprises tumor blood vessels. Tumor blood vessels differ from normal blood vessels and can be distinguished by histology, e.g., by an irregular shape and dilation; and may comprise tumor cells and endothelial cells.

10 *Exogenous proteins*

One or more of the exogenous proteins may have post-translational modifications characteristic of eukaryotic cells, e.g., mammalian cells, e.g., human cells. In some embodiments, one or more of the exogenous proteins are glycosylated, phosphorylated, or both. In vitro detection of glycoproteins is routinely accomplished on SDS-PAGE gels and Western
15 Blots using a modification of Periodic acid-Schiff (PAS) methods. Cellular localization of glycoproteins may be accomplished utilizing lectin fluorescent conjugates known in the art. Phosphorylation may be assessed by Western blot using phospho-specific antibodies.

Post-translation modifications also include conjugation to a hydrophobic group (e.g., myristoylation, palmitoylation, isoprenylation, prenylation, or glypiation), conjugation to a
20 cofactor (e.g., lipoylation, flavin moiety (e.g., FMN or FAD), heme C attachment, phosphopantetheinylation, or retinylidene Schiff base formation), diphthamide formation, ethanolamine phosphoglycerol attachment, hypusine formation, acylation (e.g. O-acylation, N-acylation, or S-acylation), formylation, acetylation, alkylation (e.g., methylation or ethylation), amidation, butyrylation, gamma-carboxylation, malonylation, hydroxylation, iodination,
25 nucleotide addition such as ADP-ribosylation, oxidation, phosphate ester (O-linked) or phosphoramidate (N-linked) formation, (e.g., phosphorylation or adenylylation), propionylation, pyroglutamate formation, S-glutathionylation, S-nitrosylation, succinylation, sulfation, ISGylation, SUMOylation, ubiquitination, Neddylation, or a chemical modification of an amino acid (e.g., citrullination, deamidation, eliminylation, or carbamylation), formation of a disulfide

bridge, racemization (e.g., of proline, serine, alanine, or methionine). In embodiments, glycosylation includes the addition of a glycosyl group to arginine, asparagine, cysteine, hydroxylysine, serine, threonine, tyrosine, or tryptophan, resulting in a glycoprotein. In
 5 glycosylation.

In some embodiments, an exogenous polypeptide described herein is at least 200, 300, 400, 500, 600, 700, or 800 amino acids in length. In some embodiments, the exogenous polypeptide is between 200-300, 300-400, 400-500, 500-600, 600-700, or 700-800 amino acids in length. In some embodiments, the exogenous polypeptide is less than 500, 450, 400, 350, or
 10 300 amino acids in length.

In embodiments, an erythroid cell described herein comprises at least 1,000, 5,000, 10,000, 15,000, 20,000, 25,000, 30,000, 50,000, 100,000, 200,000, or 500,000 copies of an exogenous polypeptide described herein. In embodiments, an erythroid cell described herein comprises about 200,000, 150,000-250,000, 100,000-300,000, or 200,000-300,000 copies of an
 15 exogenous polypeptide described herein, e.g., an exogenous polypeptide comprising 4-1BBL.

In embodiments, an exogenous polypeptide described herein forms a multimer, e.g., a dimer, trimer, tetramer, or hexamer. In embodiments, the exogenous polypeptide (e.g., comprising 4-1BBL) forms a trimer, e.g., a trimer at the surface of the erythroid cell.

In some embodiments, the erythroid cell comprises an agent, e.g., an exogenous
 20 polypeptide, e.g., a surface-exposed exogenous polypeptide, e.g., a surface-exposed polypeptide comprising a transmembrane domain, that binds a tumor antigen described herein, e.g., a tumor antigen of Table 1. In embodiments, the erythroid cell comprises an agent, e.g., an exogenous polypeptide, that comprises an antibody or other binding agent of Table 1 or Table 2, or a fragment or variant thereof. For instance, the fragment or variant could be a single domain
 25 antibody, e.g., scFv, corresponding to an antibody of Table 1 or Table 2. As another example, the fragment or variant could be an antigen-binding fragment of an antibody of Table 1 or Table 2, e.g., a light chain variable fragment, heavy chain variable fragment, or both. As another example, the fragment or variant could be an active fragment of a binding agent of Table 1 or Table 2. As another example, the fragment or variant could be an antibody having less than
 30 100% sequence identity to an antibody of Table 1 or Table 2, e.g., an antibody having at least 70%, 75%, 80%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity to the antibody of Table 1 or

Table 2 or to a light chain variable fragment, heavy chain variable fragment, or both. For instance, the fragment or variant could have the same CDRs as an antibody of Table 1 or Table 2, but one or more mutations in the framework and/or constant region. As another example, the fragment or variant could be a binding agent having less than 100% sequence identity to a binding agent of Table 1 or Table 2, e.g., a binding agent having at least 70%, 75%, 80%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity to a binding agent of Table 1 or Table 2 or to an active portion thereof.

In some embodiments, one or more of the exogenous polypeptides is a fusion protein, e.g., is a fusion with an endogenous red blood cell protein or fragment thereof. In embodiments, the exogenous polypeptide comprises a transmembrane protein, e.g., a Type I, Type II, or Type III red blood cell transmembrane protein or transmembrane fragment thereof. In embodiments, the transmembrane protein is GPA or a transmembrane fragment thereof.

In embodiments, the transmembrane domain comprises GPA or a transmembrane portion thereof, e.g., as set out in SEQ ID NO: 1 herein or a transmembrane portion thereof, or a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to any of the foregoing. In embodiments, the GPA polypeptide is C-terminal of the binding domain. In embodiments, the GPA polypeptide has a sequence of:

LSTTEVAMHTSTSSSVTKSYISSQTNDTHKRDTYAATPRAHEVSEISVRTVYPPEEETGER
VQLAHHFSEPEITLIIFGVMAGVIGTILLISYGIRRLIKKSPSDVKPLPSPD TDVPLSSVEIEN
PETS DQ (SEQ ID NO: 1)

or a transmembrane portion thereof, or a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identity to any of the foregoing. In embodiments, the GPA polypeptide is C-terminal of the antibody domain.

In embodiments, the exogenous polypeptide comprises a polypeptide of SEQ ID NO: 80 or SEQ ID NO: 81, or an amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity thereto, or a functional fragment thereof. In embodiments, the functional fragment of 4-1BBL is a 4-1BB binding fragment. In embodiments, the functional fragment of anti-PD-L1 is a PD-L1 binding fragment.

In an embodiment, the exogenous polypeptide comprises a ligand for a cellular receptor that mediates apoptosis. In an embodiment the ligand is a TRAIL receptor ligand, e.g., wild-type or mutant TRAIL polypeptides, or antibody that binds TRAIL receptors, and induces apoptosis in a target cell. In some embodiments, an enucleated erythroid cell comprises one or more (e.g.,
 5 two or three) TRAIL receptor ligands. In some embodiments, enucleated erythroid cell comprising one or more TRAIL receptor ligands further comprises a targeting moiety, e.g., a targeting moiety described herein.

TRAIL (TNF-related apoptosis inducing ligand) is a member of the TNF family that induces apoptosis. TRAIL has at least two receptors, TRAIL R1 and TRAIL R2. TRAIL receptor
 10 agonists, e.g., mutants of TRAIL that bind one or more of the receptors, or antibodies that bind one or both of TRAIL R1 or TRAIL R2 (see, e.g. Gasparian et al., Apoptosis 2009 Jun 14(6), Buchsbaum et al. Future Oncol 2007 Aug 3(4)), have been developed as a clinical therapy for a wide range of cancers. In embodiments, the enucleated erythroid cell comprises a TRAIL R1 ligand and a TRAIL R2 ligand.

15 In some embodiments, one or more of the exogenous polypeptides comprises a member of the TNF superfamily or a portion thereof. In some embodiments, the exogenous polypeptides bind to one or both of death receptors DR4 (TRAIL-R1) and DR5 (TRAIL-R2). In some embodiments, the exogenous polypeptides bind to one or more of TNFRSF10A/TRAILR1, TNFRSF10B/TRAILR2, TNFRSF10C/TRAILR3, TNFRSF10D/TRAILR4, or
 20 TNFRSF11B/OPG. In some embodiments, the exogenous polypeptides activate one or more of MAPK8/JNK, caspase 8, and caspase 3.

In some embodiments, a TRAIL polypeptide is a TRAIL agonist having a sequence of any of SEQ ID NOS: 2-6 herein, or a sequence with at least 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity thereto. Sequence identity is measured, e.g., by BLAST (Basic Local
 25 Alignment Search Tool). SEQ ID Nos. 2-6 are further described in Mohr et al. BMC Cancer (2015) 15:494), which is herein incorporated by reference in its entirety.

SEQ ID NO: 2

Soluble TRAIL variant DR4-1

30 MAMMEVQGGPSLGQTCVLIVIFTVLLQSLCAVTVYVYFTNELKQMQDKYSKSGIACFLKEDDSYWDPNDEESMNSPC
 WQVKWQLRQLVRKMILRTSEETISTVQEKQONISPLVRERGPQRVAAHITGTRRRSNTLSSPNSKNEKALGRKINSW

ESSRSGHSFLSNLHLRNGELVIEHKGFFYYIYSQTYFRFQEEIKENTKNDKQMVQYIYKYTSYPDPILLMKSARNSCW
SKDAEYGLYSIYQGGIFELKENDRIFVSVTNEHLIDMDHEASFFGAFLVG

SEQ ID NO: 3

5 Soluble TRAIL variant DR4-2

MAMMEVQGGPSLGQTCVLIVIFTVLLQSLCVAVTYVYFTNELKQMMDKYSKSGIACFLKEDDSYWDPNDEESMNSPC
WQVKWQLRQLVRKMILRTSEETISTVQEKQQNISPLVRERGPQRVAAHITGTRGRSNTLSSPNSKNEKALGRKINSW
ESSRSGHSFLSNLHLRNGELVIEHKGFFYYIYSQTYFRFQEEIKENTKNDKQMVQYIYKYTSYPDPILLMKSARNSCW
SKDAEYGLYSIYQGGIFELKENDRIFVSVTNEHLIDMDHEASFFGAFLVG

10

SEQ ID NO: 4

Soluble TRAIL variant DR4-3

15 MAMMEVQGGPSLGQTCVLIVIFTVLLQSLCVAVTYVYFTNELKQMMDKYSKSGIACFLKEDDSYWDPNDEESMNSPC
WQVKWQLRQLVRKMILRTSEETISTVQEKQQNISPLVRERGPQRVAAHITGTRRRSNTLSSPNSKNEKALGIKINSW
ESSRSGHSFLSNLHLRNGELVIEHKGFFYYIYSQTYFRFQEEIKENTKNDKQMVQYIYKYTDYPDPILLMKSARNSCW
SKDAEYGLYSIYQGGIFELKENDRIFVSVTNEHLIDMDHEASFFGAFLVG

SEQ ID NO: 5

Soluble TRAIL variant DR5-1

20 MAMMEVQGGPSLGQTCVLIVIFTVLLQSLCVAVTYVYFTNELKQMMDKYSKSGIACFLKEDDSYWDPNDEESMNSPC
WQVKWQLRQLVRKMILRTSEETISTVQEKQQNISPLVRERGPQRVAAHITGTRGRSNTLSSPNSKNEKALGRKINSW
ESSRSGHSFLSNLHLRNGELVIEHKGFFYYIYSQTYFRFQEEIKENTKNDKQMVQYIYKYTSYPDPILLMKSARNSCW
SKDAEYGLYSIYQGGIFELKENDRIFVSVTNEHLIDMHHEASFFGAFLVG

25 SEQ ID NO: 6

Soluble TRAIL variant DR5-2

30 MAMMEVQGGPSLGQTCVLIVIFTVLLQSLCVAVTYVYFTNELKQMMDKYSKSGIACFLKEDDSYWDPNDEESMNSPC
WQVKWQLRQLVRKMILRTSEETISTVQEKQQNISPLVRERGPQRVAAHITGTRGRSNTLSSPNSKNEKALGRKINSW
ESSRSGHSFLSNLHLRNGELVIEHKGFFYYIYSQTYFRFQERIKENTKNDKQMVQYIYKYTSYPDPILLMKSARNSCW
SKDAEYGLYSIYQGGIFELKENDRIFVSVTNEHLIDMHHEASFFGAFLVG

In some embodiments, the TRAIL receptor ligand comprises an antibody, e.g., an antibody fragment.. In embodiments, the antibody recognizes one or both of TRAIL R1 and TRAIL R2. The antibody may be, e.g., Mapatumumab (human anti-DR4 mAb), Tigatuzumab

(humanized anti-DR5 mAb), Lexatumumab (human anti-DR5 mAb), Conatumumab (human anti-DR5 mAb), or Apomab (human anti-DR5 mAb). In some embodiments, erythroid cell comprises at least one antibody that binds a TRAIL receptor and at least one TRAIL polypeptide.

In some embodiments, the erythroid cell comprises a binding agent with targeting
 5 activity. In embodiments, the binding agent also has anti-cancer activity, e.g., the same exogenous polypeptide is an anti-cancer agent and a targeting agent. In embodiments, the erythroid cell comprises a second agent, e.g., a second exogenous polypeptide, having anti-cancer activity. In embodiments, the targeting agent binds at or near a cancer cell, e.g., solid tumor cell, and the second agent (e.g., second polypeptide) has an anti-cancer function. In some
 10 embodiments the site of action is tumor vasculature. In embodiments, the targeting agent binds a marker of neovasculature, e.g. binds an integrin such as α vB1, α vB3, or α vB5, or α 4b1 integrins, e.g. a synthetic peptide knottin (Kim et al, JACS 2016, 137(1)) or an endogenous or natural ligand, e.g. echistatin, RGD, EETI2.5F, or VCAM-1, or binds prostate-specific membrane antigen, which is also found abundantly on neovasculature. In some embodiments, the targeting
 15 agent binds a cancer cell marker such as CD269 (expressed, e.g., in multiple myeloma cells) or CD123 (expressed, e.g., in ALM cells), CD28 (expressed, e.g., in T cells; CD28 can be bound by CD80/CD86), NY-ESO-1 (expressed, e.g., in ovarian cancer).

In embodiments, the anti-cancer agent comprises an enzyme, e.g., asparaginase, methionine gamma lyase (MGL), serine dehydrogenase, or fragment or variant thereof, that
 20 degrades metabolites that are selectively required by tumor cells to grow. The anti-cancer agent may be an inhibitor of angiogenesis, e.g. an inhibitor of angiopoietin or an inhibitor of VEGF or VEGFR to prevent further growth of blood vessels. The anti-cancer agent may be an immunostimulatory molecule to activate T cells, e.g., a costimulatory molecule (e.g., a costimulatory molecule of Table 5), an immune checkpoint inhibitor (see, e.g., Table 4) or a
 25 cytokine or a T cell activation ligand. The anti-cancer agent may bind an immune effector cell, e.g. a T cell or an inflammatory macrophage and may capture and bring the effector cell into proximity of the tumor. The anti-cancer agent may be a direct mediator of cell killing, e.g. TRAIL or FAS-L or other death ligands, or a toxin. In some embodiments, the anti-cancer agent comprises an agonist of a TRAIL receptor, e.g., an agonistic antibody. In embodiments, the anti-
 30 cancer agent is a pro-apoptotic agent. In embodiments, the anti-cancer agent comprises an

adjuvant. For any of these anti-cancer agents, the net result is a red cell therapeutic (RCT) that localizes to a tumor site and thus concentrates its anti-tumor effect in a location that increases its efficacy.

In some embodiments, the exogenous polypeptide inhibits an immune checkpoint molecule. In embodiments, the exogenous polypeptide is situated at the surface of the erythroid cell (e.g., comprises a transmembrane portion and a surface-exposed portion) and binds an immune checkpoint molecule.

In some embodiments, the exogenous polypeptide inhibits an immune checkpoint molecule. In one embodiment, the inhibitor of the immune checkpoint molecule is an inhibitory antibody (e.g., an antibody such as a monospecific antibody, monoclonal antibody, or a single chain antibody). The antibody may be, e.g., humanized or fully human. In other embodiments, the inhibitor of the immune checkpoint molecule is a fusion protein, e.g., an Fc-receptor fusion protein. In some embodiments, the inhibitor of the immune checkpoint molecule is an agent, such as an antibody, that interacts with an immune checkpoint protein. In some embodiments, the inhibitor of the immune checkpoint molecule is an agent, such as an antibody, that interacts with the ligand of an immune checkpoint receptor. In one embodiment, the inhibitor of the immune checkpoint molecule is an inhibitor (e.g., an inhibitory antibody or small molecule inhibitor) of CTLA-4 (e.g., an anti-CTLA4 antibody such as ipilimumab/Yervoy or tremelimumab). In one embodiment, the inhibitor of the immune checkpoint molecule is an inhibitor (e.g., an inhibitory antibody or small molecule inhibitor) of PD-1 (e.g., nivolumab/Opdivo®; pembrolizumab/Keytruda®; pidilizumab/CT-011). In one embodiment, the inhibitor of the immune checkpoint molecule is an inhibitor (e.g., an inhibitory antibody or small molecule inhibitor) of PD-L1 (e.g., MPDL3280A/RG7446; MEDI4736; MSB0010718C; BMS 936559). In one embodiment, the inhibitor of the immune checkpoint molecule is an inhibitor (e.g., an inhibitory antibody or Fc fusion or small molecule inhibitor) of PDL2 (e.g., a PDL2/Ig fusion protein such as AMP 224). In one embodiment, the inhibitor of the immune checkpoint molecule is an inhibitor (e.g., an inhibitory antibody or small molecule inhibitor) of B7-H3 (e.g., MGA271), B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK 1, CHK2, A2aR, B-7 family ligands, or a combination thereof.

Inhibitors of immune checkpoint molecules can be broken down into at least 4 major categories: i) agents such as antibody that block an inhibitory pathway directly on T cells or natural killer (NK) cells (e.g., PD-1 targeting antibodies such as nivolumab and pembrolizumab, antibodies targeting TIM-3, and antibodies targeting LAG-3, 2B4, CD160, A2aR, BTLA, CGEN-15049, or KIR), ii) agents such as antibodies that activate stimulatory pathways directly on T cells or NK cells (e.g., antibodies targeting OX40, GITR, or 4-1BB), iii) agents such as antibody that block a suppressive pathway on immune cells or rely on antibody-dependent cellular cytotoxicity to deplete suppressive populations of immune cells (e.g., CTLA-4 targeting antibodies such as ipilimumab, antibodies targeting VISTA, and antibodies targeting PD-L2, Gr1, or Ly6G), and iv) agents such as antibodies that block a suppressive pathway directly on cancer cells or that rely on antibody-dependent cellular cytotoxicity to enhance cytotoxicity to cancer cells (e.g., rituximab, antibodies targeting PD-L1, and antibodies targeting B7-H3, B7-H4, Gal-9, or MUC1). Such agents described herein can be designed and produced, e.g., as described in Templeton, Gene and Cell Therapy, 2015; Green and Sambrook, Molecular Cloning, 2012.

In some embodiments, the erythroid cell comprises an agent, e.g., an exogenous polypeptide, e.g., a surface-exposed exogenous polypeptide, e.g., a surface-exposed polypeptide comprising a transmembrane domain, that binds an immune checkpoint molecule of Table 4. In embodiments, the erythroid cell comprises an agent, e.g., an exogenous polypeptide, that comprises an antibody or other binding agent of Table 4, or a fragment or variant thereof. For instance, the fragment or variant could be a single domain antibody, e.g., scFv, corresponding to an antibody of Table 4. As another example, the fragment or variant could be an antigen-binding fragment of an antibody of Table 4, e.g., a light chain variable fragment, heavy chain variable fragment, or both. As another example, the fragment or variant could be an active fragment of a binding agent of Table 4. As another example, the fragment or variant could be an antibody having less than 100% sequence identity to an antibody of Table 4, e.g., an antibody having at least 70%, 75%, 80%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity to the antibody of Table 4 or to a light chain variable fragment, heavy chain variable fragment, or both. For instance, the fragment or variant could have the same CDRs as an antibody of Table 4, but one or more mutations in the framework and/or constant region. As another example, the fragment or variant could be a binding agent having less than 100% sequence identity to a binding agent of Table 4,

e.g., a binding agent having at least 70%, 75%, 80%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity to a binding agent of Table 4 or to an active portion thereof.

In some embodiments, the erythroid cell comprises an agent, e.g., an exogenous polypeptide, e.g., a surface-exposed exogenous polypeptide, e.g., a surface-exposed polypeptide comprising a transmembrane domain, that comprises a costimulatory molecule of Table 5 or a fragment or variant thereof. In embodiments, the erythroid cell comprises an agent, e.g., an exogenous polypeptide, that comprises a costimulatory molecule of Table 5 or a fragment or variant thereof. For instance, the fragment or variant could be a protein sequence having less than 100% sequence identity to a costimulatory molecule of Table 5, e.g., a costimulatory molecule having at least 70%, 75%, 80%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity to the costimulatory molecule. In embodiments, the fragment or variant is an isoform of the costimulatory molecule, e.g., isoform 1, 2, 3, 4, or 5 of a costimulatory molecule of Table 5, or a fragment or variant thereof. In embodiments, the fragment or variant is a mature form of a costimulatory molecule of Table 5, e.g., has a sequence of the processed form of the costimulatory molecule of Table 5.

In embodiments, a costimulatory molecule comprises one or more of a MHC class I molecule, BTLA, a Toll ligand receptor, OX40, CD27, CD28, CDS, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), and 4-1BB (CD137), CDS, ICAM-1, GITR, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD160, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, and CD19a, or a fragment or variant thereof.

As another example, an erythroid cell brings an immune effector cell (e.g., T cell) and a cancer cell in close proximity with one another to facilitate the killing of the cancer cell by the immune effector cell. Thus, in some embodiments, the first polypeptide binds a cell surface

marker of a cancer cell and the second polypeptide binds a cell surface marker of an immune effector cell. The first and second polypeptides may comprise, e.g., antibodies. In some embodiments, the cancer cell marker is selected from CD19 (expressed, e.g., in B cell acute leukemia), EpCAM (expressed, e.g., in CTCs), CD20 (expressed, e.g., in B cell acute leukemia),
5 CD45 (expressed, e.g., in CTCs), EGFR, HER2 (expressed, e.g., in breast cancer cells). In some embodiments, the immune cell marker is CD3.

Vehicles for polypeptides described herein

While in many embodiments herein, the one or more exogenous polypeptides are situated on or in an erythroid cell, it is understood that any exogenous polypeptide(s) described herein
10 can also be situated on or in another vehicle. The vehicle can comprise, e.g., a cell, an erythroid cell, a corpuscle, a nanoparticle, a micelle, a liposome, or an exosome. The vehicle may be, e.g., a particle such as a nanoparticle or a particle greater than 100, 200, 300, 400, 500, 1,000, 1,500, 2,000, or 2,500 nm in diameter. For instance, in some aspects, the present disclosure provides a vehicle (e.g., a cell, an erythroid cell, a corpuscle, a nanoparticle, a micelle, a liposome, or an
15 exosome) comprising, e.g., on its surface, one or more agents described herein. In some embodiments, the one or more agents comprise an agent selected from a polypeptide of any of Table 2, Table 4, or Table 5, or a fragment or variant thereof, or an agonist or antagonist thereof, or an antibody thereto. In some embodiments, the vehicle comprises two or more agents described herein, e.g., any pair of agents described herein.

20 In some embodiments, the vehicle comprises an erythroid cell. In embodiments, the erythroid cell is a nucleated red blood cell, red blood cell precursor, or enucleated red blood cell. In embodiments, the erythroid cell is a cord blood stem cell, a CD34+ cell, a hematopoietic stem cell (HSC), a spleen colony forming (CFU-S) cell, a common myeloid progenitor (CMP) cell, a blastocyte colony-forming cell, a burst forming unit-erythroid (BFU-E), a megakaryocyte-
25 erythroid progenitor (MEP) cell, an erythroid colony-forming unit (CFU-E), a reticulocyte, an erythrocyte, an induced pluripotent stem cell (iPSC), a mesenchymal stem cell (MSC), a polychromatic normoblast, an orthochromatic normoblast, or a combination thereof. In some embodiments, the erythroid cells are immortal or immortalized cells. In some embodiments, the vehicle comprises an immune effector cell, e.g., a B cell, T cell, or NK cell. In some
30 embodiments, the cell is autologous or allogeneic.

Heterogeneous populations of cells

While in many embodiments herein, one or more (e.g., two or more) exogenous polypeptides are situated on or in a single cell, it is understood that any polypeptide or
5 combination of polypeptides described herein can also be situated on a plurality of cells. For instance, in some aspects, the disclosure provides a plurality of erythroid cells, wherein a first cell of the plurality comprises a first exogenous polypeptide and a second cell of the plurality comprises a second exogenous polypeptide. In some embodiments, the plurality of cells
10 comprises two or more polypeptides described herein, e.g., any pair of polypeptides described herein. In some embodiments, less than 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5%, 2%, or 1% of the cells in the population comprise both the first exogenous polypeptide and the second exogenous polypeptide.

Cells encapsulated in a membrane

15 In some embodiments, enucleated erythroid cells or other vehicles described herein are encapsulated in a membrane, e.g., semi-permeable membrane. In embodiments, the membrane comprises a polysaccharide, e.g., an anionic polysaccharide alginate. In embodiments, the semipermeable membrane does not allow cells to pass through, but allows passage of small molecules or macromolecules, e.g., metabolites, proteins, or DNA. In embodiments, the
20 membrane is one described in Lienert et al., "Synthetic biology in mammalian cells: next generation research tools and therapeutics" Nature Reviews Molecular Cell Biology 15, 95–107 (2014), incorporated herein by reference in its entirety. While not wishing to be bound by theory, in some embodiments, the membrane shields the cells from the immune system and/or keeps a plurality of cells in proximity, facilitating interaction with each other or each other's
25 products.

Physical characteristics of enucleated erythroid cells

In some embodiments, the enucleated erythroid cells described herein have one or more (e.g., 2, 3, 4, or more) physical characteristics described herein, e.g., osmotic fragility, cell size,
30 hemoglobin concentration, or phosphatidylserine content. While not wishing to be bound by theory, in some embodiments an enucleated erythroid cell that expresses an exogenous protein

has physical characteristics that resemble a wild-type, untreated erythroid cell. In contrast, a hypotonically loaded erythroid cell sometimes displays aberrant physical characteristics such as increased osmotic fragility, altered cell size, reduced hemoglobin concentration, or increased phosphatidylserine levels on the outer leaflet of the cell membrane.

5 In some embodiments, the enucleated erythroid cell comprises an exogenous protein that was encoded by an exogenous nucleic acid that was not retained by the cell, has not been purified, or has not existed fully outside an erythroid cell. In some embodiments, the erythroid cell is in a composition that lacks a stabilizer.

Osmotic fragility

10 In some embodiments, the enucleated erythroid cell exhibits substantially the same osmotic membrane fragility as an isolated, uncultured enucleated erythroid cell that does not comprise an exogenous polypeptide. In some embodiments, the population of enucleated erythroid cells has an osmotic fragility of less than 50% cell lysis at 0.3%, 0.35%, 0.4%, 0.45%, or 0.5% NaCl. Osmotic fragility is determined, in some embodiments, using the method of
15 Example 59 of WO2015/073587.

Cell size

In some embodiments, the enucleated erythroid cell has approximately the diameter or volume as a wild-type, untreated erythroid cell.

In some embodiments, the population of erythroid cells has an average diameter of about
20 4, 5, 6, 7, or 8 microns, and optionally the standard deviation of the population is less than 1, 2, or 3 microns. In some embodiments, the one or more erythroid cell has a diameter of about 4-8, 5-7, or about 6 microns. In some embodiments, the diameter of the erythroid cell is less than about 1 micron, larger than about 20 microns, between about 1 micron and about 20 microns, between about 2 microns and about 20 microns, between about 3 microns and about 20 microns,
25 between about 4 microns and about 20 microns, between about 5 microns and about 20 microns, between about 6 microns and about 20 microns, between about 5 microns and about 15 microns or between about 10 microns and about 30 microns. Cell diameter is measured, in some embodiments, using an Advia 120 hematology system.

In some embodiment the volume of the mean corpuscular volume of the erythroid cell is
30 greater than 10 fL, 20 fL, 30 fL, 40 fL, 50 fL, 60 fL, 70 fL, 80 fL, 90 fL, 100 fL, 110 fL, 120 fL, 130 fL, 140 fL, 150 fL, or greater than 150 fL. In one embodiment the mean corpuscular volume

of the erythroid cell is less than 30 fL, 40 fL, 50 fL, 60 fL, 70 fL, 80 fL, 90 fL, 100 fL, 110 fL, 120 fL, 130 fL, 140 fL, 150 fL, 160 fL, 170 fL, 180 fL, 190 fL, 200 fL, or less than 200 fL. In one embodiment the mean corpuscular volume of the erythroid cell is between 80-100, 100-200, 200-300, 300-400, or 400-500 femtoliters (fL). In some embodiments, a population of erythroid
5 cells has a mean corpuscular volume set out in this paragraph and the standard deviation of the population is less than 50, 40, 30, 20, 10, 5, or 2 fL. The mean corpuscular volume is measured, in some embodiments, using a hematological analysis instrument, e.g., a Coulter counter.

Hemoglobin concentration

In some embodiments, the enucleated erythroid cell has a hemoglobin content similar to a
10 wild-type, untreated erythroid cell. In some embodiments, the erythroid cells comprise greater than 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9% or greater than 10% fetal hemoglobin. In some embodiments, the erythroid cells comprise at least about 20, 22, 24, 26, 28, or 30 pg, and optionally up to about 30 pg, of total hemoglobin. Hemoglobin levels are determined, in some embodiments, using the Drabkin's reagent method of Example 33 of WO2015/073587.

Phosphatidylserine content

In some embodiments, the enucleated erythroid cell has approximately the same
phosphatidylserine content on the outer leaflet of its cell membrane as a wild-type, untreated erythroid cell. Phosphatidylserine is predominantly on the inner leaflet of the cell membrane of wild-type, untreated erythroid cells, and hypotonic loading can cause the phosphatidylserine to
20 distribute to the outer leaflet where it can trigger an immune response. In some embodiments, the population of erythroid cells comprises less than about 30, 25, 20, 15, 10, 9, 8, 6, 5, 4, 3, 2, or 1% of cells that are positive for Annexin V staining. Phosphatidylserine exposure is assessed, in some embodiments, by staining for Annexin-V-FITC, which binds preferentially to PS, and measuring FITC fluorescence by flow cytometry, e.g., using the method of Example 54 of
25 WO2015/073587.

Other characteristics

In some embodiments, the population of erythroid cells comprises at least about 50%, 60%, 70%, 80%, 90%, or 95% (and optionally up to 90 or 100%) of cells that are positive for GPA. The presence of GPA is detected, in some embodiments, using FACS.

30 In some embodiments, the enucleated erythroid cells have a half-life of at least 30, 45, or 90 days in a subject.

In some embodiments, a population of cells comprising erythroid cells comprises less than about 10, 5, 4, 3, 2, or 1% echinocytes.

In some embodiments, an erythroid cell is enucleated. In some embodiments, a cell, e.g., an erythroid cell, contains a nucleus that is non-functional, e.g., has been inactivated.

5

Universal donor erythroid cells

In some embodiments, erythroid cells described herein are autologous and/or allogeneic to the subject to which the cells will be administered. For example, erythroid cells allogeneic to the subject include one or more of blood type specific erythroid cells (e.g., the cells can be of the same blood type as the subject) or one or more universal donor erythroid cells. In some
10 embodiments, the enucleated erythroid cells described herein have reduced immunogenicity compared to a reference cell, e.g., have lowered levels of one or more blood group antigens.

Where allogeneic cells are used for transfusion, a compatible ABO blood group can be chosen to prevent an acute intravascular hemolytic transfusion reaction. The ABO blood types
15 are defined based on the presence or absence of the blood type antigens A and B, monosaccharide carbohydrate structures that are found at the termini of oligosaccharide chains associated with glycoproteins and glycolipids on the surface of the erythrocytes (reviewed in Liu et al., Nat. Biotech. 25:454-464 (2007)). Because group O erythrocytes contain neither A nor B antigens, they can be safely transfused into recipients of any ABO blood group, e.g., group A, B, AB, or O recipients. Group O erythrocytes are considered universal and may be used in all blood
20 transfusions. Thus, in some embodiments, an erythroid cell described herein is type O. In contrast, group A erythroid cells may be given to group A and AB recipients, group B erythroid cells may be given to group B and AB recipients, and group AB erythroid cells may be given to AB recipients.

25 In some instances, it may be beneficial to convert a non-group O erythroid cell to a universal blood type. Enzymatic removal of the immunodominant monosaccharides on the surface of group A and group B erythrocytes may be used to generate a population of group O-like erythroid cells (See, e.g., Liu et al., Nat. Biotech. 25:454-464 (2007)). Group B erythroid cells may be converted using an α -galactosidase derived from green coffee beans. Alternatively
30 or in addition, α -N-acetylgalactosaminidase and α -galactosidase enzymatic activities derived from *E. meningosepticum* bacteria may be used to respectively remove the immunodominant A

and B antigens (Liu et al., Nat. Biotech. 25:454-464 (2007)), if present on the erythroid cells. In one example, packed erythroid cells isolated as described herein, are incubated in 200 mM glycine (pH 6.8) and 3 mM NaCl in the presence of either α -N-acetylgalactosaminidase and α -galactosidase (about 300 μ g/ml packed erythroid cells) for 60 min at 26° C. After treatment, the erythroid cells are washed by 3-4 rinses in saline with centrifugation and ABO-typed according to standard blood banking techniques.

While the ABO blood group system is the most important in transfusion and transplantation, in some embodiments it can be useful to match other blood groups between the erythroid cells to be administered and the recipient, or to select or make erythroid cells that are universal for one or more other (e.g., minor) blood groups. A second blood group is the Rh system, wherein an individual can be Rh+ or Rh-. Thus, in some embodiments, an erythroid cell described herein is Rh-. In some embodiments, the erythroid cell is Type O and Rh-.

In some embodiments, an erythroid cell described herein is negative for one or more minor blood group antigens, e.g., Le(a-b-) (for Lewis antigen system), Fy(a-b-) (for Duffy system), Jk(a-b-) (for Kidd system), M-N- (for MNS system), K-k- (for Kell system), Lu(a-b-) (for Lutheran system), and H-antigen negative (Bombay phenotype), or any combination thereof. In some embodiments, the erythroid cell is also Type O and/or Rh-. Minor blood groups are described, e.g., in Agarwal et al "Blood group phenotype frequencies in blood donors from a tertiary care hospital in north India" Blood Res. 2013 Mar; 48(1): 51-54 and Mitra et al "Blood groups systems" Indian J Anaesth. 2014 Sep-Oct; 58(5): 524-528, each of which is incorporated herein by reference in its entirety.

Methods of manufacturing enucleated erythroid cells

Methods of manufacturing enucleated erythroid cells comprising an exogenous polypeptide are described, e.g., in WO2015/073587 and WO2015/153102, each of which is incorporated by reference in its entirety.

In some embodiments, hematopoietic progenitor cells, e.g., CD34+ hematopoietic progenitor cells, are contacted with a nucleic acid or nucleic acids encoding one or more exogenous polypeptides, and the cells are allowed to expand and differentiate in culture.

The nucleic acid may be, e.g., DNA or RNA. A number of viruses may be used as gene transfer vehicles including retroviruses, Moloney murine leukemia virus (MMLV), adenovirus,

adeno-associated virus (AAV), herpes simplex virus (HSV), lentiviruses such as human immunodeficiency virus 1 (HIV 1), and spumaviruses such as foamy viruses, for example.

In some embodiments, the cells are produced using conjugation, e.g., sortagging, e.g., as described in WO2014/183071 or WO2014/183066, each of which is incorporated by reference in its entirety.

In some embodiments, the erythroid cells are expanded at least 1000, 2000, 5000, 10,000, 20,000, 50,000, or 100,000 fold (and optionally up to 100,000, 200,000, or 500,000 fold).

Number of cells is measured, in some embodiments, using an automated cell counter.

In some embodiments, the population of erythroid cells comprises at least 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 98% (and optionally up to about 80, 90, or 100%) enucleated erythroid cells. In some embodiments, the population of erythroid cells contains less than 1% live enucleated cells, e.g., contains no detectable live enucleated cells. Enucleation is measured, in some embodiments, by FACS using a nuclear stain. In some embodiments, at least 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, or 80% (and optionally up to about 70, 80, 90, or 100%) of erythroid cells in the population comprise one or more (e.g., 2, 3, 4 or more) of the exogenous polypeptides. Expression of the polypeptides is measured, in some embodiments, by FACS using labeled antibodies against the polypeptides. In some embodiments, at least 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, or 80% (and optionally up to about 70, 80, 90, or 100%) of erythroid cells in the population are enucleated and comprise one or more exogenous polypeptides. In some embodiments, the population of erythroid cells comprises about $1 \times 10^9 - 2 \times 10^9$, $2 \times 10^9 - 5 \times 10^9$, $5 \times 10^9 - 1 \times 10^{10}$, $1 \times 10^{10} - 2 \times 10^{10}$, $2 \times 10^{10} - 5 \times 10^{10}$, $5 \times 10^{10} - 1 \times 10^{11}$, $1 \times 10^{11} - 2 \times 10^{11}$, $2 \times 10^{11} - 5 \times 10^{11}$, $5 \times 10^{11} - 1 \times 10^{12}$, $1 \times 10^{12} - 2 \times 10^{12}$, $2 \times 10^{12} - 5 \times 10^{12}$, or $5 \times 10^{12} - 1 \times 10^{13}$ cells.

Methods of treatment with compositions herein, e.g., erythroid cells

Methods of administering erythroid cells comprising (e.g., expressing) exogenous agent (e.g., polypeptides) are described, e.g., in WO2015/073587 and WO2015/153102, each of which is incorporated by reference in its entirety.

In embodiments, the erythroid cells described herein are administered to a subject, e.g., a mammal, e.g., a human. Exemplary mammals that can be treated include without limitation, humans, domestic animals (e.g., dogs, cats and the like), farm animals (e.g., cows, sheep, pigs, horses and the like) and laboratory animals (e.g., monkey, rats, mice, rabbits, guinea pigs and the

like). The methods described herein are applicable to both human therapy and veterinary applications.

In some embodiments, the erythroid cells are administered to a patient every 1, 2, 3, 4, 5, or 6 months.

5 In some embodiments, a dose of erythroid cells comprises about $1 \times 10^9 - 2 \times 10^9$, $2 \times 10^9 - 5 \times 10^9$, $5 \times 10^9 - 1 \times 10^{10}$, $1 \times 10^{10} - 2 \times 10^{10}$, $2 \times 10^{10} - 5 \times 10^{10}$, $5 \times 10^{10} - 1 \times 10^{11}$, $1 \times 10^{11} - 2 \times 10^{11}$, $2 \times 10^{11} - 5 \times 10^{11}$, $5 \times 10^{11} - 1 \times 10^{12}$, $1 \times 10^{12} - 2 \times 10^{12}$, $2 \times 10^{12} - 5 \times 10^{12}$, or $5 \times 10^{12} - 1 \times 10^{13}$ cells.

In some aspects, the present disclosure provides a method of treating a disease or condition described herein, comprising administering to a subject in need thereof a composition described herein, e.g., an erythroid cell described herein. In some embodiments, the disease or condition is a cancer, e.g., a cancer described herein. In some aspects, the disclosure provides a use of an erythroid cell described herein for treating a disease or condition described herein, e.g., a cancer. In some aspects, the disclosure provides a use of an erythroid cell described herein for manufacture of a medicament for treating a disease or condition described herein, e.g., a cancer.

15 Types of cancer include acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML), anal cancer, bile duct cancer, bladder cancer, bone cancer, bowel cancer, brain tumors, breast cancer, cancer of unknown primary, cancer spread to bone, cancer spread to brain, cancer spread to liver, cancer spread to lung, carcinoid, cervical cancer, choriocarcinoma, chronic lymphocytic leukaemia (CLL), chronic myeloid leukaemia (CML), colon cancer, colorectal cancer, endometrial cancer, eye cancer, gallbladder cancer, gastric cancer, gestational trophoblastic tumors (GTT), hairy cell leukaemia, head and neck cancer, Hodgkin lymphoma, kidney cancer, laryngeal cancer, leukaemia, liver cancer, lung cancer, lymphoma, B-cell lymphoma, melanoma skin cancer, mesothelioma, men's cancer, molar pregnancy, mouth and oropharyngeal cancer, myeloma, nasal and sinus cancers, nasopharyngeal cancer, non-Hodgkin lymphoma (NHL), oesophageal cancer, ovarian cancer, pancreatic cancer, penile cancer, prostate cancer, rare cancers, rectal cancer, salivary gland cancer, secondary cancers, skin cancer (non-melanoma), soft tissue sarcoma, stomach cancer, testicular cancer, thyroid cancer, unknown primary cancer, uterine cancer, vaginal cancer, and vulval cancer.

In some embodiments, the cancer is a highly immunogenic cancer, e.g., the cancer has (e.g., as determined by analysis of a cancer biopsy) one or more of the following characteristics: (a) tumor infiltrating lymphocytes (TIL), e.g., at least 1, 5, 10, 100 TIL per 1000 tumor cells; (b)

mutations, e.g., 0.1 or more somatic mutations per megabase of tumor genomic DNA; (c) neoantigens, e.g., 1 or more neoantigen with one or more endogenous T cell receptor and/or one or more idiotype clone that recognizes a processed and presented moiety of the neoantigen; (d) tertiary lymphoid structures; (e) high expression of inflammatory gene expression, e.g., 2-fold
 5 increased expression of cytokines above baseline expression in non-cancerous tissue; and (f) immune cells exhibiting immunosuppressive phenotype, e.g. dendritic cells lacking cytokine expression. Methods of assessing these characteristics of the cancer are known (see, e.g., Clin Cancer Res. 2000 May;6(5):1875-81; Nature. 2013 Aug 22;500(7463):415-21. doi: 10.1038/nature12477. Epub 2013 Aug 14; Nature. 2014 Nov 27;515(7528):577-81. doi: 10.1038/nature13988; Trends Immunol. 2014 Nov;35(11):571-80. doi: 10.1016/j.it.2014.09.006.
 10 Epub 2014 Oct 22; Front Immunol. 2013 Dec 11;4:438. doi: 10.3389/fimmu.2013.00438; Eur J Cancer. 2009 Jan;45(2):228-47. doi: 10.1016/j.ejca.2008.10.026.

In some embodiments, the tumor cell is in a primary tumor. In some embodiments, the
 15 tumor cell is other than a circulating tumor cell.

In some embodiments, the tumor comprises a mutation in a gene of Table 8. In some embodiments, the mutation in a gene of Table 8 is a mutation specified in the third column of Table 8. In embodiments, the cancer comprises at least two different mutations, e.g., a mutation in a gene in Table 8 (e.g., a mutation specified in the third column of Table 8) and a second
 20 mutation. In embodiments, the second mutation is a mutation in a gene in Table 8, e.g., a mutation specified in the third column of Table 8. In embodiments, some cells in the tumor comprise the mutation and other cells do not.

In some embodiments, the tumor does not comprise p53-null cells. In embodiments, the patient having the tumor is not heterozygous for a p53-null mutation.

25 The tumor mutation status can be determined by a number of methods, such as PCR, microarray, or nucleic acid sequencing, e.g., high throughput DNA sequencing.

In some embodiments, the tumor comprises a tumor antigen chosen from one or more of: CD19; CD123; CD22; CD30; CD171; CS-1 (also referred to as CD2 subset 1, CRACC, SLAMF7, CD319, and 19A24); C-type lectin-like molecule-1 (CLL-1 or CLECL1); CD33;

epidermal growth factor receptor variant III (EGFRvIII); ganglioside G2 (GD2); ganglioside GD3 (aNeu5Ac(2-8)aNeu5Ac(2-3)bDGalp(1-4)bDGlc(1-1)Cer); TNF receptor family member B cell maturation (BCMA); Tn antigen ((Tn Ag) or (GalNAc α -Ser/Thr)); prostate-specific membrane antigen (PSMA); Receptor tyrosine kinase-like orphan receptor 1 (ROR1); Fms-Like Tyrosine Kinase 3 (FLT3); Tumor-associated glycoprotein 72 (TAG72); CD38; CD44v6; Carcinoembryonic antigen (CEA); Epithelial cell adhesion molecule (EPCAM); B7H3 (CD276); KIT (CD117); Interleukin-13 receptor subunit alpha-2 (IL-13Ra2 or CD213A2); Mesothelin; Interleukin 11 receptor alpha (IL-11Ra); prostate stem cell antigen (PSCA); Protease Serine 21 (Testisin or PRSS21); vascular endothelial growth factor receptor 2 (VEGFR2); Lewis(Y) antigen; CD24; Platelet-derived growth factor receptor beta (PDGFR-beta); Stage-specific embryonic antigen-4 (SSEA-4); CD20; Folate receptor alpha; Receptor tyrosine-protein kinase ERBB2 (Her2/neu); Mucin 1, cell surface associated (MUC1); epidermal growth factor receptor (EGFR); neural cell adhesion molecule (NCAM); Prostase; prostatic acid phosphatase (PAP); elongation factor 2 mutated (ELF2M); Ephrin B2; fibroblast activation protein alpha (FAP); insulin-like growth factor 1 receptor (IGF-I receptor), carbonic anhydrase IX (CAIX); Proteasome (Prosome, Macropain) Subunit, Beta Type, 9 (LMP2); glycoprotein 100 (gp100); oncogene fusion protein consisting of breakpoint cluster region (BCR) and Abelson murine leukemia viral oncogene homolog 1 (Abl) (bcr-abl); tyrosinase; ephrin type-A receptor 2 (EphA2); Fucosyl GM1; sialyl Lewis adhesion molecule (sLe); ganglioside GM3 (aNeu5Ac(2-3)bDGalp(1-4)bDGlc(1-1)Cer); transglutaminase 5 (TGS5); high molecular weight-melanoma-associated antigen (HMWMAA); o-acetyl-GD2 ganglioside (OAcGD2); Folate receptor beta; tumor endothelial marker 1 (TEM1/CD248); tumor endothelial marker 7-related (TEM7R); claudin 6 (CLDN6); thyroid stimulating hormone receptor (TSHR); G protein-coupled receptor class C group 5, member D (GPRC5D); chromosome X open reading frame 61 (CXORF61); CD97; CD179a; anaplastic lymphoma kinase (ALK); Polysialic acid; placenta-specific 1 (PLAC1); hexasaccharide portion of globoH glycosphingolipid (GloboH); mammary gland differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); Hepatitis A virus cellular receptor 1 (HAVCR1); adrenoceptor beta 3 (ADRB3); pannexin 3 (PANX3); G protein-coupled receptor 20 (GPR20); lymphocyte antigen 6 complex, locus K 9 (LY6K); Olfactory receptor 51E2 (OR51E2); TCR Gamma Alternate Reading Frame Protein (TARP); Wilms tumor protein (WT1); Cancer/testis antigen 1 (NY-ESO-1); Cancer/testis antigen 2 (LAGE-1a); Melanoma-

associated antigen 1 (MAGE-A1); ETS translocation-variant gene 6, located on chromosome 12p (ETV6-AML); sperm protein 17 (SPA17); X Antigen Family, Member 1A (XAGE1); angiopoietin-binding cell surface receptor 2 (Tie 2); melanoma cancer testis antigen-1 (MAD-CT-1); melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; tumor protein p53 (p53); p53 mutant; prostatein; survivin; telomerase; prostate carcinoma tumor antigen-1 (PCTA-1 or Galectin 8), melanoma antigen recognized by T cells 1 (MelanA or MART1); Rat sarcoma (Ras) mutant; human Telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoints; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease, serine 2 (TMPRSS2) ETS fusion gene); N-Acetyl glucosaminyl-transferase V (NA17); paired box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN); Ras Homolog Family Member C (RhoC); Tyrosinase-related protein 2 (TRP-2); Cytochrome P450 1B1 (CYP1B1); CCCTC-Binding Factor (Zinc Finger Protein)-Like (BORIS or Brother of the Regulator of Imprinted Sites), Squamous Cell Carcinoma Antigen Recognized By T Cells 3 (SART3); Paired box protein Pax-5 (PAX5); proacrosin binding protein sp32 (OY-TES1); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchor protein 4 (AKAP-4); synovial sarcoma, X breakpoint 2 (SSX2); Receptor for Advanced Glycation Endproducts (RAGE-1); renal ubiquitous 1 (RU1); renal ubiquitous 2 (RU2); legumain; human papilloma virus E6 (HPV E6); human papilloma virus E7 (HPV E7); intestinal carboxyl esterase; heat shock protein 70-2 mutated (mut hsp70-2); CD79a; CD79b; CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR or CD89); Leukocyte immunoglobulin-like receptor subfamily A member 2 (LILRA2); CD300 molecule-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); EGF-like module-containing mucin-like hormone receptor-like 2 (EMR2); lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); and immunoglobulin lambda-like polypeptide 1 (IGLL1).

In some embodiments, the tumor is resistant to, e.g., has been treated with and does not respond to, a therapy comprising an antibody of Table 1 or Table 2, or a fragment or variant thereof. For instance, the fragment or variant could be a single domain antibody, e.g., scFv, corresponding to an antibody of Table 1 or Table 2. As another example, the fragment or variant could be an antigen-binding fragment of an antibody of Table 1 or Table 2, e.g., a light chain

variable fragment, heavy chain variable fragment, or both. As another example, the fragment or variant could be an active fragment of a binding agent of Table 1 or Table 2. As another example, the fragment or variant could be an antibody having less than 100% sequence identity to an antibody of Table 1 or Table 2, e.g., an antibody having at least 70%, 75%, 80%, 95%,
5 96%, 97%, 98%, 99%, or 99.5% identity to the antibody of Table 1 or Table 2 or to a light chain variable fragment, heavy chain variable fragment, or both. For instance, the fragment or variant could have the same CDRs as an antibody of Table 1 or Table 2, but one or more mutations in the framework and/or constant region. As another example, the fragment or variant could be a binding agent having less than 100% sequence identity to a binding agent of Table 1 or Table 2,
10 e.g., a binding agent having at least 70%, 75%, 80%, 95%, 96%, 97%, 98%, 99%, or 99.5% identity to a binding agent of Table 1 or Table 2 or to an active portion thereof.

In some embodiments, the erythroid cell comprises a chemotherapeutic agent. In some embodiments, the erythroid cell is administered in combination with a chemotherapeutic agent, e.g., the erythroid cell is administered before, after, or simultaneously with the chemotherapeutic
15 agent. In some embodiments, the erythroid cell and the chemotherapeutic agent are admixed, and in some embodiments, they are in separate preparations. The erythroid cell and the chemotherapeutic agent may be administered through the same route of administration (e.g., intravenous) or different routes of administration (e.g., intravenous and oral).

In embodiments, the chemotherapeutic is a microtubule inhibitor, DNA replication
20 inhibitor, photosensitizer, or enzyme inhibitor. In embodiments, the chemotherapeutic is a microtubule inhibitor (e.g., blocks microtubule assembly for instance a vinca alkaloid, or blocks microtubule disassembly such as a taxane or epothilone). In embodiments, the chemotherapeutic is a DNA replication inhibitor (e.g., an antimetabolite such as an antimetabolite of folic acid, a purine, a pyrimidine, or DNA; a topoisomerase inhibitor such as an
25 inhibitor of Topoisomerase I or II; or a DNA crosslinker such as an alkylating agent, platinum-based agent, nonclassical DNA crosslinker, or intercalator). In embodiments, the photosensitizer is a porphyrin derivative.

More specifically, in some embodiments, the vinca alkaloid is chosen from vinblastine, Vincristine, Vinflunine, Vindesine, or Vinorelbine. In some embodiments, the taxane is chosen
30 from Cabazitaxel, Docetaxel, Larotaxel, Ortataxel, Paclitaxel, or Tese-taxel. In some

embodiments, the folic acid antimetabolite is selected from a Dihydrofolate reductase inhibitor (e.g., Aminopterin, Methotrexate, Pemetrexed, or Pralatrexate) or a Thymidylate synthase inhibitor (e.g., Raltitrexed or Pemetrexed). In some embodiments, the purine antimetabolite is selected from an Adenosine deaminase inhibitor (e.g., Pentostatin), a halogenated/ribonucleotide reductase inhibitor (e.g., Cladribine, Clofarabine, Fludarabine), or a Thiopurine (e.g., Thioguanine or Mercaptopurine). In some embodiments, the pyrimidine antimetabolite is chosen from a Thymidylate synthase inhibitor (e.g., Fluorouracil, Capecitabine, Tegafur, Carmofur, or Floxuridine), a DNA polymerase inhibitor (e.g., Cytarabine), a Ribonucleotide reductase inhibitor (e.g., Gemcitabine) or a Hypomethylating agent (e.g., Azacitidine or Decitabine). In some embodiments, the Deoxyribonucleotide antimetabolite is a Ribonucleotide reductase inhibitor (e.g., Hydroxycarbamide). In embodiments, the topoisomerase inhibitor is an inhibitor of topoisomerase I, e.g., a Camptotheca compound, e.g., Camptothecin, Cositecan, Belotecan, Gimatecan, Exatecan, Irinotecan, Lurtotecan, Silatecan, Topotecan, or Rubitecan. In embodiments, the topoisomerase inhibitor is an inhibitor of topoisomerase II, e.g., a Podophyllum compound (e.g., Etoposide or Teniposide). In embodiments, the topoisomerase inhibitor has intercalation activity, e.g., an Anthracycline (e.g., Aclarubicin, Daunorubicin, Doxorubicin, Epirubicin, Idarubicin, Amrubicin, Pirarubicin, Valrubicin, or Zorubicin) or an Anthracenedione (e.g., Mitoxantrone or Pixantrone).

In embodiments, the alkylating agent is a Nitrogen mustard, e.g., Mechlorethamine, a Cyclophosphamide (e.g., Ifosfamide or Trofosfamide), a Chlorambucil (e.g., Melphalan or Prednimustine), Bendamustine, Uramustine, or Estramustine. In embodiments, the alkylating agent is a Nitrosourea, e.g., Carmustine, Lomustine, Fotemustine, Nimustine, Ranimustine, or Streptozocin. In embodiments, the alkylating agent is an Alkyl sulfonate, e.g., a Busulfan (e.g., Mannosulfan or Treosulfan). In embodiments, the alkylating agent is an Aziridine, e.g., Carboquone, ThioTEPA, Triaziquone, or Triethylenemelamine. In embodiments, the platinum-based agent is chosen from Carboplatin, Cisplatin, Dicycloplatin, Nedaplatin, Oxaliplatin, or Satraplatin. In embodiments, the nonclassical DNA crosslinker is a Hydrazine (e.g., Procarbazine), a Triazene (e.g., Dacarbazine or Temozolomide), Altretamine, Mitobronitol, or Pipobroman. In embodiments, the intercalator is a Streptomyces compound, e.g., (Actinomycin, Bleomycin, Mitomycin, or Plicamycin).

In embodiments, the photosensitizer is selected from an Aminolevulinic acid / Methyl aminolevulinate, Efaproxiral, a porphyrin derivative (e.g., Porfimer sodium Talaporfin, Temoporfin, or Verteporfin).

In embodiments, the enzyme inhibitor is selected from a Farnesyltransferase inhibitor (e.g., Tipifarnib), a CDK inhibitor (e.g., Alvocidib, Seliciclib, or Palbociclib), a Proteasome inhibitor (e.g., (Bortezomib, Carfilzomib, or Ixazomib), a Phosphodiesterase inhibitor (e.g., Anagrelide), an IMP dehydrogenase inhibitor (e.g., Tiazofurin), a Arachidonate 5-lipoxygenase inhibitor (e.g., Masoprocol), a PARP inhibitor (e.g., Olaparib or Rucaparib), an HDAC inhibitor (e.g., Belinostat, Panobinostat, Romidepsin, or Vorinostat) or a Phosphoinositide 3-kinase inhibitor, e.g., (Idelalisib).

In embodiments, the chemotherapeutic is a receptor antagonist. In embodiments, the receptor antagonist is chosen from an Endothelin receptor antagonist (e.g., Atrasentan), a Retinoid X receptor antagonist (e.g., Bexarotene), or a Sex steroid (e.g., Testolactone). In embodiments, the chemotherapeutic is selected from Amsacrine, Trabectedin, a Retinoid (e.g., Alitretinoin or Tretinoin), Arsenic trioxide, an Asparagine deplete (e.g., Asparaginase of Pegaspargase). Celecoxib, Demecolcine, Elesclomol, Elsamitrucin, Etoglucid, Lonidamine, Lucanthone, Mitoguazone, Mitotane, Oblimersen, Omacetaxine mepesuccinate, or Eribulin.

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TABLES

Table 1. Therapeutic anti-cancer antibodies. This table comprises antibodies that have received regulatory approval and/or have entered clinical trials.

Antibody	Target	Exemplary indications
rituximab	CD20	Lymphoma e.g., NHL, leukemia
alemtuzumab	CD52	Leukemia, e.g., B cell leukemia
Trastuzumab, e.g., ado-trastuzumab, e.g., ado-trastuzumab emtansine	HER2/neu	Solid tumors, e.g., breast cancer, e.g., HER2-positive breast cancer, e.g., breast cancer with HER2 overexpression
nimotuzumab	EGFR	Solid tumors, e.g., squamous cell carcinoma or

		glioma
cetuximab	EGFR	Solid tumors, e.g., squamous cell carcinoma or colorectal carcinoma
bevacizumab	VEGF	Solid tumors, e.g., colon cancer, lung cancer, renal cancer, ovarian cancer, glioma, breast cancer
bavituximab	phosphatidylserine	Solid tumors, e.g., lung cancer e.g. non-small cell lung cancer, pancreatic cancer, hepatocellular carcinoma, melanoma, rectal cancer, prostate cancer
gemtuzumab, e.g., gemtuzumab ozogamicin	CD33	Leukemia, e.g., acute myelogenous leukemia
ibritumomab, e.g., ibritumomab tiuxetan	CD20	Lymphoma, e.g., NHL, e.g., B cell non-Hodgkin's lymphoma
Tositumomab, e.g., tositumomab-I-131	CD20	Lymphoma, e.g., NHL,
panitumumab	EGFR	Solid tumors, e.g., colorectal cancer
ofatumumab	CD20	Leukemia, e.g., CLL, lymphoma e.g., NHL, e.g., DLBCL
ipilimumab	CTLA-4	Solid tumors, e.g., melanoma, bladder cancer, prostate cancer, and lung cancer e.g. NSCLC and SCLC
brentuximab, e.g., brentuximab vedotin	CD30	Lymphoma, e.g., Hodgkin lymphoma and sALCL
pertuzumab	Her2	Solid tumors, e.g., breast cancer, e.g., HER2-positive breast cancer, e.g., breast cancer with HER2 overexpression
obinutuzumab	CD20	Leukemia, e.g., CLL, and lymphoma, e.g., follicular lymphoma
durvalumab	PD-L1	Solid tumors, e.g., bladder cancer and lung cancer

		e.g., NSCLC
nivolumab	PD-1	Solid tumors, e.g., renal cell carcinoma, melanoma or lung cancer e.g., NSCLC
pembrolizumab	PD-1	Solid tumors, e.g., HNSCC, melanoma, or lung cancer e.g., NSCLC
olatumab	PDGF-R α	Solid tumors, e.g., soft tissue sarcoma
atezolizumab	PD-L1	Solid tumors, e.g., lung cancer, e.g., NSCLC
elotuzumab	SLAMF7 (CD319)	Multiple myeloma
necitumumab	EGFR	Solid tumors, e.g., lung cancer, e.g., NSCLC
daratumumab	CD38	Multiple myeloma
ramucirumab	VEGFR2 (KDR)	Solid tumors, e.g., colorectal cancer
dinutuximab	GD2	Solid tumors, e.g., neuroblastoma
blinatumomab	CD19, CD3	Leukemia, e.g., ALL
siltuximab	IL-6	solid tumors, e.g., renal cell cancer, prostate cancer
denosumab	RANK ligand	Solid tumors, e.g., giant cell tumor of bone
catumaxomab	EpCAM, CD3	Solid tumors, e.g., head and neck cancer
enoblituzumab	B7H3	Solid tumors, e.g., NSCLC, SCCHN, Melanoma, Urothelial Cancer
tremelimumab	CTLA4	Solid tumors, e.g., Melanoma, mesothelioma
lirilumab	KIR2D subgroup	Leukemia, e.g., AML, CLL; solid tumors
pidilizumab	PD1	Lymphoma, e.g., DLBCL; multiple myeloma
avelumab	PDL1	Solid tumors, e.g., Nasopharyngeal Cancer, Endometrial Cancer, Merkel Cell Carcinoma, Ovarian Cancer, Peritoneal Cancer, Fallopian Tube Cancer; Leukemia e.g., AML

Table 2. Antibody VH and VL sequences. The sequences in this table were obtained from publically available sources and if there is a discrepancy between a sequence herein and the sequence of the named antibody, the actual antibody controls.

Antibody	Sequence
rituximab	>Rituximab heavy chain chimeric QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTP GRGLEWIGAIYPGNGDTSYNQKFKGKATLTADKSSSTAYMQLS SLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKG PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKKAEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK (SEQ ID NO: 7) >Rituximab light chain chimeric QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKP WIYATSNLASGVPVRFSGSGSGTSYSLTISRVEAEDAATYYCQQ WTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCL LNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSST LTLISKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 8)
alemtuzumab	>1CE1:H CAMPATH-1H:Heavy Chain 1 QVQLQESGPGLVRPSQTLSTCTVSGFTFTDFYMNWVRQPPGR GLEWIGFIRDKAKGYTTEYNPSVKGRVTMLVDTSKNQFSLRLSS VTAADTAVYYCAREGHTAAPFDYWGGSLVTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK

	KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHE ALHNHYTQKSLSLSPGK (SEQ ID NO: 9) >1CE1:L CAMPATH-1H:Light Chain 1 DIQMTQSPSSLSASVGDRVTITCKASQNIDKYLNWYQQKPGKA PKLLIYNTNNLQGTGVPSTRFSGSGSGTDFTFTISSLPEDIATYYCL QHISRPRTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNMFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLS TLTSLKADYEKHKVYACEVTHQGLSSPVTKSFNR (SEQ ID NO: 10)
Trastuzumab, e.g., ado-trastuzumab, e.g., ado-trastuzumab emtansine	>Anti-HER2 Light chain (1 and 2) DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKA PKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISLQPEDFATYYCQ QHYYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNMFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLS TLTSLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 11) >Anti-HER2 Heavy chain (1 and 2) EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGK GLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD KKVEPPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS

	TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK (SEQ ID NO: 12)
nimotuzumab	>pdb13gkwlHeavy chain QVQLQQSGAEVKKPGSSVKVSCKASGYTFTNYYIYWVRQAPG QGLEWIGGINPTSGGSNFNEKFKTRVTITADESSTTAYMELSSLR SEDTAFYFCTRQGLWFDSDGRGFDGWGQGTTVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD KKVP (SEQ ID NO: 13) >pdb13gkwlLight chain DIQMTQSPSSLSASVGDRVTITCRSSQNIVHSNGNTYLDWYQQT PGKAPKLLIYKVSNRFSGVPSRFSGSGSGTDFTFTISSLQPEDIA YYCFQYSHVPWTFGQGTKLQITREVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 14)
cetuximab	>Cetuximab heavy chain QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGK GLEWLGVIWSSGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQ SNDTAIYYCARALTYDYEFAYWGQGLTVTVSAASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK (SEQ ID NO: 15)

	>Cetuximab light chain DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRL LIKYASESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNN NWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTL TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 16)
bevacizumab	>"Bevacizumab light chain" DIQMTQSPSSLSASVGDRVTITCSASQDISNYLNWYQQKPGKAP KVLIIYFTSSLHSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQ YSTVPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLS TLTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 17) >"Bevacizumab heavy chain" EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYGMNWVRQAPG KGLEWVGWINTYTGEPTYAADFKRRFTFSLDTSKSTAYLQMNS LRAEDTAVYYCAKYPHYYGSSHWFYFDVWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNT KVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSS VMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 18)
bavituximab	>8734_Hlbavituximab H-GAMMA-1 (VH(1-120)+CH1(121- 218)+HINGE-REGION(219-233)+CH2(234-343)+CH3(344- 450)) 450 MW 49410.6 MW 49410.6 EVQLQQSGPELEKPGASVKLSCKASGYSFTGYNMNWVKQSHG

	KSLEWIGHIDPYYGDTSYNQKFRGKATLTVDKSSSTAYMQLKS LTSEDSAVYYCVKGGYYGHWYFDVWGAGTTVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD KKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 19) >8734_Llbavituximab L-KAPPA (V-KAPPA(1-107)+C-KAPPA(108-208)) 208 MW 22655.0 MW 22655.0 DIQMTQSPSSLSASLGERVSLTCRASQDIGSSLNWLQQGPDGTIKRLIYATSSLD SGVPKRFSGRSGSDYSLTISSEDFVDYYCLQYVSSPPTFGAGTKLELKRADAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 20)
gemtuzumab, e.g., gemtuzumab ozogamicin	> Light Chain No. 1 QIVLTQSPAISASPGKEVTITCSASSSISYMHWFQQKPGTSPKLWIYTTSNLASGVPARFSGSGSGTSYSLTISRMEAEDAATYYCHQRSTYPLTFGSGTKLELK (SEQ ID NO: 21) > Light Chain No. 2 DIQMTQSPSTLSASVGDRVTITCRASQSINTWLAWYQQKPGKAPKLLMYKASSLESQVPSRFISGSGSGTEFTLTISSLQPDDFATYYCQQYNDSKMFQGQGTKVEVK (SEQ ID NO: 22) > Heavy Chain No. 1 QVQLQQSGAELAKPGASVKMSCKASGYTFTSYRMHWVKQRP GQGLEWIGYINPSTGYTEYNQKFKDKATLTADKSSSTAYMQLS SLTFEDSAVYYCARGGGVFDYWGQGTTTLTVSS (SEQ ID NO: 23)

	> Heavy Chain No. 2 QVQLVQSGAEVKKPGSSVKVSCKASGGTFSRSAIIWVRQAPGQ GLEWMGGIVPMFGPPNYAQKFQGRVTITADESTNTAYMELSSL RSEDTAFYFCAGGYGIYSPEEYNGGLVTVSS (SEQ ID NO: 24)
ibritumomab, e.g., ibritumomab tiuxetan	>Ibritumomab tiuxetan heavy chain QAYLQQSGAELVRPGASVKMSCKASGYTFTSYNMHWVKQTPR QGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYMQLSS LTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSAPSVYP LAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFP AVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEP RGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMSLSPIVTCVV VDVSEDDPDVQISWVNNVEVHTAQTQTHREDYNSTLRVVSAL PIQHQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVY VLPPPEEEMTKKQVTLTCMVTDFMPEDIYVEWTNNGKTELNY KNTEPVLDSDGSYFMYSKLRVEKKNWVERNSYSCSVVHEGLH NHHTTKSFSR (SEQ ID NO: 25) >Ibritumomab tiuxetan light chain QIVLSQSPAILSASPGEKVTMTCRASSSVSYMHWYQQKPGSSPK PWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQ QWSFNPPFTFGAGTKLELKRADAAPTVFIFPPSDEQLKSGTASVV CLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN (SEQ ID NO: 26)
Tositumomab, e.g., tositumomab-I-131	Mouse-Human chimeric Anti-CD20 Heavy Chain 1 QAYLQQSGAELVRPGASVKMSCKASGYTFTSYNMHWVKQTPR QGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYMQLSS LTSEDSAVYFCARVVYYSNSYWFYFDVWGTGTTVTVSGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKA PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV

	VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK (SEQ ID NO: 27) >Mouse-Human chimeric Anti-CD20 Light Chain 1 QIVLSQSPAILSASPGEKVTMTCRASSSVSYMHYQKPGSSPK PWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQ QWSFNPPTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR (SEQ ID NO: 28) >Mouse-Human chimeric Anti-CD20 Heavy Chain 2 QAYLQQSGAELVRPGASVKMSCKASGYTFTSYNMHWVKQTPR QGLEWIGAIYPGNGDTSYNQKFKGKATLTVDKSSSTAYMQLSS LTSEDSAVYFCARVYYYSNSYWFYFDVWGTGTTVTVSGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKA PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK (SEQ ID NO: 29) >Mouse-Human chimeric Anti-CD20 Light Chain 2 QIVLSQSPAILSASPGEKVTMTCRASSSVSYMHYQKPGSSPK PWIYAPSNLASGVPARFSGSGSGTSYSLTISRVEAEDAATYYCQ QWSFNPPTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR (SEQ ID NO: 30)
panitumumab	>pd5sx5Heavy chain J

	QVQLQESGPGLVKPSSETLSLTCTVSGGSVSSGDYYWTWIRQSPG KGLEWIGHIYYSGNTNYNPSLKSRLTISIDTSKTQFSLKLSSVTA ADTAIYYCVRDRVTGAFDIWGQGTMTVTVSSASTKGPSVFPLAP CSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVTVTPSSNFGTQTYTCNVDPHKPSNTKVDKTVR KC (SEQ ID NO: 31) >pdbl5sx5lK Light chain DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAP KLLIYDASNLETGVPSRFSGSGSGTDFTFTISSLPEDIATYFCQH FDHLPLAFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCL LNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSS LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 32)
ofatumumab	>Ofatumumab Heavy Chain EVQLVESGGGLVQPGRSLRLSCAASGFTFNDYAMHWVRQAPG KGLEWVSTISWNSGSIQYADSVKGRFTISRDNKKSLYLQMNS LRAEDTALYYCAKDIQYGNYYYGMDVWGQGTITVTVSSASTK GPSVFPLAPGSSKSTSGTAALGCLVKDYFPEPVTVSWNSGALTS GVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNT KVDKKVEP (SEQ ID NO: 33) >Ofatumumab Light Chain EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAP RLLIYDASNRATGIPARFSGSGSGTDFTLTISLSEPEDFAVYYCQ QRSNWPITFGQGRLEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSS TLTSLKADYEKHKVYACEVTHQGLSSPVTKSFNR (SEQ ID NO: 34)
ipilimumab	>Ipilimumab heavy chain QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYTMHWVRQAPG KGLEWVTFISYDGNNKYYADSVKGRFTISRDNKNTLYLQMNS

	LRAEDTAIYYCARTGWLGPFDYWQGTLTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK (SEQ ID NO: 35) >Ipilimumab light chain EIVLTQSPGTLSSLSPGERATLSCRASQSVGSSYLAWYQQKPGQA PRLLIYGAFSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQ QYGSSPWTFTGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 36)
brentuximab, e.g., brentuximab vedotin	>Brentuximab vedotin - heavy chain QIQLQQSGPEVVKPGASVKISCKASGYTFTDYYITWVKQKPGQ GLEWIGWIYPGSGNTKYNEKFKGKATLTVDTSSSTAQFMQLSSLT SEDVAVYFCANYGNYWFAYWGQGTQVTVSAASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPG (SEQ ID NO: 37) >Brentuximab vedotin - light chain DIVLTQSPASLAVSLGQRATISCKASQSVDFDGD SYMNWYQQK PGQPPKVLIIAASNLESGIPARFSGSGSGTDFTLNIHPVEEEDAA

	TYYCQQSNEDPWTFGGGKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 38)
pertuzumab	>Amino acid sequence for pertuzumab light chain DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ QYYIYPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSLSS TLTLTKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 39) >Amino acid sequence for pertuzumab heavy chain EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPG KGLEWVADVNPNSGGSIYNQRFKGRFTLSVDRSKNTLYLQMNS LRAEDTAVYYCARNLGPSFYFDYWGQGLTVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPG (SEQ ID NO: 40)
obinutuzumab	>Amino acid sequence of the light chains of obinutuzumab (SEQ ID NO: 41) 1 DIVMTQTPLSLPVTPGEPASISCRSSKSLHSNGITYLYWYLQKPGQSPQ 51 LLIYQMSNLVSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELP 101 YTFGGGKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK 151 VQWKVDNALQSGNSQESVTEQDSKDSYSLSSSTLTLSKADYEKHKVYACE 201 VTHQGLSSPVTKSFNRGEC 219 > Amino acid sequence of the heavy chains of obinutuzumab (SEQ ID NO: 42)

	1 QVQLVQSGAEVKKPGSSVKVSCASGYAFSYSWINWVRQAPGQGLEWMGR 51 IFPGDGD TDYNGKFKGRVTITADKSTSTAYMELSSLRSED TAVYYCARNV 101 FDGYWL VYWGQGLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKD 151 YFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTY 201 ICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK 251 DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS 301 TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV 351 YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVL 401 DSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK 449
durvalumab	>Heavy chain (SEQ ID NO: 43) EVQLVESGGG LVQPGGSLRL SCAASGFTFS RYWMSWVRQA PGKGLEWVAN 50 IKQDGSEKYY VDSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCAREG 100 GWFGELAFDY WGQGLVTVS SASTKGPSVF PLAPSSKSTS GGTAALGCLV 150 KDYFPEPVT VSWNSGALTSG VHTFPAVLQS SGLYSLSSV TVPSSSLGTQ 200 TYICNVNHKP SNTKVDKRV E PKSCDKTHTC PPCPAPEFEG GPSVFLFPPK 250 PKDTLMISRT PEVTCVVVDV SHEDPEVKFN WYVDGVEVHN AKTKPREEQY 300 NSTYRVVSVL TVLHQDWLNG KEYKCKVSNK ALPASIEKTI SKAKGQPREP 350 QVYTLPPSRE EMTKNQVSLT CLVKGFYPSD IAVEWESNGQ PENNYKTTTP 400 VLDSGSFFL YSKLTVDKSR WQQGNVFCFS VMHEALHNHY TQKSLSLSPG 450 K 451 >Light chain (SEQ ID NO: 44) EIVLTQSPGT LSLSPGERAT LSCRASQ RVS SSYLAWYQQK PGQAPRLLIY 50 DASSRATGIP DRFSGSGSGT DFTLTISRLE PEDFAVYYCQ QYGLSPWTFG 100 QGTKVEIKRT VAAPSVFIFP PSDEQLKSGT ASVVCLLNNF YPREAKVQWK 150 VDNALQSGNS QESVTEQDSK DSTYSLSSL TLSKADYEH K VYACEVTHQ 200 GLSSPVTKSF NRGEK 215
nivolumab	>Heavy Chain Sequence QVQLVESGGGVVQPGRSLRLDCKASGITFSNSGMHWVRQAPG KGLEWVAVIWDGSKRYYADSVKGRFTISRDN SKNTLFLQMN SLRAEDTAVYYCATNDDYWGQGLVTVSSASTKGPSVFPLAPC SRSTSESTAALGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQ SSGLYSLSSVTVPSSSLG TKTYTCNV DHKPSNTKVDKRVE SKY GPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVL HQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPP SQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTP

	VLDS DGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQ KSLSLSLGK (SEQ ID NO: 45) >Light Chain Sequence EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAP RLLIYDASN RATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQ QSSNWPRTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 46)
pembrolizumab	>Heavy Chain Sequence QVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYWVRQAP GQGLEWMGGINPSNGGTNFNEKFKNRVTLTDSSTTTAYMELK SLQFDDTAVYYCARRDYRFDMGFDYWGQGTTVTVSSASTKGP SVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTKYTCNV DHKPSNTKV DKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEV TCVVDVDSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYR VVS VLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPR EPQVYTLPPS QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTTPVLDS DGSFFLYSRLTVDKSRWQEGNVFSCSVMHEA LHNHYTQKSLSLSLGK (SEQ ID NO: 47) >Light Chain Sequence EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKP GQAPRLLIYLASYLESGV PARFSGSGSGTDFTLTISSLEPEDFAV YYCQHSRDLPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKST YLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 48)
olaratumab	>Heavy chain QLQLQESGPG LVKPSETLSL TCTVSGGSIN SSSYYWGWL R QSPGKGLEWI 50 GSFFYTGSTY YNPSLRSLT ISVDTSKNQF SLMLSSVTAA DTAVYYCARQ 100

	STYYYGSGNY YGWFDWRDQG TLVTVSSAST KGPSVFPLAP SSKSTSGGTA 150 ALGCLVKDYF PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVVTVP 200 SSLGTQTYIC NVNHKPSNTK VDKRVEPKSC DKTHTCPPCP APELLGGPSV 250 FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK 300 PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK 350 GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPEN 400 YKTPPVLDSDGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS 450 LSLSPGK 457 (SEQ ID NO: 49) >Light chain EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQK GQAPRLIYD 50 ASNRATGIPA RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ RSNWPPAFGQ 100 GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV 150 DNALQSGNSQ ESVTEQDSKD STYLSSTLT LSKADYEKHK VYACEVTHQG 200 LSSPVTKSFN RGEK 214 (SEQ ID NO: 50)
atezolizumab	>Heavy Chain Sequence EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGK GLEWVAWISPYGGSTYYADSVKGRFTISADTSKNTAYLQMNSL RAEDTAVYYCARRHWPGGFDYWGGQGLTVTVSSASTKGPSVF LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK (SEQ ID NO: 51) >Light Chain Sequence DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKA PKLLIYSASFLYSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQ QYLYHPATFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 52)
elotuzumab	>Elotuzumab heavy chain

	EVQLVESGGGLVQPGGSLRLSCAASGFDPSRYWMSWVRQAPG KGLEWIGEINPDSSTINYAPSLKDKFIISRDNANKNSLYLQMNSLR AEDTAVYYCARPDGNYWYFDVWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK (SEQ ID NO: 53) >Elotuzumab light chain DIQMTQSPSSLSASVGDRVTITCKASQDVGIAVAWYQQKPGKV PKLLIYWASTRHTGVPDRFSGSGSGTDFTLTISSLQPEDVATYYC QQYSSYPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSL STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 54)
necitumumab	>Sequence A QVQLQESGPGLVKPSQTLSTCTVSGGSISSGDYYWSWIRQPPG KGLEWIGYIYYSGSTDYNPSLKSRTMSVDTSKNQFSLKVNSVT AADTAVYYCARVSIFGVGTFDYWGQGTLVTVSSASTKGPSVLP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK (SEQ ID NO: 55) >Sequence A'

	QVQLQESGPGLVKPSQTLSTCTVSGGSISSGDYYWSWIRQPPG KGLEWIGYIYYSGSTDYNPSLKSRTMSVDTSKNQFSLKVNST AADTAVYYCARVSIFGVGTFDYWGQGTLLTVSSASTKGPSVLP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK (SEQ ID NO: 56) >Sequence B EIVMTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAP RLLIYDASNRTGIPARFSGSGSGTDFTLTISSELPEDFAVYYCH QYGSTPLTFGGGTAEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLS TLTSLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 57) >Sequence B' EIVMTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAP RLLIYDASNRTGIPARFSGSGSGTDFTLTISSELPEDFAVYYCH QYGSTPLTFGGGTAEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLS TLTSLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 58)
daratumumab	Heavy chain EVQLLESGGG LVQPGGSLRL SCAVSGFTFN SFAMSWVRQA PGKGLEWVSA 50 ISGSGGGTYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYFCAKDK 100 ILWFGPEVFD YWGQGTLLTV SSASTKGPSV FPLAPSSKST SGGTAALGCL 150 VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTPSSSLGT 200 QTYICNVNHK PSNTKVDKRV EPKSCDKTHT CPPCPAPELL GGPSVFLFPP 250 KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ 300

	YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE 350 PQVYTLPPSR EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP 400 PVLDSGDSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP 450 GK 452 (SEQ ID NO: 59) Light chain EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLIYD 50 ASNRATGIPA RFGSGSGTD FTLTISSLEP EDFAVYYCQQ RSNWPPTFGQ 100 GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV 150 DNALQSGNSQ ESVTEQDSKD STYLSSTLT LSKADYEKHK VYACEVTHQG 200 LSSPVTKSFN RGEC 214 (SEQ ID NO: 60)
ramucirumab	>9098_HramucirumabHomo sapiensH-GAMMA-1 (VH(1-116)+CH1(117-214)+HINGE-REGION(215-229)+CH2(230-339)+CH3(340-446)) 446 MW 48696.0IMW 48696.0I EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGK GLEWVSSISSSSYIYYADSVKGRFTISRDNKNSLYLQMNSLR AEDTAVYYCARVTDADFIDWGGTMVTVSSASTKGPSVFPLAPS SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK SCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK (SEQ ID NO: 61) >9098_LramucirumabHomo sapiensL-KAPPA (V-KAPPA(1-107)+C-KAPPA(109-214)) 214 MW 23124.7IMW 23124.7I DIQMTQSPSSVSASIGDRVTITCRASQGIDNWLGWYQQKPGKAP KLLIYDASNLDTGVPSTRFSGSGSGTYFTLTISSLQAEDFAVYFCQ QAKAFPPPTFGGGTKVDIKGTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 62)
dinutuximab	> dinutuximab Heavy chain

	EVQLQSGPE LEKPGASVMI SCKASGSSFT GYNMNVWRQN IGKSLEWIGA 50 IDPYYGGTSY NQKFGRATL TVDKSSSTAY MHLKSLTSED SAVYYCVSGM 100 EYWGQGTSVT VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFPEPV 150 TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG TQTYICNVNH 200 KPSNTKVDKR VEPKSCDKTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS 250 RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS 300 VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS 350 REEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF 400 FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK 443 (SEQ ID NO: 63) > dinutuximab Light chain EIVMTQSPAT LSVSPGERAT LSCRSSQSLV HRNGNTYLHW YLQKPGQSPK 50 LLIHKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTHVP 100 PLTFGAGTKL ELKRTVAAPS VFIFPPSDEQ LKSGTASVVC LLNNFYPREA 150 KVQWKVDNAL QSGNSQESVT EQDSKDYSTYS LSSTLTLSKA DYEEKHKVYAC 200 EVTHQGLSSP VTKSFNRGEC 220 (SEQ ID NO: 64) > dinutuximab beta Heavy chain EVQLQSGPE LEKPGASVMI SCKASGSSFT GYNMNVWRQN IGKSLEWIGA 50 IDPYYGGTSY NQKFGRATL TVDKSSSTAY MHLKSLTSED SAVYYCVSGM 100 EYWGQGTSVT VSSASTKGPS VFPLAPSSKS TSGGTAALGC LVKDYFPEPV 150 TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG TQTYICNVNH 200 KPSNTKVDKR VEPKSCDKTH TCPPCPAPEL LGGPSVFLFP PKPKDTLMIS 250 RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE QYNSTYRVVS 300 VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR EPQVYTLPPS 350 REEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT PPVLDSDGSF 400 FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS PGK 443 (SEQ ID NO: 65) > dinutuximab beta Light chain EIVMTQSPAT LSVSPGERAT LSCRSSQSLV HRNGNTYLHW YLQKPGQSPK 50 LLIHKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDLGV YFCSQSTHVP 100 PLTFGAGTKL ELKRTVAAPS VFIFPPSDEQ LKSGTASVVC LLNNFYPREA 150 KVQWKVDNAL QSGNSQESVT EQDSKDYSTYS LSSTLTLSKA DYEEKHKVYAC 200 EVTHQGLSSP VTKSFNRGEC 220 (SEQ ID NO: 66)
blinatumomab	>single chain variable fragment fusion protein DIQLTQSPASLAVSLGQRATISCKASQSVDYDGDSYLNWYQQIP GQPPKLLIYDASNLVSGIPPRFSGSGSGTDFTLNIHPVEKVDAAT YHCQQSTEDPWTFGGGTTKLEIKGGGGSGGGGSGGGGSGVQLQ QSGAELVRPGSSVKISCKASGYAFSSYWMNVVKQRPGQGLEW

	IGQIWPGDGDNTNYNGKFKGKATLTADESSSTAYMQLSSLASED SAVYFCARRETTTVGRYYYAMDYWGQGTTVTVSSGGGGSDIK LQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVKQRPGQGL EWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSE DSAVYYCARYYDDHYCLDYWGQGTTTLTVSSVEGGSGGSGGSG GSGGVDDIQLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQQ KSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISSEAE AATYYCQQWSSNPLTFGAGTKLELKHSHHHHHH (SEQ ID NO: 67)
siltuximab	>Heavy Chain Sequence EVQLVESGGKLLKPGGSLKLSAASGFTFSSFAMSWFRQSPEKR LEWVAEISSGGSYTYYPDTVTGRFTISRDNKNTLYLEMSSLRS EDTAMYYCARGLWGYYALDYWGQGTSTVTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK (SEQ ID NO: 68) >Light Chain Sequence QIVLIQSPAIMSASPGEKVTMTCSASSSVSYMYWYQQKPGSSPR LLIYDTSNLAGVPPVRFSGSGSGTSYSLTISRMEAEAAATYYCQ QWSGYPTYFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASV CLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 69)
denosumab	> Denosumab αOPGL-1 heavy chain sequence EVQLLESGGGLVQPGGSLRLSAAASGFTFSSYAMSWVRQAPGK GLEWVSGITGSGGSTYYADSVKGRFTISRDNKNTLYLQMNSL RAEDTAVYYCAKDPGTTVIMSWFDPWGQGTLLTVTVSSASTKGPS

	VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVD KKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT EVTCTVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTTPVLDS DGSFFLYSKLTVDKSRWQQGNV FSCSVMH EALHNHYTQKSLSLSPGK (SEQ ID NO: 70) > Denosumab αOPGL-1 light chain sequence EIVLTQSPGTLSPGERATLSCRASQSVRGRYLA WYQQKPGQA PRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVFYCQ QYGSSPRTEFGQGTKVEIKRTVAAPS VFIFPPSDEQLKSGTASVVC LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS TLTSLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 71)
Enoblituzumab	>Heavy chain QVELVESGGG LVQPGGSLRL SCAASGFTFT SYWMSWVRQA PGKGLELVSS 50 ITSYGSFTYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARNM 100 YTHFDSWGQG TLVTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF 150 PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVTVPS SSLGTQTYIC 200 NVNHKPSNTK VDKKVEPKSC DKHTHTCPPCP APELLGGPSV FLFPPKPKDT 250 LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY 300 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT 350 LPPSRDELTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPVLDS 400 DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS LSLSPGK 447 (SEQ ID NO: 72) >Light chain DIVLTQPPSV SGAPGQRVTI SCSGSSSNIG SNSVSWYQQL PGTAPKLLIY 50 DNSKRPSGVP DRFSGSKSGT SASLAITGLQ SEDEADYYCQ SRDTYGYWV 100 FGGGTKLTVL GQPKAAPSVT LFPPSSEELQ ANKATLVCLI SDFYPGAVTV 150 AWKGDSSPVK AGVETTTPSK QSNNKYAASS YLSLTPEQWK SHRSYSCQVT 200 HEGSTVEKTV APTECS 216 (SEQ ID NO: 73)
Lirilumab	>Heavy chain QVQLVQSGAE VKKPGSSVKV SCKASGGTFS FYAISWVRQA PGQGLEWMGG 50 FIPIFGAANY AQKFQGRVTI TADESTSTAY MELSSLRSDD TAVYYCARIP 100

	SGSYYYDYDM DVWGQGT TTVT VSSASTKGPS VFPLAPCSRS TSESTAALGC 150 LVKDYFPEPV TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG 200 TKTYTCNVDH KPSNTKVDKR VESKYGPPCP PCPAPEFLGG PSVFLFPPKP 250 KDTLMISRTP EVTCVVVDVS QEDPEVQFNW YVDGVEVHNA KTKPREEQFN 300 STYRVVSVLT VLHQDWLNGK EYKCKVSNKG LPSSIEKTIS KAKGQPREPQ 350 VYTLPPSQEE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTTPV 400 LDSGDSFFLY SRLTVDKSRW QEGNVFSCSV MHEALHNHYT QKSLSLGLGK 450 (SEQ ID NO: 74) >Light chain EIVLTQSPVT LSLSPGERAT LSCRASQSVS SYLAWYQQK GQAPRLIYD 50 ASNRATGIPA RFGSGSGTD FTLTISSLEP EDFAVYYCQQ RSNWMYTFGQ 100 GTKLEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV 150 DNALQSGNSQ ESVTEQDSKD STYLSSTLT LSKADYEKHK VYACEVTHQG 200 LSSPVTKSFN RGEC 214 (SEQ ID NO: 75)
pidilizumab	>Heavy chain QVQLVQSGSE LKKPGASVKI SCKASGYTFT NYGMNWVRQA PGQGLQWMGW 50 INTDSGESTY AEEFKGRFVF SLDTSVNTAY LQITSLTAED TGMFYCVRVG 100 YDALDYWGQG TLTVSSAST KGPSVFPLAP SSKSTSGGTA ALGCLVKDYF 150 PEPVTVSWNS GALTSGVHTF PAVLQSSGLY SLSSVTVPS SSLGTQTYIC 200 NVNHKPSNTK VDKRVEPKSC DKHTCPCP APELLGGPSV FLFPPKPKDT 250 LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK PREEQYNSTY 300 RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK GQPREPQVYT 350 LPPSREEMTK NQVSLTCLVK GFYPSDIAVE WESNGQPENN YKTTTPVLDS 400 DGSFFLYSKL TVDKSRWQQG NVFSCSV MHE ALHNHYTQKS LSLSPGK 447 (SEQ ID NO: 76) >Light chain EIVLTQSPSS LSASVGDRVT ITCSARSSVS YMHWFQKPG KAPKLWIYRT 50 SNLASGVPSR FSGSGGTSY CLTINSLQPE DFATYYCQR SSFPLTFGGG 100 TKLEIKRTVA APSVFIFPPS DEQLKSGTAS VVCLLNNFY REAKVQWKVD 150 NALQSGNSQE SVTEQDSKDS TYLSSTLT TL SKADYEKHKV YACEVTHQGL 200 SSPVTKSFNR GEC 213 (SEQ ID NO: 77)
avelumab	>Heavy chain EVQLLES GGG LVQPGGSLRL SCAASGFTFS SYIMMWVRQA PGKGLEWVSS 50 IYPSGGITFY ADTVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARIK 100 LGTVTVDYD W GQGT LTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK 150 DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT 200 YICNVNHKPS NTKVDKKVEP KSCDKHTTCP PCPAPELLGG PSVFLFPPKP 250 KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN 300 STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ 350 VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTTPV 400

	LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK 450 (SEQ ID NO: 78) >Light chain QSALTQPASV SGSPGQSITI SCTGTSSDVG GYNYVSWYQQ HPGKAPKLMI 50 YDVSNRPSGV SNRFSGSKSG NTASLTISGL QAEDEADYYC SSYTSSSTRV 100 FGTGTKVTVL GQPKANPTVT LFPPSSEELQ ANKATLVCLI SDFYPGAVTV 150 AWKADGSPVK AGVETTKPSK QSNNKYAASS YLSLTPEQWK SHRSYSCQVT 200 HEGSTVEKTV APTECS 216 (SEQ ID NO: 79)
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Table 3. Exemplary cancers

Acute lymphoblastic leukaemia (ALL)	Colorectal cancer	Macroglobulinemia, Waldenström	Pleuropulmonary Blastoma, Childhood
Acute myeloid leukaemia (AML)	Craniopharyngioma, Childhood	Male Breast Cancer	Pregnancy and Breast Cancer
Adrenocortical Carcinoma	Cutaneous T-Cell Lymphoma	Malignant Fibrous Histiocytoma of Bone and Osteosarcoma	Primary Central Nervous System (CNS) Lymphoma
AIDS-Related Kaposi Sarcoma	Ductal Carcinoma In Situ (DCIS)	Melanoma	Prostate Cancer
AIDS-Related lymphoma	Embryonal Tumors, Childhood	Merkel Cell Carcinoma	Rare cancers
Anal Cancer	Endometrial Cancer	Mesothelioma	Rectal Cancer
Appendix Cancer	Ependymoma, Childhood	Metastatic Squamous Neck Cancer with Occult Primary	Renal cell carcinoma
Astrocytomas, Childhood	Epithelial cancer	Midline Tract Carcinoma Involving NUT Gene	Renal Pelvis and Ureter, Transitional Cell Cancer
Atypical Teratoid/Rhabdoid Tumor,	Esophageal Cancer	Molar pregnancy	Retinoblastoma

Childhood			
Basal Cell Carcinoma	Esthesioneuroblastoma, Childhood	Mouth and oropharyngeal cancer	Rhabdomyosarcoma
Bile duct cancer	Ewing sarcoma	Multiple Endocrine Neoplasia Syndromes, Childhood	Salivary Gland Cancer
Bladder cancer	Extragenital Germ Cell Tumor	Multiple Myeloma/Plasma Cell Neoplasm	Sarcoma
Bone cancer	Extrahepatic Bile Duct Cancer	Mycosis Fungoides	Secondary cancers
Bowel cancer	Eye Cancer	Myelodysplastic Syndromes	Sézary Syndrome
Brain Stem Glioma, Childhood	Gallbladder Cancer	Myelodysplastic/Myeloproliferative Neoplasms	Skin Cancer
Brain tumors	Gastric cancer	Myeloproliferative Disorders, Chronic	Skin cancer (non melanoma)
Breast cancer	Gastrointestinal Carcinoid Tumor	Nasal Cavity and Paranasal Sinus Cancer	Small Cell Lung Cancer
Bronchial Tumors, Childhood	Germ Cell Tumor	Nasopharyngeal cancer	Small Intestine Cancer
Burkitt Lymphoma	Gestational trophoblastic tumors (GTT)	Neuroblastoma	Soft Tissue Sarcoma
Cancer of unknown primary	Glioma	Non-Hodgkin Lymphoma	Squamous Cell Carcinoma
Cancer spread to bone	Hairy cell leukaemia	Non-Small Cell Lung Cancer	Squamous Neck Cancer with Occult Primary, Metastatic
Cancer spread to brain	Head and neck cancer	Oesophageal cancer	Stomach (Gastric) Cancer
Cancer spread to liver	Heart Cancer, Childhood	Oral Cancer	Stomach cancer
Cancer spread to	Hepatocellular (Liver)	Oral Cavity Cancer	T-Cell Lymphoma,

lung	Cancer		Cutaneous - see Mycosis Fungoides and Sézary Syndrome
Carcinoid Tumor	Histiocytosis, Langerhans Cell	Oropharyngeal Cancer	Testicular cancer
Carcinoma of Unknown Primary	Hodgkin Lymphoma	Osteosarcoma (Bone Cancer)	Throat Cancer
Cardiac (Heart) Tumors, Childhood	Hypopharyngeal Cancer	Osteosarcoma and Malignant Fibrous Histiocytoma	Thymoma and Thymic Carcinoma
Central Nervous System Atypical Teratoid/Rhabdoi d Tumor, Childhood	Intraocular Melanoma	Ovarian Cancer	Thyroid Cancer
Central Nervous System Embryonal Tumors, Childhood	Islet Cell Tumors, Pancreatic Neuroendocrine Tumors	Pancreatic Cancer	Transitional Cell Cancer of the Renal Pelvis and Ureter
Central Nervous System, Childhood	Kidney cancer	Pancreatic Neuroendocrine Tumors (Islet Cell Tumors)	Unknown primary cancer
Cervical cancer	Langerhans Cell Histiocytosis	Papillomatosis, Childhood	Ureter and Renal Pelvis, Transitional Cell Cancer
Chordoma, Childhood	Laryngeal Cancer	Paraganglioma	Urethral Cancer
Choriocarcinoma	Leukemia	Parathyroid Cancer	Uterine Cancer, Endometrial
Chronic	Lip and Oral Cavity	Penile Cancer	Uterine Sarcoma

Lymphocytic Leukemia (CLL)	Cancer		
Chronic myeloid leukaemia (CML)	Liver cancer	Pharyngeal Cancer	Vaginal cancer
Chronic Myeloproliferative Disorders	Lobular Carcinoma In Situ (LCIS)	Pheochromocytoma	Vulvar Cancer
Colon cancer	Low Malignant Potential Tumor	Pituitary Tumor	Waldenström Macroglobulinemia
Lymphoma	Lung Cancer	Plasma Cell Neoplasm/Multiple Myeloma	Wilms Tumor

Table 4. Immune checkpoint molecules, and agents that bind thereto.

Immune checkpoint molecule	Antibody or other binding agent (e.g., immune checkpoint molecule inhibitors)
A2aR	Anti-Adenosine Receptor A2a Antibody, clone 7F6-G5-A2 (EMD Millipore)
2B4 (also called CD244 or SLAMF4)	CD48 (e.g., Accession Number CAG33293.1)
B-7 family ligand, e.g., B7-H3	Enoblituzumab
B7-H4	h1D11 TDC (see Leong et al., doi: 10.1021/mp5007745)
BTLA	HVEM (e.g., Accession Number AAQ89238.1)
CGEN-15049	Anti- CGEN-15049 antibody
CD160	HVEM (e.g., Accession Number AAQ89238.1)
CEACAM (e.g., CEACAM- 1, CEACAM-3 and/or CEACAM-5)	Annexin, e.g., Annexin A2 (e.g., Accession Number AAH52558.1)
CHK 1	2G1D5 mAb (Cell Signalling Technology)
CHK2	1C12 mAb #3440 (Cell Signalling Technology)

CTLA4	B7-1 (CD80; e.g., Accession Number NP_005182.1); B7-2 (CD86; e.g., Accession Number NP_787058.4); ipilimumab, tremelimumab
GAL9	Anti-human Galectin-9 Antibody 9M1-3 (BioLegend)
GR1	RB6-8C5 antibody (eBioscience)
HVEM	BTLA (e.g., Accession Number NP_861445.3), CD160 (e.g., Accession Number NP_008984.1)
KIR	Lirilumab
LAG3	BMS-986016
LAIR1	COLLAGEN; Anti-Human CD305 (eBioscience)
LY6G	1A8 antibody (eBioscience)
MUC1	Anti-MUC1 antibody EPR1023 (Abcam)
PD1	B7DC; PD-L1; nivolumab; pembrolizumab; pidilizumab
PD-L1 (also called B7H1)	B7-1 (CD80; e.g., Accession Number NP_005182.1); PD1 (e.g., Accession Number NP_005009.2); durvalumab; atezolizumab; avelumab; BMS 936559
PDL2	AMP 224
TGF beta	Antibody 1D11 (see Ueda R et al., doi: 10.1158/1078-0432.CCR-09-1067)
TIGIT	CD155 (e.g., Accession Number NP_001129240.1); CD112 (e.g., Accession Number NP_001036189.1); CD113 (e.g., Accession Number NP_001230215.1);
TIM3	GALECTIN9;
TIM4	TIM4R; CD48 (e.g., Accession Number NP_001769.2)
TIM4R	TIM4 (e.g., Accession Number NP_612388.2)
VISTA	Human VISTA Antibody Clone # 730804 (R&D Systems)

Table 5. Costimulatory molecules, and agents that bind thereto.

Costimulatory molecule	Antibody or other binding agent
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4-1BB (CD137; e.g., Accession NP_001552.2)	4-1BBL (e.g., Accession Number NP_003802.1), CD137L (e.g., Accession Number NP_003802.1)
CD2 (e.g., Accession Number NP_001315538.1)	CD48 (e.g., Accession Number NP_001769.2)
CD27 (e.g., Accession Number NP_001233.1)	CD70 (e.g., Accession Number NP_001243.1)
CD28 (e.g., Accession Number NP_006130.1)	B7-H2 (e.g., Accession Number NP_056074.1), B7-1 (CD80; e.g., Accession Number NP_005182.1)
CD30 (e.g., Accession Number NP_001234.3)	CD30L (e.g., Accession Number NP_001235.1), brentuximab
CD40 (e.g., Accession Number NP_001241.1)	CD40L (e.g., Accession Number BAA06599.1)
CD226 (e.g., Accession Number NP_006557.2)	CD155 (e.g., Accession Number NP_001129240.1), CD112 (e.g., Accession Number NP_001036189.1)
CDS	
DR3 (e.g., Accession Number NP_683866.1)	TL1A (e.g., Accession Number NP_005109.2)
GITR (e.g., Accession Number NP_004186.1)	GITRL (e.g., Accession Number NP_005083.2)
HVEM (LIGHTR) (e.g., Accession Number AAQ89238.1)	LIGHT (e.g., Accession Number NP_003798.2)
ICAM-1 (e.g., Accession Number NP_000192.2)	Anti-ICAM1 antibody [MEM-111] (Abcam)
LFA-1 (CD11a/CD18) (e.g., Accession Number NP_002200.2)	odulimomab
LIGHT (e.g., Accession Number NP_003798.2)	HVEM (e.g., Accession Number AAQ89238.1)
ICOS (CD278) (e.g., Accession Number NP_036224.1)	B7-H2 (e.g., Accession Number NP_056074.1)
OX40 (e.g., Accession Number	OX40L (e.g., Accession Number NP_003317.1)

NP_003318.1)	
TIM1 (e.g., Accession Number NP_036338.2)	TIM4 (e.g., Accession Number NP_612388.2)

Table 6: α CD20-RCTs binding to CD20 cell lines; Percent cells bound

Cell type	α CD20-RCT binding	HA-GPA control RCT binding
Raji	83.6%	7.2%
Ramos	83.0%	5.59%
Z138	43.7%	3.92%
Granta-519	76.0%	8.95%
Toledo	37.1%	5.96%
SU-DHL4	91.3%	36.1%
DOHH-2	86.7%	7.18%

Table 7: BCL-xL and BCL-2 levels in cancer cells exposed to RCT-antiCD20 or control RCT-

5 HA-GPA

Cell Type	RCT-antiCD20	RCT-HA-GPA
Raji (1:1 Ratio)	BCL-xL+/BCL-2+: 93.9% BCL-xL-/BCL-2+: 1.58% BCL-xL-/ BCL-2-: 3.46% BCL-xL+/ BCL-2-: 1.06%	BCL-xL+/BCL-2+: 98.5% BCL-xL-/BCL-2+: 0.20 BCL-xL-/ BCL-2-: 0.60 BCL-xL+/ BCL-2-: 0.67%
Raji (1:5 Ratio)	BCL-xL+/BCL-2+: 69.8% BCL-xL-/BCL-2+: 22.2% BCL-xL-/ BCL-2-: 7.27% BCL-xL+/ BCL-2-: 0.76%	BCL-xL+/BCL-2+: 98.9% BCL-xL-/BCL-2+: 0.50% BCL-xL-/ BCL-2-: 0.50% BCL-xL+/ BCL-2-: 0.14%
Ramos (1:1 Ratio)	BCL-xL+/BCL-2+: 79.1% BCL-xL-/BCL-2+: 4.56% BCL-xL-/ BCL-2-: 5.08% BCL-xL+/ BCL-2-: 11.2%	BCL-xL+/BCL-2+: 89.0% BCL-xL-/BCL-2+: 0.94% BCL-xL-/ BCL-2-: 2.31% BCL-xL+/ BCL-2-: 7.72%
Ramos (1:5 Ratio)	BCL-xL+/BCL-2+: 86.5% BCL-xL-/BCL-2+: 9.83% BCL-xL-/ BCL-2-: 1.91% BCL-xL+/ BCL-2-: 1.79%	BCL-xL+/BCL-2+: 98.9% BCL-xL-/BCL-2+: 0.67% BCL-xL-/ BCL-2-: 0.26% BCL-xL+/ BCL-2-: 0.21%

DOHH-2 (1:1 Ratio)	BCL-xL+/BCL-2+: 88.9% BCL-xL-/BCL-2+: 8.88% BCL-xL-/ BCL-2-: 1.73% BCL-xL+/ BCL-2-: 0.52%	BCL-xL+/BCL-2+: 98.6% BCL-xL-/BCL-2+: 1.01% BCL-xL-/ BCL-2-: 0.26% BCL-xL+/ BCL-2-: 0.099%
DOHH-2 (1:5 Ratio)	BCL-xL+/BCL-2+: 74.4% BCL-xL-/BCL-2+: 24.4% BCL-xL-/ BCL-2-: 1.12% BCL-xL+/ BCL-2-: 0.023%	BCL-xL+/BCL-2+: 99.2% BCL-xL-/BCL-2+: 0.77% BCL-xL-/ BCL-2-: 0.072% BCL-xL+/ BCL-2-: 0.0016%
SU-DHL4 (1:1 Ratio)	BCL-xL+/BCL-2+: 95.1% BCL-xL-/BCL-2+: 0.32% BCL-xL1/ BCL-2-: 1.11% BCL-xL+/ BCL-2-: 3.49%	BCL-xL+/BCL-2+: 98.8% BCL-xL-/BCL-2+: 0.74% BCL-xL-/ BCL-2-: 0.37% BCL-xL+/ BCL-2-: 0.14%
SU-DHL4 (1:5 Ratio)	BCL-xL+/BCL-2+: 93.0% BCL-xL-/BCL-2+: 5.54% BCL-xL-/ BCL-2-: 1.14% BCL-xL+/ BCL-2-: 0.33%	BCL-xL+/BCL-2+: 98.3% BCL-xL-/BCL-2+: 1.13% BCL-xL-/ BCL-2-: 0.46% BCL-xL+/ BCL-2-: 0.085%

Table 8: Genes mutated in certain cancers

Gene	Exemplary cancers	Exemplary mutations
ABCB1	AML; breast cancer	3853C>T
ABCB1	AML	2677G>T/A
ABCC2		
ABCC4		
ABCG2		q141K
ABL1	Leukemia (<i>e.g.</i> , chronic myeloid leukemia (CML), acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL))	Codon 315
ABL1	CML, ALL, T-ALL	Translocation with BCR, ETV6, NUP214
ABL2	AML	Translocation with ETV6
ACSL3	prostate	Translocation with ETV1
ACVR1B	Pancreas, breast	
AF15Q14	AML	Translocation with MLL
AF1Q	ALL	Translocation with MLL
AF3p21	ALL	Translocation with MLL
AF5q31	ALL	Translocation with MLL
AKAP9	papillary thyroid	Translocation with BRAF
AKT1	breast cancer, colorectal cancer, ovarian cancer	
AKT2	Ovarian cancer, pancreatic cancer	
AKT3	Melanoma, glioma, uterine cancer, prostate cancer, oral cancer, ovarian cancer	

ALK	ALCL, NSCLC, Neuroblastoma, Lymphoma (e.g., non-Hodgkin lymphoma, anaplastic large-cell lymphoma (ALCL)), inflammatory myofibroblastic tumor	Translocation with NPM1, TPM3, TFG, TPM4, ATIC, CLTC, MSN, ALO17, CARS, or EML4
ALK	ALCL, NSCLC, Neuroblastoma, Lymphoma (e.g., non-Hodgkin lymphoma, anaplastic large-cell lymphoma (ALCL)), inflammatory myofibroblastic tumor	Translocation with NPM1, TPM3, TFG, TPM4, ATIC, CLTC, MSN, ALO17, CARS, or EML4
ALO17	ALCL	Translocation with ALK
ALOX12B	Mutiple myeloma (MM)	
APC	Colorectal cancer, medulloblastoma, mismatch repair cancer syndrome	Codon 1114, 1338, 1450, or 1556
AR	Prostate cancer	
ARAF	Angioimmunoblastic T-cell lymphoma, ehrlich ascites tumor	
ARFRP1	Breast cancer	
ARHGEF12	AML	Translocation with MLL
ARHH	NHL	Translocation with BCL6
ARID1A	Neuroblastoma, acute lymphoblastic leukemia (ALL), neuroendocrine tumor	
ARNT	AML	Translocation with ETV6
ASPCR1	alveolar soft part sarcoma	Translocation with TFE3
ASXL1	Mutiple myeloma (MM)	
ATF1	malignant melanoma of soft parts , angiomatoid fibrous histiocytoma	Translocation with EWSR1 or FUS
ATIC	ALCL	Translocation with ALK
ATM	Leukemia (e.g., T-cell prolymphocytic leukemia (T-PLL)), lymphoma, medulloblastoma, glioma, breast cancer	Biallelic inactivation
ATR	Pyothorax-associated lymphoma, T-cell lymphoma, breast cancer	Biallelic inactivation
ATRX	Pancreatic neuroendocrine tumors	
AURKA	Laryngeal squamous cell carcinoma, ovarian cancer, bladder cancer, head and neck squamous cell carcinoma (HNSCC), laryngeal carcinoma, esophageal squamous cell carcinoma (ESCC), pancreatic cancer	
AURKB	Colorectal cancer, astrocytoma, ependymal tumor, glioma, esophageal squamous cell carcinoma (ESCC), acute myeloid leukemia (AML)	
AXL	Non small cell lung cancer (NSCLC), MM	
BACH1	Breast	
BAP1	Uveal melanoma, breast, NSCLC	

BARD1	Breast	
BCL10	MALT	Translocation with IGH
BCL11A	B-CLL	Translocation with IGH
BCL11B	T-ALL	Translocation with TLX3
BCL2	Lymphoma, colorectal adenocarcinoma, esophageal squamous cell carcinoma (ESCC), synovial sarcoma, leukemia	
BCL2	NHL, CLL	Translocation with IGH
BCL2A1	Pulmonary granuloma, gastric adenoma, burkitt lymphoma, parotid adenoma, kaposi sarcoma, gastric cancer, colon cancer	
BCL2L1	Head and neck squamous cell carcinoma, glioblastoma, mesothelioma, pancreatic cancer, adenocarcinoma lung	
BCL2L2	Brain cancer, leukemia, lymphoma, colorectal adenocarcinoma, colorectal cancer, adenoma, cervical squamous cell carcinoma	
BCL3	CLL	Translocation with IGH
BCL5	CLL	Translocation with MYC
BCL6	Lymphoma, leukemia	
BCL6	NHL, CLL	Translocation with IG loci, ZNFN1A1, LCP1, PIM1, TFRC, MHC2TA, NACA, HSPCB, HSPCA, HIST1H4I, IL21R, POU2AF1, ARHH, EIF4A2, or SFRS3
BCL7A	BNHL	Translocation with MYC
BCL9	B-ALL	Translocation with IGH or IGL
BCOR	Breast	
BCORL1	Breast	
BCR	CML, ALL, AML	Translocation with ABL1, FGFR1, or JAK2
BIRC3	MALT	Translocation with MALT1
BLM	Leukemia, lymphoma, skin squamous cell , other cancers	
BRAF	Lung cancer, non-Hodgkin lymphoma, colorectal cancer, thyroid cancer, melanoma, breast cancer	Codon 594 (e.g., D594G) or 600 (e.g., V600E)
BRAF	melanoma, colorectal, papillary thyroid, borderline ov, Non small-cell lung cancer (NSCLC), cholangiocarcinoma, pilocytic astrocytoma	Translocation with AKAP9 or KIAA1549
BRCA1	Breast cancer, ovarian cancer	Biallelic inactivation
BRCA2	Breast cancer, ovarian cancer, pancreatic cancer	Biallelic inactivation
BRD3	lethal midline carcinoma of young people	Translocation with NUT
BRD4	lethal midline carcinoma of young people	Translocation with NUT
BRIP1	Acute myeloid leukemia (AML), leukemia,	

	breast	
BTG1	BCLL	Translocation with MYC
C12orf9	lipoma	Translocation with LPP
C15orf21	prostate	Translocation with ETV1
C17orf39	Breast	
C1orf144		
CANT1	prostate	Translocation with ETV4
CARD11	Lymphoma	
CARS	ALCL	Translocation with ALK
CASP8	Breast	
CBFA2T1	AML	Translocation with MLL or RUNX1
CBFA2T3	AML	Translocation with RUNX1
CBFB	AML	Translocation with MYH11
CBL	Lymphoma, leukemia	
CBL	AML, JMML, MDS	Translocation with MLL
CCND1	CLL, B-ALL, breast	Translocation with IGH or FSTL3
CCND2	NHL, CLL, Retinoblastoma, mantle cell lymphoma, T-cell acute lymphoblastic leukemia (T-ALL), Burkitt lymphoma, testicular germ cell tumor, ovarian granulosa cell tumor, multiple myeloma	Translocation with IGL
CCND3	MM, Retinoblastoma, mantle cell lymphoma, anaplastic large cell lymphoma, lymphoma (non-hodgkins), B-cell lymphoma, laryngeal squamous cell carcinoma, indolent lymphoma, null cell adenoma	Translocation with IGH
CCNE1	Breast cancer, ovarian cancer, bladder cancer, retinoblastoma	
CD22	NSCLC, breast	
CD74	NSCLC	Translocation with ROS1
CD79A	Diffuse large B-cell lymphoma (DLBCL)	
CD79B	DLBCL	
CDC73	Parathyroid	
CDH11	aneurysmal bone cysts, Gastric cancer, lobular carcinoma, squamous cell carcinoma, invasive ductal carcinoma, invasive lobular carcinoma	Translocation with USP6
CDH2	Melanoma, malignant mesothelioma, pleural mesothelioma, desmoplastic melanoma, lung adenocarcinoma, endometrioid tumor, mesothelioma, bladder cancer, esophageal squamous cell carcinoma (ESCC)	
CDH20	Breast cancer	
CDH5	Granuloma, epithelioid sarcoma	

CDK12	Ovarian	
CDK4	Melanoma	
CDK6	ALL	Translocation with MLLT10
CDK8	Colon cancer, lung cancer, rectal cancer, acute lymphoblastic leukemia (ALL)	
CDKN1B	Breast	
CDKN2A	melanoma, pancreatic cancer, Li-Fraumeni syndrome, lung cancer (<i>e.g.</i> , non-small cell lung cancer (NSCLC)), squamous cell carcinoma, retinoblastoma, astrocytoma	
CDKN2B	Leukemia, retinoblastoma, laryngeal squamous cell carcinoma	
CDKN2C	Thyroid carcinoma, pituitary adenoma, oligodendroglioma, pancreatic endocrine tumor, multiple myeloma, hepatoblastoma, lymphoid tumor, multiple endocrine neoplasia type 1, anaplastic oligodendroglioma	
CDX2	AML	Translocation with ETV6
CEBPA	Leukemia (<i>e.g.</i> , acute myeloid leukemia (AML), acute myeloid leukemia (AML), monoblastic leukemia), retinoblastoma	
CEP1	MPD, NHL	Translocation with FGFR1
CHCHD7	salivary adenoma	Translocation with PLAG1
CHEK1	Leukemia, colon cancer	
CHEK2	Breast cancer	
CHIC2	AML	Translocation with ETV6
CHN1	extraskeletal myxoid chondrosarcoma	Translocation with TAF15
CHUK	Colorectal	
CIC	soft tissue sarcoma	Translocation with DUX4
CLTC	ALCL, renal	Translocation with ALK, TFE3
CLTCL1	ALCL	Translocation
CMKOR1	lipoma	Translocation with HMGA2
COL1A1	dermatofibrosarcoma protuberans, aneurysmal bone cyst	Translocation with PDGFB or USP6
COX6C	uterine leiomyoma	Translocation with HMGA2
CRBN	Upper aerodigestive tract	
CREB1	clear cell sarcoma, angiomatoid fibrous histiocytoma	Translocation with EWSR1
CREB3L2	fibromyxoid sarcoma	Translocation with FUS
CREBBP	Acute lymphoblastic leukemia (ALL), AML, DLBCL, B-cell non-Hodgkin's lymphoma (B-NHL)	
CREBBP	AL, AML	Translocation with MLL, MORE, or RUNXBP2

CRKL	Leukemia, lymphoma	
CRLF2	Leukemia	
CRTC3	salivary gland mucoepidermoid	Translocation with MAML2
CSF1R	NSCLC	
CTCF	Breast	
CTNNA1	Breast	
CTNNB1	Colorectal cancer, ovarian cancer, prostate cancer, liver cancer (<i>e.g.</i> , hepatoblastoma (HB), hepatocellular carcinoma (HCC)), pilomatixoma, medulloblastoma, salivary gland pleiomorphic adenomas	Codon 32, 33, 34, 37, 41, or 45
CTNNB1	colorectal, ovarian, hepatoblastoma, others, pleomorphic salivary adenoma	Translocation with PLAG1
CUL4A	Leukemia	
CUL4B	Leukemia	
CYP17A1	Breast	
CYP1B1	breast cancer	CYP1B1*3
CYP2C19		CYP2C19*17
CYP2C19		CYP2C19*17
CYP2C8		461delV
CYP2C8		K399R
CYP2D6		CYP2D6: 3183 G>A
CYP2D6		CYP2D6: 2988 G>A
CYP2D6		CYP2D6: 2850 C>T
CYP2D6		CYP2D6: 2613-2615 del AGA
CYP2D6		CYP2D6: 2549 del A
CYP2D6		CYP2D6: 1846 G>A
CYP2D6		CYP2D6: 1707 del T
CYP2D6		CYP2D6: 1659G>A
CYP2D6		CYP2D6: 1023 C>T
CYP2D6		CYP2D6: 100C>T
CYP3A4		CYP3A4*16B
CYP3A4		CYP3A4*1B
CYP3A5		
D10S170	papillary thyroid, CML	Translocation with RET or PDGFRB
DAXX	Pancreatic neuroendocrine tumors	
DDIT3	liposarcoma	Translocation with FUS
DDR2	NSCLC	
DDX10	AML*	Translocation with NUP98
DDX5	prostate	Translocation with ETV4
DDX6	B-NHL	Translocation with IGH

DEK	AML	Translocation with NUP214
DIS3	MM	
DNMT3A	Testicular germ cell tumor, lymphosarcoma, hepatocellular carcinoma, salivary gland tumor	
DOT1L	Leukemia	
DPYD		DPYD*2A
DPYD		DPYD*5
DPYD		496A>G
DPYD		DPYD*9A
DUX4	soft tissue sarcoma	Translocation with CIC
EGFR	Lung cancer, squamous cell carcinoma, glioblastoma, glioma, colorectal cancer	Codon 719, 746-750, 768, 790 (e.g., T790M), 858 (e.g., L858R), or 861
EIF4A2	NHL	Translocation with BCL6
ELF4	AML	Translocation with ERG
ELK4	prostate	Translocation with SLC45A3
ELKS	papillary thyroid	Translocation with RET
ELL	AL	Translocation with MLL
ELN	B-ALL	Translocation with PAX5
EML4	NSCLC	Translocation with ALK
EMSY	Breast	
EP300	Colorectal, breast, pancreatic, AML, ALL, DLBCL	
EP300	colorectal, breast, pancreatic, AML	Translocation with MLL or RUNXBP2
EP300	colorectal, breast, pancreatic, AML	Translocation with MLL or RUNXBP2
EPHA3	Rhabdomyosarcoma, lymphoma, prostate cancer, hepatocellular carcinoma, leukemia, melanoma	
EPHA5	Glioblastoma, breast cancer, astrocytoma, Wilms' tumor, glioma	
EPHA6	Breast cancer	
EPHA7	Glioblastoma multiforme (GBM), colon cancer, duodenal cancer, parathyroid tumor, prostate cancer	
EPHB1	Colorectal cancer, embryonal carcinoma, gastric cancer, teratocarcinoma, mucinous carcinoma	
EPHB4	Head and neck squamous cell carcinoma (HNSCC), brain cancer, endometrial cancer, ovarian cancer	
EPHB6	Neuroblastoma, melanoma, non-small cell lung cancer (NSCLL)	
EPS15	ALL	Translocation with MLL
ERBB2	Gastric cancer, glioma, ovarian cancer, lung cancer	Amplification

ERBB3	Breast cancer, non-small cell lung cancer (NSCLC), pancreatic cancer, invasive ductal carcinoma, lung adenocarcinoma, endometrioid carcinoma, pilocytic astrocytoma	
ERBB4	Breast cancer, medulloblastoma, cervical squamous cell carcinoma, prostate cancer, leukemia	
ERCC2	Skin basal cell, skin squamous cell, melanoma	2251A>C
ERG	Ewing sarcoma, prostate, AML, Prostate cancer, Ewing's sarcoma, leukemia, prostate cancer	Translocation with EWSR1, TMPRSS2, ELF4, FUS, or HERPUD1
ESR1	Breast cancer, endometrial cancer, endometrial adenocarcinoma, leiomyoma, mammary ductal carcinoma	
ESR2		
ETV1	Prostate cancer, breast cancer, Ewing's sarcoma, desmoplastic small round cell tumor, myxoid liposarcoma, clear cell sarcoma	Translocation with EWSR1, TMPRSS2, SLC45A3, C15orf21, HNRNPA2B1, or ACSL3
ETV4		
ETV4	Breast cancer, ovarian cancer, squamous cell carcinoma tongue, Ewing's sarcoma, Prostate carcinoma	Translocation with EWSR1, TMPRSS2, DDX5, KLK2, or CANT1
ETV5	Ganglioglioma, brain tumor	
ETV5	Prostate	Translocation with TMPRSS2 or SCL45A3
ETV6	leukemia, congenital fibrosarcoma, multiple leukemia and lymphoma, secretory breast, secretory carcinoma, MDS, ALL, myelodysplastic syndrome	Translocation with NTRK3, RUNX1, PDGFRB, ABL1, MN1, ABL2, FACL6, CHIC2, ARNT, JAK2, EVI1, CDX2, STL, HLXB9, MDS2, PER1, SYK, TTL, FGFR3, or PAX5
EVI1	AML, CML	Translocation with RUNX1, ETV6, PRDM16, or RPN1
EWSR1	Ewing sarcoma, desmoplastic small round cell tumor, ALL, clear cell sarcoma, sarcoma, myoepithelioma, extraskeletal myxoid chondrosarcoma, myxoid liposarcoma, angiomatoid fibrous histiocytoma	Translocation with FLI1, ERG, ZNF278, NR4A3, FEV, ATF1, ETV1, ETV4, WT1, ZNF384, CREB1, POU5F1, or PBX1
EZH2	Prostate cancer, gallbladder adenocarcinoma, breast cancer, bladder cancer, gastric cancer, Ewing's sarcoma	
FACL6	AML, AEL	Translocation with ETV6
FAM46C	MM	
FANCA	Leukemia	
FANCC	AML, leukemia	
FANCD2	AML, leukemia	
FANCE	AML, leukemia	
FANCG	AML, leukemia	

FANCL	AML, leukemia	
FBXW7	Colorectal cancer, endometrial cancer, T-cell acute lymphoblastic leukemia (T-ALL)	
FCGR2B	ALL	Translocation
FCGR3A		V158F
FEV	Ewing sarcoma	Translocation with EWSR1 or FUS
FGF1	Breast	
FGF10	Breast	
FGF12	Breast	
FGF14	Breast	
FGF19	Breast	
FGF23	Breast	
FGF3	Breast	
FGF4	Breast	
FGF6	Breast	
FGF7	Breast	
FGFR1		
FGFR1	MPD, NHL, Leukemia, lymphoma	Translocation with BCR, FOP, ZNF198, or CEP1
FGFR1OP	MPD, NHL	Translocation with FGFR1
FGFR2	Breast cancer, prostate cancer	
FGFR3	bladder, MM, T-cell lymphoma, cervical cancer,	Translocation with IGH or ETV6
FGFR4	Pituitary tumor, prostate cancer, lung cancer, astrocytoma, rhabdomyosarcoma, pituitary adenoma, fibroadenoma	
FGFR4		GLY388ARG
FIP1L1	idiopathic hypereosinophilic syndrome	Translocation with PDGFRA
FLI1	Ewing sarcoma, Breast cancer, prostate cancer	Translocation with EWSR1
FLT3	Leukemia (<i>e.g.</i> , acute myeloid leukemia (AML), acute promyelocytic leukemia, acute lymphoblastic leukemia)	Codon 835
FLT4	Lung cancer, Kaposi's sarcoma, gastric cancer, lymphangioma, squamous cell carcinoma	
FNBP1	AML	Translocation with MLL
FOXL2	Granulosa-cell tumour of the ovary	Codon 134
FOXO1A	alveolar rhabdomyosarcomas	Translocation with PAX3
FOXO3A	AL	Translocation with MLL
FOXP1	ALL	Translocation with PAX5
FOXP4	Lymphoma, brain tumor	
FSTL3	B-CLL	Translocation with CCND1

FUS	liposarcoma, AML, Ewing sarcoma, angiomatoid fibrous histiocytoma, fibromyxoid sarcoma	Translocation with DDIT3, ERG, FEV, ATF1, or CREB3L2
FVT1	B-NHL	Translocation with IGK
GAS7	AML*	Translocation with MLL
GATA1	Megakaryoblastic leukemia of Downs Syndrome	
GATA2	AML, Chronic Myeloid Leukemia (CML, blast transformation)	
GATA3	Breast	
GMPS	AML	Translocation with MLL
GNA11	Breast cancer	
GNAQ	Uveal melanoma	
GNAS	Pituitary adenoma	
GOLGA5	papillary thyroid	Translocation with RET
GPHN	AL	Translocation with MLL
GPR124	Colon cancer	
GRAF	AML, MDS	Translocation with MLL
GRIN2A	Malignant melanoma	
GSK3B	NSCLC	
GSTP1		I105V
GSTP1		A114V
GUCY1A2	Breast cancer	
HCMOGT-1	JMML	Translocation with PDGFRB
HEAB	AML	Translocation with MLL
HEI10	uterine leiomyoma	Translocation with HMGA2
HERPUD1	prostate	Translocation with ERG
HGF	MM	
HIP1	CMML	Translocation with PDGFRB
HIST1H4I	NHL	Translocation with BCL6
HLA-A	MM	
HLF	ALL	Translocation with TCF3
HLXB9	AML	Translocation with ETV6
HMGA1	microfollicular thyroid adenoma, various benign mesenchymal tumors	Translocation
HMGA2	lipoma	Translocation with LHFP, RAD51L1, LPP, HEI10, COX6C, CMKOR1, or NFIB
HNRNPA2B1	prostate	Translocation with ETV1
HOOK3	papillary thyroid	Translocation with RET
HOXA11	CML	Translocation with NUP98
HOXA13	AML	Translocation with NUP98

HOXA3	Breast cancer	
HOXA9	AML*	Translocation with NUP98 or MSI2
HOXC11	AML	Translocation with NUP98
HOXC13	AML	Translocation with NUP98
HOXD11	AML	Translocation with NUP98
HOXD13	AML*	Translocation with NUP98
HRAS	Hurthle cell thyroid carcinoma, bladder cancer, melanoma, colorectal cancer	Codon 12, 13, 61
HSP90AA1	Lymphoma, myeloma	
HSPCA	NHL	Translocation with BCL6
HSPCB	NHL	Translocation with BCL6
IDH1	Glioblastoma multiforme (GBM)	
IDH2	Glioblastoma multiforme (GBM)	
IGF1	Breast	
IGF1R	Ewing's sarcoma, breast cancer, uveal melanoma, adrenocortical carcinoma, pancreatic cancer	
IGF2	Breast	
IGF2R	Gastrointestinal tumor, liver cancer	
IGH	MM, Burkitt lymphoma, NHL, CLL, B-ALL, MALT, MLCLS	Translocation with MYC, FGFR3, PAX5, IRTA1, IRF4, CCND1, BCL9, BCL8, BCL6, BCL2, BCL3, BCL10, BCL11A, LHX4, DDX6, NFKB2, PAFAH1B2, or PCSK7
IGK	Burkitt lymphoma, B-NHL	Translocation with MYC or FVT1
IGL	Burkitt lymphoma	Translocation with BCL9, MYC, or CCND2
IKBKE	Breast cancer	
IKZF1	Lymphoma, leukemia	
IL2	intestinal T-cell lymphoma	Translocation with TNFRSF17
IL21R	NHL	Translocation with BCL6
IL7R	T-cell acute lymphoblastic leukemia (T-ALL)	
INHBA	Erythroleukemia, barrett metaplasia, esophageal adenocarcinoma, granulosa cell tumor, sex cord-stromal tumor, lung adenocarcinoma, pheochromocytoma, krukensberg tumor, ovarian cancer	
INSR	NSCLC, glioblastoma, gastric	
IRF4	Multiple myeloma (MM)	
IRF4	MM	Translocation with IGH
IRS2	Hyperinsulinemia, uterine leiomyosarcoma	
IRTA1	B-NHL	Translocation with IGH
ITK	peripheral T-cell lymphoma	Translocation with SYK

ITPA		
JAK1	Leukemia, ovarian cancer, breast cancer	
JAK2	Leukemia (<i>e.g.</i> , chronic lymphoblastic leukemia (CLL), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), acute myelogenous leukemia (AML))	Codon 617
JAK2	ALL, AML, MPD, CML	Translocation with ETV6, PCM1, or BCR
JAK3	Acute lymphoblastic leukemia (ALL)	
JAZF1	endometrial stromal tumours	Translocation with SUZ12
JUN	Skin cancer, leukemia	
KDM4C	Ovarian, breast	
KDM5A	AML	Translocation with NUP98
KDM6A	Renal, oesophageal squamous cell carcinoma (SCC), MM	
KDR	Non-small cell lung cancer (NSCLC), angiosarcoma	
KEAP1	NSCLC	
KIT	Gastrointestinal stromal tumor (GIST), testicular tumor, leukemia (<i>e.g.</i> , acute myeloid leukemia (AML)), mast cell tumor, mesenchymal tumor, adenoid cystic carcinoma, lung cancer (<i>e.g.</i> , small cell lung cancer), lymphoma (<i>e.g.</i> , Burkitt lymphoma)	Codon 816
KLHL6	Chronic lymphocytic leukaemia (CLL)	
KLK2	prostate	Translocation with ETV4
KRAS	Leukemia (<i>e.g.</i> , acute myelogenous leukemia (AML), juvenile myelomonocytic leukemia (JMML)), colorectal cancer, lung cancer	Codon 12, 13 (<i>e.g.</i> , G13D), or 61
KTN1	papillary thyroid	Translocation with RET
LAF4	ALL, T-ALL	Translocation with MLL or RUNX1
LASP1	AML	Translocation with MLL
LCK	T-ALL	Translocation with TRB
LCP1	NHL	Translocation with BCL6
LCX	AML	Translocation with MLL
LHFP	lipoma	Translocation with HMGA2
LIFR	salivary adenoma	Translocation with PLAG1
LMO1	T-ALL, neuroblastoma	Translocation with TRD
LMO2	T-ALL	Translocation with TRD
LPP	lipoma, leukemia	Translocation with HMGA2, MLL, or C12orf9
LRP1B	Lung cancer, gastric cancer, esophageal cancer	
LRP2		

LRP6	NSCLC, malignant melanoma	
LRRK2	Ovarian, NSCLC	
LTK	Lymphoma, breast cancer	
LYL1	T-ALL	Translocation with TRB
MAF	MM	Translocation with IGH
MAFB	MM	Translocation with IGH
MAGED1	MM	
MALT1	MALT	Translocation with BIRC3
MAML2	salivary gland mucoepidermoid	Translocation with MECT1 or CRTC3
MAN1B1		
MAP2K1	Prostate cancer, gastric cancer	
MAP2K2	Pancreatic cancer, intestinal tumor	
MAP2K4	Pancreatic cancer, breast cancer, colorectal cancer	
MAP3K1	Breast	
MAP3K13	Breast	
MCL1	Multiple myeloma, leukemia, lymphoma	
MDM2	Sarcoma, glioma, colorectal cancer	
MDM4	Glioblastoma multiforme (GBM), bladder cancer, retinoblastoma	
MDS1	MDS, AML	Translocation with RUNX1
MDS2	MDS	Translocation with ETV6
MECT1	salivary gland mucoepidermoid	Translocation with MAML2
MEN1	Parathyroid tumor	
MET	Gastric cancer, hepatocellular carcinoma (HCC), hereditary papillary renal carcinoma (HPRC), lung cancer (<i>e.g.</i> , non-small cell lung cancer), papillary thyroid carcinoma, glioma, esophageal adenocarcinoma, osteosarcoma, endometrial cancer, squamous cell carcinoma, melanoma, breast cancer	
MHC2TA	NHL	Translocation with BCL6
MITF	Melanoma	
MKL1	acute megakaryocytic leukemia	Translocation with RBM15
MLF1	AML	Translocation with NPM1
MLH1	Colorectal cancer, endometrial cancer, ovarian cancer, CNS cancer	
MLL	Leukemia (<i>e.g.</i> , acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML))	

MLL	AML, ALL	Translocation with MLL, MLLT1, MLLT2, MLLT3, MLLT4, MLLT7, MLLT10, MLLT6, ELL, EPS15, AF1Q, CREBBP, SH3GL1, FBNP1, PNUTL1, MSF, GPHN, GMPS, SSH3BP1, ARHGEF12, GAS7, FOXO3A, LAF4, LCX, SEPT6, LPP, CBFA2T1, GRAF, EP300, PICALM, or HEAB
MLL2	Medulloblastoma, renal	
MLLT1	AL	Translocation with MLL
MLLT10	AL	Translocation with MLL, PICALM, or CDK6
MLLT2	AL	Translocation with MLL
MLLT3	ALL	Translocation with MLL
MLLT4	AL	Translocation with MLL
MLLT6	AL	Translocation with MLL
MLLT7	AL	Translocation with MLL
MLST8	Breast	
MN1	AML, meningioma	Translocation with ETV6
MN1	AML, meningioma	Translocation with ETV6
MPL	Myeloproliferative disorder (MPD)	
MRE11A	Breast cancer, lymphoma	
MSF	AML	Translocation with MLL
MSH2	Colorectal cancer, endometrial cancer, ovarian cancer	Biallelic inactivation
MSH6	Colorectal cancer	
MSI2	CML	Translocation with HOXA9
MSN	ALCL	Translocation with ALK
MTCP1	T cell prolymphocytic leukemia	Translocation with TRA
MTHFR		677C>T
MTOR	Lymphoma lung cancer, renal cancer, clear cell carcinoma, glioma	
MUC1	B-NHL	Translocation with IGH
MUTYH	Colorectal cancer	
MYB	adenoid cystic carcinoma	Translocation with NFIB
MYC	chronic lymphocytic leukemia (CLL), Burkitt lymphoma, plasmacytoma, breast cancer	Amplification
MYC	Burkitt lymphoma, amplified in other cancers, B-CLL	Translocation with IGK, BCL5, BCL7A, BTG1, TRA, or IGH
MYCL1	Small cell lung cancer (SCLC)	
MYCN	Neuroblastoma	
MYD88	Activated B cell-like-DLBCL (ABC-DLBCL)	
MYH11	AML	Translocation with CBFB
MYH9	ALCL	Translocation with ALK

MYST3	Breast	
MYST4	AML	Translocation with CREBBP
NACA	NHL	Translocation with BCL6
NCOA1	alveolar rhabdomyosarcoma	Translocation with PAX3
NCOA2	AML	Translocation with RUNXBP2
NCOA4	papillary thyroid	Translocation with RET
NCOR1	Breast	
NF1	Leukemia (<i>e.g.</i> , juvenile myelomonocytic leukemia (JMML)), neurofibroma,	
NF2	Meningioma, acoustic neuroma, renal cancer	
NFE2L2	NSCLC, head and neck squamous cell carcinoma (HNSCC)	
NFIB	adenoid cystic carcinoma, lipoma	Translocation with MYB or HGMA2
NFKB1	Breast	
NFKB2	B-NHL	Translocation with IGH
NFKBIA	Breast	
NIN	MPD	Translocation with PDGFRB
NKX2-1	Lung cancer, thyroid cancer, adenocarcinoma	
NONO	papillary renal cancer	Translocation with TFE3
NOTCH1	Squamous cell carcinoma, leukemia (<i>e.g.</i> , acute lymphoblastic leukemia (ALL)), medullary thyroid carcinoma, lymphoma (<i>e.g.</i> , thymic lymphoma, T-cell lymphoma)	Codon 1575 or 1601
NOTCH1	T-ALL	Translocation with TRB
NOTCH2	Marginal zone lymphoma, DLBCL	
NOTCH3	NSCLC, breast	
NOTCH4	NSCLC, breast	
NPM1	Lymphoma (<i>e.g.</i> , non-Hodgkin lymphoma, anaplastic large cell lymphoma, anaplastic lymphoma), leukemia (<i>e.g.</i> , acute promyelocytic leukemia, acute myelogenous leukemia (AML))	
NPM1	NHL, APL, AML	Translocation with ALK, RARA, or MLL1
NQO1	breast cancer	NQO1*2
NR4A3	extraskeletal myxoid chondrosarcoma	Translocation with EWSR1
NRAS	Leukemia (<i>e.g.</i> , juvenile myelomonocytic leukemia (JMML), acute myeloid leukemia (AML), acute lymphoblastic leukemia), melanoma, colon cancer	Codon 12, 13, 61 (<i>e.g.</i> , Q61K or Q61H)
NRP2		
NSD1	AML	
NSD1	AML	Translocation with NUP98

NTRK1	papillary thyroid	Translocation with TPM3, TPR, or TFG
NTRK2	Renal, NSCLC	
NTRK3	Congenital fibrosarcoma, secretory breast cancer	
NTRK3	congenital fibrosarcoma, Secretory breast	Translocation with ETV6
NUMA1	APL	Translocation with RARA
NUP214	AML, T-ALL	Translocation with DEK, SET, or ABL1
NUP93	Breast	
NUP98	AML	Translocation with HOXA9, NSD1, WHSC1L1, DDX10, TOP1, HOXD13, PMX1, HOXA13, HOXD11, HOXA11, RAP1GDS1, or HOXC11
NUT	lethal midline carcinoma of young people	Translocation with BRD4 or BRD3
OLIG2	T-ALL	Translocation with TRA
OMD	aneurysmal bone cysts	Translocation with USP6
PAFAH1B2	MLCLS	Translocation with IGH
PAK3	Lung cancer	
PAK7	NSCLC, malignant melanoma	
PALB2	Wilms tumor, medulloblastoma, AML, breast	
PAX3	alveolar rhabdomyosarcoma	Translocation with FOXO1A or NCOA1
PAX5	Non-Hodgkin Lymphoma (NHL), acute lymphoblastic leukemia (ALL, <i>e.g.</i> , B-cell ALL)	
PAX5	NHL, ALL, B-ALL	Translocation with IGH, ETV6, PML, FOXP1, ZNF521, or ELN
PAX7	alveolar rhabdomyosarcoma	Translocation with FOXO1A
PAX8	follicular thyroid	Translocation with PPARG
PBRM1	Clear cell renal carcinoma, breast	
PBX1	pre B-ALL, myoepithelioma	Translocation with TCF3 or EWSR1
PCM1	papillary thyroid, CML, MPD	Translocation with RET or JAK2
PCSK7	MLCLS	Translocation with IGH
PDE4DIP	MPD	Translocation with PDGFRB
PDGFB	DFSP	Translocation with COL1A1
PDGFRA	Gastrointestinal stromal tumor (GIST), leukemia (<i>e.g.</i> , chronic eosinophilic leukemia (CEL), acute lymphocytic leukemia (ALL)), mesenchymal tumor	
PDGFRA	GIST, idiopathic hypereosinophilic syndrome	Translocation with FIP1L1
PDGFRB	Myeloproliferative disorder (MPD), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), chronic myelomonocytic leukemia (CMML)	

PDGFRB	MPD, AML, CMML, CML	Translocation with ETV6, TRIP11, HIP1, RAB5EP, H4, NIN, HCMOGT-1, or PDE4DIP
PDK1	NSCLC	
PER1	AML, CMML	Translocation with ETV6
PHLPP2	Ovarian, glioblastoma, NSCLC	
PHOX2B	Neuroblastoma	
PICALM	TALL, AML,	Translocation with MLLT10 or MLL
PIK3C2G	NSCLC	
PIK3C3	NSCLC	
PIK3CA	Colorectal cancer, breast cancer, ovarian cancer, hepatocellular carcinoma, head and neck squamous cell carcinoma (HNSCC), anaplastic thyroid carcinoma, endometrial cancer, gallbladder adenocarcinoma, glioblastoma	Codon 88, 542, 545, 546, 1047 (e.g., H1047R), or 1049
PIK3CG	NSCLC	
PIK3R1	Glioblastoma, ovarian cancer, colorectal cancer	
PIK3R2	NSCLC	
PIM1	NHL	Translocation with BCL6
PKHD1	Pancreatic cancer	
PLAG1	salivary adenoma	Translocation with TCEA1, LIFR, CTNNB1, or CHCHD7
PLCG1	Head and neck cancer, leukemia	
PML	APL, ALL	Translocation with RARA or PAX5
PMX1	AML	Translocation with NUP98
PNRC1	MM	
PNUTL1	AML	Translocation with MLL
POU2AF1	NHL	Translocation with BCL6
POU5F1	sarcoma	Translocation with EWSR1
PPARG	follicular thyroid	Translocation with PAX8
PRCC	papillary renal	Translocation with TFE3
PRDM1	DLBCL	
PRDM16	MDS, AML	Translocation with EVI1
PRKAR1A	Adrenal gland, thyroid	
PRKAR1A	papillary thyroid	Translocation with RET
PRKDC	Glioma, glioblastoma, gastric cancer, ovarian cancer	
PRO1073	renal cell carcinoma (childhood epithelioid)	Translocation with TFEB
PRSS8	Breast	
PSIP2	AML	Translocation with NUP98
PTCH1	Skin basal cell , medulloblastoma	

PTCH2	Malignant melanoma	
PTEN	Head and neck squamous cell carcinomas (HNSCC), endometrial cancer, glioma, prostate cancer, glioblastoma, colon cancer, breast cancer	Codon 130, 173, 233, or 267; biallelic inactivation
PTK2	NSCLC, glioblastoma	
PTK2B	NSCLC, breast	
PTPN11	Juvenile myelomonocytic leukemia (JMML), acute myeloid leukemia (AML), myelodysplastic syndromes (MDS)	
PTPRD	Lung cancer, cutaneous squamous cell carcinoma, glioblastoma, neuroblastoma	
RAB5EP	CMML	Translocation with PDGFRB
RAD50	Breast	
RAD51	Breast	
RAD51L1	lipoma, uterine leiomyoma	Translocation with HMGA2
RAF1	pilocytic astrocytoma	Translocation with SRGAP3
RANBP17	ALL	Translocation with TRD
RAP1GDS1	T-ALL	Translocation with NUP98
RARA	Leukemia	
RARA	APL	Translocation with PML, ZNF145, TIF1, NUMA1, or NPM1
RB1	Retinoblastoma, bladder cancer, osteosarcoma, lung cancer (<i>e.g.</i> , small cell lung cancer, non-small cell lung cancer), leukemia (<i>e.g.</i> , acute lymphoblastic leukemia (ALL))	
RBM15	acute megakaryocytic leukemia	Translocation with MKL1
REL	Hodgkin Lymphoma	
RET	Colorectal cancer, medullary thyroid carcinoma, multiple neoplasia type 2B, pheochromocytoma, multiple neoplasia type 2A, thyroid papillary carcinoma, thyroid cancer, retinoblastoma	Codon 918
RET	medullary thyroid, papillary thyroid, pheochromocytoma	Translocation with H4, PRKAR1A, NCOA4, PCM1, GOLGA5, TRIM33, KTN1, TRIM27, or HOOK3
RHEB	NSCLC, colorectal	
RICTOR	Colon cancer, lymphoma, glioma, breast cancer	
ROCK1	Breast	
ROS1	glioblastoma, NSCLC	Translocation with GOPC or ROS1
RPL22	AML, CML	Translocation with RUNX1
RPN1	AML	Translocation with EVI1
RPTOR	Breast cancer, prostate cancer	

RUNX1	Acute myeloid leukemia (AML), pre-B-cell acute lymphoblastic leukemia (preB-ALL), T-cell acute lymphoblastic leukemia (T-ALL)	
RUNX1	AML, preB- ALL, T-ALL	Translocation with RPL22, MDS1, EVI1, CBFA2T3, CBFA2T1, ETV6, or LAF4
RUNXBP2	AML	Translocation with CREBBP, NCOA2, or EP300
RUNXT1	NSCLC, colorectal	
SEPT6	AML	Translocation with MLL
SET	AML	Translocation with NUP214
SETD2	Clear cell renal carcinoma	
SF3B1	MDS, CML, ALL, pancreatic, breast	
SFPQ	papillary renal cell	Translocation with TFE3
SFRS3	follicular lymphoma	Translocation with BCL6
SH2B3	Myelodysplastic syndrome (MDS)	
SH3GL1	AL	Translocation with MLL
SIL	T-ALL	Translocation with TAL1
SLC19A1		
SLC22A2		Ala270Ser
SLC45A3	prostate	Translocation with ETV1, ETV5, ELK4, or ERG
SLCO1B3		
SMAD2	esophageal squamous cell carcinoma (ESCC)	
SMAD3	Skin cancer, choriocarcinoma	
SMAD4	Pancreatic cancer, colon cancer	
SMARCA4	Non-small cell lung cancer (NSCLC)	
SMARCB1	Malignant rhabdoid	
SMO	Skin basal cell cancer	
SOCS1	DLBCL	
SOD2	breast cancer	V16A
SOX10	Oligodendroglioma	
SOX2	Embryonal carcinoma, germ cell tumor	
SPEN	Adenoid cystic carcinoma	
SPOP	Malignant melanoma	
SRC	Sarcoma, colon cancer, breast cancer	
SRGAP3	pilocytic astrocytoma	Translocation with RAF1
SS18	synovial sarcoma	Translocation with SSX1 or SSX2
SS18L1	synovial sarcoma	Translocation with SSX1
SSH3BP1	AML	Translocation with MLL
SSX1	synovial sarcoma	Translocation with SS18

SSX2	synovial sarcoma	Translocation with SS18
SSX4	synovial sarcoma	Translocation with SS18
STAG2	Glioblastoma	
STAT3	Breast	
STAT4	Breast	
STK11	Non-small cell lung cancer (NSCLC), pancreatic cancer	
STK12	PNET, NSCLC	
STL	B-ALL	Translocation with ETV6
SUFU	Medulloblastoma	
SULT1A1		
SUZ12	endometrial stromal tumours	Translocation with JAZF1
SYK	MDS, peripheral T-cell lymphoma	Translocation with ETV6, ITK
TAF15	extraskelatal myxoid chondrosarcomas, ALL	Translocation with TEC, CHN1, or ZNF384
TAL1	lymphoblastic leukemia/biphasic	Translocation with TRD, or SIL
TAL2	T-ALL	Translocation with TRB
TBX22	Breast cancer	
TBX23	Breast	
TBX3	Breast	
TCEA1	salivary adenoma	Translocation with PLAG1
TCF12	extraskelatal myxoid chondrosarcoma	Translocation with TEC
TCF3	pre B-ALL	Translocation with PBX1, HLF, or TFPT
TCL1A	T-CLL	Translocation with TRA
TCL6	T-ALL	Translocation with TRA
TET2	Myelodysplastic syndromes (MDS)	
TFE3	papillary renal, alveolar soft part sarcoma, renal	Translocation with SFPQ, ASPSCR1, PRCC, NONO, or CLTC
TFEB	renal (childhood epithelioid)	Translocation with ALPHA
TFG	papillary thyroid, ALCL, NSCLC	Translocation with NTRK1 or ALK
TFPT	pre-B ALL	Translocation with TCF3
TFRC	NHL	Translocation with BCL6
TGFBR2	Lung cancer, gastric cancer, colon cancer	
THRAP3	aneurysmal bone cysts	Translocation with USP6
TIF1	APL	Translocation with RARA
TLX1	T-ALL	Translocation with TRB or TRD
TLX3	T-ALL	Translocation with BCL11B
TMPRSS2	prostate	Translocation with ERG, ETV1, ETV4, or ETV5
TMPT		TPMT*3B
TNFAIP3	Marginal zone B-cell lymphomas, Hodgkin's lymphoma, primary mediastinal B cell lymphoma	

TNFRSF14	Follicular lymphoma	
TNFRSF17	Intestinal T-cell lymphoma	
TNFRSF17	intestinal T-cell lymphoma	Translocation with IL2
TNKS	NSCLC	
TNKS2	Melanoma, breast	
TOP1	AML	Translocation with NUP98
TP53	TP53 is frequently mutated or inactivated in about 60% of cancers, <i>e.g.</i> , esophageal squamous cell carcinoma, Li-Fraumeni syndrome, head and neck squamous cell carcinomas (HNSCC), lung cancer, hereditary adrenocortical carcinoma, astrocytoma, squamous cell carcinoma, bladder cancer, colorectal cancer, glioblastoma, retinoblastoma	Codon 175, 245, 248, 273, or 306
TPM3	papillary thyroid, ALCL	Translocation with NTRK1 or ALK
TPM4	ALCL	Translocation with ALK
TPMT		
TPR	papillary thyroid	Translocation with NTRK1
TRA	T-ALL	Translocation with ATL, OLIG2, MYC, TCL1A, TCL6, MTCPI1, or TCL6
TRB	T-ALL	Translocation with HOX11, LCK, NOTCH1, TAL2, or LYL1
TRD	T-cell leukemia	Translocation with TAL1, HOX11, TLX1, LMO1, LMO2, or RANBP17
TRIM27	papillary thyroid	Translocation with RET
TRIM33	papillary thyroid	Translocation with RET
TRIP11	AML	Translocation with PDGFRB
TRRAP	Colorectal, glioblastoma	
TSC1	Hamartoma, renal cell cancer	
TSC2	Hamartoma, renal cell cancer	
TTL	ALL	Translocation with ETV6
TYK2	NSCLC, breast	
TYMS		28bp tandem repeat
TYMS		6bp deletion
UGT1A1		
UMPS		Gly213Ala
USP6	aneurysmal bone cysts	Translocation with COL1A1, CDH11, ZNF9, or OMD
USP9X	Leukemia	
VHL	Renal cancer, hemangioma, pheochromocytoma	Biallelic inactivation
WHSC1	MM	Translocation with IGH

WHSC1L1	AML	Translocation with NUP98
WT1	Wilms' tumor, desmoplastic small round cell tumor	
XBP1	MM	
XPO1	Chronic lymphocytic leukaemia (CLL)	
ZNF145	APL	Translocation with RARA
ZNF198	MPD, NHL	Translocation with FGFR1
ZNF217	Breast	
ZNF278	Ewing sarcoma	Translocation with EWSR1
ZNF331	follicular thyroid adenoma	Translocation
ZNF384	ALL	Translocation with EWSR1, or TAF15
ZNF521	ALL	Translocation with PAX5
ZNF703	Breast	
ZNF9	aneurysmal bone cysts	Translocation with USP6
ZNFN1A1	ALL, DLBL	Translocation with BCL6

Table 9. Additional exogenous polypeptide sequences

Agent	Sequence
4-1BBL	ACPWAVSGARASPGSAASPRREGPELSPDDPAGLLDLRQGMFAQLVAQN VLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAAGVYYVFFQLEL RRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQ GRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGLPSP RSE (SEQ ID NO: 80)
Anti-PD-L1 scFv	VQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHVVRQAPGKGLEWVAWI SPYGGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHW PGGFDYWGGTLVTVSSGGGGSGGGGSGGGGSIQMTQSPSSLSASVGDRT TITCRASQDVSTAVAWYQQKPKAPKLLIYSASFLYSGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIK (SEQ ID NO: 81)

EXAMPLES

- 5 **Example 1: Erythroid cells expressing an exogenous polypeptide comprising a binding agent to a tumor antigen bind to and promote apoptosis in cells expressing the tumor antigen, and penetrate solid tumors**

Enucleated erythroid cells were produced which express on their surface a fusion protein comprising, from N-to-C terminus, a rituximab CD20 scFv domain, an HA epitope tag, and full-

length GPA (including the extracellular, transmembrane, and intracellular domains) (RCT-antiCD20). Control enucleated erythroid cells were produced which express on their surface just the HA epitope tag fused to the N terminus of full-length GPA (RCT-HA-GPA). Method for producing erythroid cells expressing an exogenous protein are described, e.g., in

5 WO2015/073587.

The RCT-antiCD20 bound to numerous CD20-expressing cell lines, as shown by flow cytometry (see Table 6). Amount of binding correlated with the levels of CD20 of the cell lines (data not shown).

10 Binding of the RCT-antiCD20 to Ramos CD20-expressing cells was also visualized by immunofluorescence. Cells were stained for CD20 (e.g., on Ramos cells), HA (a tag on α CD20-RCTs and control RCT-HA-GPA), and DNA (using DAPI). A co-culture of CD20+ Ramos cells and RCT-antiCD20 showed extensive colocalization of CD20 and HA, indicating the RCTs were binding the CD20+ cells and inducing hyper-crosslinking of CD20 (data not shown). In contrast, a co-culture of CD20+ Ramos cells and control RCT-HA-GPA showed minimal colocalization
15 of CD20 and HA.

It was further assessed whether the interaction between RCT-antiCD20 and CD20-expressing cells could induce apoptosis, by co-culturing RCT-antiCD20 cells with a panel of CD20+ human lymphoma cell lines, representing a variety of lymphoma subtypes; DoHH2 (follicular lymphoma), Ramos (Burkitt's lymphoma), Granta-519 (Mantle Cell Lymphoma) and
20 SU-DHL-4 (diffuse large B cell lymphoma). In all cases, RCT-antiCD20 co-culture resulted in increased apoptosis relative to RCT-HA-GPA or soluble Rituximab monoclonal antibody alone (Fig. 1). While not wishing to be bound by theory, direct tumor cell killing *in vitro* was hypothesized to be more effective than monoclonal antibody alone due to the hyper-crosslinking of CD20 on the lymphoma cell.

25 The performance of RCT-antiCD20 relative to soluble rituximab was quantified. In the apoptosis experiment described above, the number of anti-CD20 molecules on the RCT-antiCD20 was measured by quantitative flow cytometry. In samples with 1:1 ratio of RCT-antiCD20 to lymphoma cells, there were approximately 11.6-fold fewer molecules of anti-CD20 on the RCT than in the soluble antibody condition, despite similar levels of induced apoptosis. In

samples with 1:5 ratio of RCT-antiCD20 to lymphoma cells, there were approximately 2.3-fold fewer molecules of anti-CD20 on the RCT than in the soluble antibody condition, despite much stronger induction of apoptosis by RCT-antiCD20. Thus, even though the soluble antibody was present at markedly higher levels than the erythroid cell-mounted antibody, the erythroid cells showed a much stronger effect.

Rituximab is thought to kill CD20+ cells via caspase-mediated apoptosis, so this pathway was examined in CD20+ cells treated with anti-CD20 antibody alone or RCT-antiCD20. RCT-antiCD20 cells or control RCT-HA-GPA were added to CD20-expressing target cells. Co-cultures were treated with Z-VAD-FMK, an inhibitor of caspase-mediated apoptosis, or DMSO as a control. Apoptosis of the target cells was measured. As shown in Fig. 2, RCT-antiCD20 robustly promoted apoptosis in Ramos cells even in the presence of the inhibitor of caspase-mediated apoptosis. This surprising result indicates that RCT-antiCD20 kills CD20+ cancer cells through a mechanism different from that of the corresponding antibody alone. The result implies that in patients who are relapsed or refractory to an antibody such as an anti-CD20 antibody, e.g., due to an impaired caspase-dependent apoptosis pathway, can nevertheless be sensitive to that antibody presented on an erythrocyte.

To further characterize the mechanism of RCT-antiCD20 triggered apoptosis, BCL-xL and BCL-2 levels were studied. RCT-antiCD20 or control RCT-HA-GPA cells were cultured with each of three cancer cell lines (Raji, Ramos, or DoHH2). The surface levels of BCL-xL and BCL-2 on the cancer cells were measured by flow cytometry. In each line, RCT-antiCD20 but not control RCT-HA-GPA reduced BCL-xL and BCL-2 levels (see Table 7). Inhibition of the BCL2 and BCLxl anti-apoptotic pathways was dose dependent.

RCT-antiCD20 was also tested for ability to penetrate solid tumors. Because an erythroid cell is much larger than an antibody, it was not expected that an erythroid cell would be able to efficiently penetrate a solid tumor. (See, e.g., Newick et al. DOI: 10.1146/annurev-med-062315-120245, Thurber et al. doi: 10.1016/j.addr.2008.04.012) Xenograft tumors were formed by subcutaneous injection of Ramos cells into immunodeficient mice. The mice were intravenously administered mRBC-antiCD20 or control mRBC-HA-GPA. The mRBC-antiCD20 showed extensive tumor penetration, e.g., by H&E staining and HA staining, compared to mRBC-HA-GPA (Fig. 3). In particular, the mRBC-antiCD20 entered non-necrotic regions of the tumor,

whereas mRBC-HA-GPA was generally restricted to the vasculature or necrotic regions of the tumor (Fig. 3). Specific tumor penetration was also shown using fluorescently labeled RCT-antiCD20 in comparison to control mRBC-HA-GPA (Fig. 4). The mean fluorescent intensity of mRBC-antiCD20 inside the tumor was more than twice as high as the control mRBC-HA-GPA.

5 These data support the therapeutic use of erythroid cells comprising a tumor-binding moiety such as an anti-CD20 antibody. They also support the use of a tumor-binding moiety such as an anti-CD20 antibody as a targeting tool to direct the erythroid cells into tumors, e.g., CD20 positive tumors.

10 **Example 2: Erythroid cells expressing immune checkpoint inhibitors prevent checkpoint inhibition of T cells**

Enucleated erythroid cells were produced which express on their surface a fusion protein comprising, from N-to-C terminus, an scFv antibody domain, an epitope tag (either HA or Flag), and full-length GPA (extracellular, transmembrane, and cytoplasmic domains. The scFv domains
15 were specific binders for PD-1 (RCT-antiPD1), PD-L1 (RCT-antiPDL1), or CTLA4 (RCT-antiCTLA4). The anti-PD-1 antibody domain is pembrolizumab-based. The anti-PD-L1 antibody domain is atezolizumab-based. The anti-CTLA4 antibody domain is ipilimumab-based.

Robust expression of anti-PD-L1 and anti-CTLA4 polypeptides was observed in a flow cytometry assay, with over 95.7% of cells expressing anti-PD-L1 after transfection with a vector
20 encoding anti-PD-L1. Similarly, over 95.2% of cells expressed anti-CTLA4 after transfection with a vector encoding anti-CTLA4.

The cells were tested in a Jurkat/IL-2 assay. Jurkat T lymphocytes typically secrete IL-2, and also express PD-1, PD-L1, and CTLA4 upon stimulation. Co-culture of the Jurkat cells with NHL cells (Z138) triggers the immune checkpoint through PD-1, PD-L1, and CTLA4 signalling,
25 resulting in downregulation of the Jurkat cells IL-2 secretion. A test agent, such as a RCT, is added to the co-culture. The test agent's ability to inhibit the immune checkpoint, thereby preserving T cell activity, is detected by the maintenance of high IL-2 secretion levels.

A 60% inhibition in IL-2 secretion upon co-culture with Z-138 (NHL) and Jurkat cells was observed in the Jurkat IL-2 assay (Fig. 5). Untransduced control RCT do not rescue the
30 inhibitory effect. However, RCT-antiPDL1 showed a rescue and restoration of T cell IL-2

secretion, as did RCT-antiCTLA4 (Fig. 5). This experiment indicates that an RCT expressing an immune checkpoint inhibitor can promote an immune response by preventing checkpoint inhibition of T cells.

The ability of RCT-antiPD1 and RCT-antiPDL1 to elicit activation in a standard antigen recall assay was assayed. PBMC from a CMV positive donor were stimulated with CMV peptide. Memory T cells sensitive to immune checkpoint inhibition were tested for activation and gamma interferon secretion by co-culture with RCT-antiPD-1 or RCT-antiPD-L1 in comparison to control PBMCs or control RCT. A 3-4 fold increase was demonstrated in interferon-gamma secretion of peripheral blood mononuclear cells (PBMC) in an antigen recall assay (Fig. 6).

Control RCT-HA-GPA did not increase interferon-gamma levels. RCT-antiPDL1 showed a 3-6 fold increase in interferon-gamma secretion in this assay (data not shown). This experiment indicates that an RCT expressing an immune checkpoint inhibitor can promote an immune response by preventing checkpoint inhibition of T cells.

RCT-antiPDL1 were tested for the ability to promote PBMC proliferation. Co-culture of PBMC with RCT-antiPDL1 resulted in a 3.6-fold increase in PBMC cells compared to PBMC alone. Co-culture of PBMC with control RCT-HA-GPA resulted in only a 2.3-fold increase. This experiment indicates an additional mechanism by which an RCT expressing an immune checkpoint inhibitor can promote the immune response.

Example 3: Erythroid cells expressing costimulatory molecules promote T cell activity

Enucleated erythroid cells were produced which express on their surface a fusion protein comprising, from N-to-C terminus, a 4-1BBL domain, an epitope tag (HA or Flag), and full-length GPA (extracellular, transmembrane, and cytoplasmic domains) (RCT-41BBL). 41-BB-L is a co-stimulatory protein that is expressed on antigen presenting cells and binds the 41-BB receptor on T-cells.

Binding of RCT-41-BB-L to recombinant 41-BB was determined using flow cytometry.

The RCT-41BBL were assayed by co-culture with Jurkat T cells overexpressing 4-1BB and NFκB-Luc2P (an NFκB-regulated luciferase reporter construct). Upon binding of 4-1BBL,

T cells generally show elevated NF κ B signalling, increased proliferation, increased secretion of IL-2 and interferon-gamma, and protection against activation induced cell death. In the assay, RCT-41BBL stimulated NF κ B activation 30-fold compared with controls, as measured by luciferase activity (Fig. 7). Untransduced enucleated erythroid cells did not raise luciferase levels.

In addition, co-culture of PBMCs with RCT-41BBL showed a 1.7 fold increase in T-cell proliferation compared to PBMCs alone.

These experiments indicate that enucleated erythroid cells can successfully present a costimulatory molecule in a manner that promotes PBMC proliferation and activates a T cell intracellular signalling pathway responsible for immune cell activation.

Example 4: Erythroid cells co-expressing a binding agent to a tumor antigen and TRAIL ligand promote apoptosis in cancer cells expressing the tumor antigen

When erythroid cells were engineered to simultaneously express anti-CD20 as well as Trail ligand (an apoptosis inducing agent) using methods described above, co-culture of Ramos cells with RCT-antiCD20, RCT-Trail, and RCT-antiCD20 +Trail (co-expressed) exert 32%, 47% and 76% apoptosis respectively after 48 hours, suggesting a synergistic effect of the co-expressing RCTs on tumor cell killing.

Example 5: Erythroid cells expressing costimulatory molecules promote T cell activity

Erythroid cells were generated that express different amounts of 4-1BB-L-HA by electroporation of increasing amounts of mRNA for 4-1BB-L-HA into RCT in the maturation phase, as described herein, as indicated in the table below. Greater amounts of electroporated mRNA led to greater expression of the encoded protein, both when measured as a percent of expressing cells and copies that are expressed per cell. Table 10 summarizes the expression level, based on 2 duplicates.

Table 10

mRNA	% cell expressing	Copies of 4-1BB-L-
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(ug/condition)	4-1bB-L-HA	HA per cell
No EP	0	0
0.025	74	42902
0.05	87.7	100500
0.1	91.75	274766
0.2	92	609145
0.4	90.6	874017
0.6	87.5	1015250

After electroporation the erythroid cells were tested for activity in a NFkB luciferase assay. In brief, erythroid cells were incubated with commercially available Jurkat cells that express 4-1BB and NFkB under the luciferase promoter (Promega). In this system, increased
5 luciferase activity as measured by a commercially available kit (Promega) indicates activation of NFkB. 100,000 Jurkat 4-1BB NFkB/Luc cells were incubated with 50,000 RCT expressing different amounts of 4-1BB-L on the cell membrane. Cells were incubated for 6 hours, after which luciferase activity was measured. The data presented in Figure 8 shows a robust increase in luciferase activity is initially dose dependent and then plateaus after 200,000 copies of 4-1BB-
10 L that are expressed per cell. This data indicates that under these assay conditions, maximal luciferase activity is reached at about 200,000 copies of 4-1BB-L per cell.

In another experiment, 4-1BB-L RCT were compared to the anti-4-1BB antibody Utomilumab for ability to promote T cell activity. 100,000 Jurkat 4-1BB NFkB/Luc cells were incubated with 4-1BB-L RCT or control RCT (un-transduced) at increasing cell numbers
15 (12,500, 50,000, 100,000, 200,000, or 400,000). Jurkat 4-1BB NFkB/Luc cells were also incubated with increasing concentration of the 4-1BB agonist Utomilumab (concentration ranging from 0.001nM to 100nM) with or without a secondary anti human IgG that was used for cross linking of Utomilumab, at a concentration ranging from 0.0025nM to 250nM. The results indicate that Utomilumab alone did not increase NFkB activity in this assay, nor did the
20 secondary antibody alone or the control RCT in any of the concentrations tested. High concentrations of Utomilumab (0.1nM and above) that was crossed linked by a secondary anti human IgG antibody led to a 4-6 fold increase in luciferase activity, suggesting NFkB promoter activation. Incubation of the Jurkat 4-1BB NFkB/Luc cells with 4-1BB-L RCT induced a more robust activation of NFkB, starting at 12,500 RCT, that led to 20 fold NFkB activation (Fig. 9).
25 Maximal activity was seen with 200,000 RCT incubated with the Jurkat 4-1BB NFkB/Luc cells,

and led to 90-fold increase in luciferase activity and hence, NFkB activity. Thus, the erythroid cells expressing 4-1BBL lead to a dramatically higher (about 15-fold higher) induction of T cell activity compared to a cross-linked antibody. Without wishing to be bound by theory, trimerization of 4-1BBL at the erythroid cell surface may contribute to its high activity.

5 4-1BB-L RCT were also shown to increase PBMC proliferation. Human peripheral blood mononuclear cells (PBMC) from 3 different donors were obtained from Astrate biologics and were analyzed in a proliferation assay. PBMCs were labelled with Cell Trace Far Red (CTFR, Thermo-Fisher) according to the manufacturer protocol. 100000 CTFR labelled PBMCs were left untreated or stimulated with 0.5ug/mL CD3 antibody. Cells were incubated with
10 12,500, 25,000 or 50,000 RCT (control or expressing 4-1BB-L), or increasing doses of Utomilumab (1nM, 10nM or 100nM) or isotype control antibody, with or without a secondary anti human IgG antibody (2.5nM, 25nM or 250nM). Cells were incubated for 5 days and analyzed in flow cytometry. As shown in Figure 10, Utomilumab with cross linking antibody led to a small increase in proliferation of CD4 and CD8 T cells. On the other hand, incubation of
15 CTFR PBMC with RCT 4-1BB-L led to a dose dependent 2-2.5 fold increase in CD4 proliferation, and dose dependent 4-7 fold increase in proliferation of CD8 cells. The data represents proliferation of PBMCs from 3 different human donors, and the calculation of fold increase in CD4 and CD8 proliferation was relative to the proliferation of the PBMCs from the same donor with CD3 antibody. This experiment indicates that RCT 4-1BB-L promotes
20 proliferation of both CD4+ T cells and CD8+ T cells, with a more robust effect in CD8+ cells than CD4+ cells.

 4-1BB-L RCT were also shown to increase cytokine secretion. Human PBMC from 3 different donors were obtained from Astrate biologics and were analyzed in a cytokine secretion assay. 100,000 PBMCs were left untreated or stimulated with 0.5ug/mL CD3 antibody. Cells
25 were incubated with 12,500, 25,000, 50,000 or 100,000 RCT (control or 4-1BB-L), or increasing doses of Utomilumab (1nM, 10nM, 100nM or 1uM) or isotype control antibody, with or without a secondary anti human IgG antibody (2.5nM, 25nM, 250nM or 2.5uM). Cells were incubated for 5 days and analyzed for cytokine secretion in flow cytometry based cytokine panel (Biolegend legendplex human inflammation panel) according to the manufacturer's protocol.
30 Data presented in Figure 11 indicates that Utomilumab alone led to a maximal secretion of 400

pg/mL of IFN γ and 600 pg/mL with the secondary antibody cross-linking, while incubation of PBMC with RCT-4-1bB-L led to 1500 pg/mL of IFN γ . A similar effect is seen with TNF α secretion, where incubation of PBMCs with RCT-4-1BB-L led a robust increase in TNF α secretion that is not seen either with Utomilumab alone or with Utomilumab and secondary
5 antibody.

Example 6: Erythroid cells comprising 41BBL slow tumor growth in vivo

An MC38 mouse model system for colon cancer was used to test the effects of erythroid cells comprising 4-1BBL on tumor growth. Without being bound by theory, 41BBL is thought
10 to slow tumor growth by eliciting diverse immune effector responses on both the innate and adaptive immune arms. The most potent responses stimulate CD8 $^{+}$ cytotoxic T cells to proliferate and increase their effector potential through increased interferon gamma production and expression of multiple granzymes. In contrast, published preclinical data using multiple 4-1BB agonists have shown little or no single agent antitumor activity in the MC38 or other
15 models (Chen, et al, 2014; Kudo-Saito, et al, 2006; Kocak, et al, Canc Res 2006; Tirapu, et al, Int J Cancer 2004; John, et al, Canc Res 2012).

Cells were conjugated with 4-1BBL such that 94.7% of the cells were labelled with m4-1BB-L. The amount of molecules labelled per cell was quantified using flow cytometry. For dosing animals, there were an average of 1.1×10^9 m4-1BB-L mRBCs administered per dose with
20 an average of 36,200 m4-1BB-L molecules per cell corresponding to 0.084 mg/kg m4-1BB-L per dose.

Fourteen female C57/B6 aged 6-8 weeks mice were inoculated s.c. in left flank with 5×10^5 MC-38 cells. Animals' weights and condition were recorded daily, and tumors were measured 3 times per week.

25 Tumors were measured three times a week by measuring each tumor in 2 dimensions. Tumor volumes were calculated using the standard formula: $(L \times W^2)/2$. The mean tumor weight and standard error of the mean was calculated for each group at each time point.

The observed anti-tumor activity of m4-1BB-L mRBC compared to untreated controls is shown in Figure 12 and demonstrates a reduction in tumor growth in mice treated with m4-1BB-L mRBC. Tumor volume distributions demonstrated statistically significant differences between the groups as early as day 5 of the study and up until day 9 ($P < 0.05$, T test).

5 Body weight was recorded daily. Changes in body weight were calculated for each mouse relatively to the body weight recorded on day 1. Treatment was well tolerated as indicated by overall increase in body weight for most mice. Mice that showed some decrease in body weight, did not lose more than 5% of the total body weight throughout the study.

10 These data support significant efficacy and potency of cellular presentation of 4-1BB-L via erythroid cells.

Example 7: Erythroid cells expressing an exogenous polypeptide comprising a binding agent to a tumor antigen penetrate solid tumors

15 Enucleated erythroid cells were conjugated with anti-PD-L1 at their surface and tested for the ability to infiltrate tumors in mice.

20 Mice were inoculated with B16F10 cells SC. Tumors were allowed to grow to 400 cubic mm before dosing. Murine RBC were conjugated with fragments antibody (Fab) from anti murine PD-L1 and isotype control. Conjugated murine RBC were labelled with CTFR according to the manufacturer's protocol. Cells were infused into the animals. One day after infusion, tumors were collected. Tumors were sectioned and stained with anti CD31 to visualize tumor vasculature and DAPI to visualize nuclei. Stained sections were scanned and pictures were taken. Using Halo software, the tumor areas and vasculature areas were identified. Total cell counts of labelled RBC in these two areas were taken for both isotype control and anti-PD-L1. The ratio between the RBC found in the tumor and the RBC found in the vessels was calculated. The data presented in Figure 13 suggests that the ratio between RBC in the vessels and RBC in the tumor is 1 (average of measurement in tumors from 8 mice) for the isotype control conjugated RBC, indicating similar amounts in the tumor and the vasculature. The ratio between RBC in the vessel and RBC in the tumor is 1.7 for the anti PD-L1 treated mice, indicating enrichment of RBC in

the tumor in the anti-PD-L1 group in comparison with the isotype control mice. The difference in ratio between the 2 groups was statistically significant with $P < 0.01$ (student T test).

While not wishing to be bound by theory, tumors expressing higher levels of PD-L1 may respond better to RCTs comprising anti-PD-L1 than tumors that express lower levels of PD-L1.

5 The B16F10 cells expressed about 300,000 copies per cell of PD-L1 when stimulated with IFN-gamma at 10 ng/ul. In contrast, CT26 cells expressed about 150,000 copies per cell of PD-L1 and A20 cells expressed about 100,000 copies per cell of PD-L1 under the same conditions. PD-L1 copy number was measured using a Quantum Simply Cellular kit (Bangs Laboratories). Erythroid cells comprising an anti-PD-L1 antibody at their surface showed greater binding to the
10 IFN-gamma treated B16F10 cells and CT26 than to the A20 cells, consistent with greater levels of PD-L1 expression on the tumor cells leading to increased binding of the erythroid cells to the tumor cells.

Example 8: Erythroid cells expressing two agents promote greater immune effector cell stimulation than either polypeptide expressed alone.

15 Human peripheral blood mononuclear cells (PBMC) from 1 donor were obtained from Astrate biologics and were analyzed in an IL-2 cytokine secretion assay. 500,000 PBMCs were stimulated with 5ug/mL CD3 antibody. Cells were incubated with 500,000 RCT control cells or RCTs expressing anti-PD-L1 or 4-1BB-L. In parallel, a combination of 500,000 RCT-anti PD-L1 and 500,000 RCT expressing 4-1BB-L was tested. Also in parallel, 500,000 cells expressing
20 both anti PD-L1 and 4-1BB-L were tested. After 24 hours, supernatant was collected and IL-2 ELISA was performed to evaluate IL-2 secretion to the media as a result of incubation with RCT-anti PD-L1 and RCT 4-1BB-L. RCT anti PD-L1 led to 4 fold increase in IL-2 secretion as compared to PBMCs alone. RCT 4-1BB-L led to a 10 fold increased secretion of 4-1BB-L, whereas the combination of RCT anti PD-L1 and RCT-4-1BB-L led to a 13 fold increase in IL-2
25 secretion. The same 13-fold increase was measured with anti PD-L1 and 4-1BB-L were co-expressed on RCT as when PD-L1 and 4-1BB-L were expressed in a non-overlapping population of cells; however the co-expressing cells achieved this effect at half the dose (500,000 cells) compared to the non-overlapping population of cells (1,000,000 cells total).

This experiment indicates that erythroid cells expressing two agents produced a greater immune effector cell stimulation (measured by cytokine secretion) than either polypeptide expressed alone. The two agents used here were a costimulatory molecule (4-1BBL) and an agent that binds an immune checkpoint molecule (anti-PD-L1). Furthermore, the effect was greater when the two agents were expressed on the same cell than when the two agents were expressed in different populations of cells.

Example 9: RCT expressing anti-cancer agents promote signalling, proliferation, cytokine secretion, and antigen recall

The Table of Figure 14 summarizes data generated with RCT expressing 4-1BB-L, OX40-L, GITR-L, and ICOS-L. Signaling activity refers to an NFkB/Luc assay as described in Example 5 for 4-1BB-L, OX40-L and GITR-L (with Jurkat cells expressing NFkB/Luc and receptor for 4-1BB, OX40 or GITR respectively). The proliferation assay and cytokine secretion assays were performed as described in Example 5. The antigen recall assay was performed using 200,000 PBMCs from a CMV-positive donor (Astarte), which were stimulated using lysate of CMV-infected cells (Astarte) at 2ug/mL. PBMCs were then incubated with 100,000 RCT expressing the indicated exogenous protein for 4 days. Cells were analyzed for the presence of memory T cells population with flow cytometry to detect cells that are CD4+ and CD45RO+. Supernatant from these experiments was collected and analyzed for IFNg using ELISA. These experiments indicate that erythroid cells expressing a variety of costimulatory molecules induce T cell activation.

CLAIMS

What is claimed is:

1. A method of delivering an agent, e.g., a binding agent, to an extravascular site in a subject, comprising:

administering to the subject's bloodstream an effective amount of a preparation comprising a plurality of enucleated erythroid cells, each cell of the plurality comprising, on its surface, an exogenous polypeptide comprising a binding agent, e.g., an antibody, against an antigen present at the extravascular site, e.g., a tumor antigen,
thereby delivering the agent, e.g., binding agent to the extravascular site.

2. A method of treating a subject having a resistant, e.g., a refractory or relapsed cancer, comprising:

administering (e.g., to the subject's bloodstream) to the subject a preparation comprising a plurality of enucleated erythroid cells, each cell of the plurality comprising, on its surface, an exogenous polypeptide comprising a binding agent, e.g., an antibody, against a tumor cell antigen, in an amount sufficient to treat the cancer,
thereby treating the cancer.

3. A method of treating a vascularized solid tumor in a subject comprising:
administering to the subject's bloodstream a preparation comprising a plurality of enucleated erythroid cells, each cell of the plurality comprising, on its surface, an exogenous polypeptide comprising a binding agent, e.g., an antibody, against a tumor cell antigen, in an amount sufficient to treat the vascularized solid tumor
thereby treating the vascularized solid tumor.

4. The method of any of the preceding claims, wherein the exogenous polypeptide comprises a transmembrane domain and an antibody.

5. The method of any of the preceding claims, wherein the antibody is an scFv.
6. The method of any of the preceding claims, wherein the antibody is an anti-CD20 antibody and the tumor antigen is CD20.
7. The method of any of the preceding claims, wherein the antibody is an anti-PD-L1 antibody and the tumor antigen is PD-L1.
8. The method of any of the preceding claims, wherein the exogenous polypeptide is present at a copy number of greater than 10^4 , 10^5 , or 10^6 .
9. The method of any of the preceding claims, wherein the erythroid cell further comprises a second exogenous polypeptide.
10. The method of any of the preceding claims, wherein the cancer lacks a functional caspase apoptosis pathway.
11. The method of any of the preceding claims, wherein the cancer comprises a mutation of Table 8.
12. The method of any of the preceding claims, wherein the cancer is resistant to an antibody therapeutic of Table 1.
13. The method of any of the preceding claims, wherein the plurality of enucleated erythroid cells is administered in an amount and for a time effective to result in one of (or more, e.g., 2 or more, 3 or more, 4 or more of): (a) reduced tumor size, (b) reduced rate of tumor growth, (c) increased tumor cell death (d) reduced tumor progression, (e) reduced number of metastases, (f) reduced rate of metastasis, (g) decreased tumor recurrence (h) increased survival of subject, (i) increased progression free survival of subject.

14. The method of any of the preceding claims, wherein the plurality of enucleated erythroid cells is administered in an amount and for a time effective to result in apoptosis, e.g., caspase-independent apoptosis and/or caspase-dependent apoptosis, of target cancer cells, e.g., B cells.

15. The method of any of the preceding claims, wherein the antibody is an anti-CD20 antibody and the plurality of enucleated erythroid cells induces hypercrosslinking of CD20 on the surface of the cancer cell.

16. The method of any of the preceding claims, wherein the ratio of (i) the enucleated erythroid cells expressing the exogenous protein resident in the tumor to (ii) the number of enucleated erythroid cells expressing the exogenous protein in the subject's bloodstream is greater than 1:1, e.g., at the peak of erythroid cell accumulation in the tumor.

17. The method of any of the preceding claims, wherein the ratio of (i) the enucleated erythroid cells expressing the exogenous protein to (ii) endogenous erythroid cells in the tumor is at least 2:1.

18. The method of any of the preceding claims, wherein the amount of binding agent, e.g., antibody, resident in the tumor is at least 2-fold greater than the amount of an otherwise similar binding agent, e.g., antibody, that is not associated with an erythroid cell, e.g., a free antibody.

19. The method of any of the preceding claims, wherein the persistence of the enucleated erythroid cells expressing the exogenous protein in the tumor is increased by at least 2-fold, as compared with an otherwise similar enucleated erythroid cell that lacks the binding agent.

20. The method of any of the preceding claims, wherein the subject is a human subject.