PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

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PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	27 FEB 2020
Applicant's or agent's file reference 47533-743601	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2019/063383	26 November 2019	30 November 2018

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C07K 14/705; C12N 5/00; C12N 9/22; C12N 15/113 (2020.01)
CPC - C07K 2319/03; C07K 2319/33; C12N 5/0636; C12N 15/113; C12N 2310/20 (2020.01)

Applicant INTIMA BIOSCIENCE, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	29 January 2020	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

☒ the international application in the language in which it was filed.

☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a)).

3. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:

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

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

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

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

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

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

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

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

5. Additional comments:

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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 11-85, 94, 109-185, 190, 194

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international search *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 11-85, 94, 109-185, 190, 194 are so unclear that no meaningful opinion could be formed *(specify)*:

Claims 11-85, 94, 109-185, 190, and 194 are multiple dependent claims not drafted in accordance with the second and third sentences of Rule 6.4(a).

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. 11-85, 94, 109-185, 190, 194

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing in the form of an Annex C/ST.25 text file, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ furnish a sequence listing on paper or in the form of an image file complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in the form and manner acceptable to it; or the sequence listing furnished did not comply with the standard provided for in Annex C of the Administrative Instructions.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ See Supplemental Box for further details.

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Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-10, 86-93, 95-108, 186-189, 191-193	YES
	Claims	None	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-10, 86-93, 95-108, 186-189, 191-193	NO
Industrial applicability (IA)	Claims	1-10, 86-93, 95-108, 186-189, 191-193	YES
	Claims	None	NO

2. Citations and explanations:

Claims 1-10 and 86-93 lack an inventive step under PCT Article 33(3) as being obvious over Stcube & Co., Inc. et al. (hereinafter Stcube).

Regarding Claim 1, Stcube discloses a method of screening a plurality of single candidate genes (Provided herein are in vitro and in vivo screening methods for identifying gene targets for cancer therapy, Para. [0032]), said method comprising: a. expressing an exogenous cellular receptor, or a functional fragment thereof, in a plurality of populations of immune cells, wherein each population comprises a plurality of immune cells (In some embodiments, the T cells are isolated from a subject. The subject can be a mammal. In some embodiments, the T cells are isolated form a mouse.... In some embodiments, the mouse is genetically engineered to expresses a T-cell receptor, Para. [0051]); b. introducing into each of said populations of immune cells a CRISPR system that comprises: i. a guide nucleic acid that binds a portion of a single candidate gene, wherein said single candidate gene is different for each of said populations of immune cells; and ii. an exogenous nuclease, or a nucleic acid encoding said exogenous nuclease; thereby generating a plurality of populations of engineered immune cells that comprise a genomic disruption in said single candidate gene, wherein said genomic disruption that suppresses expression of said single candidate gene (The modified T cell can have a candidate gene knocked-down or knocked out, Para. [0026]; The unmodified T cells can be genetically modified using methods described herein or otherwise known in the art to produce modified T cells.... In some embodiments, the gene in the isolated T cells is knocked-out or knocked-down by using CAS9 (CRISPR), Para. [0054]); c. performing an in vitro assay that comprises contacting said plurality of engineered immune cells with a plurality of cells expressing a cognate antigen of said exogenous cellular receptor or said functional fragment thereof in vitro (In other embodiments, provided herein are methods of assessing the development of T cell effector function in response to antigen-specific activation. Assessing the cytotoxic activity of T cells, elicited by antigen-specific stimulation in vitro, allows one to assess the functional cytolytic potential of effector cells, Para. [0104]; In some embodiments, the antigen is a protein that is not naturally present in the target cells, Para. [0106]; By way of example, some vectors used in recombinant DNA techniques allow entities, such as a segment of DNA (such as a heterologous DNA segment, such as a heterologous cDNA segment), to be transferred into a target cell, Para. [0183]); and d. obtaining a readout from said in vitro assay, to thereby determine an effect of said genomic disruption that suppresses expression of said single candidate gene on said plurality of populations of engineered immune cells (In certain embodiments, evaluation of cytolytic activity by 51Cr release, can be used to investigate T cell-specific effector function and its modulation/inhibition by immunomodulatory cells, while preserving the simplicity of the assay and allowing the investigator to work with numerous variables and rare cell populations. Chromium release assay can provide a more specific functional readout, Para. [0104]).
Stcube fails to explicitly disclose said plurality of populations are separated.
However, it would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Stcube to include separate populations of cells comprising different single gene modifications, since separating parts that were once integral involves only routine skill in the art. The motivation for doing so would be to separately and independently test different immune cells comprising different genetic modifications to determine which modifications can enhance or reduce antigen responses to determine optimal treatments for cancer.

Regarding Claim 2, Stcube discloses the method of claim 1, wherein said readout comprises determining a level of cytolytic activity of each of said plurality of separate populations of engineered immune cells (Assessing the cytotoxic activity of T cells, elicited by antigen-specific stimulation in vitro, allows one to assess the functional cytolytic potential of effector cells, Para. [0104]).

Regarding Claim 3, Stcube discloses the method of claim 2, wherein said level of cytolytic activity is determined by a chromium release assay, an electrical impedance assay, time-lapse microscopy, or a co-culture assay (Chromium release assay can provide a more specific functional readout, Para. [0104]).

Regarding Claim 4, Stcube discloses the method of claim 1, wherein said readout comprises determining a level of proliferation of each of said plurality of separate populations of engineered immune cells (The in vitro screening methods can measure activities including, but not limited to proliferation, Para. [0097]).

Regarding Claim 5, Stcube discloses the method of claim 4, wherein said level of proliferation is determined by a Carboxyfluorescein Succinimidyl Ester (CFSE) assay, microscopy, an electrical impedance assay, or flow cytometry (In another embodiment, the proliferation is determined by flowcytometric analysis of T cells labeled with carboxyfluorescein succinimidyl ester (CFSE), Para. [0122]).

Regarding Claim 6, Stcube discloses the method of claim 1, wherein said readout comprises determining a level of a factor expressed by each of said plurality of separate populations of engineered immune cells (The activity can also be the immunity of effector T cells, including such as cytotoxic activity or cytokine production activity, Para. [0027]).

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Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Regarding Claim 7, Stcube discloses the method of claim 6, wherein said factor is a protein (The activity can also be the immunity of effector T cells, including such as cytotoxic activity or cytokine production activity, Para. [0027]).

Regarding Claim 8, Stcube discloses the method of claim 7, wherein said protein is secreted from said population of engineered immune cells (The activity can also be the immunity of effector T cells, including such as cytotoxic activity or cytokine production activity, Para. [0027]; In addition to surface markers, different subtypes of T cells can also have distinct function and cytokine secretion profiles, Para. [0023]).

Regarding Claim 9, Stcube discloses the method of claim 7, wherein said protein is a cytokine or chemokine (The activity can also be the immunity of effector T cells, including such as cytotoxic activity or cytokine production activity, Para. [0027]; In addition to surface markers, different subtypes of T cells can also have distinct function and cytokine secretion profiles, Para. [0023]).

Regarding Claim 10, Stcube discloses the method of claim 7, wherein said protein is a cell surface protein (The activity can also be the immunity of effector T cells, Para. [0027]; Differential expression of markers on the cell surface provide valuable clues to the diverse nature and function of T cells, and can serve as useful tools for the isolation of T cells or specific subtypes of T cells, Para. [0023]).

Regarding Claim 86, Stcube discloses a composition comprising a plurality of populations of immune cells (These examples are meant to be illustrative of pharmaceutical compositions and dosage forms provided herein, but are not in any way limiting, Para. [0212]; Accordingly, in some embodiments, provided herein are in vitro methods for screening a target gene for cancer therapy including culturing separately... (b) modified T cells and suppressor cells; wherein the modified T cells have a candidate gene knocked-down or knocked-out, Para. [0093]; The modified T cell can overexpress or underexpress a gene as compared to a wild type T cell. The modified T cell can have a candidate gene knocked-down or knocked out. A population of modified T cell can be a population of T cells with different genetic modification. For example, a population of modified T cell can each have different genetic signatures with at most one gene knocked-down or knocked-out as compared to a wild type T cell, Para. [0026]), wherein each population of immune cells comprises a plurality of immune cells that i) express an exogenous cellular receptor (In some embodiments, the T cells are isolated from a subject. The subject can be a mammal. In some embodiments, the T cells are isolated form a mouse.... In some embodiments, the mouse is genetically engineered to expresses a T-cell receptor, Para. [0051]); and ii) comprise a CRISPR system that comprises a guide nucleic acid that binds a portion of a single candidate gene, wherein said single candidate gene is different for each of said populations of immune cells; and an exogenous nuclease, or a nucleic acid encoding said exogenous nuclease (The modified T cell can have a candidate gene knocked-down or knocked out, Para. [0026]; The unmodified T cells can be genetically modified using methods described herein or otherwise known in the art to produce modified T cells.... In some embodiments, the gene in the isolated T cells is knocked-out or knocked-down by using CAS9 (CRISPR), Para. [0054]).
Stcube fails to explicitly disclose said plurality of populations are separated.
However, it would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Stcube to include separate populations of cells comprising different single gene modifications, since separating parts that were once integral involves only routine skill in the art. The motivation for doing so would be to separately and independently test different immune cells comprising different genetic modifications to determine which modifications can enhance or reduce antigen responses to determine optimal treatments for cancer.

Regarding Claim 87, Stcube discloses the composition of claim 86, wherein said population of said plurality of immune cells of each separate population comprises a genomic disruption in said single candidate gene (To identify a target for cancer therapy, i.e., molecules implicated in regulating the inhibitory mechanisms of suppressor cells, naïve T cells are isolated from a non-tumor bearing genetically or non-genetically engineered mouse and placed into a 96 well plate.... The knock-down or knock-out of a particular candidate gene is done one well at a time, Para. [0213]).

Regarding Claim 88, Stcube discloses the composition of claim 87, but fails to explicitly disclose wherein at least 70%, 80%, or 90% of said plurality of immune cells of each separate population comprises a genomic disruption in said single candidate gene.
However, it would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Stcube to include generating a population of immune cells having at least 70% genomic disruption of a target gene, since the general conditions of the claim are disclosed in the prior art, discovering the optimum or workable ranges involves only routine skill in the art. The motivation for doing so would be sufficiently knockdown expression of a target gene in an isolated population to ensure results observed from assays are representative of interruption of the target gene and not a false positive or false negative result.

Regarding Claim 89, Stcube discloses the composition of claim 86, wherein each of said separate populations of immune cells are contained with separate compartments of one or more arrays (The method can include culturing separately... modified T cells and suppressor cells; wherein the modified T cells have a candidate gene knocked-down or knocked-out, Para. [0107]; In one embodiment, the method is conducted in arrayed format in a high throughput setting, Para. [0124]).

Regarding Claim 90, Stcube discloses the composition of claim 86, but fails to explicitly disclose wherein said plurality of separate populations of immune cells comprises at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 5000, 10000, 50000, or 100000 separate populations of immune cells.
However, it would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Stcube to include using at least 10 cells per separate population, since the general conditions of the claim are disclosed in the prior art, discovering the optimum or workable ranges involves only routine skill in the art. The motivation for doing so would be to use a sufficient number of cells to generate a detectable response for determining if interruption of a target gene affects T cell responses.

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Regarding Claim 91, Stcube discloses a composition comprising a plurality of cell populations (These examples are meant to be illustrative of pharmaceutical compositions and dosage forms provided herein, but are not in any way limiting, Para. [0212]; Accordingly, in some embodiments, provided herein are in vitro methods for screening a target gene for cancer therapy including culturing separately... (b) modified T cells and suppressor cells; wherein the modified T cells have a candidate gene knocked-down or knocked-out, Para. [0093]; The modified T cell can overexpress or underexpress a gene as compared to a wild type T cell. The modified T cell can have a candidate gene knocked-down or knocked out. A population of modified T cell can be a population of T cells with different genetic modification. For example, a population of modified T cell can each have different genetic signatures with at most one gene knocked-down or knocked-out as compared to a wild type T cell, Para. [0026]) that each comprise i) a plurality of immune cells that express an exogenous cellular receptor (In some embodiments, the T cells are isolated from a subject. The subject can be a mammal. In some embodiments, the T cells are isolated form a mouse.... In some embodiments, the mouse is genetically engineered to expresses a T-cell receptor, Para. [0051]) and ii) cells that express a cognate antigen of said exogenous cellular receptor (In other embodiments, provided herein are methods of assessing the development of T cell effector function in response to antigen-specific activation. Assessing the cytotoxic activity of T cells, elicited by antigen-specific stimulation in vitro, allows one to assess the functional cytolytic potential of effector cells, Para. [0104]; In some embodiments, the antigen is a protein that is not naturally present in the target cells, Para. [0106]; By way of example, some vectors used in recombinant DNA techniques allow entities, such as a segment of DNA (such as a heterologous DNA segment, such as a heterologous cDNA segment), to be transferred into a target cell, Para. [0183]); wherein each of said plurality of immune cells comprises an altered genome sequence of a single candidate gene, and wherein said single candidate gene is different for each of said cell populations (The modified T cell can have a candidate gene knocked-down or knocked out, Para. [0026]; The unmodified T cells can be genetically modified using methods described herein or otherwise known in the art to produce modified T cells.... In some embodiments, the gene in the isolated T cells is knocked-out or knocked-down by using CAS9 (CRISPR), Para. [0054]).
Stcube fails to explicitly disclose said plurality of populations are separated.
However, it would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Stcube to include separate populations of cells comprising different single gene modifications, since separating parts that were once integral involves only routine skill in the art. The motivation for doing so would be to separately and independently test different immune cells comprising different genetic modifications to determine which modifications can enhance or reduce antigen responses to determine optimal treatments for cancer.

Regarding Claim 92, Stcube discloses the composition of claim 91, but fails to explicitly disclose wherein at least 70%, 80%, or 90% of said plurality of immune cells of each separate cell population comprises said altered genome sequence of said single candidate gene. However, it would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Stcube to include generating a population of immune cells having at least 70% genomic disruption of a target gene, since where the general conditions of the claim are disclosed in the prior art, discovering the optimum or workable ranges involves only routine skill in the art. The motivation for doing so would be sufficiently knockdown expression of a target gene in an isolated population to ensure results observed from assays are representative of interruption of the target gene and not a false positive or false negative result.

Regarding Claim 93, Stcube discloses the composition of claim 91, wherein each of said separate cell populations are contained with separate compartments of one or more arrays (The method can include culturing separately... modified T cells and suppressor cells; wherein the modified T cells have a candidate gene knocked-down or knocked-out, Para. [0107]; In one embodiment, the method is conducted in arrayed format in a high throughput setting, Para. [0124]).

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In case **the space in any of the preceding boxes is not sufficient.**
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Claims 186-188 lack an inventive step under PCT Article 33(3) as being obvious over Dana-Farber Cancer Institute, Inc. (hereinafter Dana-Farber).

Regarding Claim 186, Dana-Farber discloses a composition comprising a plurality of populations of cancer cells (In one aspect, an in vivo method of identifying a cancer cell modulator of response to an anti-cancer immunotherapy, comprising a) obtaining at least a first population of cancer cells, wherein the cancer cells are syngeneic to a first and second subject, wherein the first subject is immunocompetent and the second subject is immuno-incompetent; b) obtaining at least a second population of cancer cells, wherein the at least first population of cancer cells are genetically engineered to comprise at least one genetic modification, Para. [0004]; In still another embodiment, the reporter is used to identify and/or isolate the genetically modified cancer cells in the at least second population of cancer cells, Para. [0007]; In one embodiment, the cells are isolated from the subject. The term "isolated" refers to a product, compound, or composition which is separated from at least one other product, compound, or composition with which it is associated in its naturally occurring state, whether in nature or as made synthetically. In other embodiments, "isolated" means that desired cells are physically separated from other cell populations, Para. [0156]), wherein each population of cancer cells comprises a plurality of cancer cells that i) expresses an antigen (Target cells may be transduced in any way that allows the virus to contact the target cells in which delivery of a sequence containing a gene of interest is desired, Para. [0103]; Such cell-based immunotherapies can be further modified to express one or more gene products to further modulate immune responses, such as... to express tumor-associated antigen (TAA) antigens, Para. [0136]); and ii) comprise a CRISPR system that comprises a guide nucleic acid that binds a portion of a single candidate gene, wherein said single candidate gene is different for each of said separate populations of cancer cells; and an exogenous nuclease, or a nucleic acid encoding said exogenous nuclease (In another embodiment, the at least first population of cells is contacted with a vector comprising an exogenous nucleic acid, wherein the vector 1) integrates into a chromosome or 2) exists as an extrachromosomal nucleic acid compartment of the cell, and expresses exogenous nucleic acids or proteins in the cell to generate the at least one genetic modification in the at least second population of cancer cells.... In another embodiment, the exogenous nucleic acid comprises a) an engineered, non-naturally occurring Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) guide RNA that hybridizes with a target nucleic acid sequence of interest and/or b) a nucleotide sequence encoding a Type-II Cas9 protein, optionally wherein the cells are transgenic for Cas9, Para. [0007]; A syngeneic transplant can be "congenic" if the transferred cells and cells of the subject differ in defined loci, such as a single locus, typically by inbreeding. The term "congenic" refers to deriving from, originating in, or being members of the same species, where the members are genetically identical except for a small genetic region, typically a single genetic locus (i.e., a single gene). A "congenic transplant" refers to transfer of cells or organs from a donor to a recipient, where the recipient is genetically identical to the donor except for a single genetic locus, Para. [0024]).
Dana-Farber fails to explicitly disclose said cell populations are separated.
However, it would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Dana-Farber to include separate populations of cells comprising different single gene modifications, since separating parts that were once integral involves only routine skill in the art. The motivation for doing so would be to separately and independently test different immune cells comprising different genetic modifications to determine which modifications can enhance or reduce antigen responses to determine optimal treatments for cancer.

Regarding Claim 187, Dana-Farber discloses the composition of claim 186, wherein said population of said plurality of cancer cells of each separate population comprises a genomic disruption in said single candidate gene (The term "congenic" refers to deriving from, originating in, or being members of the same species, where the members are genetically identical except for a small genetic region, typically a single genetic locus (i.e., a single gene). A "congenic transplant" refers to transfer of cells or organs from a donor to a recipient, where the recipient is genetically identical to the donor except for a single genetic locus, Para. [0024]).

Regarding Claim 188, Dana-Farber discloses the composition of claim 187, but fails to explicitly disclose wherein at least 70%, 80%, or 90% of said plurality of cancer cells of each separate population comprises a genomic disruption in said single candidate gene.
However, it would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Dana-Farber to include generating a population of immune cells having at least 70% genomic disruption of a target gene, since where the general conditions of the claim are disclosed in the prior art, discovering the optimum or workable ranges involves only routine skill in the art. The motivation for doing so would be sufficiently knockdown expression of a target gene in an isolated population to ensure results observed from assays are representative of interruption of the target gene and not a false positive or false negative result.

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Claims 95, 100-108, and 191-193 lack an inventive step under PCT Article 33(3) as being obvious over Stcube & Co., Inc. et al. (hereinafter Stcube) in view of Dana-Farber Cancer Institute, Inc. (hereinafter Dana-Farber).

Regarding Claim 95, Stcube discloses a method of screening a plurality of single candidate genes (Provided herein are in vitro and in vivo screening methods for identifying gene targets for cancer therapy, Para. [0032]), said method comprising: a. obtaining a plurality of populations of cells that express an antigen, wherein each population comprises a plurality of cells (In other embodiments, provided herein are methods of assessing the development of T cell effector function in response to antigen-specific activation. Assessing the cytotoxic activity of T cells, elicited by antigen-specific stimulation in vitro, allows one to assess the functional cytolytic potential of effector cells, Para. [0104]; In some embodiments, the antigen is a protein that is not naturally present in the target cells, Para. [0106]; By way of example, some vectors used in recombinant DNA techniques allow entities, such as a segment of DNA (such as a heterologous DNA segment, such as a heterologous cDNA segment), to be transferred into a target cell, Para. [0183]); c. performing an in vitro assay that comprises contacting in vitro said plurality of engineered cells with a plurality of immune cells that express a cellular receptor, or functional fragment thereof, that binds to said antigen (In other embodiments, provided herein are methods of assessing the development of T cell effector function in response to antigen-specific activation. Assessing the cytotoxic activity of T cells, elicited by antigen-specific stimulation in vitro, allows one to assess the functional cytolytic potential of effector cells, Para. [0104]); and d. obtaining a readout from said in vitro assay, to thereby determine an effect of said genomic disruption that suppresses expression of said single candidate gene on said plurality of populations of engineered cancer cells or said immune cells that express a cellular receptor, or functional fragment thereof, that binds to said antigen (In certain embodiments, evaluation of cytolytic activity by 51Cr release, can be used to investigate T cell-specific effector function and its modulation/inhibition by immunomodulatory cells, while preserving the simplicity of the assay and allowing the investigator to work with numerous variables and rare cell populations. Chromium release assay can provide a more specific functional readout, Para. [0104]).
Stcube fails to explicitly disclose said antigen-expressing cells are separated and are cancer cells; and b. introducing into each of said separate populations of cancer cells a CRISPR system that comprises: i. a guide nucleic acid that binds a portion of a single candidate gene, wherein said single candidate gene is different for each of said separate populations of cancer cells; and ii. an exogenous nuclease, or a nucleic acid encoding said exogenous nuclease; thereby generating a plurality of separate populations of engineered cancer cells that comprise a genomic disruption in said single candidate gene, wherein said genomic disruption suppresses expression of said single candidate gene; and said engineered cells of c. and d. are cancer cells.
However, it would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Stcube to include separate populations of cells comprising different single gene modifications, since separating parts that were once integral involves only routine skill in the art. The motivation for doing so would be to separately and independently test different immune cells comprising different genetic modifications to determine which modifications can enhance or reduce antigen responses to determine optimal treatments for cancer.
Dana-Farber teaches populations of cancer cells expressing antigens genetically modified at a single locus (In one aspect, an in vivo method of identifying a cancer cell modulator of response to an anti-cancer immunotherapy, comprising a) obtaining at least a first population of cancer cells, wherein the cancer cells are syngeneic to a first and second subject, wherein the first subject is immunocompetent and the second subject is immuno-incompetent; b) obtaining at least a second population of cancer cells, wherein the at least first population of cancer cells are genetically engineered to comprise at least one genetic modification, Para. [0004]; In another embodiment, the at least first population of cells is contacted with a vector comprising an exogenous nucleic acid, wherein the vector 1) integrates into a chromosome or 2) exists as an extrachromosomal nucleic acid compartment of the cell, and expresses exogenous nucleic acids or proteins in the cell to generate the at least one genetic modification in the at least second population of cancer cells.... In another embodiment, the exogenous nucleic acid comprises a) an engineered, non-naturally occurring Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) guide RNA that hybridizes with a target nucleic acid sequence of interest and/or b) a nucleotide sequence encoding a Type-II Cas9 protein, optionally wherein the cells are transgenic for Cas9, Para. [0007]; A syngeneic transplant can be "congenic" if the transferred cells and cells of the subject differ in defined loci, such as a single locus, typically by inbreeding. The term "congenic" refers to deriving from, originating in, or being members of the same species, where the members are genetically identical except for a small genetic region, typically a single genetic locus (i.e., a single gene). A "congenic transplant" refers to transfer of cells or organs from a donor to a recipient, where the recipient is genetically identical to the donor except for a single genetic locus, Para. [0024]; Such cell-based immunotherapies can be further modified to express one or more gene products to further modulate immune responses, such as expressing cytokines like GM-C SF, and/or to express tumor-associated antigen (TAA) antigens, Para. [0136]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Stcube with the teaching of Dana-Farber for the purpose of creating populations of cancer cells genetically modified at a single locus that can be used to determine the effects of said cancer cells when contacted by immune cells expressing a cognate receptor for the cancer antigen to determine if the disrupted gene is a potential target for cancer therapy.

Regarding Claim 100, modified Stcube discloses the method of claim 95. Stcube further discloses wherein said readout comprises determining a level of cytolytic activity of said plurality of immune cells (Assessing the cytotoxic activity of T cells, elicited by antigen-specific stimulation in vitro, allows one to assess the functional cytolytic potential of effector cells, Para. [0104]).

Regarding Claim 101, modified Stcube discloses the method of claim 100. Stcube further discloses wherein said level of cytolytic activity is determined by a chromium release assay, an electrical impedance assay, time-lapse microscopy, or a co-culture assay (Chromium release assay can provide a more specific functional readout, Para. [0104]).

Regarding Claim 102, modified Stcube discloses the method of claim 95. Stcube further discloses wherein said readout comprises determining a level of proliferation of said plurality of immune cells (The in vitro screening methods can measure activities including, but not limited to proliferation, Para. [0097]).

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Regarding Claim 103, modified Stcube discloses the method of claim 102. Stcube further discloses wherein said level of proliferation is determined by a Carboxyfluorescein Succinimidyl Ester (CFSE) assay, microscopy, an electrical impedance assay, or flow cytometry (In another embodiment, the proliferation is determined by flowcytometric analysis of T cells labeled with carboxyfluorescein succinimidyl ester (CFSE), Para. [0122]).

Regarding Claim 104, modified Stcube discloses the method of claim 95. Stcube further discloses wherein said readout comprises determining a level of a factor expressed by said plurality of immune cells (The activity can also be the immunity of effector T cells, including such as cytotoxic activity or cytokine production activity, Para. [0027]).

Regarding Claim 105, modified Stcube discloses the method of claim 104. Stcube further discloses wherein said factor is a protein (The activity can also be the immunity of effector T cells, including such as cytotoxic activity or cytokine production activity, Para. [0027]).

Regarding Claim 106, modified Stcube discloses the method of claim 105. Stcube further discloses wherein said protein is secreted from said population of engineered immune cells (The activity can also be the immunity of effector T cells, including such as cytotoxic activity or cytokine production activity, Para. [0027]; In addition to surface markers, different subtypes of T cells can also have distinct function and cytokine secretion profiles, Para. [0023]).

Regarding Claim 107, modified Stcube discloses the method of claim 104. Stcube further discloses wherein said protein is a cytokine or chemokine (The activity can also be the immunity of effector T cells, including such as cytotoxic activity or cytokine production activity, Para. [0027]; In addition to surface markers, different subtypes of T cells can also have distinct function and cytokine secretion profiles, Para. [0023]).

Regarding Claim 108, modified Stcube discloses the method of claim 104. Stcube further discloses wherein said protein is a cell surface protein (The activity can also be the immunity of effector T cells, Para. [0027]; Differential expression of markers on the cell surface provide valuable clues to the diverse nature and function of T cells, and can serve as useful tools for the isolation of T cells or specific subtypes of T cells, Para. [0023]).

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Regarding Claim 191, Stcube discloses a composition comprising a plurality of cell populations (These examples are meant to be illustrative of pharmaceutical compositions and dosage forms provided herein, but are not in any way limiting, Para. [0212]; Accordingly, in some embodiments, provided herein are in vitro methods for screening a target gene for cancer therapy including culturing separately... (b) modified T cells and suppressor cells; wherein the modified T cells have a candidate gene knocked-down or knocked-out, Para. [0093]; The modified T cell can overexpress or underexpress a gene as compared to a wild type T cell. The modified T cell can have a candidate gene knocked-down or knocked out. A population of modified T cell can be a population of T cells with different genetic modification. For example, a population of modified T cell can each have different genetic signatures with at most one gene knocked-down or knocked-out as compared to a wild type T cell, Para. [0026]) that each comprise: i) a plurality of cells that express an antigen (In other embodiments, provided herein are methods of assessing the development of T cell effector function in response to antigen-specific activation. Assessing the cytotoxic activity of T cells, elicited by antigen-specific stimulation in vitro, allows one to assess the functional cytolytic potential of effector cells, Para. [0104]; In some embodiments, the antigen is a protein that is not naturally present in the target cells, Para. [0106]; By way of example, some vectors used in recombinant DNA techniques allow entities, such as a segment of DNA (such as a heterologous DNA segment, such as a heterologous cDNA segment), to be transferred into a target cell, Para. [0183]); and ii) cells that express a cellular receptor, or functional fragment thereof, that binds to said antigen (In some embodiments, the T cells are isolated from a subject. The subject can be a mammal. In some embodiments, the T cells are isolated form a mouse.... In some embodiments, the mouse is genetically engineered to expresses a T-cell receptor, Para. [0051]); wherein each of said plurality of cells comprises an altered genome sequence of a single candidate gene, and wherein said single candidate gene is different for each of said cell populations (The modified T cell can have a candidate gene knocked-down or knocked out, Para. [0026]; The unmodified T cells can be genetically modified using methods described herein or otherwise known in the art to produce modified T cells.... In some embodiments, the gene in the isolated T cells is knocked-out or knocked-down by using CAS9 (CRISPR), Para. [0054]). Stcube fails to explicitly disclose said cell populations are separated, and said plurality of cells expressing an antigen are cancer cells. However, it would have been obvious to one of ordinary skill in the art at the time of the invention to modify the method of Stcube to include separate populations of cells comprising different single gene modifications, since separating parts that were once integral involves only routine skill in the art. The motivation for doing so would be to separately and independently test different immune cells comprising different genetic modifications to determine which modifications can enhance or reduce antigen responses to determine optimal treatments for cancer.
Dana-Farber teaches population of cancer cells expressing a cancer antigen and comprise a modified gene (In one aspect, an in vivo method of identifying a cancer cell modulator of response to an anti-cancer immunotherapy, comprising a) obtaining at least a first population of cancer cells, wherein the cancer cells are syngeneic to a first and second subject, wherein the first subject is immunocompetent and the second subject is immuno-incompetent; b) obtaining at least a second population of cancer cells, wherein the at least first population of cancer cells are genetically engineered to comprise at least one genetic modification, Para. [0004]; In another embodiment, the at least first population of cells is contacted with a vector comprising an exogenous nucleic acid, wherein the vector 1) integrates into a chromosome or 2) exists as an extrachromosomal nucleic acid compartment of the cell, and expresses exogenous nucleic acids or proteins in the cell to generate the at least one genetic modification in the at least second population of cancer cells.... In another embodiment, the exogenous nucleic acid comprises a) an engineered, non-naturally occurring Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) guide RNA that hybridizes with a target nucleic acid sequence of interest and/or b) a nucleotide sequence encoding a Type-II Cas9 protein, optionally wherein the cells are transgenic for Cas9, Para. [0007]; A syngeneic transplant can be "congenic" if the transferred cells and cells of the subject differ in defined loci, such as a single locus, typically by inbreeding. The term "congenic" refers to deriving from, originating in, or being members of the same species, where the members are genetically identical except for a small genetic region, typically a single genetic locus (i.e., a single gene). A "congenic transplant" refers to transfer of cells or organs from a donor to a recipient, where the recipient is genetically identical to the donor except for a single genetic locus, Para. [0024]; Such cell-based immunotherapies can be further modified to express one or more gene products to further modulate immune responses, such as expressing cytokines like GM-C SF, and/or to express tumor-associated antigen (TAA) antigens, Para. [0136]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Stcube with the teaching of Dana-Farber for the purpose of creating populations of cancer cells genetically modified at a single locus that can be used to determine the effects of said cancer cells when contacted by immune cells expressing a cognate receptor for the cancer antigen to determine if the disrupted gene is a potential target for cancer therapy.

Regarding Claim 192, modified Stcube discloses the composition of claim 191. Stcube fails to explicitly disclose wherein at least 70%, 80%, or 90% of said population of said plurality of cancer cells of each separate cell populations comprises said altered genome sequence of said single candidate gene.
It would have been obvious to one of ordinary skill in the art at the time the invention was made to generate a population of immune cells having at least 70% genomic disruption of a target gene, since where the general conditions of the claim are disclosed in the prior art, discovering the optimum or workable ranges involves only routine skill in the art. The motivation for doing so would be sufficiently knockdown expression of a target gene in an isolated population to ensure results observed from assays are representative of interruption of the target gene and not a false positive or false negative result.

Regarding Claim 193, modified Stcube discloses the composition of claim 191. Stcube further discloses wherein each of said separate cell populations are contained with separate compartments of one or more arrays (The method can include culturing separately... modified T cells and suppressor cells; wherein the modified T cells have a candidate gene knocked-down or knocked-out, Para. [0107]; In one embodiment, the method is conducted in arrayed format in a high throughput setting, Para. [0124]).

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Claims 96-99 lack an inventive step under PCT Article 33(3) as being obvious over Stcube & Co., Inc. et al. (hereinafter Stcube) in view of Dana-Farber Cancer Institute, Inc. (hereinafter Dana-Farber) and Vivia Biotech, S.L. (hereinafter Vivia Biotech).

Regarding Claim 96, modified Stcube discloses the method of claim 95. Stcube fails to explicitly disclose wherein said readout comprises determining a level of cell death of each of said separate populations of engineered cancer cells.
Vivia Biotech teaches determining levels of cell death of cancer cells using flow cytometry (Described herein is a cell-based screening platform that incorporates both automated sample preparation and automated evaluation by flow cytometry that is useful as a personalized medicine test because of its rapid data acquisition, analysis, and reporting of results, Para. [0002]; In an embodiment, a method for analyzing cellular responsiveness to drugs is provided, comprising: obtaining a sample of a tissue from a hematological neoplasm that has been withdrawn from a patient; dividing the sample of tissue into at least 35 aliquots; combining the at least 35 aliquots each having a drug composition; and measuring apoptosis in at least one cell population in each of the at least 35 aliquots, Para. [0015]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Stcube with the teaching of Vivia Biotech for the purpose of determining if a genetic modification of a cancer cell is a potential therapeutic target based on the level of cell death when contacted with a therapeutic composition.

Regarding Claim 97, modified Stcube discloses the method of claim 96. Stcube fails to explicitly disclose wherein said level of cell death is determined by flow cytometry or microscopy.
Vivia Biotech teaches determining levels of cell death of cancer cells using flow cytometry (Described herein is a cell-based screening platform that incorporates both automated sample preparation and automated evaluation by flow cytometry that is useful as a personalized medicine test because of its rapid data acquisition, analysis, and reporting of results, Para. [0002]; In an embodiment, a method for analyzing cellular responsiveness to drugs is provided, comprising: obtaining a sample of a tissue from a hematological neoplasm that has been withdrawn from a patient; dividing the sample of tissue into at least 35 aliquots; combining the at least 35 aliquots each having a drug composition; and measuring apoptosis in at least one cell population in each of the at least 35 aliquots, Para. [0015]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Stcube with the teaching of Vivia Biotech for the purpose of determining if a genetic modification of a cancer cell is a potential therapeutic target based on the level of cell death when contacted with a therapeutic composition.

Regarding Claim 98, modified Stcube discloses the method of claim 95. Stcube fails to explicitly disclose wherein said readout comprises determining a time to which a certain percentage of cells each of said separate populations of engineered cancer cells are killed.
Vivia Biotech teaches determining levels of cell death of cancer cells over a period of time using flow cytometry (Described herein is a cell-based screening platform that incorporates both automated sample preparation and automated evaluation by flow cytometry that is useful as a personalized medicine test because of its rapid data acquisition, analysis, and reporting of results, Para. [0002]; In an embodiment, a method for analyzing cellular responsiveness to drugs is provided, comprising: obtaining a sample of a tissue from a hematological neoplasm that has been withdrawn from a patient; dividing the sample of tissue into at least 35 aliquots; combining the at least 35 aliquots each having a drug composition; and measuring apoptosis in at least one cell population in each of the at least 35 aliquots, Para. [0015]; One advantage is that the methods can analyze cellular responses to a large number of variables, including many drug compositions and different incubation times, Para. [0083]; FIG. 8 also indicates that the methods described herein are useful for measuring the ability of different drug compositions to induce apoptosis at different time periods, Para. [0140]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Stcube with the teaching of Vivia Biotech for the purpose of determining if a genetic modification of a cancer cell is a potential therapeutic target based on the level of cell death when contacted with a therapeutic composition.

Regarding Claim 99, modified Stcube discloses the method of claim 98. Stcube fails to explicitly disclose wherein said level of cell death is determined by flow cytometry or microscopy.
Vivia Biotech teaches determining levels of cell death of cancer cells over a period of time using flow cytometry (Described herein is a cell-based screening platform that incorporates both automated sample preparation and automated evaluation by flow cytometry that is useful as a personalized medicine test because of its rapid data acquisition, analysis, and reporting of results, Para. [0002]; In an embodiment, a method for analyzing cellular responsiveness to drugs is provided, comprising: obtaining a sample of a tissue from a hematological neoplasm that has been withdrawn from a patient; dividing the sample of tissue into at least 35 aliquots; combining the at least 35 aliquots each having a drug composition; and measuring apoptosis in at least one cell population in each of the at least 35 aliquots, Para. [0015]; One advantage is that the methods can analyze cellular responses to a large number of variables, including many drug compositions and different incubation times, Para. [0083]; FIG. 8 also indicates that the methods described herein are useful for measuring the ability of different drug compositions to induce apoptosis at different time periods, Para. [0140]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Stcube with the teaching of Vivia Biotech for the purpose of determining if a genetic modification of a cancer cell is a potential therapeutic target based on the level of cell death when contacted with a therapeutic composition.

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Claim 189 lacks an inventive step under PCT Article 33(3) as being obvious over Dana-Farber Cancer Institute, Inc. (hereinafter Dana-Farber) in view of Stcube & Co., Inc. et al. (hereinafter Stcube).

Regarding Claim 189, Dana-Farber discloses the composition of claim 186. Dana-Farber fails to explicitly disclose wherein each of said separate populations of cancer cells are contained with separate compartments of one or more arrays.
Stcube teaches separating populations of cells comprising different mutated loci into different compartments in an array (The method can include culturing separately... modified T cells and suppressor cells; wherein the modified T cells have a candidate gene knocked-down or knocked-out, Para. [0107]; In one embodiment, the method is conducted in arrayed format in a high throughput setting, Para. [0124]).
It would have been obvious to one of ordinary skill in the art at the time of the invention to modify Dana-Farber with the teaching of Stcube for the purpose of separating cells comprising different individual genetic modifications to determine if a response to a genetically modified cancer cell is associated with a specific gene.

Claims 1-10, 86-93, 95-108, 186-189, and 191-193 meet the criteria set out in PCT Article 33(4), and thus have industrial applicability because the subject matter claimed can be made or used in industry.