

cells (Beagley KW, Husband AJ. Crit Rev Immunol. 1998;18(3):237-254). V δ 2 T cells that generally coexpress V γ 9 are abundant in the peripheral blood and lymphatic system.

The ability of $\gamma\delta$ T-cells to recognize an antigen on diseased cells directly and to exhibit inherent ability to kill tumor cells renders $\gamma\delta$ T-cells an attractive therapeutic tool. Adoptive transfer of V γ 9V δ 2 T cells has yielded limited objective clinical responses for investigational treatment of cancer (Kondo et al, Cytotherapy, 10:842- 856, 2008; Lang et al, Cancer Immunology, Immunotherapy: CII, 60: 1447-1460, 2011; Nagamine et al , 2009; Nicol et al, British Journal of Cancer, 105:778-786, 2011; Wilhelm et al, Blood. 2003 Jul 1;102(1):200-6), indicating the need to isolate and test clinically new $\gamma\delta$ T-cell populations.

The ability to selectively expand $\gamma\delta$ T-cell subset populations having potent anti-tumor activity with improved purity and in clinically-relevant levels is highly desirable. Although antibodies and cytokine cocktails have been used to propagate a more diverse set of $\gamma\delta$ T cells, activation of specific $\gamma\delta$ T-cell subsets to sufficient purity and clinically-relevant levels, was not achieved (Dokouhaki et al, 2010; Kang et al, 2009; Lopez et al, 2000; Kress, 2006). Therefore, clinically-relevant methods of expanding specific $\gamma\delta$ T cell subsets *ex vivo*, and the cells produced thereby, are greatly needed.

SUMMARY OF THE INVENTION

The present ~~invention, in one aspect, disclosure~~ provides *ex vivo* methods for producing enriched $\gamma\delta$ T cell populations. The enriched $\gamma\delta$ T cell populations can be produced from isolated mixed cell populations by a method comprising contacting the mixed cell population, or a purified fraction thereof, with one or more agents that selectively expand δ 3 T cells by binding to an epitope specific to a δ 3 TCR. The expanded T cell populations can be used, *e.g.*, individually or in combination with other T cell populations, in methods of treatment by administering to a subject in need thereof. In some cases, the agents are antibodies or fragments thereof.

~~In a first aspect, the present invention provides~~Herein described is an agent that selectively binds an epitope specific to a δ 3 $\gamma\delta$ TCR. In some embodiments, the agent is an antibody or a fragment thereof. In some embodiments, the agent is an activating agent that selectively binds an activating epitope specific to a δ 3 $\gamma\delta$ TCR.

In one aspect, there is provided an antibody or fragment thereof that binds an epitope specific to a $\delta 3$ $\gamma\delta$ TCR, wherein the antibody or fragment thereof binds essentially the same epitope as, or competes for binding with: an antibody selected from the group consisting of $\delta 3$ -08, $\delta 3$ -20, $\delta 3$ -23, $\delta 3$ -31, $\delta 3$ -42, $\delta 3$ -47, and $\delta 3$ -58.

In one aspect, there is provided an antibody or fragment thereof that binds an epitope specific to a $\delta 3$ $\gamma\delta$ TCR, wherein the antibody or fragment thereof comprises: (i) a heavy chain variable region/light chain variable region (HCVR/LCVR) sequence pair selected from the group consisting of SEQ ID NOs: 1/8, 2/9, 3/10, 4/11, 5/12, 6/13, and 7/14; or (ii) the six complementarity determining regions (CDRs) of a HCVR/LCVR sequence pair selected from the group consisting of SEQ ID NOs: 1/8, 2/9, 3/10, 4/11, 5/12, 6/13, and 7/14.

In another aspect, the present disclosure refers to a nucleic acid encoding any one of the antibodies or fragments thereof as described herein, wherein the nucleic acid is operably linked to a heterologous promoter.

In another aspect, the present disclosure refers to a host cell comprising any one of the antibodies or fragments thereof as described herein or a nucleic acid as described herein.

In another aspect, the present disclosure refers to a method of making an antibody or fragment thereof that binds an epitope specific to a $\delta 3$ $\gamma\delta$ TCR, the method comprising culturing the host cell as described herein under conditions sufficient to produce the antibody or fragment thereof.

In another aspect, the present disclosure refers to an ex vivo method for producing an enriched $\gamma\delta$ T cell population, comprising contacting a first cell population comprising $\gamma\delta$ T cells with one or more antibodies or fragments thereof as described herein.

In another aspect, the present disclosure refers to an ex vivo method for producing an enriched $\gamma\delta$ T cell population, comprising: a) contacting a first cell population comprising $\gamma\delta$ T cells with one or more first activating agents that activate and expand $\gamma\delta$ T cells, thereby producing an expanded first $\gamma\delta$ T cell population; and b) contacting the expanded first $\gamma\delta$ T cell population with one or more second activating agents that activate and expand $\gamma\delta$ T cells, thereby producing the enriched $\gamma\delta$ T cell population, wherein, at least one of the one or more first activating agents or at least one of the one or more second activating agents is an antibody or fragment thereof as described herein.

In another aspect, the present disclosure refers to an enriched $\gamma\delta$ T cell population produced by the method as described herein.

In another aspect, the present disclosure refers to a composition comprising the enriched $\gamma\delta$ T cell population as described herein; and the antibody or fragment thereof as described herein.

In another aspect, the present disclosure refers to the use of an enriched $\delta\beta$ $\gamma\delta$ T cell population as described herein or the composition as described herein in the manufacture of a medicament for treating cancer, an inflammatory disease, or an autoimmune disease in a subject in need thereof.

In another aspect, the present disclosure refers to an enriched $\delta\beta$ $\gamma\delta$ T cell population as described herein or the composition as described herein for use in therapy.

In some embodiments, the agent (*e.g.*, antibody or fragment thereof) binds the same epitope, or essentially the same epitope, as (or competes with) an antibody selected from the

an Fc region of the agent (*e.g.*, antibody or fragment thereof) is bound to an Fc-receptor expressed by the APC. In some cases, the APC is an artificial APC (aAPC). In some cases, the APC is in a subject, isolated, in an *ex vivo* or *in vitro* culture, or is in a container.

In some cases, the agent (*e.g.*, antibody or fragment thereof) binds to a region distal from the γ -chain binding interface of V δ 3. In some cases, the agent (*e.g.*, antibody or fragment thereof) binds to β strand D and E of the V δ 3 of the $\gamma\delta$ TCR according to IMGT nomenclature. In some cases, the agent (*e.g.*, antibody or fragment thereof) binds to β strand C'' and D and a loop between β strands E and F of the V δ 3 of the $\gamma\delta$ TCR according to IMGT nomenclature. In some cases, the agent (*e.g.*, antibody or fragment thereof) binds to β strand A, B, D, and E of the V δ 3 of the $\gamma\delta$ TCR according to IMGT nomenclature.

In some cases, the agent (*e.g.*, antibody or fragment thereof) binds to amino acids K79 and H88 of V δ 3 (IMGT nomenclature). In some cases, the agent (*e.g.*, antibody or fragment thereof) binds to amino acids R75 and R95 of V δ 3 (IMGT nomenclature). In some cases, the agent (*e.g.*, antibody or fragment thereof) binds to amino acids K79, E18, and H88 of V δ 3 (IMGT nomenclature). In some cases, the agent (*e.g.*, antibody or fragment thereof) binds to amino acids E67, D70, R75, R95, and E97 of V δ 3 (IMGT nomenclature). In some cases, the agent (*e.g.*, antibody or fragment thereof) binds to amino acids K3, K79, and H88 of V δ 3 (IMGT nomenclature). In some cases, the agent (*e.g.*, antibody or fragment thereof) binds to amino acids K79, L82, and H88 of V δ 3 (IMGT nomenclature). Typically, the agent binds the specified amino acids or groups thereof in the context of a V δ 3 $\gamma\delta$ TCR, such as a V δ 3 $\gamma\delta$ TCR expressed on the surface of a V δ 3 $\gamma\delta$ T cell.

In [a second another](#) aspect, ~~the present invention provided~~s is a nucleic acid encoding any one of the foregoing agents (*e.g.*, antibodies or fragments thereof).~~In some cases, the nucleic acid is~~ operably linked to a heterologous promoter. In [a third another](#) aspect, the present invention provides a host cell comprising any one of the foregoing agents or any one of the foregoing nucleic acids. In some cases, the host cell is an artificial antigen presenting cell (aAPC).

In [a fourth another](#) aspect, ~~the present invention provided~~d is a method of making an agent (*e.g.*, antibody or fragment thereof) that binds an epitope specific to a δ 3 $\gamma\delta$ TCR, the method comprising culturing any one of the foregoing host cells under conditions sufficient to produce the antibody or fragment thereof.

In ~~a-fifth~~another aspect, ~~the-present-invention~~provided d is an *ex vivo* method for producing an enriched $\gamma\delta$ T cell population, comprising contacting a first cell population comprising $\gamma\delta$ T cells with one or more of the foregoing agents (*e.g.*, antibodies or fragments thereof). In some embodiments, the first cell population is an isolated mixed cell population comprising $\alpha\beta$ T cells and $\gamma\delta$ T cells (*e.g.*, $\delta 3$ $\gamma\delta$ T cells). In some embodiments, the first cell population is selected from a peripheral blood sample, a leukapheresis sample, a cord blood sample, a tumor sample, or a tissue sample. In some cases, the first cell population is a sample isolated from a single donor. In some cases, the first cell population is a sample isolated from multiple donors. In some cases, the first cell population is a PBMC sample. In some cases, the first cell population is a sample of PBMCs isolated from a single donor. In some cases, the first cell population is a sample of PBMCs isolated from multiple donors.

In some embodiments, the method comprises directly contacting the isolated mixed cell population with the one or more of the foregoing agents (*e.g.*, antibodies or fragments thereof). In some embodiments, the first cell population is a population comprising one or more engineered $\gamma\delta$ T cells. In some embodiments, the first cell population is a first population of expanded $\gamma\delta$ T cells. In some embodiments, the method comprises a first $\gamma\delta$ T cell expansion and a second $\gamma\delta$ T cell expansion. In some embodiments, the method comprises producing an enriched $\gamma\delta$ T cell population comprising at least 10^8 $\delta 3$ $\gamma\delta$ T cells. In some embodiments, the enriched $\gamma\delta$ T cell population comprising at least 10^8 $\delta 3$ $\gamma\delta$ T cells is produced within 12 to 21 days. In some embodiments, the method comprises expanding the $\gamma\delta$ T cells in the first cell population at least 1,000-fold. In some embodiments, the at least 1,000-fold expansion is achieved within 12 to 21 days.

In ~~a-sixth~~another aspect, ~~the-present-invention~~provided d is an *ex vivo* method for producing an enriched $\gamma\delta$ T cell population, comprising: a) contacting a first cell population comprising $\gamma\delta$ T cells with one or more first activating agents that activate and expand $\gamma\delta$ T cells, thereby producing an expanded first $\gamma\delta$ T cell population; and b) contacting the expanded first $\gamma\delta$ T cell population with one or more second activating agents that activate and expand $\gamma\delta$ T cells, thereby producing the enriched $\gamma\delta$ T cell population, wherein, at least one of the one or more first activating agents or at least one of the one or more second activating agents is an agent (*e.g.*, antibody or fragment thereof) according to the first aspect. In some embodiments, the method comprises isolating the expanded first $\gamma\delta$ T cell population after a) and before b). In some

embodiments, a) comprises culturing the first cell population in the presence of an antigen presenting cell (APC) and the one or more first activating agents. In some embodiments, b) comprises culturing the activated $\gamma\delta$ T cell population in the presence of an antigen presenting cell (APC) and the one or more second activating agents.

In some embodiments, the first cell population is an isolated mixed cell population comprising $\alpha\beta$ T cells and $\gamma\delta$ T cells. In some embodiments, the first cell population is selected from a peripheral blood sample, a leukapheresis sample, a cord blood sample, a tumor sample, or a tissue sample. In some embodiments, the first cell population is a population of engineered $\gamma\delta$ T cells. In some embodiments, the method comprises genetically modifying the expanded first $\gamma\delta$ T cell population or genetically modifying the enriched $\gamma\delta$ T cell population. In some embodiments, the method comprises producing an enriched $\gamma\delta$ T cell population comprising at least 10^8 $\delta 3$ $\gamma\delta$ T cells. In some embodiments, the enriched $\gamma\delta$ T cell population comprising at least 10^8 $\delta 3$ $\gamma\delta$ T cells is produced within 12 to 21 days.

In some embodiments, the method comprises expanding the $\gamma\delta$ T cells in the first cell population at least 1,000-fold. In some embodiments, the at least 1,000-fold expansion is achieved within 12 to 21 days.

In some embodiments, at least one of the one or more first activating agents is structurally identical to at least one of the one or more second activating agents. In some embodiments, at least one of the one or more first activating agents is structurally different than at least one of the one or more second activating agents. In some embodiments, at least one of the one or more first activating agents or at least one of the one or more second activating agents is immobilized. In some embodiments, the immobilized activating agent is immobilized on a surface of a culture vessel. In some embodiments, the immobilized activating agent is immobilized on a surface of an antigen presenting cell (APC). In some embodiments, the immobilized activating agent is the agent (*e.g.*, antibody or fragment thereof) that binds the epitope specific to a $\delta 3$ $\gamma\delta$ TCR.

In ~~a-seventh~~another aspect, ~~the present invention provided~~is an expanded $\gamma\delta$ T cell population (*e.g.*, before positive or negative selection for $\delta 3$ $\gamma\delta$ T cells), wherein greater than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, or 80% of the expanded $\gamma\delta$ T cells are $\delta 3$ $\gamma\delta$ T cells. In some embodiments, from 10% to 80% of the expanded $\gamma\delta$ T cells are $\delta 3$ $\gamma\delta$ T cells (*e.g.*, before positive or negative selection for $\delta 3$ $\gamma\delta$ T cells). In some embodiments, from 10% to 60% of the

expanded $\gamma\delta$ T cells are $\delta 3 \gamma\delta$ T cells (*e.g.*, before positive or negative selection for $\delta 3 \gamma\delta$ T cells). In some embodiments, from 10% to 40% of the expanded $\gamma\delta$ T cells are $\delta 3 \gamma\delta$ T cells (*e.g.*, before positive or negative selection for $\delta 3 \gamma\delta$ T cells). In some embodiments, from 20% to 80% of the expanded $\gamma\delta$ T cells are $\delta 3 \gamma\delta$ T cells (*e.g.*, before positive or negative selection for $\delta 3 \gamma\delta$ T cells). In some embodiments, from 20% to 60% of the expanded $\gamma\delta$ T cells are $\delta 3 \gamma\delta$ T cells (*e.g.*, before positive or negative selection for $\delta 3 \gamma\delta$ T cells). In some embodiments, from 20% to 50% of the expanded $\gamma\delta$ T cells are $\delta 3 \gamma\delta$ T cells (*e.g.*, before positive or negative selection for $\delta 3 \gamma\delta$ T cells). In some embodiments, from 20% to 40% of the expanded $\gamma\delta$ T cells are $\delta 3 \gamma\delta$ T cells (*e.g.*, before positive or negative selection for $\delta 3 \gamma\delta$ T cells).

In some embodiments, the expanded $\gamma\delta$ T cell population comprises polyclonal TCR diversity. In some embodiments, greater than 30%, 40%, 50%, 60%, or 70% of the $\delta 3 \gamma\delta$ T cells in the expanded $\gamma\delta$ T cell population express the phenotype CD45RA⁺/CD27⁺ and/or CD45RA⁻/CD27⁺. In some embodiments, from 20% to 70% of the $\delta 3 \gamma\delta$ T cells in the expanded $\gamma\delta$ T cell population express the phenotype CD45RA⁺/CD27⁺ and/or CD45RA⁻/CD27⁺. In some embodiments, greater than 30%, 40%, 50%, 60%, or 70% of the $\delta 1^+ \delta 2^- \gamma\delta$ T cells in the expanded $\gamma\delta$ T cell population express the phenotype CD45RA⁺/CD27⁺ and/or CD45RA⁻/CD27⁺. In some embodiments, from 20% to 70% of the $\delta 1^+ \delta 2^- \gamma\delta$ T cells in the expanded $\gamma\delta$ T cell population express the phenotype CD45RA⁺/CD27⁺ and/or CD45RA⁻/CD27⁺.

In some embodiments, the expanded $\gamma\delta$ T cell population is derived from a tissue such as skin. In some embodiments, the expanded $\gamma\delta$ T cell population is derived from tumor infiltrating lymphocytes. In some embodiments, the expanded $\gamma\delta$ T cell population comprises $\gamma\delta$ T-cells that express an endogenous or heterologous tumor recognition moiety. In some embodiments, the population comprises a therapeutically effective amount (*e.g.*, $>10^8$) of $\gamma\delta$ T-cells. In some embodiments, the $\gamma\delta$ T cell population comprises anti-tumor cytotoxicity that is independent of NKp30 activity, NKp44 activity, and/or NKp46 activity. In some embodiments, the $\gamma\delta$ T cell population does not comprise NKp30 activity-dependent anti-tumor cytotoxicity, NKp44 activity-dependent anti-tumor cytotoxicity, and/or NKp46 activity-dependent anti-tumor cytotoxicity. In some embodiments, less than 40% of the $\gamma\delta$ T cells express (*e.g.*, as protein or mRNA) a detectable level of NKp30, NKp44, and/or NKp46.

In ~~an eighth~~another aspect, ~~the present invention~~provided is an expanded $\gamma\delta$ T cell population, which is produced by any one of the foregoing methods. In ~~a ninth~~another aspect, ~~the present invention~~

provided is a method of treating cancer, an inflammatory disease, or an autoimmune disease in a subject in need thereof, the method comprising administering a therapeutically effective amount of $\delta 3$ $\gamma\delta$ T cells to the subject, administering a therapeutically effective amount of a $\gamma\delta$ T cell population that is enriched in $\delta 3$ $\gamma\delta$ T cells to the subject, or administering to the subject a therapeutically effective amount of a $\gamma\delta$ T cell population obtained by a method described herein.

In another aspect, provided is use of a therapeutically effective amount of $\delta 3$ $\gamma\delta$ T cells in the manufacture of a medicament for treating cancer, an inflammatory disease, or an autoimmune disease in a subject in need thereof.

In ~~a tenth~~ another aspect, ~~the present invention~~ provided is a method of treating cancer, an inflammatory disease, or an autoimmune disease in a subject in need thereof, the method comprising: a) providing a therapeutically effective amount of $\delta 3$ $\gamma\delta$ T cells isolated from any one or more of the foregoing expanded $\gamma\delta$ T cell populations; and administering the therapeutically effective amount of $\delta 3$ $\gamma\delta$ T cells to the subject. In some embodiments, the method further comprises admixing the expanded $\gamma\delta$ T cell population with a different expanded $\gamma\delta$ T cell population to form an admixed population and administering the admixed population to the subject.

In some embodiments, the different expanded $\gamma\delta$ T cell population comprises an amount of expanded $\delta 1$ $\gamma\delta$ T cells, and/or an amount of expanded $\delta 2$ $\gamma\delta$ T cells. In some embodiments, the different expanded $\gamma\delta$ T cell population comprises $> 5\%$ $\delta 1$ (e.g., $> 10\%$, 20% , 30% , 40% , 50% , 60% , 70% , 80% , or 90% $\delta 1$) or $> 5\%$ $\delta 2$ (e.g., $> 10\%$, 20% , 30% , 40% , 50% , 60% , 70% , 80% , or 90% $\delta 2$) $\gamma\delta$ T cells. In some embodiments, the different expanded $\gamma\delta$ T cell population comprises from about, or at least about, 60% to about 90% $\delta 1$ $\gamma\delta$ T cells, or from about, or at least about, 60% to about 80% $\delta 1$ $\gamma\delta$ T cells. In some embodiments, the different expanded $\gamma\delta$ T cell population comprises from about, or at least about, 60% to about 90% $\delta 2$ $\gamma\delta$ T cells, from about, or at least about, 70% to about 80% $\delta 2$ $\gamma\delta$ T cells, or from about, or at least about, 80% to about 90% $\delta 2$ $\gamma\delta$ T cells.

In some embodiments, the admixed $\gamma\delta$ T cell population comprises an amount of $\delta 1$ $\gamma\delta$ T cells, and/or an amount of $\delta 2$ $\gamma\delta$ T cells, and an amount of $\delta 3$ $\gamma\delta$ T cells. In some embodiments, the admixed $\gamma\delta$ T cell population comprises $> 5\%$ $\delta 1$ (e.g., $> 10\%$, 20% , 30% , 40% , 50% , 60% , 70% , 80% , or 90% $\delta 1$) or $> 5\%$ $\delta 2$ (e.g., $> 10\%$, 20% , 30% , 40% , 50% , 60% , 70% , 80% , or 90% $\delta 2$) $\gamma\delta$ T cells. In some embodiments, the admixed $\gamma\delta$ T cell population comprises $> 5\%$ $\delta 3$ (e.g.,

> 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% $\gamma\delta$ T cells. In some embodiments, the admixed $\gamma\delta$ T cell population comprises from about, or at least about, 5% to about 90% $\gamma\delta$ T cells, from about, or at least about, 10% to about 90% $\gamma\delta$ T cells, from about, or at least

Raji cells. Untransduced V δ 3 $\gamma\delta$ T cells exhibited slight cytotoxicity against the CD20+ Raji cells at Effector:Target (E/T) ratios >1 and moderate cytotoxic activity at E/T ratios > 5. Anti-CD20 CAR transduced V δ 3 $\gamma\delta$ T cells exhibited moderate cytotoxic activity at an E/T ratio of 0.5 and strong cytotoxic activity at E/T ratios >0.75.

Fig. 12 illustrates an amino acid sequence of V δ 3, numbered according to IMGT nomenclature. [Fig. 12 discloses SEQ ID NO: 15.](#)

Fig. 13 illustrates 3 different projections of a 3D model of antigen binding hot spots identified by fine epitope mapping via mutagenesis as described in Example 7.

Figs. 14A-C illustrate IMGT Collier de Perles, with residues shown by fine epitope mapping to contribute to antigen binding highlighted by dark double circles. **A.** K79 and H88 contribute to antigen binding of δ 3-23, δ 3-42, and δ 3-58. **B.** E67 and D70 contribute to antigen binding of δ 3-31; R75 and R95 also contribute to antigen binding of δ 3-31; the combination of E67, D70, R75, R95, and E97 contributes to antigen binding of δ 3-31 and δ 3-42. **C.** E18 contributes to antigen binding of δ 3-58; and K3 contributes to antigen binding of δ 3-08. [Figs. 14A-C each disclose SEQ ID NO: 15.](#)

DETAILED DESCRIPTION OF THE INVENTION

While various embodiments of the invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions may occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which the inventions described herein belong. For purposes of interpreting this specification, the following definitions will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa. In the event that any definition set forth conflicts with any document incorporated herein by reference, the definition set forth below shall control.

The term “ $\gamma\delta$ T cells (gamma delta T cells)” as used herein refers to a subset of T-cells that express a distinct T-cell receptor (TCR), $\gamma\delta$ TCR, on their surface, composed of one γ -chain

methodologies that are described in the publications, which might be used in connection with the presently described inventions. The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors described herein are not entitled to antedate such disclosure by virtue of prior invention or for any other reason. It is to be understood that, if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art, in Australia or any other country.

The present invention is explained in more detail by the following non-limiting examples.

In the claims which follow and in the preceding description of the invention, except where the context requires otherwise due to express language or necessary implication, the word “comprise” or variations such as “comprises” or “comprising” is used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the presence or addition of further features in various embodiments of the invention.

EXAMPLES

Example 1. Generation of Antibodies that Specifically Bind $\delta 3$ TCR

$\gamma\delta$ TCR activators in the form of murine antibodies were produced by immunization of recombinant human soluble $\gamma 2\delta 3$ TCR- FC, comprising the mature ECD of the $\gamma\delta$ TCR chains fused to human IgG1FC. Three strains of mice (Balb/c, CD-1 and FVB) were inoculated with human recombinant $\gamma\delta$ TCR to provide hybridomas secreting high affinity, murine monoclonal antibody activators.

The $\gamma\delta$ TCR-Fc fusion construct was generated by PCR amplification. $\gamma 2\delta 3$ TCR chains were amplified from RNA isolated from tumor infiltrating $\gamma\delta$ lymphocytes extracted from a fresh specimen of malignant ovary cells from a metastasis to the omentum. Soluble $\delta 3$ TCR-FC was purified from the supernatant of transiently transfected HEK 293 cells. 10 μ g of soluble TCR was emulsified with an equal volume of TITERMAX® Gold (Sigma Aldrich) or Imject Alum Adjuvant (Thermo Fisher) and used for the immunization of each mouse. The resulting emulsions were then injected into three mice (1 each: Balb/c, CD-1 and FVB) via the footpad route.

Solid-phase ELISA assays were used to screen mouse sera for mouse IgG antibodies specific for human $\gamma\delta 3$ TCR. Briefly, 96 well plates (VWR International, Cat. #610744) were coated with recombinant $\gamma\delta 3$ TCR- at 1 $\mu\text{g}/\text{ml}$ in ELISA coating buffer overnight. After washing with PBS containing 0.02% (v/v) Tween 20, the wells were blocked with 3% (w/v) BSA in PBS, 200 $\mu\text{L}/\text{well}$ for 1 hour at room temperature (RT). Mouse serum was titrated (1:100, 1:200, 1:400, and 1:800) and added to the $\gamma\delta$ TCR coated plates at 50 $\mu\text{L}/\text{well}$ and incubated at RT for 1 hour. The plates are washed and then incubated with 50 $\mu\text{L}/\text{well}$ HRP-labeled goat anti-mouse IgG diluted 1:10,000 in 3% BSA-PBS or 2% FCS in PBS for 1 hour at RT. Again the plates were washed and 40 $\mu\text{L}/\text{well}$ of a TMB substrate solution (Thermo Scientific 34028) was added