

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

MILTENYI BIOMEDICINE GmbH and MILTENYI BIOTEC INC.
Petitioner

v.

FRED HUTCHINSON CANCER CENTER
Patent Owner

IPR Trial No. IPR2023-
U.S. Patent No. 9,987,308
Issue Date: June 5, 2018

Title: Method and Compositions for Cellular Immunotherapy

DECLARATION OF DR. JONATHAN BRAMSON

**IN SUPPORT OF PETITION FOR INTER PARTES REVIEW OF
U.S. PATENT NO. 9,987,308**

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I. INTRODUCTION

1. I, Jonathan Bramson, Ph.D., have been retained as an expert witness on behalf of Miltenyi Biomedicine GmbH and Miltenyi Biotec Inc. (collectively, “Petitioner”) in connection with its petition (“Petition”) for inter partes review of U.S. Patent 9,987,308 (“the ’308 patent”) (Ex. 1001). I understand that the ’308 patent is owned by the Fred Hutchinson Cancer Center (“Patent Owner”).

2. I understand that this declaration is being submitted in support of Petitioner’s Petition of claims 1-16 and 18-31 (“Challenged Claims”) of the ’308 patent, which issued from U.S. Application No. 14/006,641 on June 5, 2018. The ’308 patent names Stanley R. Riddell and Michael Hudecek as inventors.

3. I have reviewed the ’308 patent and in my review, considered the teachings of the scientific literature before March 23, 2011.

4. I am being compensated at my usual and customary rate of \$425 per hour. My compensation is in no way dependent on the outcome of this case.

5. In the last 4 years, I served as an expert in, and was deposed and/or testified in connection with, the cases listed in Appendix B.

II. EDUCATION AND EXPERIENCE

6. A copy of my curriculum vitae, which describes my qualifications in greater detail and includes a list of publications that I authored in the past 30 years, is attached as Appendix A.

7. I am an expert in the field of engineered T-cell therapy, including chimeric antigen receptor (“CAR”)-T cells. I have significant experience designing and constructing CAR-T cells and other T-cell immunotherapies. I also have significant experience developing novel immunotherapies, including CAR T-cell therapies.

8. I received my B.Sc. in Biochemistry from McGill University in 1991. I received my Ph.D. in Experimental Medicine from McGill University in 1994.

9. I am currently Vice Dean of Research at the Faculty of Health Sciences, McMaster University. Prior to that, I served as Assistant Dean, Research Infrastructure, at the Faculty of Health Sciences, as well as Director at the Fraunhofer-McMaster Project Center. I am, and have been since 2021, a professor in the Department of Medicine at McMaster University. Before joining Medicine, I was a professor in Pathology and Molecular Medicine at McMaster from 2009 – 2020, having been a member of that department since joining McMaster as an Assistant Professor in 1999. For a period of over 5 years (2009 – 2014), I also served as Director at the McMaster Immunology Research Centre. Before entering academia, I was a research scientist and project leader at Inex Pharmaceuticals for two years (1997 – 1999).

10. I am currently a chair officer for three committees, councils, or panels, those being Triumvira Immunologics Scientific Advisory Board, Chronic Pain

Centre of Excellence Board of Directors, and CIHR Operating Grant Review Panel Cancer Biology-Therapeutics 2. I am an active member of five others, those being NIH Immuno-oncology Research Grant Review Panel, McMaster Innovation Park Board of Directors, Council of Ontario Faculties of Medicine Research Committee, Association of Faculties of Medicine of Canada Research Council, and the Canadian Cancer Immunotherapy Consortium. In the past, I have been a member, organizer, or consultant for numerous others, for which details can be found in my curriculum vitae.

11. My most recent speaking experience was at the 13th Annual Myeloma Canada Scientific Roundtable, which took place in Montreal in November 2022. I presented on the status of engineered cell therapy research and clinical trials, including academic and commercial CAR-T cells, at McMaster University and our partner hospital, Hamilton Health Sciences Corporation. Before that, for the year of 2022, I spoke in six other conferences, including three in Canada and one in Pennsylvania. Due to the COVID-19 pandemic, I only spoke in two conferences in 2021, but before that I made an effort to speak in at least 4-5 conferences a year, preferably more. All of my topics circled around gene therapy, and later in my career I focused exclusively on engineered T cell cancer immunotherapy. I have been speaking and giving oral presentations on these topics since 2001.

12. Since 2012, I have served as an expert consultant to various biotechnology companies. In these roles, I have provided expert advice on program development.

13. I have co-authored various publications on CAR-T cells, including those targeting CD19, HER2, and ROR1. I have also consulted about the impact of immunophenotypes on T-cell adoptive transfer.

14. I have supported clinical trials of engineered T cells (NCT03880279, NCT04727151).

III. MATERIALS CONSIDERED

15. In forming the opinions in this declaration, I considered the materials cited in this declaration from the perspective of the general knowledge in the art before March 23, 2011.

16. I also relied on my education and knowledge, as well as my experience in academic and clinical research projects, including those discussed in Section II.

17. I reserve the right to amend and supplement any of the opinions in this declaration as needed in response to new information and evidence made available to me, additional analysis that leads me to conclude that supplementation is necessary, new issues that may arise, any discovery, arguments, evidence, or testimony presented in this inter partes review, and any Patent Trial and Appeal Board (“Board”) decisions or orders.

IV. TECHNOLOGY BACKGROUND

18. Below I provide a brief background of the state of the art before March 23, 2011 to support my opinions on anticipation and obviousness discussed in Sections X-XIII below. A person of ordinary skill in the art (“POSA”) also would have been aware of additional references related to T cells and CARs.

A. T Cells

19. Lymphocytes are a type of white blood cell, and T cells are a type of lymphocyte. T cells work as part of the immune system by detecting foreign invaders and producing helpful cytokines in response. Cytokines are protein-based molecules that effect an immune response. *See, e.g.*, Ex. 1018, [0005], [0016], [0009] (“transferred T cells ... directly recognize and lyse tumor targets, produce cytokines that recruit and activate antigen non-specific anti-tumor effector cells ...”); Ex. 1001, 2:1-31.

20. T cells are commonly distinguished based on the presence or absence of surface proteins, CD4 and CD8. T cells that express the CD4 membrane glycoprotein (“CD4”) are typically referred to as CD4⁺ T cells. Based on their functional attributes, CD4⁺ cells have also been historically described as “helper T cells”. T cells that express the CD8 membrane glycoprotein (“CD8”), are typically referred to as CD8⁺ T cells. Based on their functional attributes, CD8⁺ cells have also been historically described as “cytotoxic T cells.” *See, e.g.*, Ex. 1001, 7:7-11,

7:66-8:3, 9:4-13, 9:57-10:5, 10:66-11:17, 11:43-51, 26:45-48. A glycoprotein is a protein with an attached carbohydrate group. In immunology, cells that express a high level of a membrane protein can be described with the name of the protein followed by a “+” sign (*e.g.*, CD4⁺ or CD8⁺ T cells). Immunologists generally use a “-” sign if a cell expresses a low level of a protein or complete absence thereof (*e.g.*, CD4⁻ or CD8⁻ T cells). *See, e.g.*, Ex. 1018, [0018], [0037]; Ex. 1007, 227-28; Ex. 1001, 25:35-44.

B. Subpopulations of T Cells

21. Both CD4⁺ and CD8⁺ T cells include several categories of T-cell subpopulations that differ in phenotype, function, and homing to their respective *in vivo* targets. Although there are many subpopulations of T cells, the subpopulations most relevant to the '308 patent are “naïve” T cells, “central-memory” T cells, “effector-memory” T cells, and “effector” T cells. *See, e.g.*, Ex. 1011, 525; Ex. 1007, 227-28; Ex. 1020, 64, Ex. 1027, 12; Ex. 1001, 1:49-67.

22. Naïve T cells (“T_N”) are mature T cells that have not yet encountered antigenic material to which they can bind. An antigen is a substance capable of stimulating an immune response. Naïve T cells are activated by stimulation from antigenic material, most commonly short peptides known as epitopes. Once stimulated, T_N cells activate, proliferate (*i.e.*, multiply), and produce cytokines. *See, e.g.*, Ex. 1007, 227-28; Ex. 1018, [0070]; Ex. 1001, 7:30-37, 11:52-67.

23. Upon activation, T_N cells may differentiate into antigen-specific central-memory T cells (“ T_{CM} ”), effector-memory T cells (“ T_{EM} ”), or effector T cells (“ T_E ”). T_{CM} and T_{EM} also have the capacity to differentiate into T_E cells upon secondary exposure to epitopes from their target antigen. *See, e.g.*, Ex. 1011, 525; Ex. 1001, 1:59-62; 7:12-42.

24. Generally speaking, T_E cells are the T-cell subpopulation primarily responsible for killing or eliminating infected cells from the body. However, they are short-lived. Although T_E cells typically die after their target antigen clears from the body, T_{CM} and T_{EM} cells linger, and are capable of responding in the event of re-exposure to the antigen. After re-exposure to an antigen, T_{CM} can differentiate into T_{CM} -derived T_E cells. *See, e.g.*, Ex. 1011, 525 (“ T_{CM} and T_{EM} cells . . . respond to antigen re-exposure by differentiating into a new wave of T_E cells.”); *id.* (“some of the progeny of clonally derived T_E cells retain intrinsic programming of the parental cell of origin”); *see also* Ex. 1001, 1:59-62 (“ $CD8^+$ T_{CM} and T_{EM} both differentiate into cytolytic effector T cells (T_E) that express a high level of granzymes and perforin, but are short-lived.”). T_{EM} cells differentiate into T_{EM} -derived T_E cells. *See, e.g., id.* There are intrinsic differences between T_{CM} -derived, and T_{EM} -derived, T_E cells, including greater persistence in T_{CM} -derived T_E cells. *See* Ex. 1011, 525 (“T-cell clones derived from T_{CM} , but not those derived from T_{EM} cells were capable of persisting long-term”). T_{EM} -derived T_E cells die rapidly after transfer. *See*

Ex. 1007, 228 (“T cells derived from T_{EM} precursors die rapidly after cell transfer and do not establish persistent T cell memory.”).

25. A T cell’s persistence refers to its ability to survive and continue to perform immune system functions and proliferate continually. T_{CM} cells live, and thus persist, longer than T_E cells. *See, e.g.*, Ex. 1015, 3360, 3363-64; Ex. 1026, 445-47, Ex. 1016, 2995, 2999-3002; Ex. 1007, 226-30; Ex. 1011, 525; Ex. 1017, 926-27; Ex. 1001, 1:31-48, 8:49-52, 27:4-7.

C. Markers of T-Cell Subpopulations

26. T-cell subpopulations such as T_N, T_{CM}, T_{EM}, and T_E may be defined by their expression of various proteins expressed on the cell surface. These proteins may be referred to as “T-cell markers,” and can be used by researchers to understand what kinds of T cells make up a given composition. The collection of a T-cell’s markers is referred to as the T cell’s immunophenotype. *See, e.g.*, Ex. 1003, 2966-67; Ex. 1027, 2-3, 7; Ex. 1028, S184; ; Ex. 1001, 7:12-42, 10:66-11:21.

27. There are many T-cell markers, but the ’308 patent claims recite only a subset of them, including CD45RA, CD45RO, and CD62L. All were well-known T-cell markers prior to March 23, 2011. *See, e.g.*, Ex. 1007, 227-28; Ex. 1004, 788; Ex. 1001, 7:12-42.

28. In fact, as of March 23, 2011, researchers understood that those markers could be used to identify T-cell populations. According to Berger, C. *et al.*,

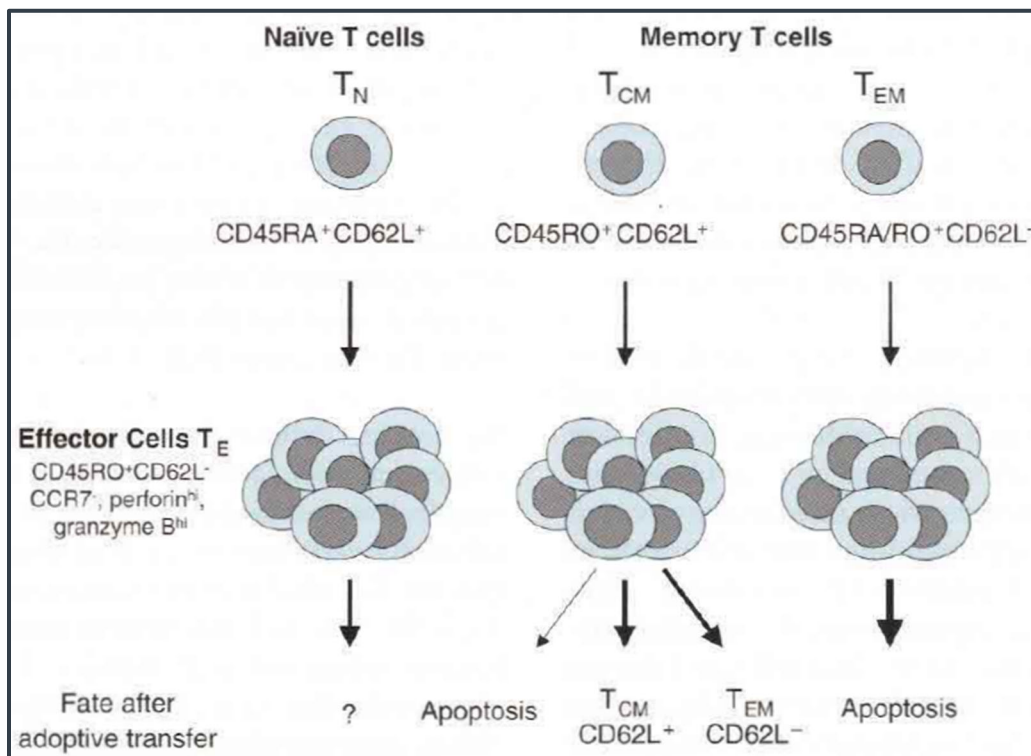
“Adoptive Transfer of Virus-Specific and Tumor-Specific T Cell Immunity,” CURR.

OPIN. IN IMMUNOL., 21: 224-32 (2009) (Ex. 1007) (“Berger”):

The T lymphocyte pool from which T cells for adoptive immunotherapy could potentially be isolated contains CD45RA⁺ CD62L⁺ naïve (T_N), CD45RO⁺ CD62L⁺ central memory (T_{CM}), and CD62L⁻ effector memory (T_{EM}) subsets that differ in phenotype, function, and homing.

Ex. 1007, 227.

29. A POSA would have understood that a T_N cell was CD45RA⁺ CD62L⁺, a T_{CM} cell was CD45RO⁺ CD62L⁺, and T_{EM} or T_E cell were both CD62L⁻, as depicted below:



Id., 228.

30. However, these were not the only markers associated with T_N, T_{CM}, and T_{EM} cells. In fact, multiple markers are used to define T-cell subpopulations, meaning that each subpopulation could be associated with more than one marker. *See, e.g.*, Ex. 1007, 227-28; Ex. 1003, 2967; Ex. 1001, 10:66-11:17. This is consistent with how the '308 patent defines immunophenotypes for T_N, T_{CM}, T_{EM}, and T_E cells. In the '308 patent, each of these T-cell subpopulations is associated with immunophenotypes based on expression of CD45RA, CD45RO, CD62L, and other T-cell markers. *See, e.g.*, Ex. 1001, 7:7-53, 10:66-11:17, 15:25-50.

D. Flow Cytometry and Cell Sorting

31. Generally speaking, cells are transparent and microscopic, meaning that to visualize them, researchers need to use specialized techniques and machinery to detect them. *See, e.g.*, Ex. 1016, 2996-2997; Ex. 1025, 4099-4101; Ex. 1018, [0104]; Ex. 1001, 10:16-24.

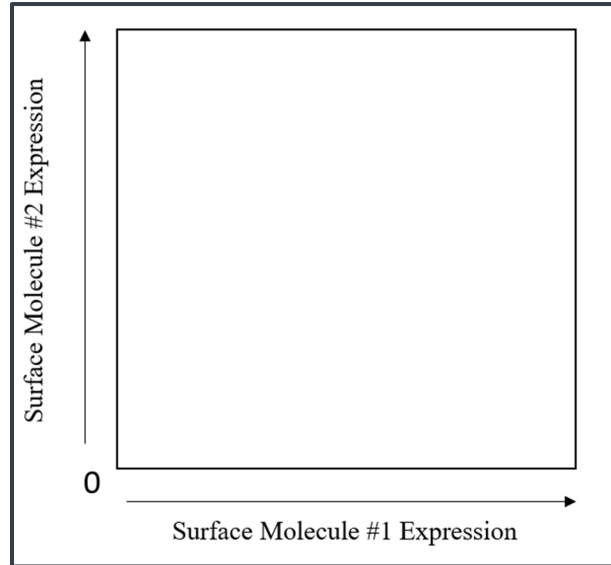
32. One method of detecting T-cell markers, and characterizing the T cells by their markers to define immunophenotypes, is through the use of a flow cytometry where cells are labeled with fluorescent tags that bind to specific markers on cell surfaces allowing the presence of specific markers on specific cells to be analyzed using an instrument known as a flow cytometer. *See, e.g.*, Ex. 1003, 2963, 2966; Ex. 1027, 3-5, 12-15; Ex. 1018, [0104], [0109]; Ex. 1015, 3361, 3363; Ex. 1014, 1099; Ex. 1020, 61-62; Ex. 1004, 788-89; Ex. 1001, 10:16-24.

33. As an example of using flow cytometry to characterize a T cell population, to determine how many T cells express CD62L within a population of T cells, a scientist would combine the T cell population with a fluorescent reagent that is capable of binding specifically to CD62L. After the reagent binds to CD62L-expressing T cells and fails to bind to any non-CD62L-expressing cells that are present, the resulting mixture would then be placed within a machine called a flow cytometer. A flow cytometer analyzes each cell one-by-one by casting laser beams on each cell as they pass through a component of the cytometer, known as the flow cell. The fluorescent reagent(s) attached to T cells will emit photons following excitation by the laser beams and those photons are captured by the flow cytometer to provide a direct measure of the amount of fluorescent reagent(s) bound to each T cell. Using the FACS method, T cells with specific fluorescent properties (*e.g.*, those that bound fluorescent antibodies directed against CD62L) can be directed to a collection tube, which separates them from the T cells that do not express CD62L. *See, e.g., id.*

34. Scientists routinely use flow cytometry to quantify T cells expressing on their surface any T-cell marker-of-interest, provided that an appropriate fluorescent, specifically binding reagent is available. *See, e.g., id.*

35. Before March 23, 2011, fluorescent reagents that could specifically bind to CD4, CD8, CD45RA, CD45RO, and CD62L were readily available to researchers. *See, e.g., id.*

36. A typical plot of hypothetical flow cytometry data is shown below. It is a two-dimensional dot plot with the x- and y-axes each representing expression of a distinct, hypothetical cell surface molecule—Surface Molecule #1 and Surface Molecule #2:

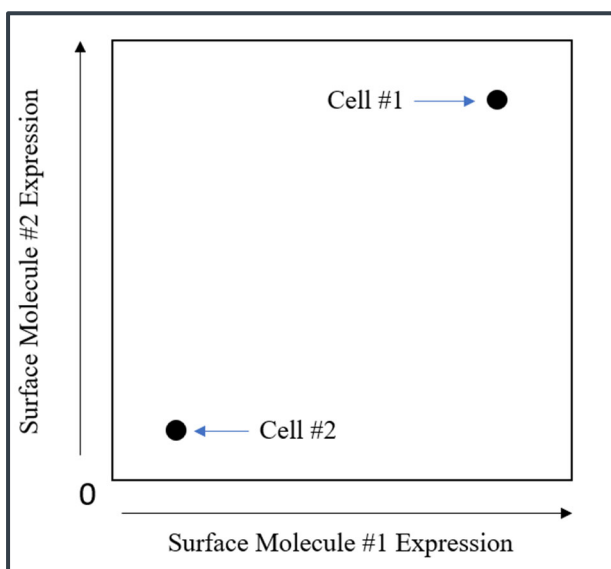


See, e.g., id.

37. In a flow cytometry dot plot, each dot represents a cell, and the position of each dot on the plot shows how much of each tested cell surface marker is expressed by the cell. Here, cells that express more Surface Molecule #1 will be represented by a dot that is further to the right on the plot than a dot corresponding to a cell that expresses less Surface Molecule #1. Similarly, cells that express more

Surface Molecule #2 will be represented by a dot that is higher on the plot than a dot corresponding to a cell that expresses less Surface Molecule #2. *See, e.g., id.*

38. In the figure below, Cell #1 has high expression of both Surface Molecules #1 and #2 because its corresponding dot is high on the plot, and far to the right. Cell #2, on the other hand, expresses those molecules only at a low level because its dot is lower on the plot, and far to the left:

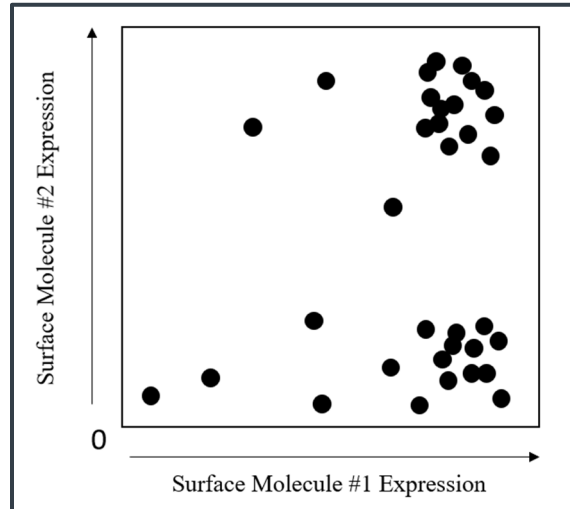


See, e.g., id.

39. A typical flow cytometry plot of a population of cells may have thousands to hundreds of thousands of dots. This provides a visualization of the population's expression of the two surface molecules in question. *See, e.g., id.*

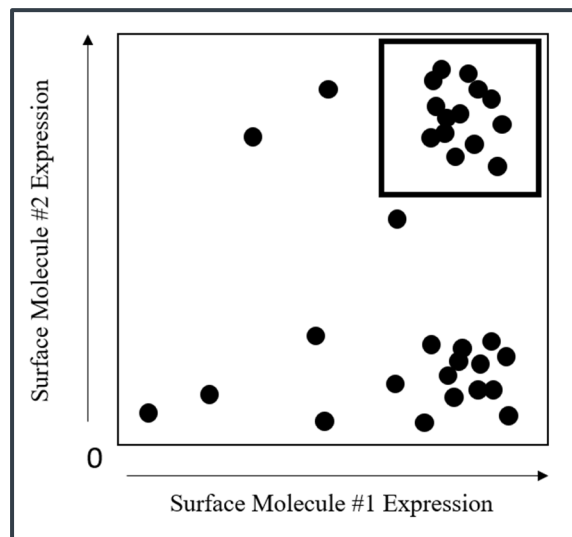
40. The following hypothetical plot shows a population in which most of the cells either express both Surface Molecules #1 and #2 at a high level (the group

of dots at the upper-right of the plot), or express only Surface Molecule #1 (the group of dots at the lower-right of the plot):



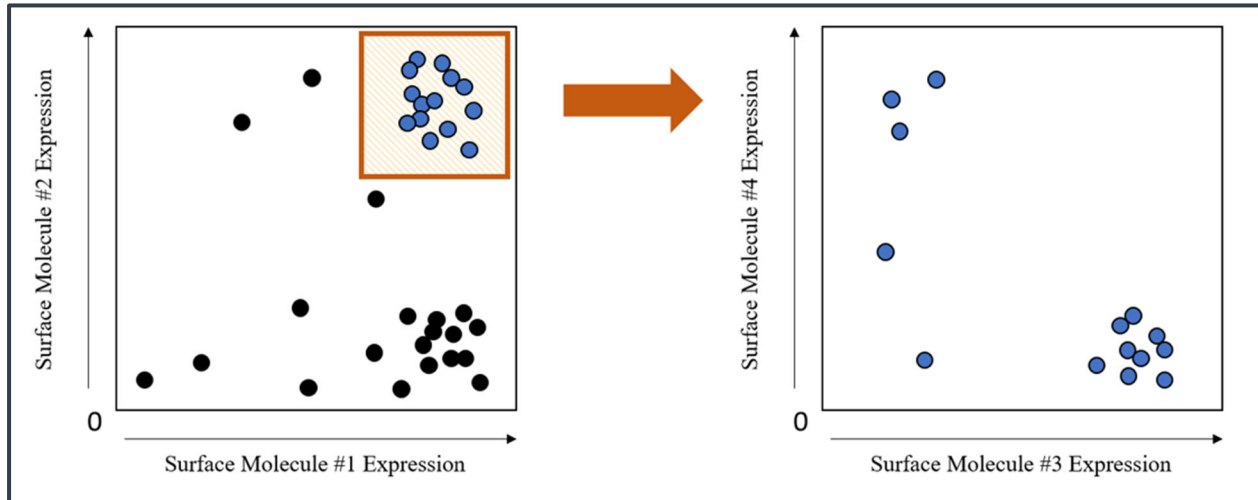
See, e.g., id.

41. Flow cytometers allow a user to visualize a cell population's plot and draw a boundary, using specific software, around a subpopulation of cells, as shown below:



See, e.g., id.

42. The user can instruct the software to isolate the cells within the drawn boundary for further analysis, a process called “gating.” Further analysis can include additional analysis of other surface molecules (here, Surface Molecule #3 and Surface Molecule #4), as shown below:



See, e.g., id.

43. In this example, the large arrow from left to right indicates that the plot on the right is a further analysis of the selected (boxed) group of cells on the left. A majority of cells that express high levels of both Surface Molecules #1 and #2 (the group of dots on the upper-right of the left-hand plot) also express a high level of Surface Molecule #3, but only a low level of Surface Molecule #4 (the group of dots on the lower-right of the right-hand plot). *See, e.g., id.*

44. Overall, a flow cytometer allows for visualization and measurement of expression of multiple surface molecules-of-interest in a population of cells on one or more dot plots. Those cells can be sorted into subpopulations as needed. *See, e.g.,*

id. A specific application of a flow cytometer is fluorescence-activated cell sorting (FACS), which allows the operator to physically isolate cells, a process known as “sorting,” using fluorescent tags that bind to specific markers on cell surfaces. *See, e.g.,* Ex. 1003, 2963, 2966; Ex. 1027, 3-5, 12-15; Ex. 1018, [0104], [0109]; Ex. 1015, 3361, 3363; Ex. 1014, 1099; Ex. 1020, 61-62; Ex. 1004, 788-89; Ex. 1001, 10:16-24. As an example of using FACS to isolate a specific T cell population, a scientist would combine the T cell population with a fluorescent reagent that is capable of binding specifically to CD62L, as in the example for flow cytometry. Processing and gating is identical. However, in the case of FACS, T cells with specific fluorescent properties (*e.g.,* those that bound fluorescent antibodies directed against CD62L) can be directed to a collection tube, which separates them from the T cells that do not express CD62L. *See, e.g., id.* An alternate strategy to isolate cells based on marker expression is magnetic-activated cell sorting (MACS). MACS also allows the operator to physically isolate cells, but instead of using fluorescent reagents to identify T cell markers, the operator employs magnetic tags that bind to specific markers on cell surfaces. Applying a magnetic field causes magnetically tagged cells to be separated from the overall population. As an example of using MACS to isolate a specific T cell population, a scientist would combine the T cell population with a magnetic reagent that is capable of binding specifically to CD62L. Rather than selecting cells through gating using software, as in the case of

flowcytometry, the operator places the T cell mixture in a magnetic field which will retain all the cells that bound the magnetic reagent (ie. CD62L-expressing cells). By retaining the cells in the magnetic field, non-magnetic impurities (ie. cells that do not express CD62L) can be washed away. The remaining T cells can be removed from the magnetic field to permit recovery of the desired population.

E. Adoptive Immunotherapy

45. By the 2000s, researchers were developing therapeutic compositions containing T cells genetically modified to express receptors that target an antigen associated with a disorder, such as cancer. *See, e.g.*, Ex. 1010, 645-48; Ex. 1018, [0056]; Ex. 1016, 2995, 2998; Ex. 1004, 792; Ex. 1001, 1:31-34, 2:11-40, 11:52-67. To manufacture these compositions, researchers would collect a subject's T cells, modify them to target a specific antigen-of-interest, and propagate, *i.e.*, grow, those cells to clinically acceptable numbers. The cells then would be mixed in, for example, a simple saline solution that could be infused intravenously into a patient by a clinician, a technique known as adoptive immunotherapy. *See, e.g.*, M.C. Jensen *et al.*, "CD19-Specific Redirected Immune Cells," U.S. Patent Application 2004/0126363 A1 (Ex. 1018) ("Jensen"), [0162] ("Processed cells are resuspended in 100 ml of 0.9% NaCl with 2% human serum albumin in a bag for [sic] suitable for clinical re-infusion.").

46. By March 23, 2011, researchers understood that T cells with certain immunophenotypes, and certain combinations of T cells, performed better than others in adoptive immunotherapies. For example, there were “nonhuman experiments in which adoptive transfer of *ex vivo* propagated macaque CD28⁺ CD95⁺ CD62L⁺ T_{CM} resulted in longer *in vivo* persistence compared with infusion of numerically expanded effector T cells [T_E (sic)].” Singh, H. *et al.*, “Redirecting Specificity of T-cell Populations for CD19 Using the *Sleeping Beauty* System,” *CANCER RES.* 68(8): 2961-71 (2008) (Ex. 1003), 2970. In addition, “[t]here [was] convincing clinical data that the presence of CD4⁺ T-helper cells improves the persistence of CD8⁺ antigen-specific T cells.” *Id.* Moreover, it was known that a composition that contained equivalent numbers of CD4⁺ and CD8⁺ T cells exhibited optimal efficacy. *See* Moeller, M. *et al.*, “Adoptive Transfer of Gene-engineered CD4⁺ Helper T Cells Induces Potent Primary and Secondary Tumor Rejection,” *BLOOD* 106: 2995-3003 (2005) (Ex. 1016), 3001 (“Optimal antitumor efficacy was achieved with the addition of equivalent numbers of engineered CD4⁺ and CD8⁺ T cells.”).

47. Before March 23, 2011, researchers thus understood at least three ways to optimize CAR T-cell compositions: (1) Use both CD4⁺ and CD8⁺ T cells in the composition; (2) Include them at equivalent numbers; and (3) Use T_{CM} cells (which were CD62L⁺) in the composition rather than T_{EM} and T_E cells (which were CD62L⁻

). *See, e.g.*, Ex. 1003, 2962, 2970; Ex. 1016, 3001; Ex. 1017, 926-27; Ex. 1001, 24:61-64, 27:1-7.

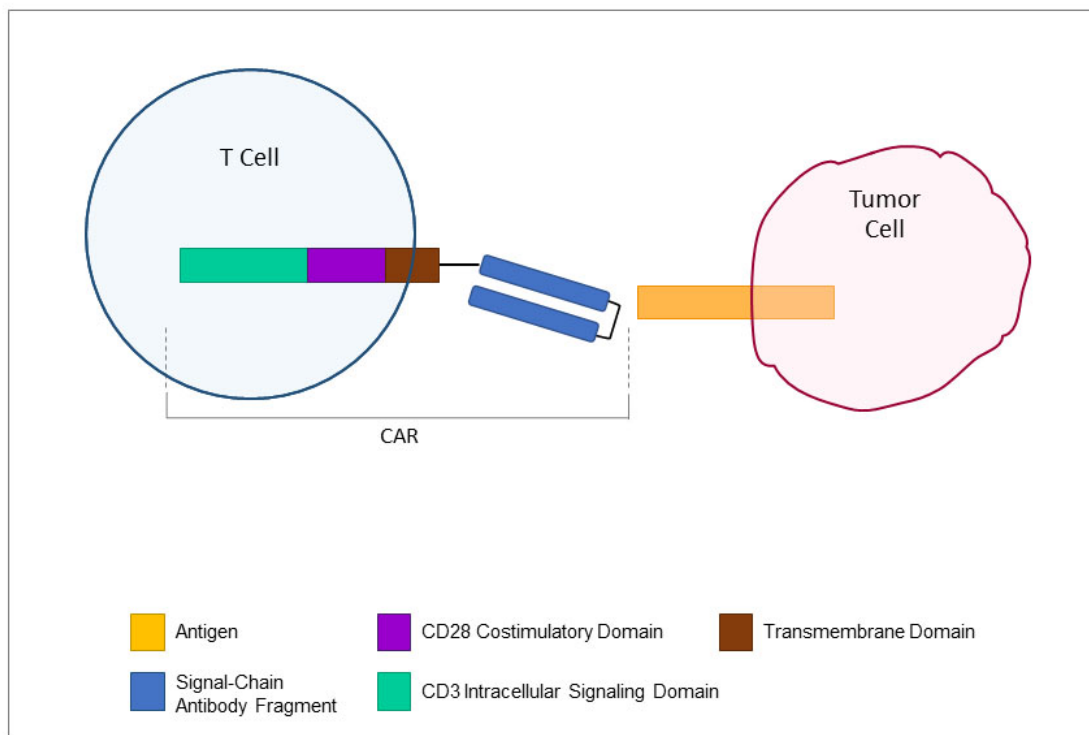
F. Chimeric Antigen Receptors

48. Naturally occurring transmembrane receptors on a T cell typically include (1) an extracellular binding domain, (2) a transmembrane domain, and (3) at least one intracellular signaling domain. *See, e.g.*, Ex. 1010, 645. A ligand¹ may interact with the extracellular binding domain, which then transmits a signal to the cell through the intracellular signaling domain. The intracellular signaling domain communicates with other intracellular signaling components to effect a response to the ligand interacting with the extracellular ligand-binding domain. *See, e.g.*, Ex. 1003, 2961; Ex. 1004, 786; Ex. 1001, 2:11-31, 12:36-47; Ex. 1013, 650.

49. Using the simple design of a single-pass transmembrane receptor, scientists in the 1990s developed chimeric receptors that have been known under different names, including chimeric immune receptors (CIRs) or chimeric antigen receptors (CARs); CAR is the preferred acronym in present day. A CAR includes binding, transmembrane, and signaling domains from two or more proteins, hence the term “chimeric.” *See, e.g.*, Ex. 1010, 645. In the following example, the external binding domain is a single-chain variable-fragment (“scFv”) of an antibody that

¹ A ligand is a molecule that binds to a receptor.

binds an antigen (*e.g.*, a type of ligand) on a tumor cell and the internal signaling domains are from CD3- ζ , an endogenous T-cell domain, and CD28:



50. Researchers can force a cell to express a CAR by incorporating into the cell nucleic acids that encode the CAR molecule. The CAR-encoding nucleic acids (*e.g.*, expression vectors) tend to be DNA-based and can be incorporated into a cell through various means, including electroporation of plasmid DNA and viral transduction. *See, e.g.*, Ex. 1003, 2961-62; Ex. 1011, 522-23; Ex. 1026, 445, 447; Ex. 1001, 5:36-39, 13:22-43. Relevant to the Petition, electroporation generally involves subjecting a mixture of cells with intact cell membranes and CAR-encoding

plasmids to an electrical shock. The shock causes holes (*i.e.*, pores) to form briefly in the cell membranes, allowing the plasmids to enter the cells. The cells then can express the CAR encoded by the plasmids. *See, e.g.*, Ex. 1003, 2961, 2963; Ex. 1001, 13:22-38. Engineering T cells via viral transduction involves infecting cells with modified, non-replicating viruses, commonly retroviruses and lentiviruses, that introduce their proviral genomes into the genome of the cell of interest. The protein of interest, *e.g.*, a CAR, is encoded by the proviral genome leading to permanent expression of the CAR by the infected cell.

51. Scientists have developed CAR T-cell therapies targeting diseases including infections (*e.g.*, human immunodeficiency virus (HIV)) and cancers (*e.g.*, B-cell malignancies). *See, e.g.*, Ex. 1004, 785; Ex. 1003, 2961; Ex. 1001, 12:54-63. B-cell malignancies include leukemias, which affect the bone marrow and blood, and lymphomas, which affect the lymphatic system. *See, e.g.*, Ex. 1011, 517-18; Ex. 1003, 2961-63; Ex. 1001, 18:6-10, 31:21-28.

52. CD19 is a B-cell marker and long has been considered a therapeutic target to allow selective destruction of B cells while preserving other cell types. To create CAR T-cell therapies targeting B-cell malignancies, scientists obtain a patient's T cells and genetically transform those T cells into CAR-modified T cells that target CD19. The resulting anti-CD19 CAR T cells are now specifically equipped to identify and kill CD19⁺ cells, which, in a B-cell malignancy like

leukemia, encompass the population of cells that cause the disease. *See, e.g.*, Ex. 1003, 2961; Ex. 1011, 518-19, 523-24; Ex. 1001, 21:22-40.

V. PERSON OF ORDINARY SKILL IN THE ART

53. A POSA is a person skilled in the art of developing genetically engineered T-cell therapies as of March 23, 2011, which I understand to be the priority date for purposes of the Petition. The person would possess a relatively high level of skill and have at least a Ph.D., together with several years of experience in researching and publishing academic articles concerning T-cell therapies. A POSA would also be knowledgeable about laboratory techniques related to engineering and testing the function of genetically modified T cells.

VI. THE '308 PATENT

54. The '308 patent includes claims to an adoptive cellular immunotherapy composition containing CAR-modified CD4⁺ and CAR-modified CD8⁺ T lymphocytes, where a percentage of the T lymphocytes are surface positive for specific phenotypic markers.

55. Claim 1 is shown below:

An adoptive cellular immunotherapy composition containing chimeric antigen receptor-modified CD4⁺ T lymphocytes and chimeric antigen receptor-modified CD8⁺ T lymphocytes, wherein:

(a) the chimeric antigen receptor-modified CD4⁺ T lymphocytes contain a chimeric antigen receptor that specifically binds to an antigen and at least 50% of the chimeric antigen receptor-modified CD4⁺ helper

T lymphocytes in the composition are surface positive for CD62L and/or CD45RA, and

(b) the chimeric antigen receptor-modified CD8⁺ T lymphocytes contain a chimeric antigen receptor that specifically binds to the antigen and at least 50% of CD8⁺ cytotoxic T lymphocytes in the composition are surface positive for CD62L and/or CD45RO.

Ex. 1001, cl.1.

56. The dependent claims include additional details regarding antigens to which the CAR binds, CAR components, and varying percentages of surface positivity for phenotypic markers.

57. The specification describes numerous permutations of CD4⁺ and CD8⁺ T-cell subpopulations. For example, the specification states that CD4⁺ T cells can be T_N, T_{CM}, T_{EM}, or bulk (*i.e.*, unsorted) cells:

In some embodiments, the CD4⁺ T helper lymphocyte cell is selected from the group consisting of naïve CD4⁺ T cells, central memory CD4⁺ T cells, effector memory CD4⁺ T cells or bulk CD4⁺ T cells. In a specific embodiment, CD4⁺ helper lymphocyte cell is a naïve CD4⁺ T cell, wherein the naïve CD4⁺ T cell comprises a CD45RO⁻, CD45RA⁺, CD62L⁺ CD4⁺ T cell.

Id., 17:24-30.

58. The specification also states that CD8⁺ T cells can be T_N, T_{CM}, T_{EM}, or bulk (*i.e.*, unsorted) cells:

In yet other embodiments, the CD8⁺ T cytotoxic lymphocyte cell is selected from the group consisting of naïve CD8⁺ T cells, central memory CD8⁺ T cells, effector memory CD8⁺ T cells or bulk CD8⁺ T cells. In a specific embodiment, the CD8⁺ cytotoxic T lymphocyte cell

is a central memory T cell wherein the central memory T cell comprises a CD45RO⁺, CD62L⁺, CD8⁺ T cell.

Id., 17:30-37.

59. The specification repeatedly emphasizes one particular T-cell composition: CD4⁺ T_N cells combined with CD8⁺ T_{CM} cells. *See, e.g., id.*, 17:37-39 (“In a specific embodiment, the CD8⁺ cytotoxic T lymphocyte cell is a central memory T cell and the CD4⁺ helper T lymphocyte cell is a naïve CD4⁺ T cell.”). This is consistent with the specification’s examples, which emphasize this one type of CAR-modified CD4⁺ T cells as “possess[ing] favorable characteristics for use in adoptive immunotherapy.” *Id.*, 27:33-34.

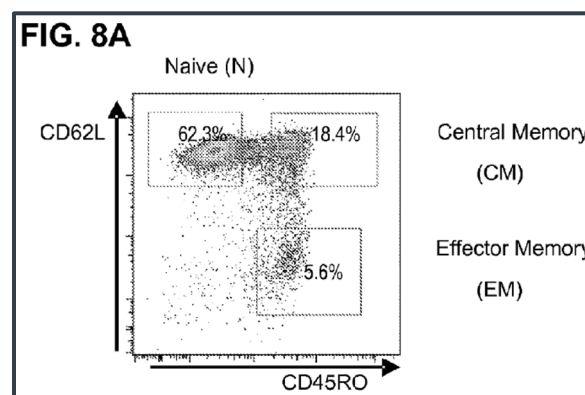
60. According to the examples, “maximum proliferation of the CD8⁺ CAR CTL in response to stimulation . . . was observed after co-culture of CD4⁺ N [naïve] CAR T cells with CD8⁺ CM CAR CTL.” *Id.*, 27:21-25. According to the specification, a composition containing CD4⁺ T_N cells and CD8⁺ T_{CM} cells exhibited the most proliferation of CD8⁺ T cells, which is important because CD8⁺ T cells tend to be the most cytolytic (*i.e.*, cell-killing) T cells.

61. The specification examples also state that “sort purified N [naïve], rather than CM [central memory], EM [effector memory] or bulk CD4⁺ T cells may be best suited to augment the effector function of CD8⁺ CTL.” *Id.*, 27:29-31. Stated differently, according to the specification, CD4⁺ T_N cells enhance the cytolytic capacity of CD8⁺ T_{CM} cells.

62. However, the claims cover more than just a combination of CD8⁺ T_{CM} cells and CD4⁺ T_N cells. Claim 1 states that the CAR-modified CD4⁺ T cells in the claimed composition be “surface positive for CD62L and/or CD45RA.” Claim 1’s CAR-modified CD4⁺ T cells thus can be (1) CD62L⁺, (2) CD45RA⁺, or (3) CD62L⁺ CD45RA⁺.

63. According to the specification, both CD4⁺ T_{CM} and CD4⁺ T_N cells are surface positive for CD62L. *See id.*, 11:48-49 (“In some embodiments, **central memory CD4⁺** cells are **CD62L positive** and CD45RO positive.”) (emphasis added); *see also id.*, 7:12-19; 11:46-48 (“In some embodiments, **naïve CD4⁺** T lymphocytes are CD45RO⁻, CD45RA⁺, **CD62L⁺ CD4⁺** T cell.”); *see also id.*, 7:30-33.

64. Figure 8A of the ’308 patent confirms this interpretation of the specification. Figure 8A is a FACS plot of CD4⁺ T cells:



Id., 11. In Figure 8A, the boxes at the top of plot are T_{CM} on the right and T_N on the left. The CD4⁺ T_{CM} are CD62L⁺, as are the CD4⁺ T_N.

65. The specification describes CD4⁺ T_N cells as expressing both CD62L and CD45RA. *See id.*, 15:28-30 (“in some embodiments, *naïve* CD4⁺ cells are CD45RO⁻, **CD45RA⁺**, **CD62L⁺** CD4⁺ T cells”); 7:30-33 (“‘Naïve’ T cells as used herein refers to a non antigen experienced T lymphocyte that expresses CD62L and CD45RA, and does not express or has decreased expression of CD45RO⁻ as compared to central memory cells.”).

66. Claim 1’s CAR-modified CD4⁺ T cells can be either T_{CM} or T_N according to the ’308 patent’s definitions of those CD4⁺ subpopulations.

67. Claim 1 also requires that its CAR-modified CD8⁺ T cells be “surface positive for CD62L and/or CD45RO.” Claim 1’s CAR-modified CD8⁺ T cells thus can be (1) CD62L⁺, (2) CD45RO⁺, or (3) CD62L⁺ CD45RO⁺.

68. Similar to the above for CD4⁺ cells, according to the specification, both CD8⁺ T_{CM} and CD8⁺ T_N cells are surface positive for CD62L. *See id.*, 1:54-57 (“CD8⁺ T_{CM} express **CD62L** and CCR7 at the cell surface”) (emphasis added); 7:12-14 (“‘**Central memory**’ T cell (or ‘T_{CM}’) as used herein refers to an antigen experienced CTL that expresses **CD62L** and CD45RO on the surface thereof”); 7:33-37 (“In some embodiments, *naïve* CD8⁺ T lymphocytes are characterized by the expression of phenotypic markers of naïve T cells including **CD62L** . . .”) (emphasis added); 11:14-17 (“In some embodiments, *naïve* CD8⁺ T lymphocytes are characterized by the expression of phenotypic markers of naïve T cells including

CD62L, CCR7, CD28, CD3, CD127, and CD45RA.”) (emphasis added), 9:66-10:2 (“In some embodiments, the CD8⁺ cytotoxic T lymphocyte cell is a *central memory* T cell wherein the *central memory* T cell comprises a CD45RO⁺, **CD62L**⁺, CD8⁺ T cell.”) (emphasis added), 15:46-48 (“In some embodiments, *naïve* CD8⁺ T lymphocytes are characterized by CD8⁺, **CD62L**⁺, CD45RO⁺, CCR7⁺, CD28⁺, CD127⁺, and CD45RO⁺ [sic].”) (emphasis added), 11:10-12 (“In some embodiments, *central memory* T cells are CD45RO⁺, **CD62L**⁺, CD8⁺ T cells.”) (emphasis added). The specification describes CD8⁺ T_{CM} cells as having both CD62L⁺ and CD45RO⁺. *See id.*, 11:10-12 (“In some embodiments, *central memory* T cells are **CD45RO**⁺, **CD62L**⁺, CD8⁺ T cells.”) (emphasis added); 7:12-16 (“‘Central memory’ T cell (or ‘T_{CM}’) as used herein refers to an antigen experienced CTL that *expresses CD62L and CD45RO* on the surface thereof, and does not express or has decreased expression of CD45RA as compared to naïve cells.”).

69. Therefore, claim 1’s CAR-modified CD8⁺ T cells can be either T_{CM} or T_N according to the ’308 patent’s definitions of those CD8⁺ subpopulations.

70. In addition, a POSA would have understood that claim 1’s CAR-modified CD8⁺ T cells also could be T_{EM} or T_E cells. As discussed above, claim 1’s CAR-modified T cells can be either (1) CD62L⁺, (2) CD45RO⁺, or (3) CD62L⁺ CD45RO⁺. Regarding (2), a POSA would have understood from Berger that CD45RO⁺ T cells include T_{EM} and T_E cells. *See* Ex. 1007, 228 (showing an

immunophenotype for T_{EM} cells as “CD45RA/**RO**⁺ CD62L⁻” and an immunophenotype for T_E cells as “**CD45RO**⁺ CD62L⁻ CCR7⁻, perforin^{hi}, granzyme B^{hi}.”).

VII. CLAIM CONSTRUCTION

71. I understand that the terms of the claims are to be given their ordinary and customary meaning as understood by one of ordinary skill in the art in light of the specification and the prosecution history of the patent.

A. “adoptive cellular immunotherapy composition”

72. I have been informed by counsel that “adoptive cellular immunotherapy composition” is in the preamble of the Challenged Claims.

73. I have been informed by counsel that a preamble limits the claimed invention if it recites essential structure or steps, or if it is necessary to give life, meaning, and vitality to the claim.

74. I have been informed by counsel that a preamble is not limiting where a patentee defines a structurally complete invention in the claim body and uses the preamble only to state a purpose or intended use for the invention.

75. The specification shows that the preamble is an intended use. The title of the '308 patent is: “Method and Compositions **For** Cellular Immunotherapy.” Ex. 1001, 1:1-2 (emphasis added); *see also id.*, 18:19-23. Additionally, the “Field of the Invention” states that “embodiments of the invention relate to methods and

compositions *for carrying out* cellular immunotherapy.” *Id.* , 1:25-27 (emphasis added). The specification also includes “methods of performing cellular immunotherapy” by “administering a composition.” *Id.*, 16:26-46.

76. The ’308 patent claims, without the preamble phrase “adoptive cellular immunotherapy composition,” recite a structurally complete invention because they recite a composition with specific characteristics in the body of the claims. The preamble phrase “adoptive cellular immunotherapy” is not used in the body of any Challenged Claim and is found only in the preambles. *See id.*, 31:45-34:47. In fact, the claimed compositions are not different if “adoptive cellular immunotherapy composition” is deleted from the claims. Therefore, the preamble phrase “adoptive cellular immunotherapy composition” is not essential or necessary.

77. I have been informed by counsel that a preamble phrase is generally not limiting unless there is clear reliance on the preamble phrase during prosecution to distinguish the claimed invention from the prior art.

78. I have reviewed the prosecution history of the ’308 patent. During prosecution, the applicant did not argue that “adoptive cellular immunotherapy composition” distinguished the claims from prior art. Ex. 1008, 17-32, 43-53, 59-68.

79. Therefore, it is my opinion that the term “adoptive cellular immunotherapy composition” is non-limiting on the scope of the Challenged Claims because it is a statement of purpose, the term is not necessary to give meaning or

provide structure to the claims, and the applicant did not rely of the term to distinguish the Challenged Claims from prior art.

80. The '308 patent does not include an express definition statement for the term “adoptive cellular immunotherapy composition.”

81. If the Board finds that the preamble phrase “adoptive cellular immunotherapy composition” is limiting, it is my opinion that “adoptive cellular immunotherapy composition” should be construed to mean “composition for adoptive cellular immunotherapy formulated to be suitable for administration to a mammal.”

82. The specification states the following:

In some embodiments, the cells are *formulated* by first harvesting them from their culture medium, and then washing and concentrating the cells in a medium and container system *suitable for administration* (a “pharmaceutically acceptable” carrier) in a treatment-effective amount. . . .

A treatment-effective amount of cells in the composition is at least 2 cells (for example, 1 CD8⁺ central memory T cell and 1 CD4⁺ helper T cell subset) or is more typically greater than 10² cells, and up to 10⁶, up to and including 10⁸ or 10⁹ cells and can be more than 10¹⁰ cells. The number of cells will depend upon the ultimate use for which the composition is intended as will the type of cells included therein.

Ex. 1001, 18:26-42 (emphasis added).

83. The specification also states that T cells of the invention “may be from any mammalian, preferably primate, species, including monkeys, dogs, and humans.” *Id.*, 6:66-7:1.

84. A POSA would understand that the '308 patent does not impute a specific therapeutic aspect to its claimed compositions because the patent does not provide anything other than generalized statement for “a treatment-effective amount” of cells in its compositions. *See id.*, 18:26-42. Specifically, the “treatment-effective amount” of cells will differ “depend[ing] upon the ultimate use for which the composition is intended.” *Id.*, 18:41-42.

85. Therefore, it is my opinion that if the preamble phrase “adoptive cellular immunotherapy composition” is limiting, “adoptive cellular immunotherapy composition” should be construed to mean “composition for adoptive cellular immunotherapy formulated to be suitable for administration to a mammal.”

B. “and/or”

86. A POSA would understand the term “and/or,” as it appears in the Challenged Claims, to cover embodiments having the element preceding “and/or,” the element following “and/or,” or both the element preceding “and/or” and the element following “and/or.”

87. Claim 1, for example, recites that the composition’s CD4⁺ T cells “are surface positive for CD62L *and/or* CD45RA”. Under the above meaning of “and/or,” the composition’s CD4⁺ T cells could be surface positive for CD62L, CD45RA, or both CD62L and CD45RA.

88. Claim 1 also recites that the composition's CD8⁺ T cells "are surface positive for CD62L *and/or* CD45RO." Therefore, the composition's CD8⁺ T cells would satisfy this limitation if surface positive for CD62L, CD45RO, or both CD62L and CD45RO.

VIII. PRIOR ART

A. Adoptive Cellular Immunotherapy Compositions With CD45RA⁺, CD45RO⁺, and/or CD62L⁺ Immunophenotypes Were Known

1. Singh

89. The adoptive cellular immunotherapy composition claimed in the '308 patent was disclosed in 2008 by researchers at University of Texas M.D. Anderson Cancer Center, University of Minnesota, and Children's Hospital Los Angeles. These researchers disclosed the composition in the April 15, 2008 issue of the scientific journal, *CANCER RESEARCH*. See Singh, H. *et al.*, "Redirecting Specificity

of T-cell Populations for CD19 Using the *Sleeping Beauty* System,” *CANCER RES.* 68(8): 2961-71 (2008) (Ex. 1003).²

90. Singh’s authors recognized that adoptive immunotherapies needed “an approach that when coupled with nonviral gene transfer shortens the culture time to generate T cells with durably expressed transgene and ***maintains a desired T-cell immunophenotype.***” *Id.*, 2961 (emphasis added). At the time, researchers understood that it was important to maintain a T-cell composition’s immunophenotype because “[n]ext-generation clinical trials using genetically modified T cells are expected to infuse predefined populations of T cells with defined characteristics such as the inclusion of both CD4⁺ and CD8⁺ T cells and T_{CM}.” *Id.*, 2970.

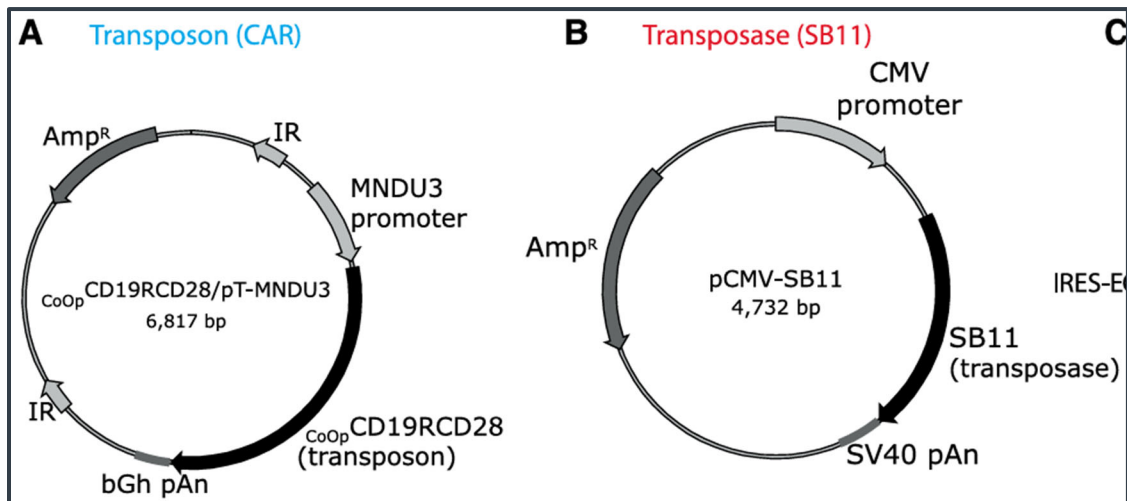
91. Singh’s authors understood that including both CD4⁺ and CD8⁺ T cells in an adoptive cellular immunotherapy composition would improve the persistence

² I understand that Singh is prior art to the ’308 patent under 35 U.S.C. § 102(b). *CANCER RESEARCH* was, and still is, a widely available and respected journal published by American Association for Cancer Research as of the date of that publication. Based on my familiarity with academic publishing, this journal in particular, and the state of the art at the time, Singh appears to have been published on the date displayed in its publication citation.

(i.e., survival) of the cells that make up the composition. *See id.* (“There is convincing clinical data that the presence of CD4⁺ T-helper cells improves the persistence of CD8⁺ antigen-specific T cells.”).

92. Singh’s authors knew that including CD62L-expressing T_{CM} in an adoptive cellular immunotherapy composition would improve persistence as well. *See id.* (“These data are supported by nonhuman experiments in which adoptive transfer of *ex vivo* propagated macaque CD28⁺CD95⁺CD62L⁺ T_{CM} resulted in longer *in vivo* persistence compared with infusion of numerically expanded effector T cells.”).

93. The manufacturing process reported in Singh for adoptive cellular immunotherapy compositions involved “introducing DNA plasmids from the *Sleeping Beauty* transposon/transposase system to directly express a CD19-specific CAR in memory and effector T cells without drug selection.” *Id.*, 2961. The *Sleeping Beauty* system utilized two DNA plasmids: one expressing a transposon encoding a CD19-specific CAR, and the other expressing a transposase, as shown below:



Id., 2962. A transposon is a DNA sequence capable of integrating into a subject's genome with the assistance of a particular enzyme—a transposase.

94. The CD19-specific CAR that Singh's authors used was referred to as "CD19RCD28." It was derived from another CAR named CD19R, which "recognizes CD19 via the scFv of a murine CD19-specific monoclonal antibody (mAb) fused to a chimeric CD3- ζ -derived activation endodomain." *Id.*, 2964.

95. CD19RCD28 included the CD19-specific scFv of CD19R. *See* Kowolik, C.M. *et al*, "CD28 Costimulation Provided Through a CD19-Specific Chimeric Antigen Receptor Enhances *In Vivo* Persistence and Antitumor Efficacy of Adoptively Transferred T Cells," *CANCER RES.* 66(22): 10995-11004 (2006) (Ex. 1009) ("Kowolik"), 10996 ("CD19RCD28 was composed of a CD19-specific single-chain variable fragment (scFv).") (parentheticals omitted) (emphasis added).

96. CD19RCD28 also included “chimeric CD3- ζ and CD28.” Ex. 1003, 2961 (“CD19RCD28 . . . provides CD19-dependent signaling through CD3- ζ and CD28.”); *see also* Ex. 1009, 10996 (“CD19RCD28 was composed of a CD19-specific single-chain variable fragment (scFv) . . . fused to a modified human IgG4 hinge and Fc region fused to residues 153 to 179 of intracellular human CD28 molecule, which included the transmembrane and a modified intracellular domain, fused to residues 31 to 142 of the human cytoplasmic CD3- ζ chain”) (parentheticals omitted).

97. Singh incorporated the *Sleeping Beauty* system into human T cells isolated from umbilical cord blood or peripheral blood mononuclear cells (PBMC) after consent and cultured them in culture medium. *See* Ex. 1003, 2963 (“Human T cells were isolated by density gradient centrifugation over Ficoll-Paque-Plus, from umbilical cord blood or peripheral blood mononuclear cells (PBMC) after consent, and were cultured in culture medium.”) (parentheticals omitted).

98. A POSA would have understood that T-cell samples that needed to be acquired “with consent” were from a human subject. The general practice is that informed consent is required before obtaining and using a sample from a subject, such as a T-cell sample.

99. Singh incorporated the *Sleeping Beauty* plasmids into T cells using electroporation. *See id.*, 2963 (“On day 0, PBMCs and umbilical cord blood

mononuclear cells were suspended in 100 μ L of Amaxa Nucleofector solution and mixed with 5 μ g of supercoiled $\text{CoOpCD19RC28/pT-MNDU3}$ and 5 μ g pCMV-SB11 DNA plasmids, transferred to a cuvette, and electroporated”) (parentheticals omitted).

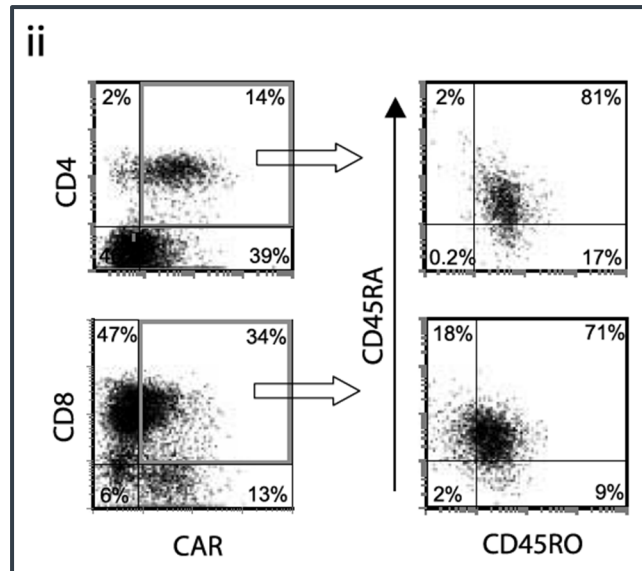
100. In line with Singh’s understanding that using both CD4^+ and CD8^+ T cells in its compositions would be beneficial, Singh reported that the CD4^+ and CD8^+ T cells in its compositions expressed the CAR. *See id.*, 2965 (“peripheral blood- and umbilical cord blood-derived electroporated CD4^+ and CD8^+ T cells readily expressed the CAR transposon.”).

101. Singh’s manufacturing process also involved expanding CD19 CAR-expressing T cells in the presence of CD19^+ artificial antigen presenting cells (“aAPCs”). *Id.*, 2962 (“We report that electrotransfer of a two-component DNA *SB* system into primary human T cells from umbilical cord blood and peripheral blood results in efficient and stable CAR gene transfer, which can be numerically expanded to clinically meaningful numbers within 4 weeks on CD19^+ artificial antigen-presenting cell (aAPC)”). “Expanding” a T-cell population means stimulating the T cells within the population to multiply. An aAPC is a cell that has been engineered to express an antigen it would not normally express. One way to generate aAPCs is to incorporate DNA expressing an antigen-of-interest into cells such that the cells express that antigen. Singh’s authors made aAPCs by

electroporating K562 cells with a DNA sequence encoding a CD19 antigen. *See id.*, 2963 (“K562 cells were electroporated with DNA plasmids to enforce expression of all of the following: truncated CD19, 4-1BBL, and MICA fused to GFP.”) (emphasis added).

102. Singh explained that this method “results in rapid outgrowth of CD4⁺ and CD8⁺ T cells expressing CAR to redirect specificity for CD19⁺ tumor cells.” *Id.*, 2961. Singh stated that its method would “greatly facilitate trial design infusing CD4⁺ and CD8⁺ CAR⁺ T cells that have desired immunophenotype, including T_{CM}.” *Id.*, 2962. As Singh pointed out, its method “provides for robust antigen-driven expansion of CD4⁺ and CD8⁺ CAR⁺ T cells to clinically meaningful numbers.” *Id.*, 2964. For example, “after 28 to 35 days, the efficiency of . . . gene transfer improved CAR expression by ~ 49 to 60-fold.” *Id.*, 2965.

103. After CAR transduction and five weeks of *ex vivo* T-cell expansion, Singh’s authors analyzed the immunophenotypes of the resulting T-cell compositions using FACS analysis. Figure 3.B.ii of Singh presents a two-step analysis:



Id., 2966.

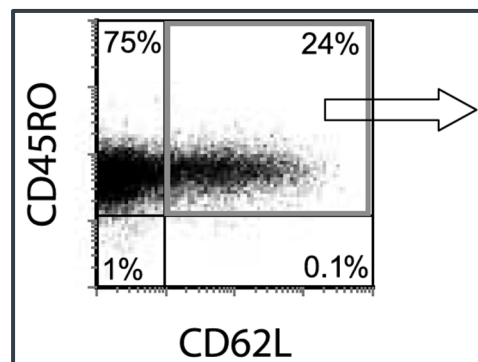
104. As noted earlier, a flow cytometer analyzes each cell on an individual basis. A given cell will have bound different amounts of fluorochrome based on the presence/absence of specific proteins on its surface (CD4, CD8, CAR, CD45RA and CD45RO in the example above). The flow cytometer exposes each cell to a laser which will stimulate the fluorochromes attached to the cell and the number of photons released will be a direct reflection of the presence/absence and abundance of a given marker. The fluorescent signal is then scaled by the flow cytometer's software across several orders of magnitude (often called "decades") and is commonly presented in two-dimensional plots with logarithmic axes as shown above. A highly trained flow cytometry expert will use a variety of criteria to set cut-off values for positive/negative cells and the immunophenotype is defined by the presence/absence of specific markers.

105. The two left-most panels represent the first step, where Singh's authors analyzed CAR expression (x-axes) in conjunction with either CD4 or CD8 expression (y-axes). The upper-left panel shows that 14% of cells in the sample were CAR-modified, CD4⁺ T cells. The lower-left panel shows that 34% of cells in the sample were CAR-modified, CD8⁺ T cells.

106. The two right-most panels represent the second step, where Singh's authors isolated CAR-modified, CD4⁺ or CD8⁺ T cells, and analyzed them for CD45RA or CD45RO expression. In the upper-right panel, Figure 3.B.ii shows that 83% (2% + 81%) of Singh's CAR-modified CD4⁺ T cells were CD45RA⁺. The lower-right panel shows that 80% (71% + 9%) of the CAR-modified CD8⁺ T cells were CD45RO⁺.

107. Singh did not specifically analyze CD62L expression in its CAR-modified, CD4⁺ or CD8⁺, populations.

108. Figure 3.B.ii of Singh also presents the immunophenotype of Singh's T-cell compositions in terms of CD45RO expression:



Id. CD45RO expression is measured on the y-axis, and all the dots above the horizontal line represent CD45RO⁺ T cells. Here, 99% (75% + 24%) of the T cells in Singh's compositions were CD45RO⁺.

109. Singh described its manufacturing process for T-cell compositions as “a cost-effective gene transfer system that encourages reiterative changes to expression vector and/or CAR design to be used in proof-of-concept clinical trials.” *Id.*, 2969. A POSA would have understood this to mean that Singh's manufacturing process could be used with expression vectors other than those used in Singh as well as CARs other than that used in Singh.

110. Having disclosed their manufacturing process, Singh's authors provided suggestions for future studies—clinical trials involving T-cell compositions containing both CD4⁺ and CD8⁺, and of the T_{CM} subpopulation. *Id.*, 2970 (“[n]ext-generation clinical trials using genetically modified T cells are expected to infuse predefined populations of T cells with defined characteristics such as the inclusion of both CD4⁺ and CD8⁺ T cells and T_{CM}.”). *Id.*

111. The authors offered two rationales for these future clinical trials. For one, Singh explained that “[t]here [was] convincing clinical data that the presence of CD4⁺ T-helper cells improves the persistence of CD8⁺ antigen-specific T cells.” *Id.* A POSA would have understood this to mean that CD8⁺ T cells survive longer in the presence of CD4⁺ T cells.

112. Another rationale related to the importance of CD62L expression in adoptive cellular immunotherapies. Singh explained that “nonhuman experiments in which adoptive transfer of *ex vivo* propagated macaque CD28⁺CD95⁺CD62L⁺ T_{CM} resulted in longer *in vivo* persistence compared with infusion of numerically expanded effector T cells.” *Id.*, 2970. A POSA would have understood this to mean that CD62L⁺ T_{CM} survived longer *in vivo* than expanded T_E cells. In fact, Singh emphasized that its manufacturing process “supports the outgrowth of T cells with [T_{CM}-associated] phenotypes as our CAR⁺ T cells maintain expression of CD27, CD28, CD45RO, CD95, and CD62L.” *Id.*

113. Singh repeatedly explained that CD62L-positivity is a marker for T_{CM}. *See, e.g., id.* (“[c]linical trials will be needed to determine whether adoptive transfer of these CAR⁺ T cells with an apparent central memory immunophenotype (CD28⁺ CD95⁺ CD62L⁺) results in long-term *in vivo* persistence of genetically modified T cells.”).

114. It was known that T_{CM} were an optimal T-cell subpopulation for adoptive immunotherapy. *See* Yang, S., *et al*, “*In Vitro* Generated Anti-Tumor T Lymphocytes Exhibit Distinct Subsets Mimicking *In Vivo* Antigen Experienced Cells,” *CANCER IMMUNOL IMMUNOTHER.* 60(5): 739-49 (2011) (“Ex. 1027”)

(“Yang I”)³, 1 (“a large percentage of *in vitro* generated anti-tumor T lymphocytes mimic a Tcm-like phenotype (based on phenotype, effector function, and increased persistence *in vivo*), which suggests that these Tcm-like cultured T cells may be optimal for adoptive immunotherapy”), 7 (“The short-term *in vitro* culture generated anti-tumor T lymphocytes show[ing] a dominant Tcm phenotype that are endowed with intrinsic advantages such as *in vivo* persistence, suggesting that Tcm-like cells may be the most appropriate type of cell for adoptive cell therapy.”). In particular, T_{CM} were “believed to contain T memory stem cells, which display the capacity for long-term self-renewal.” Yang, S. *et al*, “TCR Engineered and In Vitro Expanded T Lymphocytes Recapitulate the Properties of Central Memory T Cells,” MOLECULAR THERAPY 18(Supp. 1): S184 (2010) (Ex. 1028) (“Yang II”), S18.

115. It also was known that T_{CM} could be distinguished based on CD62L expression. *See* Ex. 1028, S184 (“Based on the differentiation marker L-selectin/CD62L and the release of the cytokine IL-2, central memory T cells can be distinguished from effector memory T cells.”); *id.* (“we could expand T lymphocytes up to 1000-fold and majority of these cells showed a central memory-like phenotype illustrated by a high percentage of CD62L”).

³ I understand that Yang I published on February 9, 2011. *See* Yang I, 1.

116. At the time, researchers understood that adoptive cellular immunotherapies had the potential to cause side effects after eliminating their target cells. Adoptive cellular immunotherapies include cells that have the capacity to proliferate in a subject in response to an antigen-of-interest (*e.g.*, a cancer-associated antigen). However, there is a possibility that those cells could continue proliferating after eliminating their respective targets. Therefore, it is important that adoptive cellular immunotherapies do not proliferate in the absence of their target antigen. Singh's authors put this issue to rest with respect to their compositions, however, explaining that they "routinely culture T cells after electroporation without aAPC and [they] are yet to observe evidence of antigen-independent proliferation." Ex. 1003, 2970.

117. Singh's authors anticipated that their manufacturing process "should be appealing to investigators undertaking single-site as well as multisite trials using gene transfer of immunoreceptor(s) to redirect the specificity of T cells, including T_{CM}." *Id.* Singh also stated that its technology was "readily accessible and practiced in compliance with cGMP for phase I/II trials." *Id.* Singh proposed that its manufacturing process "will be of interest not just for generating clinical grade T cells with specificity for CD19, but for genetically modifying T cells to express CAR with alternative specificities." *Id.* A POSA would have understood this to mean that Singh's authors had developed a manufacturing process for adoptive cellular

immunotherapy compositions that would be (1) useful for expressing CARs other than CD19RCD28, and (2) ready to use in connection with clinical trials.

118. Singh concluded by noting that its authors planned to evaluate its compositions in a future clinical trial. *See id.*, 2970-2971 (“We have now incorporated *ex vivo* CAR-dependent proliferation to derive genetically modified T cells and will evaluate the CD19-specific T cells . . . in a next-generation clinical trial.”).

119. NCT00968760 denotes a clinical trial that involved at least five of Singh’s authors. *See Kebriaei, P. et al.*, “Infusing CD19-Directed T Cells to Augment Disease Control in Patients Undergoing Autologous Hematopoietic Stem-Cell Transplantation for Advanced B-Lymphoid Malignancies,” *HUMAN GENE THERAPY* 23:444-50 (2012) (Ex. 1026) (“Kebriaei”), 444 (listing as authors Helen Huls, Perry Hackett, Elizabeth Shpall, Richard Champlin, and Laurence Cooper), 445 (“The trial is listed at ClinicalTrials.gov (identifier: NCT00968760).”).

120. NCT00968760 published in 2009 on ClinicalTrials.gov. *See* CD19-specific T Cell Infusion in Patients with B-Lineage Lymphoid Malignancies After Autologous Hematopoietic Stem Cell Transplantation, ClinicalTrials.gov, https://clinicaltrials.gov/ct2/history/NCT00968760?V_1=View#StudyPageTop (Ex. 1024) (“ClinicalTrials.gov”).

121. NCT00968760 involved the same CAR, CD19RCD28, as Singh. It also utilized the same transduction vehicle, *Sleeping Beauty*. Furthermore, it used Singh's manufacturing process involving co-culture with aAPCs. *See* Ex. 1026, 447 ("This first-in-human Phase I study will assess the safety and feasibility of using the (1) second-generation CD19RCD28 CAR, (2) SB [*Sleeping Beauty*] system to express CAR in T cells"), and (3) aAPC to propagate CAR⁺ T cells").

122. Together, these facts would indicate to a POSA that some of Singh's authors took Singh's manufacturing process to the clinic and used it in NCT00968760 to target CD19-expressing cancers.

123. NCT00968760 was not the only clinical trial involving CD19-targeting CAR T-cell compositions before March 23, 2011. By at least 2010, researchers reported positive clinical results using CD19-targeting CAR-T cells. *See* Kochenderfer, J.N. *et al*, "Eradication of B-lineage Cells and Regression of Lymphoma in a Patient Treated with Autologous T Cells Genetically Engineered to Recognize CD19," BLOOD 116(20): 4099-4102 (2010) (Ex. 1025) ("Kochenderfer"), 4101 ("The prolonged elimination of CD19⁺ cells in this patient indicates in vivo antigen-specific activity of anti-CD19-CAR-expressing T cells. Our findings should encourage continued study of anti-CD19-CAR-transduced T cells.").

2. Mitsuyasu

124. Other groups had used adoptive cellular immunotherapy compositions before Singh. For example, in 2000, researchers from University of California, Los Angeles, University of Virginia, University of Pennsylvania, and various hospitals disclosed the composition claimed in the '308 patent but for one detail. Instead of using a CAR (as defined by the '308 patent), these researchers used a “chimeric immune receptor” (“CIR”). *See* Mitsuyasu, R.T. *et al.*, “Prolonged Survival and Tissue Trafficking Following Adoptive Transfer of CD4ζ Gene-modified Autologous CD4⁺ and CD8⁺ T Cells in Human Immunodeficiency Virus-infected Subjects,” BLOOD 96: 785-93 (2000) (Ex. 1004) (“Mitsuyasu”).⁴

125. I understand that Mitsuyasu was cited in an information disclosure statement during prosecution of the '308 patent. Ex. 1008, 3. I also understand that

⁴ I understand that Mitsuyasu is prior art to the '308 patent under 35 U.S.C. § 102(b). BLOOD was, and still is, a widely available and respected journal published by American Society of Hematology as of the date of that publication. Based on my familiarity with academic publishing, this journal in particular, and the state of the art at the time, Mitsuyasu appears to have been published on the date displayed in its publication citation.

the patent examiner did not discuss Mitsuyasu in his office actions. *Id.* 10-16, 33-42, 54-58.

126. Mitsuyasu describes one of the first in-human clinical trials (Phase II) of a CAR-T cell-based adoptive cellular immunotherapy. Mitsuyasu's authors drew blood from HIV-positive patients and, through a routine selection process, isolated the patients' T cells. Ex. 1004, 786 ("Lymphapheresis was performed locally using an automated cell separator Recovered cells were stimulated using magnetic beads coated with anti-CD3 and anti-CD28.") (parentheticals omitted).

127. Mitsuyasu does not report the use of any selection process for CD4⁺, CD8⁺, or CD62L⁺ T cells.

128. Mitsuyasu's authors transduced patients' T cells with a CIR. Although Mitsuyasu did not define what it means for a receptor to be a CIR, a POSA would have been aware that a CIR was equivalent to a CAR based on prior-art definitions of a CIR, such as that disclosed in Shibaguchi, H., *et al.*, "A Full Human Chimeric Immune Receptor for Retargeting T-Cells to CEA-Expressing Tumor Cells," *ANTICANCER RESEARCH* 26: 4067-72 (2006) (Ex. 1019) ("Shibaguchi"). According to Shibaguchi:

[A] CIR is based on an artificial immune receptor composed of an extracellular antigen-binding domain linked through a transmembrane motif to a cytoplasmic lymphocyte-signaling moiety. The CIR approach using antibody-derived single-chain variable fragment (scFv) as a recognition unit, is especially suited to immunotherapy of cancer.

Ex. 1019, 4067.

129. Mitsuyasu referred to its CIR as “CD4ζ.” According to Mitsuyasu:

CD4ζ is a genetically engineered, MHC-unrestricted, chimeric immune receptor composed of the zeta (ζ) subunit of the CD3 T-cell receptor (the cytoplasmic domain involved in signal transduction) fused to the transmembrane and extracellular domains of human CD4 (which targets HIV *env* expressed on the surface of infected cells).

Ex. 1004, 786.

130. After transducing patients’ T cells with the CIR, Mitsuyasu’s authors cryopreserved the resulting T-cell compositions in a solution containing Plasmalyte-A, 10% dimethylsulfoxide, 1% dextran-40, and 5% human serum albumin. *Id.* (“The final T-cell products were cryopreserved in 50-mL bags containing 6×10^9 cells/bag in Plasmalyte-A with 10% dimethylsulfoxide, 1% dextran-40, and 5% human serum albumin.”) (parentheticals omitted). A POSA would have known that Plasmalyte-A is a solution meant to mimic human plasma.

131. Before infusing the T-cell compositions into patients, Mitsuyasu’s authors performed phenotypic analysis using antibodies to CD3, CD4, CD8, CD28, CD25, and CD62L. *See id.* (“Phenotypic analysis was performed using antibodies to CD3, CD4, CD8, CD28, CD25, and CD62L.”).

132. When a patient was ready to be infused with CIR-modified T cells, Mitsuyasu’s authors thawed the cryopreserved T-cell compositions at bedside and infused them directly into the patient’s bloodstream. *See id.* (“Cryopreserved T cells

were thawed in a 37°C water bath at the patient’s bedside and infused directly through a peripheral intravenous catheter over 50 to 10 minutes per bag.”).

133. Mitsuyasu reported that its T-cell manufacturing process “yielded a mixed population of CD4⁺ and CD8⁺ T cells.” *Id.*, 791. The authors measured CAR transduction efficiency in each T-cell population using PCR. *See id.*, 788. The immunophenotypes and CAR expression of Mitsuyasu’s T-cell compositions is shown in Table 2 of Mitsuyasu, which I have reproduced below:

Table 2. T-cell manufacturing data							
Patient ID	CD4 (%) [*]	CD8 (%) [*]	CD62L (%) [*]	CD28 (%) [*]	CD25 (%) [*]	Culture days [†]	CD4ζ by PCR [‡]
N215	24	73	83	88	—	13	11
H202	62	38	96	98	88	12	17
C211	17	80	84	91	42	12	12
F209	13	83	—	85	45	12	18
S201	26	72	—	89	36	12	9
Q202	22	77	94	98	77	12	5
S206	52	46	93	96	74	17	49
S212	20	79	95	98	43	12	10
P217	31	68	94	93	39	12	13
G251	22	86	82	87	41	12	17
E207	28	69	73	94	60	12	17
T253	24	77	82	88	55	12	18
L218	13	86	85	94	36	12	15
R255	60	37	79	90	27	12	15
Y254	22	76	94	98	59	12	13
U213	7	92	84	96	43	10	9
D282	23	78	82	95	50	13	16
D212	3	96	44	90	64	14	15
T232	28	70	87	88	34	14	11
D256	12	88	59	74	58	15	15
J233	7	90	81	90	43	14	18
Y292	37	61	96	99	28	12	28
X214	8	90	64	79	49	16	38
C298	26	73	83	63	36	12	57
Mean	24	74	82	90	49	13	19
SD	15	16	13	8	16	2	13
[*] Percentage of CD3 ⁺ T cells expressing marker by FACS analysis. [†] Total number of days in culture for each T-cell lot. [‡] Transduction efficiency as measured by DNA PCR for CD4ζ.							

Id. (Table 2).

134. The vast majority of compositions that Mitsuyasu's authors tested for CD62L expression (seventeen) had CD62L-positivity above 80% despite the authors not attempting to enrich T cells for CD62L-positivity. The naturally high levels of CD62L are consistent with other art published before March 23, 2011. The Morgan group, for example, created three compositions and similarly saw that all of them

had CD62L levels above 80% despite not enriching specifically for this marker. *See* Ex. 1027, 12.

135. All of Mitsuyasu's T-cell compositions, listed under "Patient ID" in Table 2, included CD8⁺ and CD4⁺ T cells. A POSA would have understood this because Mitsuyasu reports a non-zero value for the percentages of CD4⁺ and CD8⁺ T cells in all of its compositions. In fact, Mitsuyasu explained that "adoptive immunotherapy with antigen-specific T cells is likely to require co-infusion of CD4⁺ and CD8⁺ T cells to achieve optimal in vivo survival and activity." Ex. 1004, 786.

136. Mitsuyasu observed "sustained, high-level persistence of gene-modified T cells" and concluded that it "demonstrates the feasibility of ex vivo T-cell gene therapy in HIV-infected adults and suggests the importance of providing HIV-specific T-helper function." *Id.*, 785.

B. The Modularity of Chimeric Antigen Receptors Was Known

1. Cooper

137. In 2009, the corresponding author of Singh published an entry in the Encyclopedia of Cancer. *See* Cooper, L.J.N. *et al.*, "Chimeric Antigen Receptor on

T Cells,” in ENCYCLOPEDIA OF CANCER, 2ND EDITION: 644-48 (2009) (Ex. 1010)

(“Cooper”).⁵ Cooper explained the modular structure of a CAR:

A chimeric antigen receptor (CAR) consists of an extracellular antigen-binding exodomain, typically derived from a single-chain antibody fragment (scFv) of a monoclonal antibody (mAb), a spacer (such as an antibody Fc region), a transmembrane region, and one or more intracellular signaling endodomains.

Ex. 1010, 645.

138. Cooper also explained that, because CARs have a modular structure, their components can be mixed and matched to meet the needs of a particular application:

One of the major advantages of the strategy infusing CAR⁺ T cells lies in the modular composition of the CAR molecule that combines an antigen-binding domain with signaling domains for effector-cell activation. This allows investigators to swap the CAR exodomain with a scFv or ligand that recognizes or binds to a desired cell-surface antigen.

⁵ I understand that Cooper is prior art to the '308 patent under 35 U.S.C. § 102(b). ENCYCLOPEDIA OF CANCER was, and still is, a widely available and respected reference text published by Springer as of the date of that publication. Based on my familiarity with academic publishing, this text in particular, and the state of the art at the time, Cooper appears to have been published on the date displayed in its publication citation.

Id., 648. A POSA would have understood this to mean that, for a given CAR construct specific for an antigen, the antigen-binding domain, for example, could be swapped out for an antigen-binding domain that is specific for a different antigen.

C. CARs Targeting CD19, CD20, CD22, ROR1, CEA, Her2, L1-CAM, and Mesothelin Were Known

139. Before March 23, 2011, researchers already had developed strategies to target with CARs numerous antigens-of-interest, including CD19 (*e.g.*, Singh's CD19RCD28 CAR), CD20, CD22, ROR1, CEA, Her2, L1-CAM, and mesothelin. The following references demonstrate that, at least, the idea of targeting each of these antigens was known before March 23, 2011. In some cases, researchers already had published results from the use of CARs targeting these antigens.

1. Hudecek I

140. In 2009, the inventors listed on the '308 patent, Michael Hudecek and Stanley R. Riddell, among others, published a review article. *See* Hudecek, M. *et al.*,

“Adoptive T-cell Therapy for B-cell Malignancies,” *EXPERT REV. HEMATOL.* 2(5): 517-32 (2009) (Ex. 1011) (“Hudecek I”).⁶

141. Hudecek I explained that “[o]ther surface molecules that are expressed on B-cell tumors have been targeted with CAR-modified T cells in preclinical studies, including CD19 and CD22.” Ex. 1011, 524. A POSA would have understood from this disclosure that CD19- and CD22-specific CARs were in use in preclinical studies before March 23, 2011.

142. Hudecek I also reported data from a then-recent study: “T-cell clones derived from T_{CM}, but not those derived from T_{EM} cells were capable of persisting long term, reacquiring the phenotype of memory cells and populating memory T-cell niches *in vivo* after adoptive transfer.” *Id.*, 525. A POSA would have understood this to mean that T cells derived from T_{CM}, but not T cells derived from T_{EM}, were able to persist long-term.

⁶ I understand that Hudecek I is prior art to the ’308 patent under 35 U.S.C. § 102(b). *EXPERT REVIEW OF HEMATOLOGY* was, and still is, a widely available and respected journal published by Expert Reviews Ltd. as of the date of that publication. Based on my familiarity with academic publishing, this journal in particular, and the state of the art at the time, Hudecek I appears to have been published on the date displayed in its publication citation.

2. Hudecek II

143. The inventors listed on the '308 patent, Michael Hudecek and Stanley Riddell, among others, published a conference abstract in 2009. *See* Hudecek, M. *et al.*, “CD8⁺ T Cells Engineered to Express a ROR1-Specific Chimeric Antigen Receptor Specifically Recognize ROR1 Positive B Cell Tumors,” BLOOD 114(22): 930 (2009) (Ex. 1012) (“Hudecek II”).⁷

144. Hudecek II offered a rationale for targeting ROR1 in the treatment of B-cell malignancies. *See* Ex. 1012, 383 (“Analysis of ROR1 protein expression . . . revealed uniform, stable, and restricted cell surface expression on B-CLL, suggesting this molecule is a candidate for targeted immunotherapy of B cell malignancies.”). In other words, ROR1 was an antigen known to be associated with B-cell malignancies. Hudecek II’s authors “constructed a lentiviral vector that encodes a chimeric antigen receptor . . . specific for ROR1.” *Id.* This means that

⁷ I understand that Hudecek II is prior art to the '308 patent under 35 U.S.C. § 102(b). BLOOD was, and still is, a widely available and respected journal published by American Society of Hematology as of the date of that publication. Based on my familiarity with academic publishing, this journal in particular, and the state of the art at the time, Hudecek II appears to have been published on the date displayed in its publication citation.

Hudecek II's authors already had made a ROR1-targeting CAR. Hudecek II explained that "targeting ROR1 with gene-modified T cells may have advantages over targeting B cell-lineage restricted molecules such as CD19 and CD20 that are expressed on normal mature B cells." *Id.*

3. Abken

145. In 2009, Hinrich Abken, an active contributor to research on CAR-engineered T cells, published an entry in the Encyclopedia of Cancer. *See* Abken, H., "Chimeric T Cell Receptors," in ENCYCLOPEDIA OF CANCER, 2ND EDITION, 649-52 (2009) (Ex. 1013) ("Abken").⁸

146. Abken discloses two "formats" of chimeric T-cell receptors: "The TCR Format" and "The Single Chain Format." Ex. 1013,649. The TCR Format involved "antibody derived binding regions VH and VL for antigen . . . grafted on to the

⁸ I understand that Abken is prior art to the '308 patent under 35 U.S.C. § 102(b). ENCYCLOPEDIA OF CANCER was, and still is, a widely available and respected reference text published by Springer as of the date of that publication. Based on my familiarity with academic publishing, this text in particular, and the state of the art at the time, Abken appears to have been published on the date displayed in its publication citation.

constant domain of the TCR α and β chains.” *Id.* That strategy required “simultaneous expression of two modified TCR chains in engineered T cells.” *Id.*

147. By contrast, Abken also discussed the “Single Chain Format,” which “consists of one polypeptide chain which is composed of an extracellular binding and an intracellular signaling domain.” *Id.* In the “Single Chain Format,” “[t]he antigen binding domain is derived from a single chain fragment of variable regions (scFv) antibody, [and] the signaling moiety is preferentially derived from the CD3 ζ chain of the TCR/CD3 complex.” *Id.* A POSA would understand this to mean that Abken’s “Single Chain Format” refers to a CAR without using the names “CAR” or “chimeric antigen receptor.”

148. Abken explained that a “patient’s T cells from the peripheral blood can be redirected in vitro toward the autologous tumor cells isolated from a tumor biopsy of the same patient.” *Id.*, 651. Abken disclosed that “[t]he approach is universal since by inserting the appropriate binding domain into the chimeric TCR, T cells can be redirected toward a broad variety of tumor associated antigens expressed on tumor cells of different entities, including . . . CEA . . . Her2 . . . [and] CD20”). *Id.* A POSA would understand this to mean that it was possible to make CARs targeting each of the CEA, Her2, and CD20 antigens.

4. Reckamp

149. In 2008, one of the inventors listed on Jensen, Michael C. Jensen, and others published an abstract. *See* Reckamp, K.L. *et al.*, “CE7 Epitope of L1CAM is a Potential Target for Tumor Specific T Cell Therapy in Lung Cancer,” *PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH* 49: 1099 (2008) (Ex. 1014) (“Reckamp”).⁹

150. Reckamp stated that the authors “evaluated the targeting of L1-CAM expressing lung cancer cell lines with cytolytic T cells genetically modified using SIN lentiviral vectors to express a L1-CAM specific chimeric antigen receptor composed of an scFv derived from the CE7 hybridoma that is in turn fused to IgG4:CD4Tm:CD3-ζcyto (CE7R).” Ex. 1014, 1099. Reckamp explained that “[t]he L1-CAM specific monoclonal antibody CE7 from which the CE7R CAR was

⁹ I understand that Reckamp is prior art to the '308 patent under 35 U.S.C. § 102(b). *PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH* was, and still is, a widely available and respected journal published by American Association for Cancer Research as of the date of that publication. Based on my familiarity with academic publishing, this text in particular, and the state of the art at the time, Reckamp appears to have been published on the date displayed in its publication citation.

derived binds to an epitope on the L1 adhesion molecule with high affinity.” *Id.* Reckamp also disclosed that “CE7R expressing CTL can kill L1-CAM-expressing lung cancer cells via CE7R mediated recognition and CTL activation as evidenced by dose-dependent blocking with CE7 monoclonal antibody.” *Id.* A POSA would have understood this to mean that Reckamp’s authors had developed a L1-CAM-targeting CAR. Reckamp explained L1-CAM is an antigen associated with numerous cancers. *Id.* (“L1CAM has been shown to be overexpressed in multiple tumors and associated with invasive/metastatic tumor biology.”). Reckamp concluded that “[t]hese data and future murine modeling will further validate L1-CAM as a target on human lung cancer and potential target for novel T cell therapies.” *Id.*

5. Carpenito

151. In 2009, researchers published results from a study that involved a mesothelin-targeting CAR. *See* Carpenito, C. *et al.*, “Control of Large, Established

Tumor Xenografts with Genetically Retargeted Human T Cells Containing CD28 and CD137 Domains,” PNAS 106(9): 3360-65 (2009) (Ex. 1015) (“Carpenito”).¹⁰

152. Carpenito states that “[m]esothelin is a cell-surface molecule over-expressed on a large fraction of carcinomas, and thus is an attractive target of immunotherapy.” Ex. 1015, 3360. Carpenito’s authors used “[l]entiviral vectors . . . to express a single-chain variable fragment that binds mesothelin and that is fused to signaling domains derived from T-cell receptor zeta, CD28, and CD137 (4-1BB).” *Id.* Carpenito referred to this construct as a chimeric receptor, and a POSA would have understood it to be a CAR. *See id.* (“Chimeric receptors were designed that contain the SS1 scFv that recognizes human mesothelin. . . . We created a series of SS1 scFv-based chimeric receptors that contain the TCR- ζ signal-transduction domain with the CD28 and CD137 (4-1BB) intracellular domains in tandem.”). Carpenito disclosed that “[m]esothelin proved to be an

¹⁰ I understand that Carpenito is prior art to the ’308 patent under 35 U.S.C. § 102(b). PNAS was, and still is, a widely available and respected journal published by The National Academy of Sciences of the USA as of the date of that publication. Based on my familiarity with academic publishing, this text in particular, and the state of the art at the time, Carpenito appears to have been published on the date displayed in its publication citation.

attractive target as the retargeted T cells efficiently and specifically killed a variety of tumors that express mesothelin.” *Id.*, 3363-3364.

D. Using Equivalent Numbers of CD4⁺ and CD8⁺ T Cells in Adoptive Cellular Immunotherapy Compositions Was Known

1. Moeller

153. As discussed above, Singh, Mitsuyasu, and others disclosed that adoptive cellular immunotherapy compositions that contained both CD4⁺ and CD8⁺ were more effective than compositions containing only CD8⁺ T cells. As early as 2005, researchers disclosed that optimal antitumor efficacy could be achieved using equivalent amounts of CD4⁺ and CD8⁺ T cells. *See* Moeller, M. *et al.*, “Adoptive Transfer of Gene-engineered CD4⁺ Helper T Cells Induces Potent Primary and Secondary Tumor Rejection,” *BLOOD* 106: 2995-3003 (2005) (Ex. 1016) (“Moeller”).¹¹

¹¹ I understand that Moeller is prior art to the '308 patent under 35 U.S.C. § 102(b). *BLOOD* was, and still is, a widely available and respected journal published by American Society of Hematology as of the date of that publication. Based on my familiarity with academic publishing, this text in particular, and the state of the art at the time, Moeller appears to have been published on the date displayed in its publication citation.

154. I understand that Moeller was cited in an information disclosure statement during prosecution of the '308 patent. Ex. 1008, 7. I also understand that the patent examiner did not discuss Moeller in his office actions. *Id.*, 10-16, 33-42, 54-58.

155. Moeller's authors sought "to assess whether increasing numbers of gene-engineered antigen-restrict CD4⁺ T cells could enhance an antitumor response mediated by similarly gene-engineered CD8⁺ T cells." Ex. 1016, 2995. They reported that "coinfusing equivalent numbers of engineered CD8⁺ and CD4⁺ T cells led to complete eradication of established tumor in mice, whereas transfer of mainly CD8⁺ T cells was less effective." *Id.*, 2995. Moeller concludes that "[o]ptimal antitumor efficacy was achieved with the addition of equivalent numbers of engineered CD4⁺ and CD8⁺ T cells." *Id.*, 3001.

E. Benefits of T_{CM}, which are CD62L⁺ T Cells, Was Well-Known

1. Wang

156. In 2008, after publication of Singh, researchers described in a conference abstract a rationale and means for enriching T-cell populations for CD62L⁺ T cells. *See* Wang, X. *et al.*, "The CD19 Chimeric Antigen Receptor Re-directs CMV Specific T Cells Derived from Central Memory T Cells (Bi-specific

T Cells) Against Human Acute Lymphoid Leukemia (ALL),” J IMMUNOTHER. 31(9): 926-27 (2008) (Ex. 1017) (“Wang”).¹²

157. Wang reported that “[c]entral memory T cells (T_{CM}) have a unique ability to self-renew, proliferate, and differentiate into effector T_{CM},¹³ which suggests that this T-cell subset will be most effective and persistent upon adoptive transfer.” Ex. 1017, 926. A POSA would have understood this to mean that adoptive cellular immunotherapy compositions should contain a high percentage of T_{CM} cells in order to maximize the compositions’ survival and efficacy.

158. Wang selected for T_{CM} by selecting for T cells that express CD62L using an anti-CD62L antibody. *Id.*, 927 (“we first selected Tcm using the Dreg56 (anti-CD62L) biotinylated antibody and CliniMacs selection.”). After selecting for CD62L-positivity, Wang’s starting T-cell compositions would have been

¹² I understand that Wang is prior art to the ’308 patent under 35 U.S.C. § 102(b). JOURNAL OF IMMUNOTHERAPY was, and still is, a widely available and respected journal published by Lippincott Williams & Wilkins as of the date of that publication. Based on my familiarity with academic publishing, this text in particular, and the state of the art at the time, Wang appears to have been published on the date displayed in its publication citation.

¹³ A POSA would understand that these are T_E derived from T_{CM} cells.

approximately 100% CD62L⁺. A POSA would have understood this because, in theory, the only cells that should bind specifically to an anti-CD62L antibody are CD62L-expressing cells. Wang's authors then expanded their CD62L⁺ T-cell compositions and transduced them with a CD19-specific CAR called "CD19R." *Id.*, 927 ("T cells were . . . genetically redirected to target CD19 on acute lymphoid leukemia through lentiviral transduction with a CD19-specific chimeric antigen receptor, CD19R.").

2. Yang I

159. Before March 23, 2011, researchers understood that there were advantages associated with using T_{CM} cells in adoptive immunotherapies over other subpopulations, including T_{EM} cells. Yang, for example, disclosed these advantages in a manuscript published online for the journal CANCER IMMUNOLOGY IMMUNOTHERAPY on February 9, 2011. *See* Yang, S. *et al.*, "In Vitro Generated Anti-Tumor T Lymphocytes Exhibit Distinct Subsets Mimicking In Vivo

Antigen-Experienced Cells,” *CANCER IMMUNOL IMMUNOTHER* 60:739-49 (2011) (Ex. 1027) (“Yang I”).¹⁴

160. For example, Yang disclosed that T_{CM} cells exhibited greater persistence than T_{EM} cells. *See* Ex. 1027, 745 (“The Tcm sub-population showed significant engraftment and persistence *in vivo* compared to the Tem sub-population.”). Additionally, Yang determined that secretion of a cytokine called interleukin-2 (“IL-2”) was greater in T_{CM} cells versus T_{EM} cells. *See id.*, 739 (“When the cells were sorted into populations that were CD45RO⁺/CD62L⁻ (Tem), CD45RO⁺/CD62L⁺ (Tcm), or CD45RO^{low}/CD62L⁺ (Tn) and co-cultured with antigen-matched tumor lines, the magnitude of cytokine release from these populations for . . . IL-2 (Tn > ***Tcm*** > ***Tem***) mimicked the types of immune cell responses observed *in vivo*.”) (emphasis added).

¹⁴ I understand that Yang I is prior art to the ’308 patent under 35 U.S.C. § 102(a). *CANCER IMMUNOLOGY IMMUNOTHERAPY* was, and still is, a widely available and respected journal published by Springer as of the date of that publication. Based on my familiarity with academic publishing, this journal in particular, and the state of the art at the time, Yang I was published online on the date recited on the first page of the article, February 9, 2011.

161. Furthermore, the authors found that, using a T cell engineering method that was commonly used at the time, where bulk T cells are activate with anti-CD3/anti-CD28 and transduced with a lentivirus, T cell products manufactured independently from three volunteers were more than 80% CD62L positive despite not enriching specifically for CD62L⁺ T cells. *See id.*, 742 (showing that three compositions had CD62L-positivity above 80% despite not enriching specifically for CD62L). The authors concluded that cells displaying a T_{CM}-like phenotype would be optimal for adoptive immunotherapy. *See id.*, 739 (“a large percentage of *in vitro* generated anti-tumor T lymphocytes mimic a Tcm-like phenotype (based on phenotype, effector function, and increased persistence *in vivo*), which suggests that these Tcm-like cultured T cells may be optimal for adoptive immunotherapy”).

3. Yang II

162. The same researchers disclosed similar results in an abstract published in the journal MOLECULAR THERAPY. *See* Yang, S., *et al.*, “TCR Engineered and In

Vitro Expanded T Lymphocytes Recapitulate the Properties of Central Memory T Cells,” MOLECULAR THERAPY 18(Supp. 1): S184 (2010) (Ex. 1028) (“Yang II”).¹⁵

163. In Yang II, the authors disclosed that T_{CM} cells and T_{EM} cells could be distinguished based on CD62L expression and IL-2 secretion. *See* Ex. 1028, S184 (“Based on the differentiation marker L-selectin/CD62L and the release of the cytokine IL-2, central memory T cells can be distinguished from effector memory T cells.”). Specifically, the authors reported that T_{CM} cells secrete more IL-2 than T_{EM} cells. *See id.* (“*Tem* sorted cells secrete high levels of IFN-γ and *low amounts of IL-2* while *Tcm* cells showed the opposite reactivity (*high IL-2* and low IFN-γ).”) (emphasis added).

4. Sallusto

164. Before March 23, 2011, researchers understood that T_{CM} cells secreted more of the cytokine, IL-2, as compared to T_{EM} cells. Sallusto reported this in

¹⁵ I understand that Yang II is prior art to the '308 patent under 35 U.S.C. § 102(a). MOLECULAR THERAPY was, and still is, a widely available and respected journal published by American Society of Gene & Cell Therapy as of the date of that publication. Based on my familiarity with academic publishing, this journal in particular, and the state of the art at the time, Yang II appears to have been published on the date displayed in its publication citation.

November 1999 in the journal NATURE. *See* Sallusto, F., Lenig, D., Förster, R. et al. “Two subsets of memory T lymphocytes with distinct homing potentials and effector functions,” NATURE 401:708–712 (1999) (Ex.1029) (“Sallusto”).¹⁶ Sallusto Figure 2a shows that central memory CD4⁺ T cells (CD45RA⁻CCR7⁺) produce more IL-2 than effector memory CD4⁺ T cells (CD45RA⁺CCR7⁻). Ex.1029, 709 (Figure 2a). Sallusto also shows that central memory CD8⁺ T cells (CD45RA⁻CCR7⁺) also produce more IL-2 than effector memory CD8⁺ T cells (CD45RA⁺CCR7⁻). Ex.1029, 709 (Figure 2c).

5. Sun

165. Before March 23, 2011, researchers understood that CD4⁺ T_{CM} cells had a higher capacity to proliferate as compared to CD4⁺ T_{EM} cells. Sun reported this in February 2005 in the journal The JOURNAL OF IMMUNOLOGY. *See* Sun, Y., et al. “Dysfunction of Simian Immunodeficiency Virus/Simian Human Immunodeficiency Virus-Induced IL-2 Expression by Central Memory CD4⁺ T

¹⁶ I understand that Sallusto is prior art to the '308 patent under 35 U.S.C. § 102(b). NATURE was, and still is, a widely available and respected journal published as of the date of that publication. Based on my familiarity with academic publishing, this journal in particular, and the state of the art at the time, Sallusto appears to have been published on the date displayed in its publication citation.

Lymphocytes,” J IMMUNOL. (2005) 174 (8): 4753–4760 (Ex.1032) (“Sun”).¹⁷ Sun assesses cytokine production profiles of functionally distinct subsets of CD4⁺ T lymphocytes in rhesus monkeys that are normal, infected with SHIV (simian human immunodeficiency virus), or vaccinated. Ex.1032, 4753. Sun states “ [c]entral memory T lymphocytes home to lymphoid organs, have little or no effector function, produce predominantly IL-2, and have a high capacity to proliferate.” Ex.1032, 4753. Sun shows that the percentage of IL-2 positive CD4⁺ T_{CM} cells is greater than the percentage of IL-2⁺ of T_{EM} CD4⁺ cells. Ex.1032, 4756-4758 (Figure 3, Figure 5, Figure 7).

F. Formulations for Adoptive Cellular Immunotherapy Compositions Were Known

1. Jensen

166. Before March 23, 2011, researchers knew that formulating a T-cell composition for *in vivo* use was a simple matter of mixing the T cells in a saline

¹⁷ I understand that Sun is prior art to the '308 patent under 35 U.S.C. § 102(b). The JOURNAL OF IMMUNOTHERAPY was, and still is, a widely available and respected journal published by Lippincott Williams & Wilkins as of the date of that publication. Based on my familiarity with academic publishing, this text in particular, and the state of the art at the time, Sun appears to have been published on the date displayed in its publication citation.

solution. As discussed above, Mitsuyasu’s authors used a saline solution supplemented with human serum albumin for infusion. Other researchers did the same. *See, e.g.*, M.C. Jensen *et al.*, “CD19-Specific Redirected Immune Cells,” U.S. Patent Application 2004/0126363 A1 (Ex. 1018) (“Jensen”).¹⁸

167. Jensen disclosed a clinical trial protocol involving CD19-specific CAR-expressing T cells. *See* Ex. 1018, [0159] (“The phase I pilot study determines the safety and toxicity of intravenously infused donor-derived CD8⁺ CTL clones genetically modified to express the CD19R-scFvFc:ζ chimeric immunoreceptor”). Jensen teaches a method for processing T cells into a finished dosage form for patient infusion—mixing the cells with a saline solution supplemented with human serum albumin. *See id.*, [0162] (“Processed cells are resuspended in 100 ml of 0.9% NaCl with 2% human serum albumin in a bag for [sic] suitable for clinical re-infusion.”).

IX. LEGAL STANDARDS

168. I understand that the Board will institute an inter partes review if the Board determines that there is a reasonable likelihood that Petitioner would prevail with respect to at least one of the claims challenged in the petition. I understand that once the Board has instituted trial in response to a petition, Petitioner has the burden to show by a preponderance of the evidence that the challenged claims are

¹⁸ I understand that Jensen is prior art to the ’308 patent under 35 U.S.C. § 102(b).

unpatentable. I have been informed that the preponderance of the evidence exists when it is more likely than not that the assertion in question is true. I have been informed that the relevant standard for anticipation is provided in 35 U.S.C. §§102(a)-102(b):

A person shall be entitled to a patent unless—

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent, or

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of the application for patent in the United states.

35 U.S.C. §§ 102(a)-102(b). I have been informed that the relevant standard for obviousness is provided in 35 U.S.C. § 103(a):

A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

35 U.S.C § 103(a).

169. I understand that Petitioner needs to show by a preponderance of the evidence that a POSA would have been motivated to combine the teachings of the prior art references (or the prior art references with the general knowledge of a POSA) to achieve the claimed invention, and that a POSA would have a reasonable

expectation of success in doing so. I also understand that the motivation to combine the prior art is a recognition, expressly or impliedly in the prior art or drawn from a convincing line of reasoning based on established scientific principles or legal precedent, that some advantage or expected beneficial result would have been produced by their combination.

170. I have also been informed that an invention may be considered obvious if routine optimization could have been used to arrive at the claimed invention. I understand that routine optimization includes testing an element or parameter identified as a “results-effective” variable, meaning a variable that achieves a recognized result. I also understand that routine optimization involves only using routine techniques known to a POSA.

171. I also understand that a reasonable expectation of success is found when all the claimed elements were known in the prior art and a POSA could have combined the elements as claimed by known methods with no change in their respective functions, and the combination would have yielded nothing more than predictable results to one of ordinary skill in the art.

X. GROUND 1: INDEPENDENT CLAIM 1 AND DEPENDENT CLAIMS 2-7, 14, 16, 21, AND 26-28 ARE ANTICIPATED BY SINGH

172. Before March 23, 2011, a POSA would have understood claims 1-7, 14, 16, 21, and 26-28 to be anticipated by Singh.

A. Independent Claim 1

173. Independent Claim 1 reads:

[a] “An adoptive cellular immunotherapy composition”

[b] “containing chimeric antigen receptor-modified CD4⁺ T lymphocytes and chimeric antigen receptor-modified CD8⁺ T lymphocytes,”

[c] “wherein: (a) the chimeric antigen receptor-modified CD4⁺ T lymphocytes contain a chimeric antigen receptor that specifically binds to an antigen”

[d] “and at least 50% of the chimeric antigen receptor-modified CD4⁺ helper T lymphocytes in the composition are surface positive for CD62L and/or CD45RA,

[e] “and (b) the chimeric antigen receptor-modified CD8⁺ T lymphocytes contain a chimeric antigen receptor that specifically binds to the antigen”

[f] “and at least 50% of CD8⁺ cytotoxic T lymphocytes in the composition are surface positive for CD62L and/or CD45RO.”

174. The elements of claim 1, to which reference letters [a]-[f] have been added, are addressed in two main sections below. In the first section, I explain that the prior art disclosed the components of the claimed composition (claim elements [a]-[c], [e]). In the second section, I explain that the claimed surface positivity of phenotypic markers (claim elements [d] and [f]) also was fully disclosed in the prior art.

1. Claim Limitations Directed to Components of the Claimed Composition

175. Singh discloses an adoptive cellular immunotherapy composition containing CD4⁺ and CD8⁺ T cells modified with a CD19-specific CAR (claim elements [a]-[c], [e]).

176. Singh disclosed that its authors developed “a clinically feasible approach to the efficient propagation of CAR⁺ T-cell populations, including T_{CM}.” Ex. 1003, 2961. As discussed in Section VIII.A.1 above, that approach involved electroporation of the *Sleeping Beauty* system encoding a CD19-specific CAR into T cells and growing the resulting CAR-modified T cells in the presence of CD19⁺ aAPCs.

177. Singh’s authors planned to evaluate cells generated using Singh’s approach in a clinical trial. *Id.*, 2970-2971 (“We have now incorporated *ex vivo* CAR-dependent proliferation to derive genetically modified T cells and will evaluate the CD19-specific T cells, using *SB* transposition and aAPC, in a next-generation clinical trial.”). As discussed in Section VIII.A.1 above, some of Singh’s authors did exactly that by using Singh’s manufacturing process in connection with clinical trial # NCT00968760.

a. [a] “[a]n adoptive cellular immunotherapy composition”

178. As mentioned in Section VII.A above, the term “adoptive cellular immunotherapy composition” is non-limiting on the scope of the Challenged Claims because it is a statement of purpose, the term is not necessary to give meaning or provide structure to the claims, and the applicant did not rely of the term to distinguish the Challenged Claims from prior art. However, even if it were limiting, Singh discloses this limitation.

179. A POSA would have understood that Singh disclosed a “composition for adoptive cellular immunotherapy formulated to be suitable for administration to a mammal.” A POSA would have known that Singh’s compositions included T cells from mammals; *i.e.*, human subjects. *See* Ex. 1003, 2963 (“Human T cells were isolated by density gradient centrifugation over Ficoll-Paque-Plus, from umbilical cord blood or peripheral blood mononuclear cells after consent”). Generally speaking, T cells from a particular mammal would be suitable for administration to that mammal.

180. Additionally, Singh emphasizes the *Sleeping Beauty* system’s performance in the context of the mammalian genome. *See id.*, 2962 (“The *SB* transposase mediates transposition by binding to [inverted repeats], excising a precise DNA sequence flanked by the [inverted repeats], and inserting the transposon into any of ~ 200 million TA sites in a mammalian genome.”). A POSA

would thus understand that the *Sleeping Beauty* system would be suitable for administration to a mammal.

181. Further, Singh's CAR, CD19RCD28, was human codon optimized with those codons optimally used in humans. *See id.* ("The second-generation CD19RCD28 CAR was human codon optimized (CoOp), substituting codons with those optimally used in mammals (GENEART) without altering anticipated amino acid sequence."). A POSA would understand that "human codon optimization" generally describes a process of making synonymous changes, or "mutations," to a DNA sequence to boost expression of the protein it encodes. *See id.*, 2961 ("we codon-optimized (CoOp) the CAR as reports have shown that codon optimization of genes toward human consensus codon usage increases protein expression."). Synonymous mutations are changes to a DNA sequence that do not change the amino acid sequence that the DNA sequence encodes. A POSA would know that a human codon optimized CAR would be suitable for administration to a human (*i.e.*, a mammal).

182. A POSA would understand that a composition intended for use in clinical trials would be suitable for administration to a human (*i.e.*, a mammal), and Singh disclosed that its authors intended to use their compositions in clinical trials. *See Id.*, 2970 (reporting "a new gene transfer approach for the *clinical* application of T cells with redirected specificity for desired antigens") (emphasis added); 2969

(describing Singh’s manufacturing process “to be used in proof-of-concept *clinical trials*.”) (emphasis added). Singh explains that its technology “is readily accessible and practiced in compliance with cGMP for *phase I/II trials*” *Id.*, 2970 (emphasis added). Singh confirms that clinical trials would involve adoptive transfer of its cells. *See id.* (“*Clinical trials* will be needed to determine whether *adoptive transfer* of these CAR⁺ T cells . . . results in long-term *in vivo* persistence . . .”) (emphasis added).

183. Singh discloses that its manufacturing process resulted in compositions having “clinically meaningful” numbers of T cells. *See id.*, 2962 (explaining that its cells “can be numerically expanded to *clinically meaningful* numbers”) (emphasis added). A POSA would have understood this to mean that Singh’s compositions would be suitable for administration to a human (*i.e.*, a mammal) because, at least, the composition had enough T cells to elicit a clinical effect.

184. For these reasons, a POSA would understand that Singh’s compositions were “adoptive cellular immunotherapy composition[s].”

b. [b] “containing chimeric antigen receptor-modified CD4⁺ T lymphocytes and chimeric antigen receptor-modified CD8⁺ T lymphocytes,”

185. Singh recognized the importance of infusing patients with compositions containing both CD4⁺ and CD8⁺ T cells. *See Ex. 1003*, 2970 (“There is convincing clinical data that the presence of CD4⁺ T-helper cells improves the persistence of

CD8⁺ antigen-specific T cells.”). A POSA would have understood this to mean that CD8⁺ T cells persist longer when in the presence of CD4⁺ T cells, as compared to CD8⁺ T cells alone.

186. Singh’s authors expected that future clinical trials would involve infusing compositions containing both CD4⁺ and CD8⁺ T cells. *See id.* (“Next-generation clinical trials . . . are expected to infuse predefined populations of T cells with defined characteristics such as the inclusion of both CD4⁺ and CD8⁺ T cells and T_{CM}.”).

187. Singh designed its manufacturing process as one that would facilitate clinical trial design involving infusion of both CD4⁺ and CD8⁺ T cells:

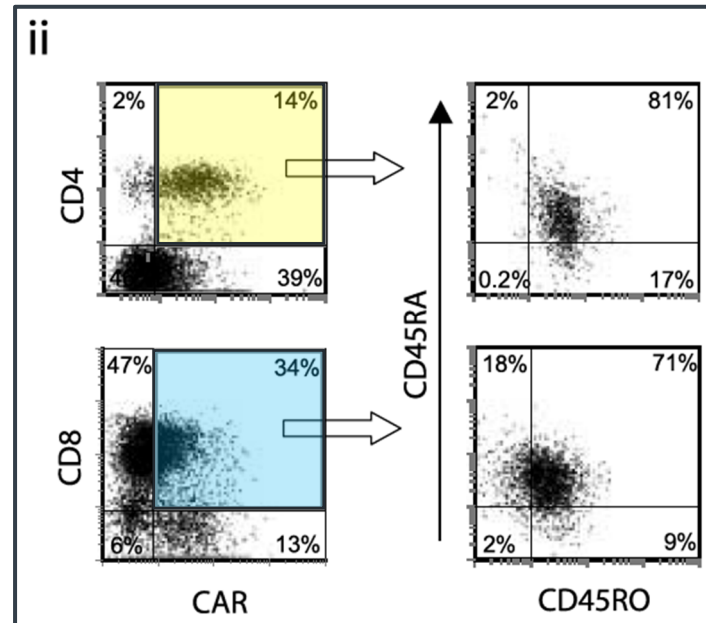
The relative ease of DNA plasmid production, electroporation, and outgrowth of stable integrants on a thawed γ -irradiated bank of aAPC can be readily transferred to the facilities operating in compliance with current good manufacturing practice (cGMP) for phase I/II trials. This is predicted to greatly facilitate trial design infusing CD4⁺ and CD8⁺ T cells that have desired immunophenotype, including T_{CM}.

Id., 2962.

188. Singh confirmed that its compositions contained both CD4⁺ and CD8⁺ T cells, and that both of those cell types had been modified with a CAR. *See Id.*, 2965 (“we observed that . . . CD4⁺ and CD8⁺ T cells readily expressed the CAR”).

189. For example, in Singh’s Figure 3.B.ii, in “T cells generated after coculture[,] CAR⁺ CD4 or CD8 cells were analyzed for the expression of CD45RA and CD45RO.” A POSA would understand this to mean that Singh’s compositions

included both CD4⁺ and CD8⁺ T cells that had been modified with a CAR. Figure 3.B.ii confirms this point:



Id., 2966 (annotated). A POSA would have understood that, in the composition analyzed in Figure 3.B.ii, CAR-modified CD4⁺ T cells made up 14% of the composition (annotated in yellow), and CAR-modified CD8⁺ T cells were 34% of the composition (annotated in blue). This would have confirmed for a POSA that Singh’s compositions included both CD4⁺ and CD8⁺ T cells that had been modified with a CAR.

190. For these reasons, a POSA would understand that Singh discloses adoptive cellular immunotherapy compositions “containing chimeric antigen receptor-modified CD4⁺ T lymphocytes and chimeric antigen receptor-modified CD8⁺ T lymphocytes.”

- c. [c] & [e] “wherein: (a) the chimeric antigen receptor-modified CD4⁺ T lymphocytes [and (b) the chimeric antigen receptor-modified CD8⁺ T lymphocytes] contain a chimeric antigen receptor that specifically binds to an antigen”

191. A POSA would have understood that the CAR in Singh’s CD4⁺ and CD8⁺ T cells specifically binds to the CD19 antigen. *See* Ex. 1003, 2961 (“We report a new approach to efficiently generating T cells with redirected specificity, introducing DNA plasmids . . . to directly express a CD19-specific CAR in memory and effector T cells”). A POSA would understand that a “CD19-specific CAR” is one that “specifically binds to” CD19.

192. Singh designated its CD19-specific CAR “CD19RCD28.” A POSA would have understood from the name alone that it targets CD19. However, Singh confirmed that CD19RCD28 conferred CD19-dependent signaling to Singh’s T cells, making them capable of specifically binding CD19⁺ tumor cells. *See id.* (“CD19RCD28 . . . provides CD19-dependent signaling”); *id.* (“this gene transfer method results in rapid outgrowth of **CD4⁺ and CD8⁺ T cells expressing CAR** to redirect ***specificity for CD19⁺ tumor cells.***”). (emphasis added)

193. For these reasons, a POSA would understand that Singh discloses adoptive immunotherapy compositions “wherein the chimeric antigen receptor-modified [CD4⁺ and CD8⁺] T lymphocytes contain a chimeric antigen receptor that specifically binds to an antigen.”

2. Claim Limitations Directed to Phenotypic Marker Surface Positivity

194. Claim 1 states that at least 50% of its CAR-modified CD4⁺ T cells “are surface positive for CD62L and/or CD45RA,” and that at least 50% of its CD8⁺ T cells “are surface positive for CD62L and/or CD45RO.” As discussed in Section VII.B above, “and/or,” as it appears in the Challenged Claims, covers embodiments having the element preceding “and/or,” the element following “and/or,” or both the element preceding “and/or” and the element following “and/or.”

195. Claim 1 states that “at least 50% of CD8⁺ cytotoxic T lymphocytes in the composition are surface positive for CD62L and/or CD45RO.” Unlike for the CAR-modified CD4⁺ helper T cells, this claim language for the “at least 50%” immunophenotype for the CD8⁺ cytotoxic T cells does not state that it is only for the CAR-modified CD8⁺ subpopulation. Regardless of whether the claim requires that the “at least 50%” immunophenotype be for the CAR-modified or overall CD8⁺ T cell population, Singh meets this limitation.

196. In Singh’s compositions, at least 50% of the CAR-modified CD4⁺ T cells were surface positive for CD45RA (satisfying claim element [d]). Additionally, at least 50% of the overall CD8⁺ T cells and the CAR-modified CD8⁺ T cells in Singh’s compositions were surface positive for CD45RO (satisfying claim element [f]).

- a. [d] & [f] “at least 50% of the chimeric antigen receptor-modified CD4⁺ helper T lymphocytes in the composition are surface positive for CD62L and/or CD45RA,” and “at least 50% of CD8⁺ cytotoxic T lymphocytes in the composition are surface positive for CD62L and/or CD45RO”

197. As mentioned above, 83% of Singh’s CAR-modified CD4⁺ helper T cells were surface positive for CD45RA, 80% of its CAR-modified CD8⁺ T cells were surface positive for CD45RO, and at least 50% of its CD8⁺ T cells were surface positive for CD45RO, thereby satisfying limitations [d] and [f]. I explain this further below.

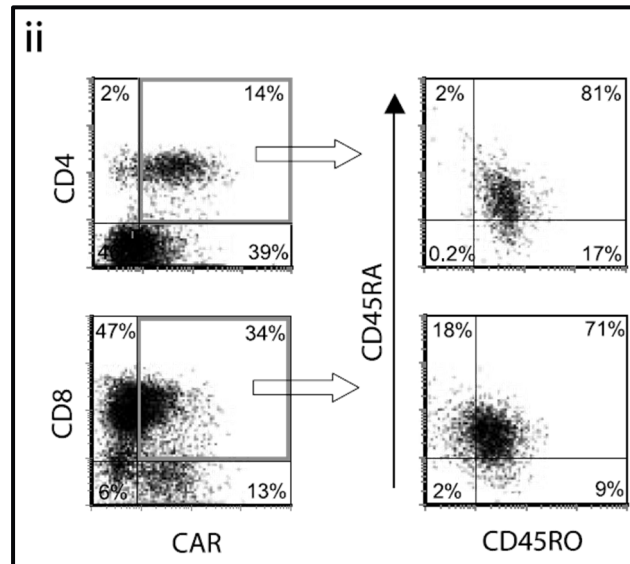
198. Singh discloses a process for transforming, expanding, and maintaining the immunophenotypes of its T cells that involved culturing its CAR-modified T cells with CD19⁺ artificial antigen-presenting cells (“aAPCs”). *See* Ex. 1003, 2966 (describing culture on γ -irradiated K562-derived artificial antigen-presenting cells expressing CD19, among other stimuli); *see also* Section VIII.A.1 *supra*.

199. The culturing method used in Singh is a routine procedure used in a laboratory setting to simulate what occurs when a T cell encounters an antigen-presenting cell (“APC”). In these specific experiments, Singh’s authors added irradiated artificial APCs—aAPCs—to its T cells to simulate antigen presentation and activation *ex vivo*.

200. After culturing their T cells, Singh’s authors assessed CD45RO and CD45RA expression in their CAR-modified T cells using FACS. Furthermore,

Singh explained that T_{CM} persist longer *in vivo*. Ex. 1003, 2968 (“These data have implications for improved *in vivo* efficacy as T_{CM} are associated with long-term persistence after adoptive transfer.”).

201. Singh’s Figure 3.B.ii shows FACS data for CD4, CD8, CD45RA, and CD45RO expression:

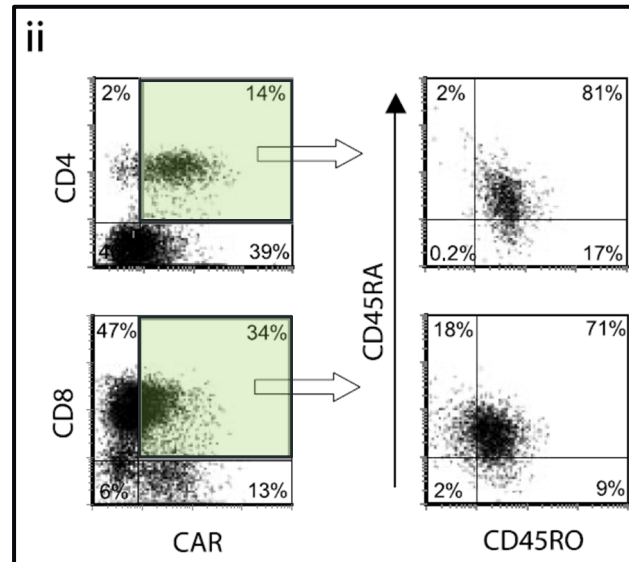


Id., 2966 (Figure 3.B.ii).

202. Singh’s Figure 3.B.ii shows FACS results on four dot plots. Each dot represents a single cell. The two dot plots on the left show a first gating step in which the authors grouped T cells by CAR expression (x-axes) and CD4 (top graph) or CD8 expression (bottom graph).

203. In the first gating step, Singh shows that 14% of cells were CAR-modified $CD4^+$ and 34% were CAR-modified $CD8^+$, as shown by the

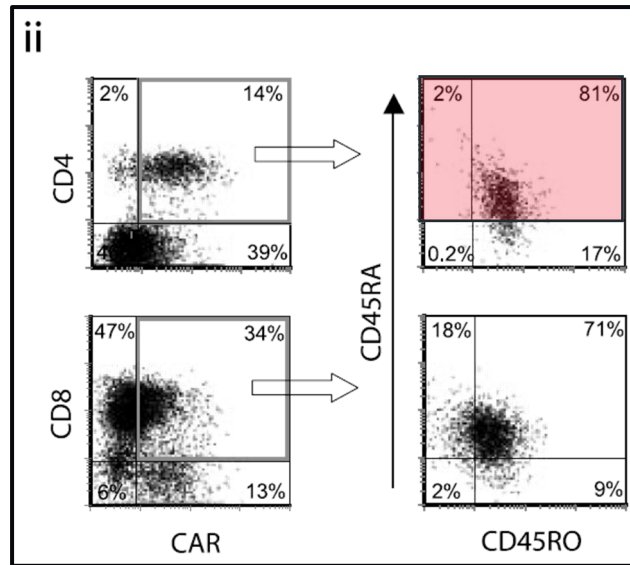
upper-right quadrant of the left-most dot plots in Figure 3.B.ii (green shading added, below):



Id. (annotated).

204. The two dot plots on the right of Figure 3.B.ii represent a second step for gating cells by CD45RO (x-axis) and CD45RA (y-axis) expression. A POSA would have understood that Singh's authors used a flow cytometry software to select CAR-modified CD4⁺ and CAR-modified CD8⁺ T cells for further analysis—here, for CD45RA and CD45RO expression.

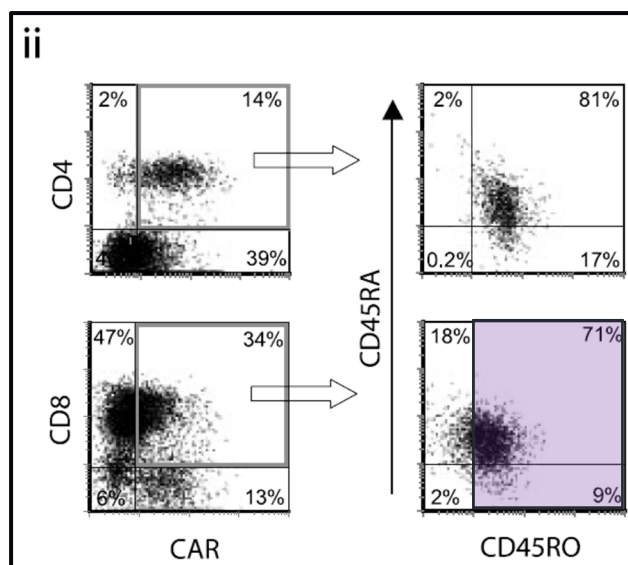
205. As shown below, the top quadrants of the upper-right dot plot indicated that, of the CD4⁺ T cells that contained the CAR modification, 83% were CD45RA⁺ (red, 2% + 81% = 83%):



Id. (annotated).

206. It is appropriate to add the subpopulation representing two percent of the composition's cells (*i.e.*, CAR-modified CD4⁺ T cells that are CD45RA⁺ and CD45RO⁻) to the subpopulation representing eighty-one percent of the population (*i.e.*, CAR-modified CD4⁺ T cells that are CD45RA⁺ and CD45RO⁺) because all cells above the horizontal line in the dot plot are CD45RA⁺. As noted earlier, an experienced flow cytometrist will establish the cut-off points that define which cells are positive/negative for a given marker and these criteria are well understood to POSAs.

207. In addition, 80% of Singh's CAR-modified CD8⁺ T cells were CD45RO⁺ (purple shading added, 9% + 71% = 80%):

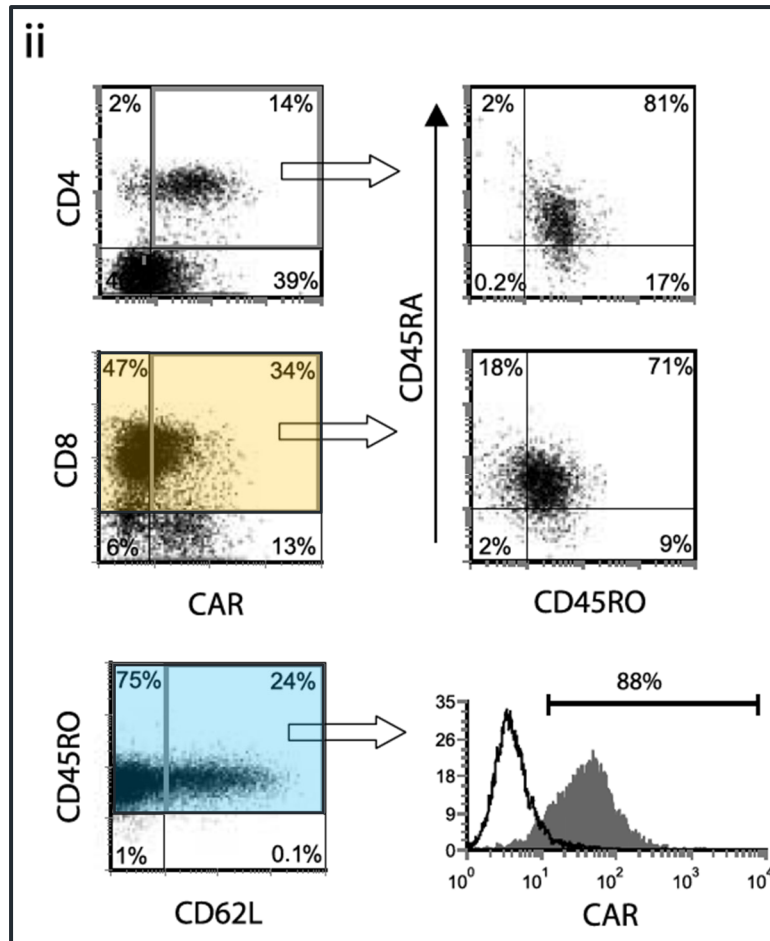


Id. (annotated).

208. It is appropriate to add the subpopulation representing nine percent of the composition's cells (*i.e.*, CAR-modified CD8⁺ T cells that are CD45RA⁻ and CD45RO⁺) to the subpopulation representing seventy-one percent of the population (*i.e.*, CAR-modified CD8⁺ T cells that are CD45RA⁺ and CD45RO⁺) because all cells to the right of the vertical line in the dot plot are CD45RO.

209. Therefore, Singh discloses that 83% of its CAR-modified CD4⁺ T cells were CD45RA⁺ and 80% of its CAR-modified CD8⁺ T cells were CD45RO⁺.

210. A POSA also would understand that Singh discloses the approximate percentage of its overall CD8⁺ T cells (CAR-modified and non-CAR-modified) in its composition that are surface positive for CD45RO. Fig. 3.B.ii includes a third FACS plot in addition to those shown above:



Id. (annotated).

211. I have annotated in orange Singh's $CD8^+$ T cells, which make up 81% of cells in its composition.

212. I have annotated in blue Singh's $CD45RO^+$ T cells, which make up 99% of cells in its composition. A POSA would understand, then, that only 1% of Singh's T cells were $CD45RO^-$.

213. Putting together the very high percentages of Singh's T cells that are $CD8^+$ and the very high percentages that are $CD45RO^+$, a POSA would recognize that, mathematically, at least 50% of Singh's T-cell population must be both $CD8^+$

and CD45RO⁺. For example, even if all CD45RO⁻ T cells are CD8⁺, a POSA would understand that roughly 99% of Singh's CD8⁺ T cells had to be CD45RO⁺. A simple calculation demonstrates this outcome. Assume that a T-cell composition contains 100 cells. Because 81% of Singh's T cells were CD8⁺, 81 cells in the composition would be CD8⁺. Assuming that all CD45RO⁻ cells are CD8⁺ (here, 1 cell), then of the 81 CD8⁺ T cells, 1 cell would be CD8⁺ CD45RO⁻ and 80 would be CD8⁺ CD45RO⁺. In percentage terms, eighty CD8⁺ CD45RO⁺ cells out of eighty-one CD8⁺ cells means that roughly 99% ($[80/81] \times 100\%$) of Singh's CD8⁺ T cells were CD45RO⁺.

214. For these reasons, a POSA would understand that Singh discloses adoptive cellular immunotherapy compositions wherein "at least 50% of the chimeric antigen receptor-modified CD4⁺ helper T lymphocytes in the compositions are surface positive for . . . CD45RA." Singh's compositions also include at least 50% of CD8⁺ cytotoxic T lymphocytes [that] are surface positive for . . . CD45RO and at least 50% of the CAR-modified CD8⁺ cytotoxic T lymphocytes that are surface positive for CD45RO.

B. Dependent Claims Directed to Antigens

1. Claims 2 and 26-28: "wherein the antigen is associated with a disease or disorder"

215. Claim 2 recites "a disease or disorder selected from a solid tumor, hematologic malignancy, melanoma, and infection with a pathogen." Claim 26

depends from claim 2 and specifies “the disease or disorder is a solid tumor, hematologic malignancy, or melanoma.” Claim 27 also depends from claim 2 and specifies “the disease or disorder is a hematologic malignancy.”

216. As explained in Section X.A.1.c, Singh’s CAR binds to the CD19 antigen. Singh teaches that CD19 is expressed on B-cell malignancies. *See* Ex. 1003, 2961 (“[W]e have previously shown that genetically modified peripheral blood- and umbilical cord blood-derived T cells can be rendered specific for **CD19, a molecule constitutively expressed on B-cell malignancies.**”) (emphasis added). A POSA would have understood that “constitutively expressed on B-cell malignancies” means always, or constantly, expressed on B-cell malignancies—the gene is always “on.” Moreover, a POSA would have known that a B-cell malignancy is a type of hematologic malignancy. *See, e.g.,* Ex. 1011, 517 (“Allogeneic HCT provides a potentially curative therapy for a variety of **hematologic malignancies, including many B-cell tumors.**”) (emphasis added). A POSA would have understood that “B-cell tumors” are “B-cell malignancies.” *See, e.g., id.*, 517 (“The success of allogeneic hematopoietic cell transplantation (HCT) for **B-cell malignancies** is evidence that **these tumors** can be eliminated by T lymphocytes.”) (emphasis added).

217. Therefore, a POSA would understand that Singh discloses an antigen—CD19—that is associated with B-cell malignancies. B-cell malignancies are a type of hematologic malignancy; thus Singh’s disclosure satisfies claims 2 and 26-27.

218. Claim 28 specifies “the antigen is a tumor associated antigen associated with a cancer selected from the group consisting of melanoma, breast cancer, squamous cell carcinoma, colon cancer, leukemia, myeloma, prostate cancer, B cell lymphoma, breast cancer, prostate cancer, and leukemia.”

219. A POSA also would have known that, because CD19 is expressed on B-cell malignancies, it is “a tumor associated antigen associated with a cancer.” A POSA would have understood that B-cell malignancies are cancers. A POSA also would have understood that B-cell malignancies encompass B-cell lymphoma and leukemia. *See id.*, 518 (“A GVT effect of allogeneic HCT has subsequently been confirmed in other ***B-cell malignancies, including*** chronic lymphocytic ***leukemia*** (B-CLL), multiple myeloma (MM) and non-Hodgkin ***lymphoma*** (NHL).”).

220. Therefore, a POSA would understand that Singh’s disclosure satisfies claim 28 as well.

2. Claims 3 and 21: “wherein the antigen is selected from ROR1, tEGFR, Her2, L1-CAM, CD19, CD20, CD22, mesothelin, and CEA”

221. Claim 3 specifies “wherein the antigen is selected from ROR1, tEGFR, Her2, L1-CAM, CD19, CD20, CD22, mesothelin, and CEA,” while claim 21 is limited to “CD19.”

222. As described in Section X.A.1.c, Singh teaches this limitation by disclosing a CAR that binds to the CD19 antigen.

223. For these reasons, a POSA would understand that Singh discloses “a chimeric antigen receptor that specifically binds to an antigen” that is CD19.

C. Dependent Claims Directed to CAR Components

1. Claims 4 and 5: “extracellular antibody variable domain or single-chain antibody fragment and an intracellular signaling module”

224. Claim 4 specifies “wherein the chimeric antigen receptor of (a) and/or (b) comprises an extracellular antibody variable domain or single-chain antibody fragment specific for the antigen and an intracellular signaling module.”

225. A POSA would have understood that a “single-chain antibody fragment specific for the antigen” could be a scFv (*i.e.* single-chain variable fragment from an antibody) that is specific for an antigen.

226. Singh discloses that its CAR, designated “CD19RCD28,” is specific for CD19. *See* Ex. 1003, 2961 (“a second-generation CAR, designated **CD19RCD28**, has been developed that provides **CD19-dependent signaling** through chimeric CD3- ζ and CD28”) (emphasis added).

227. A POSA would have understood that CD19RCD28 includes a CD19-specific scFv because Singh discloses that the CAR from which CD19RCD28 was derived, designated “CD19R,” “**recognizes CD19 via the scFv** of a murine CD19-specific monoclonal antibody.” *See id.*, 2961 (emphasis added).

228. Singh also indicates that its CD19CD28 CAR includes an intracellular signaling module. *See id.*, 2961 (“a second-generation CAR, designated CD19CD28, has been developed that provides CD19-dependent signaling through *chimeric CD3-ζ and CD28*”) (emphasis added). The ’308 patent indicates that an “intracellular signaling molecule” can comprise CD3 and CD28 intracellular domains. *See* Ex. 1001, 17:12-15 (“the intracellular signaling molecule comprises the CD3 intracellular domain, a CD28 domain, [or] a CD28 transmembrane and signaling domain linked to a CD3 intracellular domain”). A POSA would have understood that CD3-ζ is a CD3 intracellular domain.

229. For these reasons, a POSA would understand that Singh discloses a single-chain antibody fragment (*i.e.*, scFv) specific for CD19 and an intracellular signaling module that is CD3-ζ.

230. Claim 5 depends from claim 4, and specifies “wherein each of the intracellular signaling module of the chimeric antigen receptor contained by the CD4⁺ T lymphocytes and the intracellular signaling module of the chimeric antigen receptor contained by the CD8⁺ T lymphocytes, individually, comprise (a) a CD28 costimulatory domain and a CD3 intracellular signaling domain, or (b) a 4-1BB costimulatory domain and a CD3 intracellular signaling domain.”

231. As discussed with respect to claim 4, Singh’s CAR includes a CD28 costimulatory domain and a CD3 intracellular signaling domain. *See* Ex. 1003, 2961

(“a second-generation CAR, designated CD19RCD28, has been developed that provides CD19-dependent signaling through *chimeric CD3-ζ and CD28*”) (emphasis added).

232. For these reasons, a POSA would understand that Singh discloses a CAR, expressed in both CD4⁺ and CD8⁺ T cells, that has an intracellular signaling module comprising a CD28 costimulatory domain and a CD3 intracellular signaling domain.

2. Claims 7 and 16: Claims Related to Whether the CAR is the Same in the CD4⁺ and CD8⁺ T Cells

233. Claim 7 specifies “wherein the intracellular signaling domain of the chimeric antigen receptor contained by the CD8⁺ T lymphocytes is the same as the intracellular signaling domain of the chimeric antigen receptor contained by the CD4⁺ T lymphocytes.”

234. Singh transfected the same CAR—CD19RCD28—into its CD8⁺ and CD4⁺ T cells via a plasmid identified as CoOpCD19RCD28/pT-MNDU3. *See* Ex.1003, 2963 (“PBMCs and umbilical cord blood mononuclear cells were . . . mixed with . . . CoOpCD19RCD28/pT-MNDU3”); *id.*, 2965 (“[W]e observed that peripheral blood- and umbilical cord blood-derived electroporated CD4⁺ and CD8⁺ T cells readily expressed the CAR transposon.”).

235. For these reasons, a POSA would understand that Singh discloses a composition wherein the intracellular signaling domain of the CAR contained by the CD8⁺ T lymphocytes and the CD4⁺ T lymphocytes is the same.

236. Claim 16 specifies “wherein the chimeric antigen receptor contained by the CD8⁺ T lymphocytes is the same as the chimeric antigen receptor contained by the CD4⁺ T lymphocytes.”

237. As discussed above, Singh used the same CAR in its CD8⁺ and CD4⁺ T cells. For this reason, a POSA would understand that Singh discloses a composition wherein the CAR contained by the CD8⁺ T lymphocytes is the same as the CAR contained by the CD4⁺ T lymphocytes.

D. Dependent Claims Directed to Percentage Surface Positivity of T-Cell Markers

238. Claim 6 includes higher percentages of surface positivity for phenotypic markers:

The adoptive cellular immunotherapy composition according to claim 1, wherein:

(a) at least 60% of the chimeric antigen receptor-modified CD4⁺ T lymphocytes are surface positive for CD62L and/or CD45RA;

(b) at least 60% of the chimeric antigen receptor-modified CD8⁺ cytotoxic T lymphocytes are surface positive for CD62L and/or CD45RO;

(c) at least 80% of the chimeric antigen receptor-modified CD4⁺ T lymphocytes are surface positive for CD62L and/or CD45RA; *and/or*

(d) at least 80% of the chimeric antigen receptor-modified CD8⁺ cytotoxic T lymphocytes are surface positive for CD62L and/or CD45RO.

239. Claim 6 recites four claim elements connected by an “and/or.” As mentioned above, the “and/or” in claim 6 indicates that the prior art need only meet one of the four elements ((a), (b), (c), or (d)). Additionally, the “and/or” connecting the T-cell markers (*i.e.*, “CD62L and/or CD45RA,” and “CD62L and/or CD45RO”) means that the prior need only disclose the recited percent surface positivity for one of those markers.

240. As explained in Section X.A.2 above, 83% Singh’s CAR⁺ CD4⁺ T cells were CD45RA⁺ and 80% of the CAR⁺ CD8⁺ cells were CD45RO⁺. Singh’s compositions thus meet all of the elements of claim 6.

241. For these reasons, a POSA would understand that Singh discloses a composition that meets all elements of claim 6.

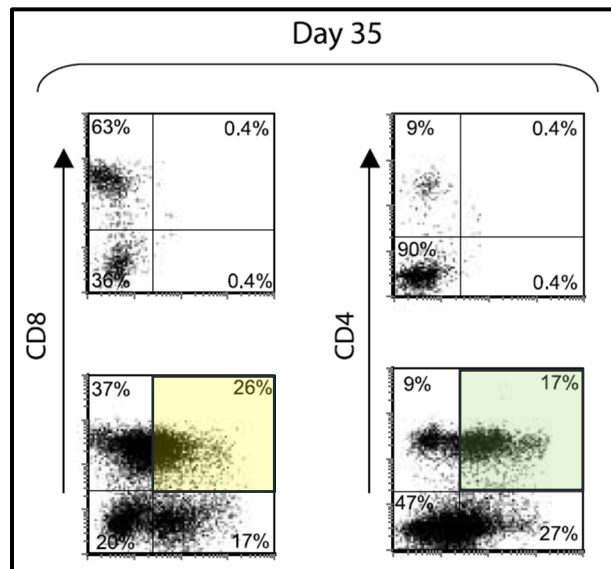
E. Dependent Claim Directed to Ratios

1. Claim 14: Composition Ratios

242. Claim 14 states: “wherein the chimeric antigen receptor-modified CD4⁺ helper T lymphocytes in the composition and the chimeric antigen receptor-modified CD8⁺ cytotoxic T lymphocytes are present in the composition at a ratio of between about 2:1 to about 1:2.”

243. A 1:2 ratio equals 0.5 (one divided by two) and a 2:1 ratio equals 2.0 (two divided by one).

244. Singh's composition comprised 17% CAR-modified CD4⁺ T cells and 26% CAR-modified CD8⁺ T cells, as shown in a FACS analysis the authors performed after culture expansion:



Ex. 1003, 2966 (Fig. 3A) (annotated, x-axes indicate CAR expression). In yellow shading, I have indicated the cells that make up the CAR-modified CD8⁺ population in Singh's composition (26% of the composition). In green shading, I have indicated the cells that make up the CAR-modified CD4⁺ population in Singh's composition (17% of the composition).

245. Seventeen percent divided by twenty-six percent equals 0.65, which falls within the scope of the claimed 0.5 – 2.0 (1:2 to 2:1) range of ratios.

246. For these reasons, a POSA would understand that Singh discloses an adoptive cellular immunotherapy composition wherein the CAR-modified CD4⁺ helper T lymphocytes in the composition and the CAR-modified CD8⁺ cytotoxic T lymphocytes are present in the composition at a ratio of between about 2:1 to about 1:2.

XI. GROUND 2: INDEPENDENT CLAIM 1 AND DEPENDENT CLAIMS 2-7, 14, 16, 21, AND 26-28 ARE RENDERED OBVIOUS BY SINGH IN VIEW OF JENSEN

247. Ground 2 differs from Ground 1 because it adds Jensen. Before March 23, 2011, a POSA would have understood claims 1-7, 14, 16, 21, and 26-28 to be obvious over Singh in view of Jensen. To the extent it is argued that Singh does not anticipate claims 1-7, 14, 16, 21, and 26-28, then Singh would render these claims obvious. A POSA would be motivated to, and have a reasonable expectation of success in, creating adoptive cellular immunotherapy composition based on the teachings of Singh discussed in Ground 1 (Section X).

A. Independent Claim 1

248. As mentioned above, “adoptive cellular immunotherapy composition” does not limit what is covered by the claims. *See* Section VII.A.

249. If, however, the preamble of claim 1 requires a “composition for adoptive cellular immunotherapy formulated to be suitable for administration to a mammal,” claim 1 still is obvious.

250. As discussed above, a POSA would understand that Singh's compositions were "compositions for adoptive cellular immunotherapy formulated to be suitable for administration to a mammal." *See* Section X.A.1.a.

251. Jensen further teaches a POSA how to further formulate Singh's T cells into an adoptive cellular immunotherapy composition.

252. Jensen discloses a clinical trial protocol utilizing CD19-specific, CAR-modified T cells. *See* Section VIII.F.1. Jensen teaches that, before infusing a T-cell composition into patients, "[p]rocessed cells are resuspended in 100 ml of 0.9% NaCl with 2% human serum albumin in a bag for [sic] suitable for clinical re-infusion." Ex. 1018, [0162]. A POSA thus would have recognized that further formulating Singh's compositions to be suitable for administration to a mammal would be as simple as diluting the T cells in a saline solution or other well-known infusion solution.

253. A POSA would have been motivated to combine Singh with Jensen because both references relate to CD19-specific CAR T-cell compositions intended for adoptive cellular immunotherapy. *See* Ex. 1003, 2970-2971 ("We . . . will evaluate the CD19-specific T cells . . . in a next-generation clinical trial."); Ex. 1018, cl.1 ("Genetically engineered CD19-specific immune cells which express and bear on the cell surface membrane a CD19-specific chimeric receptor, wherein the chimeric receptor consists of an intracellular signaling domain for an effector

function of the immune cell, at least one transmembrane domain and at least one extracellular domain, the extracellular domain comprising a CD19-specific receptor”), cl.19 (“A method of treating a CD19⁺ malignancy in a mammal which comprises infusing into the animal CD19-specific immune cells of claim 1 in a therapeutically effective amount.”).

254. Furthermore, both references relate to clinical trials. Singh explains that its compositions used then-available technologies and complied with good manufacturing practices (“GMP”) for clinical trials. *See* Ex. 1003, 2970 (“The platform we describe for producing CAR⁺ T cells should be appealing to investigators undertaking . . . trials [because it] uses technology that is readily accessible and practiced in compliance with cGMP for phase I/II trials”). Jensen describes a clinical trial protocol, and in that protocol, provides instructions for formulating a CAR T-cell composition. *See* Ex. 1018, [0162].

255. A POSA would have had a reasonable expectation of success in formulating Singh’s compositions to be suitable for administration to a mammal according to Jensen’s instructions. A POSA would have understood that doing so according to the teachings of Jensen would have been a simple, routine matter of mixing the cells with a solution of sodium chloride and human serum albumin in a bag for infusion. *See* Ex. 1018, [0162].

256. For these reasons, a POSA would have understood Singh in view of Jensen renders obvious claims 1-7, 14, 16, 21, and 26-28.

B. Claims 2-7, 14, 16, 21, and 26-28

257. As explained in Sections X.B-X.E above, Singh teaches all elements of claims 2-7, 14, 16, 21, and 26-28.

XII. GROUND 3: ALL CHALLENGED CLAIMS ARE RENDERED OBVIOUS BY SINGH IN VIEW OF JENSEN, HUDECEK I, WANG, MITSUYASU, MOELLER, ABKEN, HUDECEK II, RECKAMP, CARPENITO, YANG I, YANG II, SALLUSTO, AND SUN

258. Ground 3 differs from Grounds 1 and 2 because it adds Hudecek I, Wang, Mitsuyasu, Moeller, Abken, Hudecek II, Reckamp, Carpenito, Yang I, Yang II, Sallusto, and Sun. In Ground 1, I show that Singh teaches all elements of claims 1-7, 14, 16, 21, and 26-28. In Ground 2, I show that if the claims require a “composition for adoptive cellular immunotherapy formulated to be suitable for administration to a mammal,” a POSA would have known that, to formulate such a dosage form, it would have been a routine matter of adding the cells to an infusion solution, per Jensen.

259. In Ground 3, I use additional references—Hudecek I, Wang, Mitsuyasu, Moeller, Abken, Hudecek II, Reckamp, Carpenito, Yang I, Yang II, Sallusto, and Sun—to show that all Challenged Claims are obvious. Not all these references are necessary to teach the elements of any one of the challenged claims.

260. For example, the antigens to which claim 1's CAR must specifically bind according to claims 3 and 18-25 are taught by Singh (in view of Jensen if the preamble is limiting) in combination with one of either Hudecek I, Abken, Hudecek II, Reckamp, or Carpenito.

261. The percentages of CAR-modified T cells that are surface positive for various T-cell markers, as outlined in claims 6 and 9-13, are taught by Singh (in view of Jensen if the preamble is limiting) in combination with Wang and/or Mitsuyasu.

262. The limitations concerning the ratio of CD4⁺:CD8⁺ T cells in a composition recited in claims 14-15 are taught by Singh (in view of Jensen if the preamble is limiting) in combination with Moeller and/or Mitsuyasu.

263. A comparison between certain aspects of claim 1's composition and one of two reference populations is required in claims 8 and 29-31. Singh (in view of Jensen if the preamble is limiting) in combination with Wang, Hudecek I, Sallusto, Sun, Yang I, and/or Yang II teaches these claims.

264. None of the Challenged Claims requires more than a small number of references to teach all of its respective elements.

265. Before March 23, 2011, a POSA would have understood that all Challenged Claims are obvious over Singh in view of the combinations of references listed above.

A. Independent Claim 1

266. As discussed in Section X above, Singh discloses claim 1's composition. In Ground 2, adding Jensen provides instructions for further making a composition for adoptive cellular immunotherapy formulated to be suitable for administration to a mammal. *See* Section XI. These references would have provided a POSA with a motivation to further formulate Singh's compositions to be "suitable for administration to a mammal" with a reasonable expectation of success. *See* Sections X-XI. In addition, Mitsuyasu further renders obvious the "adoptive cellular immunotherapy" limitation because, as discussed in Section VIII.A.2, Mitsuyasu created and administered genetically engineered T-cell immunotherapy compositions to patients.

B. Dependent Claims Directed to Antigens

1. Claims 3 and 18-25

267. As explained in Section X.A.1.c above, Singh discloses a CD19-specific CAR. For this reason, a POSA would understand that Singh meets the limitations of claims 3 and 21.

268. Claims 18-20 and 22-25 recite "wherein the antigen is" followed by an antigen: either ROR1, Her2, L1-CAM, CD20, CD22, mesothelin, or CEA. At least one year before March 23, 2011, the prior art taught targeting each of these antigens with a CAR-modified T cell.

269. A POSA would have been motivated to use CARs targeting antigens other than CD19 in Singh's compositions and would have had a reasonable expectation of success in doing so (*i.e.*, a reasonable expectation of producing CAR-modified T cells targeting different antigens) because Singh proposed targeting other antigens. *See, e.g.*, Ex. 1003, 2970 ("It is anticipated that this approach will be of interest not just for generating clinical grade T cells with specificity for CD19, but for genetically modifying T cells to express CAR with alternative specificities"). A POSA would have understood this to mean that Singh's authors viewed their manufacturing process as agnostic in relation to CAR-specificity. Simply, it could be used to manufacture T-cell compositions that express a CAR specific for CD19 as well as an antigen other than CD19.

270. Claim 18 requires the antigen to be ROR1.

271. Hudecek II teaches targeting ROR1 with a CAR. *See* Ex. 1012, 383("We constructed a lentiviral vector that encodes a chimeric antigen receptor . . . specific for ROR1").

272. A POSA would have been motivated to target ROR1 because Hudecek II taught that ROR1 is a candidate for targeting B-cell malignancies. *See id.* ("Analysis of ROR1 protein expression . . . revealed uniform, stable, and restricted cell surface expression on B-CLL, suggesting this molecule is a candidate for targeted immunotherapy of B cell malignancies.").

273. There would have a reasonable expectation of success when targeting ROR1 because Hudecek II reported that ROR1 may be an even better target than CD19 for targeting B-cell malignancies. *See id.* (“targeting ROR1 with gene-modified T cells may have advantages over targeting B cell-lineage restricted molecules such as CD19 and CD20 that are expressed on normal mature B cells.”).

274. Claim 19 requires the antigen to be Her2. Claims 22 and 25 require the antigen to be CD20 and CEA, respectively.

275. Abken teaches targeting these antigens with a CAR. *See* Ex. 1013, 651 (“The approach is universal since by inserting the appropriate binding domain into the chimeric TCR, T cells can be redirected toward a broad variety of tumor associated antigens expressed on tumor cells of different entities, including . . . CEA . . . Her2 . . . [and] CD20”). Targeting CD20 was also taught by other prior-art references. *See also* Ex. 1011, 524 (“The feasibility of adoptive immunotherapy with autologous CD20-specific CAR-modified T cells has been studied in patients with NHL and mantle cell lymphoma”).

276. A POSA would have understood that the “single chain format” of the “chimeric TCR” discussed in Abken is a “chimeric antigen receptor” of the claims. *See* Section VIII.C.3.

277. A POSA would have been motivated to target Her2, CD20, and CEA with Singh’s process because Abken teaches that targeting diseases with CARs is

“universal” (*see* Ex. 1013, 651) and Singh proposed targeting other antigens. *See* Section VIII.A.1.

278. There would have been a reasonable expectation of success because Abken proposes targeting each of the recited antigens as an option for redirecting T cells and explains that clinical trials involving such CARs already had been initiated. *See* Ex. 1013, 651 (“Clinical trials have been and are currently initiated using engineered T cells equipped with chimeric TCRs in order to eliminate tumor cells.”). Indeed, Singh proposed targeting other antigens. *See* Section VIII.A.1.

279. Claim 20 requires the antigen to be L1-CAM.

280. Reckamp teaches targeting L1-CAM with a CAR. *See* Ex. 1014, 1099 (“We also evaluated the targeting of L1-CAM expressing lung cancer cell lines with cytolytic T cells genetically modified using SIN lentiviral vectors to express a L1-CAM specific chimeric antigen receptor composed of an scFv derived from the CE7 hybridoma that is in turn fused to IgG4:CD4Tm:CD3-ζcyto (CE7R).”).

281. A POSA would have been motivated to target L1-CAM because Reckamp teaches that it is an antigen expressed on numerous cancers. *See id.* (“L1CAM has been shown to be overexpressed in multiple tumors and associated with invasive/metastatic tumor biology.”). Moreover, Singh proposed targeting other antigens using its system. *See* Section VIII.A.1.

282. A POSA would have a reasonable expectation of success because Reckamp states that “[L1-CAM-targeting CAR] CE7R expressing CTL can kill L1-CAM-expressing lung cancer cells via CE7R mediated recognition and CTL activation as evidenced by dose-dependent blocking with CE7 monoclonal antibody.” *See* Ex. 1014, 1099. Again, Singh proposed targeting other antigens with its system. *See* Section VIII.A.1.

283. Claim 23 requires the antigen to be CD22.

284. Hudecek I teaches that “[o]ther surface molecules that are expressed on B-cell tumors have been targeted with CAR-modified T cells in preclinical studies, including . . . CD22.” Ex. 1011, 524. A POSA would have understood this to mean that researchers already had made a CD22-targeting CAR and had caused T cells to express it.

285. A POSA would have been motivated to target CD22 because Hudecek’s disclosure communicated that CD22 was a promising target for B-cell tumors. Additionally, Singh proposed targeting other antigens. *See* Section VIII.A.1.

286. There would have been a reasonable expectation of success because researchers already had made a CD22-targeting CAR, had caused T cells to express it, and were investigating CD22 in preclinical studies. Moreover, Singh proposed targeting other antigens with its system. *See* Section VIII.A.1.

287. Claim 24 requires the antigen to be mesothelin.

288. Carpenito teaches a mesothelin-targeting CAR. *See* Ex. 1015, 3360 (“Lentiviral vectors were used to express a single-chain variable fragment that binds mesothelin and that is fused to signaling domains derived from T-cell receptor zeta, CD28, and CD137 (4-1BB).”). A POSA would have understood this to mean that Carpenito disclosed a mesothelin-targeting CAR. *See id.* (“*Chimeric receptors* were designed that contain the *SS1 scFv that recognizes human mesothelin*. . . . We created a series of *SS1 scFv-based chimeric receptors* that contain the TCR- ζ signal-transduction domain with the CD28 and CD137 (4-1BB) intracellular domains in tandem.”). *See* Section VIII.C.5.

289. A POSA would have been motivated to target mesothelin because Carpenito teaches that mesothelin is expressed on carcinomas. *See* Ex. 1015, 3360 (“Mesothelin is a cell-surface molecule over-expressed on a large fraction of carcinomas, and thus is an attractive target of immunotherapy.”). In addition, Singh proposed targeting other antigens. *See* Section VIII.A.1.

290. There would have been a reasonable expectation of success because Carpenito reported that “the retargeted T cells efficiently and specifically killed a variety of tumors that express mesothelin.” *See* Ex. 1015, 3363-3364. A POSA would have understood this to mean that the authors had successfully made a mesothelin-targeting CAR, had caused T cells to express it, and had observed

tumor-killing capacity in the resulting cells. Also, Singh proposed targeting other antigens with its system. *See* Section VIII.A.1.

291. For these reasons, a POSA would understand that the prior art disclosed CARs that specifically bind to an antigen, including the ROR1, Her2, L1-CAM, CD19, CD20, CD22, mesothelin, and CEA antigens.

C. Claims Directed to Percentage Surface Positivity of T-Cell Markers

1. Claims 1, 6 and 9-13

292. Claim 1 requires at least 50% surface positivity for various surface markers. Claims 6 and 9-13 include additional limitations concerning the percentages.

293. As explained in Section X.A.2, 83% of Singh's CAR-modified CD4⁺ helper T lymphocytes were surface positive for CD45RA, 80% of Singh's CAR⁺ CD8⁺ T cells were surface positive for CD45RO, and at least 50% of Singh's overall CD8⁺ T cells were surface positive for CD45RO. These values establish that Singh discloses all elements of claims 1 and 6.

294. Claims 9-13 recite percentages of surface positivity for CD62L instead of for CD45RO or CD45RA. Claims 9 and 10 require that at least 50% of either the chimeric antigen receptor-modified CD4⁺ helper T lymphocytes (claim 9) or the chimeric antigen receptor-modified CD8⁺ cytotoxic T lymphocytes (claim 10) in the composition are surface positive for CD62L. Claim 11 requires that at least 50% of

both the chimeric antigen receptor-modified CD4⁺ helper T lymphocytes and the chimeric antigen receptor-modified CD8⁺ cytotoxic T lymphocytes in the composition are surface positive for CD62L. Claims 12 and 13 require at least 60% (claim 12) or at least 80% (claim 13) surface positivity for CD62L for either the CAR-modified CD4⁺ helper T lymphocytes or the CAR-modified CD8⁺ cytotoxic T lymphocytes.

295. A POSA would understand that Singh in view of Wang and/or Mitsuyasu discloses all elements of claims 9-13, and also claims 1 and 6 on the basis of percentage CD62L⁺ even apart from percentages of CD45RO⁺ and CD45RA⁺. Singh encouraged using T_{CM} cells in adoptive immunotherapies and Wang provided a rationale and means for using a high percentage of CD62L⁺ T_{CM} in such therapies. Although Mitsuyasu is not necessary for the combination of Singh and Wang to render the claims obvious, Mitsuyasu shows that a POSA could easily achieve >80% CD62L⁺ composition, as encouraged by Singh and Wang.

296. A POSA would have recognized that Singh encouraged using a composition that includes CD62L⁺ T cells in clinical trials:

Clinical experience will also be needed to assess whether the presence of CD62L (L-selectin) on genetically modified T cells enables CAR⁺ T cells to traffic to sites of minimal residual disease for B-lineage malignancies, such as secondary lymphoid organs.

Ex. 1003, 2970. A POSA would have understood this to mean that Singh's authors believed that future clinical trials would need to include CAR-modified, CD62L⁺ T cells to address malignant B cells in secondary lymphoid organs.

297. Singh did not specifically measure the percent surface positivity of CD62L in its CAR-modified CD4⁺ T cells or CAR-modified CD8⁺ T cells.

298. Nonetheless, Singh saw that CD62L was a potentially beneficial marker for its T-cell compositions because one of the authors' goals was to use high numbers of T_{CM}. *See id.*, 2961 ("We anticipated that the therapeutic efficacy of adoptive transfer of CD19-specific T cells would be improved by . . . introducing the CAR into central memory (CM) T cells.").

299. Wang is a conference abstract that discusses the same subject matter as Singh (*i.e.*, CD19-specific, CAR-T adoptive immunotherapy compositions). *See* Section VIII.E.1. Wang discloses a rationale and means for generating CD19-specific adoptive cellular immunotherapies comprised of a high percentage of T_{CM}, which are CD62L⁺. *See* Section VIII.E.1.

300. Wang's rationale for using a high percentage of T_{CM} is that they "have a unique ability to self-renew, proliferate, and differentiate into effector T_{CM}, which suggests that this T-cell subset will be most effective and persistent upon adoptive transfer." Ex. 1017, 926.

301. Other researchers in the field shared this view as well. *See* Ex. 1011, 525 (“T-cell clones derived from T_{CM}, but not those derived from T_{EM} cells were capable of persisting long term, reacquiring the phenotype of memory cells and populating memory T-cell niches *in vivo* after adoptive transfer.”); Ex. 1027, 739 (“a large percentage of *in vitro* generated anti-tumor T lymphocytes mimic a Tcm-like phenotype (based on phenotype, effector function, and increased persistence *in vivo*), which suggests that these Tcm-like cultured T cells may be optimal for adoptive immunotherapy”); Ex. 1028, S184 (“Based on the differentiation marker L-selectin/CD62L and the release of the cytokine IL-2, central memory T cells can be distinguished from effector memory T cells.”). A POSA would have understood this to mean that T cells derived from T_{CM}, but not T cells derived from T_{EM}, were able to persist long-term.

302. Wang selected for T_{CM} by using CD62L positivity as a T_{CM} marker. *See* Ex. 1017, 927 (“we first selected Tcm using the Dreg56 (anti-CD62L) biotinylated antibody”). A POSA would have known that a biotinylated antibody is an antibody conjugated with the biotin molecule. A POSA also would have understood that the only cells that would bind to an anti-CD62L antibody are cells that express CD62L. As a result, Wang’s starting T-cell population was approximately 100% CD62L⁺.

303. Wang reported that its authors expanded its starting population and transduced it with a CD19-specific CAR. *See id.*, (“T cells were expanded more than

1000-fold . . . and then genetically redirected . . . with a CD19-specific chimeric antigen receptor, CD19R.”).

304. Wang does not expressly state the percentage of CD62L⁺ T cells after expansion and transduction. However, a POSA would have understood that the goal was to maintain as high CD62L-positivity as possible, up to 100% CD62L-positivity. A POSA would have known this because, as discussed above, Wang selected for CD62L⁺ T cells as a marker for T_{CM} and suggested that T_{CM} “will be most effective and persistent upon adoptive transfer.” *Id.*, 926.

305. A POSA would have understood that Singh’s method of generating CAR-modified T cell compositions could be used to maintain a desired immunophenotype, including CD62L-positivity. Ex. 1003, 2970 (“We note that the electrotransfer of *SB* plasmids and subsequent CAR-mediated propagation on aAPC supports the outgrowth of T cells with these desired phenotypes as our CAR⁺ T cells *maintain expression of* CD27, CD28, CD45RO, CD95, and **CD62L**.”) (emphasis added).

306. As a result, a POSA would have understood that all that would have been required to see the claimed percentage of T cells expressing CD62L (in claims 1, 6, and 9-13) is to use Singh’s manufacturing process to maintain a T-cell population having a high percentage of CD62L⁺ cells, as encouraged by Wang.

307. To the extent necessary, a POSA would have known that T cells having a desired immunophenotype (*e.g.*, CD62L⁺) could be isolated using FACS or MACS, as in the case of Wang. Therefore, if a POSA did not naturally achieve the desired high rates of CD62L-positivity claimed without selection for CD62L⁺ cells, a POSA at least would have known that the claimed percentages of CD62L-positivity would have been achieved easily through routine optimization.

308. A POSA would have understood from Mitsuyasu that one could easily achieve a high level of CD62L-positive T cells, as encouraged by Singh and Wang, encompassing the claimed percentages in claims 1, 6, and 9-13. Mitsuyasu is a research article that reports results from one of the first clinical trials of engineered T cell-based adoptive cellular immunotherapies.

309. As discussed above, Mitsuyasu's authors drew blood from HIV-positive patients, isolated T cells from the blood, transduced the cells with a chimeric receptor named "CD4ζ," and expanded them. A POSA would recognize that there was no selection step for CD62L. *See* Ex. 1004, 786 ("T-cell Processing"). After expansion, Mitsuyasu's authors cryopreserved the cells in a solution meant to mimic human plasma supplemented with human serum albumin. After thawing, the authors determined expression of various T-cell markers, including CD4, CD8, and CD62L, in the final adoptive immunotherapy compositions and infused them into patients. *See* Section VIII.A.2.

310. Mitsuyasu shows that, even without selecting for T cells with CD62L expression before the engineering step, there would be some compositions that meet the claim limitation. Most of Mitsuyasu's compositions made were >80% CD62L⁺, with two compositions being 96% CD62L⁺. Oftentimes, human T cells in the peripheral circulation, which are typically employed for engineering with chimeric receptors, contain a high percentage of CD62L⁺ T cells. In Yang I, for example, the researchers saw greater than >80% CD62L⁺ for their T-cell compositions from three independent donors. *See* Ex. 1027, 742 (showing that three compositions all had CD62L-positivity above 80% despite not enriching specifically for CD62L). These authors similarly concluded that cells displaying a T_{CM}-like phenotype would be optimal for adoptive immunotherapy. *See id.*, 739 (“a large percentage of *in vitro* generated anti-tumor T lymphocytes mimic a Tcm-like phenotype (based on phenotype, effector function, and increased persistence *in vivo*), which suggests that these Tcm-like cultured T cells may be optimal for adoptive immunotherapy”). Mitsuyasu demonstrates that a POSA could easily achieve a majority CD62L⁺ composition using conventional T cell engineering methods, as encouraged by Singh and Wang.

311. Mitsuyasu not only shows that most of its compositions had more than 80% CD62L⁺ in the overall T-cell population, a POSA also would have expected that the percentage of CD62L⁺ was more than 80% in the claimed subpopulations—

CAR⁺ CD4⁺ T cells and CAR⁺ CD8⁺ T cells (and overall CD8⁺ T cells). As discussed in more detail in Ground 4 (Section XIII.A.2.a) below, a POSA would have expected that the CD4⁺, CD8⁺, and receptor-modified T-cell populations in Mitsuyasu's compositions would exhibit roughly the same rate of CD62L-positivity as the authors observed in their respective overall compositions.

312. As discussed in more detail in Ground 4 (Section XIII.A.2.a) below, Mitsuyasu's Figure 2 shows that seventeen of the tested compositions had greater than 80% CD62L-positivity. *See* Ex. 1004, 788 (showing that the following patient IDs were associated with a T-cell composition having more than 80% CD62L-positivity: N215, H202, C211, Q202, S206, S212, P217, G251, T253, L218, Y254, U213, D282, T232, J233, Y292, C298). *See* Section XIII.A.2.a below. A POSA would have expected that the receptor-modified CD4⁺ T cells, the receptor-modified CD8⁺ T cells, and the overall CD8⁺ T cells in those seventeen compositions also would have exhibited greater than 80% CD62L-positivity. *See* Section XIII.A.2.a below. This meets the limitations of claims 1, 6, 9-13.

313. Additionally, it would be impossible for less than 8 of the compositions to have CD4⁺ and CD8⁺ T cells with less than 50% CD62L positivity for the reasons discussed in Section XIII.A.2.a below.

314. Further, it would have been impossible for at least composition S206 in Mitsuyasu not to have (1) at least 50% CD62L-positivity in both of the

CAR-modified, CD4⁺ and CD8⁺, T-cell populations and the overall CD8⁺ population (relevant for claims 1, 6, 9-11), (2) at least 60% CD62L-positivity in at least one of the CAR-modified, CD4⁺ and CD8⁺, T-cell populations (relevant for claim 12) and (3) at least 80% CD62L-positivity in at least one of the CAR-modified, CD4⁺ and CD8⁺, T-cell populations (relevant for claim 13). *See* Section XIII.A.2.a below.

315. A POSA would have understood that this high level of CD62L-positivity is indicative of a corresponding high level of T_{CM}. *See* Ex. 1017, 926-927 (linking CD62L-positivity with T_{CM} cells); *see also* Ex. 1001, 7:12-19. A POSA thus would have understood from Mitsuyasu that, even absent active selection for CD62L⁺ T cells, a POSA should expect a high percentage of CD62L-positivity when developing autologous adoptive cellular immunotherapies.

316. A POSA would have been motivated to combine the teachings of Singh, Wang, and Mitsuyasu for numerous reasons. All references concern adoptive immunotherapies involving chimeric receptors. *See* Sections VIII.A.1, VIII.E.1, VIII.A.2; Ex. 1003, 2961; Ex. 1017, 926-927; Ex. 1004, 786. Furthermore, Singh and Wang teach that T_{CM} cells (which are CD62L⁺ cells) are optimal for adoptive immunotherapy compositions and should be present as a high percentage of the cells in the compositions. *See* Sections VIII.A.1, VIII.E.1 above. In addition, a POSA would have been motivated by Singh and Wang to use as high a percent

CD62L-positivity as possible. *See* Section VIII.A.1; Ex. 1017, 926-927. A POSA also would have understood from Mitsuyasu that using a high percentage of CD62L⁺ (T_{CM} in this instance) cells would optimize the persistence of a T-cell composition. *See* Ex. 1004, 791 (“CD4ζ-modified CD4⁺ and CD8⁺ T cells persisted in the blood of all patients at high levels throughout the 8-weeks observation period, and extended follow-up demonstrated maintained survival for at least 1 year in 17 of 18 subjects.”) (internal parentheticals omitted).

317. A POSA would have recognized that Singh taught to improve therapeutic efficacy of adoptive cellular immunotherapies by enriching for T_{CM}. *See* Ex. 1003 at 2961 (“We anticipated that the therapeutic efficacy of adoptive transfer of CD19-specific T cells would be improved by developing a CAR with a fully competent activation signal and introducing the CAR into central memory (CM) T cells.”); *id.* at 2962 (“This is predicted to greatly facilitate trial design infusing CD4⁺ and CD8⁺ CAR⁺ T cells that have desired immunophenotype, including T_{CM}.”). A POSA would have understood that Wang’s authors wanted to improve adoptive transfer by enriching for T_{CM} and described a means for doing so. *See* Ex. 1017, 926-927 (explaining that T_{CM} would be “most effective” for adoptive transfer and describing T_{CM} selection using an anti-CD62L antibody).

318. Specifically, Wang would have motivated a POSA to start with a T-cell population in which 100% of the cells express CD62L. Because Singh disclosed that

its expansion process could be used to maintain an immunophenotype while growing cells to clinically acceptable numbers, the POSA then could have used Singh's process to maintain high CD62L positivity throughout the expansion process. Ex. 1003, 2970 ("We note that the electrotransfer of *SB* plasmids and subsequent CAR-mediated propagation on aAPC supports the outgrowth of T cells with these desired phenotypes as our CAR⁺ T cells *maintain expression of* CD27, CD28, CD45RO, CD95, and **CD62L**."). In fact, researchers could modify T cells with CARs and select T cells for CD4, CD8, and CD62L expression at the end of the manufacturing period, so there was nothing preventing a POSA from combining the teachings of Singh, Wang, and Mitsuyasu. *See* Ex. 1003, 2963; Ex. 1017, 926-927, Ex. 1004, 786.

319. A POSA would have reasonably expected to succeed for at least two reasons. First, a POSA would have recognized that, in Mitsuyasu, seventeen tested compositions met the elements of these claims. *See* Section XIII.A.2.a *infra*. A POSA would understand from Mitsuyasu that one could easily achieve a majority CD62L⁺ composition, as encouraged by Singh and Wang. Second, as discussed above, to the extent necessary, a POSA would have known from Singh that T cells having a desired immunophenotype (*e.g.*, CD62L⁺) could be isolated using readily accessible cell sorting methods. Therefore, if a POSA did not naturally achieve the desired high rates of CD62L-positivity claimed without selection for CD62L⁺ cells,

a POSA at least would have known that the claimed percentages of CD62L-positivity would have been achieved easily through routine optimization.

320. The claimed CD62L⁺ percentages are also obvious through routine optimization. A POSA could have used FACS or MACS to isolate populations having the claimed CD62L-positivity levels. As discussed above, many prior-art references taught the benefits of CD62L positivity for adoptive cellular immunotherapy compositions. Wang selected for CD62L⁺ and targeted 100% positivity for all T-cell populations; Mitsuyasu reported CD62L⁺ up to 96% in the final T-cell immunotherapy compositions.

321. For these reasons, a POSA would understand that the prior art (Singh and Wang, or the combination of Singh, Wang, and Mitsuyasu) renders obvious an adoptive cellular immunotherapy composition with at least 80% CAR-modified CD4⁺ helper T lymphocytes, at least 80% CAR-modified CD8⁺ cytotoxic T lymphocytes, and at least 50% of CD8⁺ cytotoxic T lymphocytes, surface positive for CD62L. This renders obvious Claims 1, 6, and 9-13.

D. Dependent Claims Directed to Ratios

1. Claim 14-15

322. Claims 14 and 15 recite additional composition ratio limitations. The claims require that the CAR-modified CD4⁺ helper T lymphocytes in the composition and the CAR-modified CD8⁺ cytotoxic T lymphocytes are present in

the composition at a ratio of between about 2:1 to about 1:2 (claim 14) or about 2:1, 1:1, or 1:2 (claim 15).

323. A POSA would have known by at least 2005 that “[o]ptimal antitumor efficacy [could be] achieved with the addition of equivalent numbers of engineered CD4⁺ and CD8⁺ T cells.” Ex. 1016, 3001. Thus, from Moeller alone, a POSA would have been motivated to modify Singh’s compositions to include equal numbers of CD4⁺ T cells and CD8⁺ T cells.

324. A POSA would have been motivated to combine Singh with Moeller because both references relate to adoptive cellular immunotherapy. In addition, Moeller teaches a way to further optimize Singh’s compositions.

325. There would have been a reasonable expectation of success in making the claimed compositions because Singh disclosed an ability, through flow cytometry and FACS, to isolate T cells with desired immunophenotypes. *See* Section VIII.A.1; Ex. 1003, 2963.

326. Alternatively, a POSA would understand that Singh and Mitsuyasu teach all elements of these claims. As explained in Section X.E, Singh discloses a composition with a ratio (0.65) that falls within claim 14’s scope (0.5—2.0). And Mitsuyasu’s Table 2 shows an example of a composition, P217, with about a 1:2 CD4⁺: CD8⁺ ratio, and another composition, S206, with a ratio of about 1:1, as recited in claim 15. *See* Ex. 1004, 788 (Table 2) (showing a composition P217 with

31% CD4⁺ T cells and 68% CD8⁺ T cells and a composition S206 with 52% CD4⁺ T cells and 46% CD8⁺ T cells).

327. For these reasons, a POSA would understand that Singh and Mitsuyasu render obvious an adoptive cellular immunotherapy wherein the chimeric antigen receptor-modified CD4⁺ helper T lymphocytes and the CD8⁺ cytotoxic T lymphocytes in the composition are present at a ratio of between about 2:1 to about 1:2, and specifically in a ratio of about 1:2 and about 1:1.

E. Dependent Claims Directed to Comparisons to Other Populations

328. Claims 8 and 29-31 recite elements that require a comparison between the prior-art compositions and one of two reference populations of T cells.

1. Claim 8

329. Claim 8 recites:

The adoptive cellular immunotherapy composition according to claim 1, wherein the CD4⁺ helper T lymphocytes and the CD8⁺ cytotoxic T lymphocytes are derived from a subject and wherein the CD4⁺ helper T lymphocytes secrete a greater amount of IL-2 and/or exhibit a greater degree of proliferation when cultured in the presence of the antigen as compared to a reference population of CD4⁺ T cells that has been expanded in vitro from an effector memory T cell-enriched CD4⁺ T cell population or a bulk CD4⁺ T cell population from the subject and modified to contain the chimeric antigen receptor contained by the CD4⁺ helper T lymphocytes.

330. Claim 8 requires that the prior-art composition contain “CD4⁺ helper T lymphocytes and the CD8⁺ cytotoxic T lymphocytes [] derived from a subject.”

331. A POSA would have understood that Singh teaches deriving CD4⁺ and CD8⁺ T cells from a subject. Singh's authors isolated the T cells only after consent from a subject. *See* Ex. 1003, 2963 ("Human T cells were isolated . . . from umbilical cord blood or peripheral blood mononuclear cells (PBMC) after consent").

332. Claim 8 also requires that the CD4⁺ T cells "secrete a greater amount of IL-2 and/or exhibit a greater degree of proliferation when cultured in the presence of the antigen as compared to" one of two reference populations.

333. The following discussion focuses on proliferation and one of the reference populations: "a reference population of CD4⁺ T cells that has been expanded in vitro from an *effector memory* T cell-enriched CD4⁺ T cell population" (emphasis added).

334. A POSA would have expected the CD4⁺ T cells resulting from the combination of Singh and Wang (which satisfies claim 1, *see* Section XII.C) to proliferate more than "a reference population of CD4⁺ T cells that has been expanded in vitro from an effector memory T cell-enriched CD4⁺ T cell population."

335. The CD4⁺ T cells resulting from the combination of Wang and Singh would be primarily CD62L⁺ because the investigators recognized the benefits of T_{CM} cells. *See* Section XII.C.

336. Contrast this with the reference population, which would be primarily T_{EM} cells and T_E cells (derived from T_{EM} cells). T_{EM} cells differentiate into

T_{EM}-derived T_E cells. *See* Ex. 1011, 525 (“T_{CM} and T_{EM} cells . . . respond to antigen re-exposure by differentiating into a new wave of T_E cells.”); *id.* (“some of the progeny of clonally derived T_E cells retain intrinsic programming of the parental cell of origin”); Ex. 1001, 1:59-62 (explaining that T_{EM} “differentiate into cytolytic effector T cells (T_E) that express a high level of granzymes and perforin, but are short-lived.”).

337. A POSA would have known that T_{CM} cells persist and exhibit a greater degree of proliferation than T_{EM} and T_E cells. Ex. 1003, 2970 (“T_{CM} resulted in longer *in vivo* persistence compared with infusion of numerically expanded effector T cells.”); Ex. 1027, 748 (“The Tcm sub-population showed significant engraftment and persistence *in vivo* compared to the Tem sub-population.”); Ex. 1011, 525 (“T-cell clones derived from T_{CM}, but not those derived from T_{EM} cells were capable of persisting long-term”); Ex.1032, 4753 (“Central memory T lymphocytes...have a high capacity to proliferate. In contrast, effector memory T lymphocytes...lack significant proliferative capacity.”).

338. A POSA also would have known that T_{CM}-derived T_E cells persist longer than T_{EM}-derived T_E cells. Ex. 1011, 525 (“In this study, T-cell clones derived from T_{CM}, but not those derived from T_{EM} cells were capable of persisting long term, reacquiring the phenotype of memory cells and populating memory T-cell niches *in vivo* after adoptive transfer.”). Additionally, a POSA would have understood that

T_{EM}-derived T_E cells die rapidly after transfer. Ex. 1007, 228 (“T cells derived from T_{EM} precursors die rapidly after cell transfer and do not establish persistent T cell memory.”).

339. Therefore, the CD4⁺ T cells resulting from the combination of Singh and Wang would include majority T_{CM}, which persist and thus proliferate to a greater degree than T_{EM} and T_E. Stated differently, the reference population’s T_{EM}-enriched cells would remain T_{EM} cells or differentiate into T_E cells, and both of those cell types are outlived by the T_{CM} cells that would represent the majority of the T cell product resulting from the combination of Singh and Wang.

340. The following discussion focuses on IL-2 secretion compared against the same reference population of T_{EM}-enriched cells.

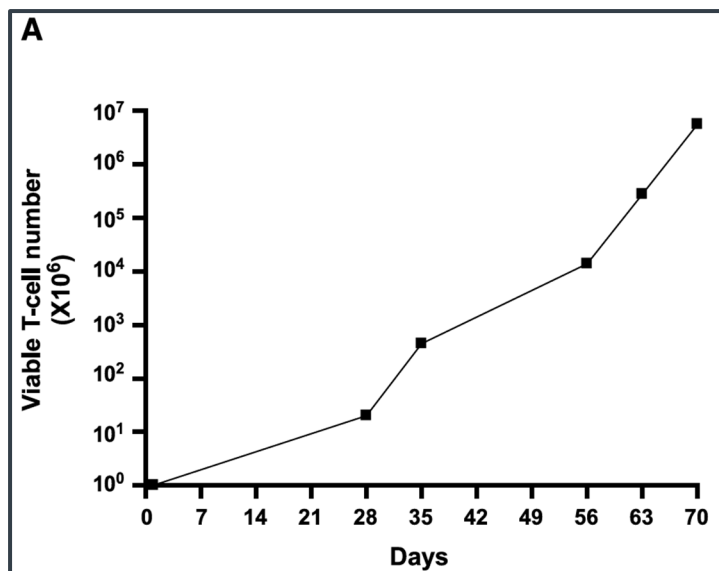
341. As discussed in the preceding paragraphs, a POSA would understand that the CD4⁺ T cells resulting from the combination of Singh and Wang would be majority T_{CM}, while the reference population would be enriched for T_{EM}. A POSA would understand that T_{CM} secrete more IL-2 than T_{EM}. Ex.1029, 709 (Fig.1) (showing CD4⁺ T_{CM} produce more IL-2 than CD4⁺ T_{EM} and CD8⁺ T_{CM} produce more IL-2 than T_{EM}); Ex.1032, 4753 (“Central memory T lymphocytes...produce predominantly IL-2.”); *Id.* at 4753 (Fig. 3) (showing greater IL-2 secretion for central memory as compared to effector memory cells); Ex. 1027, 739 (showing based on CD8⁺ T cells data that “[w]hen the cells were sorted into populations that

were CD45RO⁺/CD62L⁻ (Tem), CD45RO⁺/CD62L⁺ (Tcm), or CD45RO^{low}/CD62L⁺ (Tn) and co-cultured with antigen-matched tumor lines, the magnitude of cytokine release from these populations for . . . IL-2 (Tn > Tcm > Tem) mimicked the types of immune cell responses observed in vivo.”); Ex. 1028, S184 (“Tem sorted cells secrete high levels of IFN-γ and low amounts of IL-2 while Tcm cells showed the opposite reactivity (high IL-2 and low IFN-γ).”).

342. Therefore, a POSA would understand that the CD4⁺ T cells resulting from the combination of Singh and Wang would secrete more IL-2 than at least one of the reference populations recited in claim 8.

343. Lastly, a POSA would recognize that claim 8’s reference populations do not require the presence of an antigen. The claim language, “when cultured in the presence of the antigen,” does not appear in the clause describing the reference populations. *See* Ex. 1001, cl. 8.

344. A POSA would understand from Singh that Singh’s CD4⁺ T cells exhibit a greater degree of proliferation when cultured in the presence of an antigen compared to cells not exposed to antigen. As noted above, Singh’s authors expanded Singh’s compositions while exposing them to the CD19 antigen. *See* Section VIII.A.1. In Figure 4A, Singh shows that its CD19-exposed compositions proliferated exponentially:



Ex. 1003, 2967.

345. On the other hand, Singh reported that when culturing T cells without CD19 antigen exposure (in the absence of CD19-expressing aAPCs), Singh's authors did not observe any proliferation. *See id.*, 2970 ("to safeguard against the emergence of genetically modified T cells with autonomous growth, we routinely culture T cells after electroporation without aAPC and *we are yet to observe evidence of antigen-independent proliferation.*") (emphasis added).

346. Therefore, a POSA would understand that the cells in Singh's composition proliferate more in the presence of the CD19 antigen than at least one of the reference populations described in claim 8 in the absence of the antigen, and render this claim obvious.

2. Claim 29

347. Claim 29 recites:

The adoptive cellular immunotherapy composition according to claim 1, wherein a greater percentage of the chimeric antigen receptor-modified CD4⁺ helper T lymphocytes are surface positive for CD62L and/or CD45RA as compared to a reference population of CD4⁺ T cells that has been expanded in vitro from an effector memory-enriched CD4⁺ T cell population or a bulk CD4⁺ T cell population from the subject and modified to contain the chimeric antigen receptor contained by the CD4⁺ helper T lymphocytes.

348. Claim 29 requires a prior-art composition wherein “a greater percentage of the chimeric antigen receptor-modified CD4⁺ helper T lymphocytes are surface positive for CD62L and/or CD45RA as compared to” one of the same reference populations recited in claim 8, *i.e.*, “a reference population of CD4⁺ T cells that has been expanded in vitro from an effector memory-enriched CD4⁺ T cell population.”

349. As discussed in Section XII.C, Wang would have motivated a POSA to start with a T-cell population in which 100% of the cells express CD62L and use Singh’s process to maintain high CD62L positivity throughout expansion. The resulting composition would have expressed **high** levels of CD62L.

350. By contrast, claim 29’s “reference population of CD4⁺ T cells that has been expanded in vitro from an effector memory-enriched CD4⁺ T cell population” would express **low** levels of CD62L. This is so because a POSA would have known that the reference population would contain largely T_{EM} and T_E cells, which are both CD62L⁻. *See, e.g.*, Ex. 1007, 227 (discussing T_{EM} cells’ CD62L⁻ immunophenotype), 10; Ex. 1001, 11:12-13 (“effector T_E are negative for CD62L), 25:42-43 (listing the

immunophenotype of CD4⁺ T_{EM} as CD45RA⁻CD45RO⁺**CD62L⁻**) (emphasis added), Fig. 8A. The reference population is an “effector memory-enriched” population, which means that it will contain primarily T_{EM} cells. As discussed above, T_{EM} cells differentiate into T_E or remain as T_{EM} cells. *See* Ex. 1011, 525 (“T_{CM} and T_{EM} cells . . . respond to antigen re-exposure by differentiating into a new wave of T_E cells.”); *id.* (“some of the progeny of clonally derived T_E cells retain intrinsic programming of the parental cell of origin”); Ex. 1001, 1:59-62 (explaining that T_{EM} “differentiate into cytolytic effector T cells (T_E) that express a high level of granzymes and perforin, but are short-lived.”).

351. Therefore, a POSA would understand the composition generated by combining teachings of Wang and Singh (*i.e.*, a composition with high CD62L positivity) would have “a greater percentage of the chimeric antigen receptor-modified CD4⁺ helper T lymphocytes [that] are surface positive for CD62L” than the claimed reference population, which would have low numbers of CD62L⁺ cells.

352. A POSA would have been motivated to combine Singh and Wang with a reasonable expectation of success in achieving the claimed invention for the same reasons described in Section XII.C.

3. Claim 30

353. Claim 30 recites:

The adoptive cellular immunotherapy composition according to claim 1, wherein a greater percentage of the chimeric antigen receptor-modified CD8⁺ cytotoxic T lymphocytes are surface positive for CD62L, CD45RO and/or CCR7 as compared to a reference population of CD8⁺ T cells that has been expanded in vitro from an effector memory-enriched CD8⁺ T cell population or bulk CD8⁺ T cell population from the subject and modified to contain the chimeric antigen receptor contained by the CD8⁺ cytotoxic T lymphocytes.

354. Claim 30 requires a prior-art composition wherein “a greater percentage of the chimeric antigen receptor-modified CD8⁺ cytotoxic T lymphocytes are surface positive for CD62L, CD45RO and/or CCR7 as compared to” one of two reference populations. One reference population is “a reference population of CD8⁺ T cells that has been expanded in vitro from an *effector memory-enriched* CD8⁺ T cell population.”

355. A POSA would understand claim 30 to be obvious for similar reasons as claim 29. *See* Section XII.E.2. As discussed, the Wang taught a 100% CD62L⁺ T-cell population and Singh’s process would maintain that *high* CD62L-positivity throughout expansion. In contrast, a POSA also would have known that the reference population would contain largely T_{EM} and T_E cells that are CD62L⁻. *See, e.g.*, Ex. 1007, 227 (discussing T_{EM} cells’ CD62L⁻ immunophenotype), 228. Indeed, the ’308 patent confirms that:

“Effector memory” T cell (or “T_{EM}”) as used herein refers to an antigen-experienced CTL that *does not express or has decreased expression of CD62L* on the surface thereof as compared to central memory cells In embodiments, effector memory cells are *negative for expression [of] CD62L*.

Ex. 1001, 7:21-27 (emphasis added);

“Effector” “T_E” T cells as used herein refers to a[n] antigen experienced cytotoxic T lymphocyte cells [sic] that *do not express or have decreased expression of CD62L*.

Id., 7:38-40 (emphasis added).

356. Similar to claim 29, the composition that a POSA would have generated by combining Wang and Singh (with high CD62L positivity) necessarily would have “a greater percentage of the chimeric antigen receptor-modified CD8⁺ cytotoxic T lymphocytes [that] are surface positive for CD62L” than the claimed reference population.

357. A POSA would have been motivated to combine Singh and Wang with a reasonable expectation of success in achieving the claimed invention for the same reasons described in Section XII.C above.

4. Claim 31

358. Claim 31 recites:

The adoptive cellular immunotherapy composition according to claim 1, wherein the chimeric antigen receptor-modified CD8⁺ cytotoxic T lymphocytes proliferate when co-cultured in vitro with the CD4⁺ helper T lymphocytes in the presence of the antigen, to a greater degree as compared to the degree of proliferation of the CD8⁺ cytotoxic T lymphocytes when co-cultured, under the same conditions, with a reference population of CD4⁺ T cells expanded in vitro from an effector memory-enriched CD4⁺ T cell population or a bulk CD4⁺ T cell population from the subject and modified to contain the chimeric antigen receptor contained by the CD4⁺ helper T lymphocytes.

359. Claim 31 is met by the Wang and Singh composition (*i.e.*, a composition with high T_{CM}, which are CD62L⁺).

360. As explained in connection with claim 8, the composition resulting from Singh and Wang would include CD4⁺ T subpopulation of cells (T_{CM}) that secrete a greater amount of IL-2 and exhibit a greater degree of proliferation as compared to the reference T_{EM}-enriched population. *See* Section XII.E.1.

361. A POSA would understand from Singh that culturing CD4⁺ T cells with CD8⁺ T cells results in long-term persistence of the CD8⁺ cells. *See* Ex. 1003, 2970 (“There is convincing clinical data that the presence of CD4⁺ T-helper cells improves the persistence of CD8⁺ antigen-specific T cells.”). Indeed, Moeller disclosed that CD8⁺ and CD4⁺ T cells should be present in an equal amount. *See* Ex. 1016, 3001 (“Optimal antitumor efficacy was achieved with the addition of equivalent numbers of engineered CD4⁺ and CD8⁺ T cells.”). A POSA would have expected that the composition resulting from Singh and Wang would maintain a higher level of CD4⁺ T cells over time compared to the reference T_{EM}-enriched population for reasons discussed in connection with claim 8 (Section XII.E.1). Therefore, the POSA would have expected this higher level of CD4⁺ T cells to provide more help for CD8⁺ T cells to proliferate to a greater degree over time.

362. A POSA would have also understand the Singh and Wang composition of majority T_{CM} CD4⁺ cells to cause greater degree of proliferation of CD8⁺ T cells

for another reason. As discussed in connection with claim 8, a POSA would understand that the Singh and Wang composition of majority T_{CM} CD4⁺ cells would cause increased IL-2 secretion as compared to the reference T_{EM}-enriched population. A POSA would understand that the increased IL-2 secretion would support a greater degree of proliferation of CD8⁺ T cells.

363. A POSA would have known that increased IL-2 secretion would cause a greater degree of proliferation of CD8⁺ T cells based on several prior-art references. D'Souza, for example, states that IL-2 is the "critical...growth factor" for CD8 T-cells: "[A]ctivated CD8 T cells initially undergo IL-2-independent proliferation, but reach a critical juncture where the requirement for IL-2 as a growth factor gains prominence." See Warren N. D'Souza *et al.*, "IL-2 Is Not Required for the Initiation of CD8 T Cell Cycling but Sustains Expansion," J. IMMUNOL. 171:5727-35 (2003) ("D'Souza") (Ex.1030) 5727. Zimmerli "demonstrated a correlation between the ability to secrete IL-2 and the proliferation capacity of CD8 T cells." See Simone C. Zimmerli *et al.*, "HIV-1-specific IFN- γ /IL-2-secreting CD8 T cells support CD4-independent proliferation of HIV-1-specific CD8 T cells," PNAS 102:7239-44, (2005) ("Zimmerli") (Ex.1031) 7242. Jensen states that IL-2 supports "in vivo persistence" of CD8⁺ T-cells: "administered IL-2 is given after the third T cell infusion to support the in vivo persistence of transferred CTL." Ex. 1018

at [0159]. Hudecek I similarly states the IL-2 “improved the persistence of transferred T cells.” Ex. 1011 at 525.

364. Therefore, a POSA would understand that the Singh and Wang composition of high levels of T_{CM} CD4⁺ cells would cause greater IL-2 secretion and degree of proliferation of CD8⁺ T cells.

F. Remaining Challenged Claims

365. Claims 2, 4-5, 7, 16, and 26-28 are obvious for the same reasons discussed in Grounds 1 and 2. *See* Sections X-XI.

XIII. GROUND 4: INDEPENDENT CLAIM 1 AND DEPENDENT CLAIMS 6 AND 9-16 ARE RENDERED OBVIOUS BY MITSUYASU IN VIEW OF SINGH AND COOPER

366. Ground 4 differs from Grounds 1-3 by presenting Mitsuyasu (not Singh) as the primary reference against claims 1, 6, and 9-16.

A. Independent Claim 1 and Dependent Claims 6 and 9-13

367. The elements of claim 1, to which reference letters [a]-[f] were added in Section X.A, are addressed in two main sections below. The first section shows that the components of the claimed composition (claim elements [a]-[c], [e]) were fully disclosed in the prior art. The second section shows that the claimed surface positivity of phenotypic markers was also fully disclosed in the prior art.

1. Claim Limitations Directed to Components of the Claimed Composition

368. Mitsuyasu, in combination with Singh in view of Cooper, discloses the components of the claimed composition. In particular, Mitsuyasu describes immunophenotypes of CD4⁺ and CD8⁺ T cells modified with an engineered CIR that targets the HIV *env* protein. As noted earlier, a POSA would have understood that “CIR” and “CAR” refer to the same class of chimeric proteins, therefore, data from Mitsuyasu’s CIR-engineered T cells can be blended with data from Singh’s CAR-engineered T cells.

a. [a] “[a]n adoptive cellular immunotherapy composition”

369. As discussed above, this element of the claim describes the intended use of the claimed composition. *See* Section VII.A.

370. However, if the preamble is limiting, Mitsuyasu discloses a “composition for adoptive cellular immunotherapy formulated to be suitable for administration to a mammal.” *Id.*

371. Mitsuyasu is an article disclosing results from patient infusions of engineered T cell compositions. *See* Ex. 1004, 785 (“subjects received a single infusion of . . . autologous CD4ζ-modified CD4⁺ and CD8⁺ T cells”). A POSA would have known that Mitsuyasu discloses “an adoptive cellular immunotherapy composition.” Before infusing the compositions into patients, Mitsuyasu’s authors

diluted the T cells into an infusion solution meant to mimic human plasma supplemented with human serum albumin. *See id.*, 786 (describing a cryopreservation solution used to infuse the cells into subjects that included Plasmalyte-A and human serum albumin).

372. For these reasons, a POSA would understand that Mitsuyasu discloses an “adoptive cellular immunotherapy composition.”

b. [b] “containing chimeric antigen receptor-modified CD4⁺ T lymphocytes and chimeric antigen receptor-modified CD8⁺ T lymphocytes,”

373. Mitsuyasu discloses receptor-modified CD4⁺ and CD8⁺ T cells that bind to an antigen, and it would have been obvious to use Singh’s CAR in place of Mitsuyasu’s chimeric receptor.

374. A POSA would have known that Mitsuyasu’s subjects received CD4⁺ and CD8⁺ T cells, and that both cell types expressed a chimeric receptor known as CD4ζ. *See* Ex. 1004, 785 (“subjects received a single infusion of . . . autologous CD4ζ-modified CD4⁺ and CD8⁺ T cells”). CD4ζ is a chimeric receptor that includes the transmembrane and extracellular domains of human CD4 fused to CD3ζ as an intracellular signaling domain. Human CD4 targets the HIV env protein, which is expressed on the surface of HIV-infected cells. *See id.*, 785-786. Mitsuyasu referred to CD4ζ as a “chimeric immune receptor” (a CIR). *Id.*, 786.

375. A POSA would have understood that a CIR is the same as a CAR and the different terms reflected the preferences of the researchers. The prior art described a CIR as an “artificial receptor” that has “an extracellular antigen-binding domain linked through a transmembrane motif to a cytoplasmic lymphocyte-signaling moiety.” Shibaguchi, H., et al., “A Fully Human Chimeric Immune Receptor for Retargeting T-Cells to CEA-Expressing Tumor Cells, *Anticancer Research* 26:4067-72 (2006) (Ex. 1019) (“Shibaguchi”), 4067. The prior art defined a CAR similarly, as having “an extracellular antigen-binding exodomain . . . a spacer, a transmembrane region, and one or more intracellular signaling domains.” Ex. 1010, 4 (internal parenthetical omitted).

376. Although the field has moved toward using the acronym “CAR” instead of “CIR,” scientific publications published prior to the priority date largely used the terms interchangeably.

377. A POSA would have recognized the benefit of Mitsuyasu’s manufacturing process: T cells that exhibit “sustained, high level persistence [that] demonstrates the feasibility of ex vivo T-cell gene therapy.” Ex. 1004, 785. This benefit would have motivated a POSA to use Mitsuyasu’s method with CARs targeting other disorders.

378. A POSA would have understood that Mitsuyasu’s process could be applied to other genetically modified T cells because doing so would have been a

simple matter of using a different engineered receptor, such as Singh's anti-CD19 CAR, in place of Mitsuyasu's HIV-targeting CIR.

379. As mentioned in Sections X.A.1.b-X.A.1.c, Singh discloses CAR-modified CD4⁺ and CAR-modified CD8⁺ T cells that bind to the CD19 antigen. A POSA would have been motivated to use Singh's CAR as a substitute for Mitsuyasu's CD4ζ receptor in order to treat CD19-expressing B-cell malignancies, which would not be targeted specifically with Mitsuyasu's receptor. Therefore, a POSA would have been motivated to use Singh's CAR in Mitsuyasu's system to obtain successful clinical results, but for a different disorder, such as a cancer.

380. A POSA would have had a reasonable expectation of success in using Mitsuyasu's manufacturing process with Singh's CAR in light of the successful results reported in both Mitsuyasu and Singh. *See* Ex. 1004, 791 ("We have developed a rapid T-cell manufacturing process that reliably yields 2 to 3 x 10¹⁰ gene-modified T cells within 2 weeks. This process was successful in 24 of 25 enrolled patients and yielded a mixed population of CD4⁺ and CD8⁺ T cells with an activated cell phenotype (high expression of CD28, CD25, and CD62L)."); *see* Ex. 1003, 2969 ("what is needed, and is provided here, is a cost-effective gene transfer system that encourages reiterative changes to expression vector and/or CAR design to be used in proof-of-concept clinical trials that support hypothesis testing from the bench to the bedside and back again."). Further support for a reasonable expectation

of success would have been known to a POSA from Cooper, which is an entry from an encyclopedia that provides an overview of CARs in 2009.

381. A POSA would understand from Cooper that modifying a genetically engineered T cell to include a different binding receptor was simple substitution. *See* Ex. 1010, 7 (“One of the major advantages of the strategy of infusing CAR⁺ T cells lies in the ***modular composition*** of the CAR molecule This allows investigators to ***swap the CAR exodomain with a scFv or ligand*** that recognizes or binds to a desired cell-surface antigen.”) (emphasis added).

382. A POSA would have understood that Mitsuyasu’s receptor had an exodomain that was a ligand—CD4. A POSA also would have known that Singh’s CAR utilized an exodomain comprising a scFv. *See* Sections VIII.A.1, X.C.1. Thus, from Cooper, a POSA would have had a reasonable expectation of success swapping Mitsuyasu’s CD4 ligand for Singh’s CD19-targeting scFv.

383. For these reasons, a POSA would understand that the prior art renders obvious an adoptive cellular immunotherapy composition “containing chimeric antigen receptor-modified CD4⁺ T lymphocytes and chimeric antigen receptor-modified CD8⁺ T lymphocytes.”

- c. [c] & [e] “wherein: (a) the chimeric antigen receptor-modified CD4⁺ T lymphocytes [and (b) the chimeric antigen receptor-modified CD8⁺ T lymphocytes] contain a chimeric antigen receptor that specifically binds to an antigen”

384. A POSA would have known that Mitsuyasu’s compositions included CD4⁺ and CD8⁺ T cells that expressed a HIV(*env*)-targeting receptor. *See* Section XIII.A.1.b. A POSA also would have known that Singh’s compositions included CD4⁺ and CD8⁺ T cells that expressed a CD19-targeting CAR. *See* Section X.A.1.c. Mitsuyasu’s receptor and Singh’s CAR both specifically bind to an antigen: HIV(*env*) and CD19, respectively. As discussed in Section XIII.A.1.b, it would have been obvious to use Singh’s CAR with Mitsuyasu’s manufacturing process.

385. For these reasons, a POSA would understand that the prior art renders obvious an adoptive immunotherapy composition “wherein the chimeric antigen receptor-modified [CD4⁺ and CD8⁺] T lymphocytes contain a chimeric antigen receptor that specifically binds to an antigen.”

2. Claim Limitations Directed to Phenotypic Marker Surface Positivity

386. Mitsuyasu discloses a composition with the claimed phenotypic marker expression because it discloses CD62L surface positivity within the claimed ranges.

- a. [d] & [f] “at least 50% of the chimeric antigen receptor-modified CD4⁺ helper T lymphocytes in the composition are surface positive for CD62L and/or CD45RA,” and “at least 50% of CD8⁺ cytotoxic T lymphocytes in the composition are surface positive for CD62L and/or CD45RO”

387. Mitsuyasu shows the immunophenotypes of its T-cell compositions in

Table 2:

Patient ID	CD4 (%) [*]	CD8 (%) [*]	CD62L (%) [*]	CD28 (%) [*]	CD25 (%) [*]	Culture days [†]	CD4 ζ by PCR [‡]
N215	24	73	83	88	—	13	11
H202	62	38	96	98	88	12	17
C211	17	80	84	91	42	12	12
F209	13	83	—	85	45	12	18
S201	26	72	—	89	36	12	9
Q202	22	77	94	98	77	12	5
S206	52	46	93	96	74	17	49
S212	20	79	95	98	43	12	10
P217	31	68	94	93	39	12	13
G251	22	86	82	87	41	12	17
E207	28	69	73	94	60	12	17
T253	24	77	82	88	55	12	18
L218	13	86	85	94	36	12	15
R255	60	37	79	90	27	12	15
Y254	22	76	94	98	59	12	13
U213	7	92	84	96	43	10	9
D282	23	78	82	95	50	13	16
D212	3	96	44	90	64	14	15
T232	28	70	87	88	34	14	11
D256	12	88	59	74	58	15	15
J233	7	90	81	90	43	14	18
Y292	37	61	96	99	28	12	28
X214	8	90	64	79	49	16	38
C298	26	73	83	63	36	12	57
Mean	24	74	82	90	49	13	19
SD	15	16	13	8	16	2	13

^{*}Percentage of CD3⁺ T cells expressing marker by FACS analysis.
[†]Total number of days in culture for each T-cell lot.
[‡]Transduction efficiency as measured by DNA PCR for CD4 ζ .

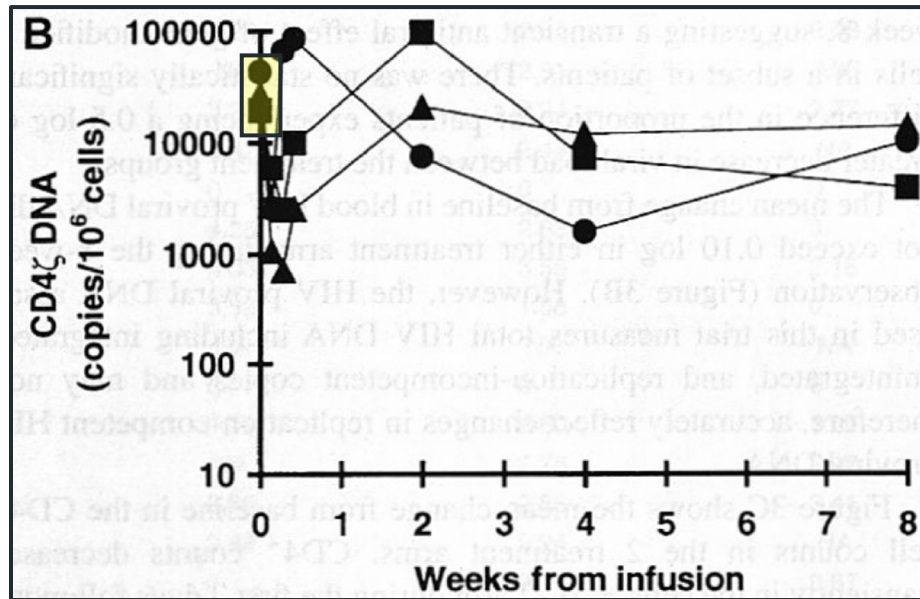
Ex. 1004, 788 (annotated). I have annotated Table 2 to show the percentages of CD4⁺, CD8⁺, CD62L⁺, and receptor-modified T cells in blue, green, yellow, and red, respectively.

388. Mitsuyasu discloses that its compositions had, on average, 82% CD62L positivity. *See id.* (“Mean” percent CD62L-positivity equals 82%). Seventeen of the tested compositions had greater than 80% CD62L positivity. *See id.* Mitsuyasu does not explicitly disclose the percentage CD62L-positivity in its subpopulations of receptor-modified CD4⁺ and CD8⁺ T cells. Nonetheless, a POSA would have recognized that Mitsuyasu’s compositions met the claimed ranges.

(1) Mitsuyasu’s Compositions Included Receptor-Modified, CD4⁺ and CD8⁺, T Cells

389. A POSA would have recognized from Mitsuyasu’s Table 2 that all of its compositions included receptor-modified CD4⁺ and CD8⁺ T cells because all compositions had non-zero values for percent CD4⁺ and percent CD8⁺ cells. *See* Ex. 1004, 788.

390. A POSA also would have seen that Mitsuyasu’s CD4ζ-encoding DNA was present in both CD4⁺ and CD8⁺ T cells at the time of infusion, as shown in Mitsuyasu’s Figure 2B below:



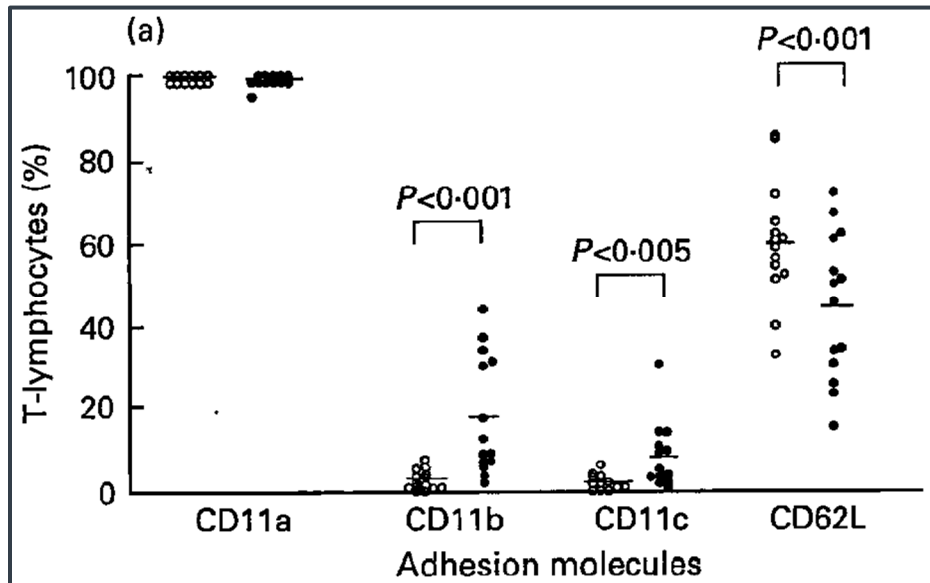
Id., 789 (annotated). In Figure 2B, which represents patient J233, Mitsuyasu represented CD4 ζ DNA in CD4⁺ and CD8⁺ T cells by squares (■) and circles (●), respectively. I have shaded in yellow above those T-cell populations at the time of infusion (*i.e.*, 0 weeks from infusion). A POSA would have recognized that CD4 ζ DNA was present at the same order of magnitude in CD4⁺ and CD8⁺ T cells.

391. A POSA would have known from Mitsuyasu that its authors administered mixtures of CAR-modified CD4⁺ and CAR-modified CD8⁺ cells to patients, not that some patients received only one type of CAR-modified cell or the other. *See id.*, 785 (“subjects received a single infusion of . . . autologous CD4 ζ -modified CD4⁺ and CD8⁺ T cells”).

(2) Percentage CD62L-Positivity Is Not Impacted by CD4 Versus CD8, and CIR, Expression

392. A POSA would understand that, while the rate of CD62L expression in Mitsuyasu's compositions would vary between CD4⁺ and CD8⁺ T cells, both populations would be expected to have substantially similar CD62L-positivity. Although, CD4⁺ T cells may have a slightly higher proportion of CD62L-positive cells. See Cavers *et al.*, "Differential Expression of β 1 and β 2 Integrins and L-Selectin on CD4⁺ and CD8⁺ T Lymphocytes in Human Blood: Comparative Analysis Between Isolated Cells, Whole Blood Samples, and Cryopreserved Preparations," CLIN EXP IMMUNOL 127:60-65 (2002) (Ex. 1020) ("Cavers"), 62 (Fig. 2a).

393. Cavers' Figure 2a is shown below:



Id., 62. Cavers' Figure 2 shows CD62L expression in blood mononuclear cells from 14 subjects, with CD4⁺ cells displayed as open circles (○) and CD8⁺ cells displayed as closed circles (●). But for a small minority of outliers, CD4⁺ and CD8⁺ T cells showed substantially similar CD62L-positivity. Therefore, a POSA would recognize that the CD4⁺ and CD8⁺ populations in Mitsuyasu's compositions would both express CD62L, and that the overall percentage of CD62L-positivity reported for all T cells would be informative of the percentage of CD62L⁺ for the CD4⁺ and CD8⁺ populations.

394. Likewise, a POSA would expect that the percentage CD62L-positivity in Mitsuyasu's compositions would not be affected by chimeric receptor expression. The rate of CD62L expression would be roughly equivalent in receptor-modified and receptor-unmodified cells. As of March 23, 2011, a POSA would have no reason to believe that CAR-positivity increases or decreases the percentage of T cells that are also CD62L-positive, nor would a POSA have a reason to believe that CD62L-positivity affects virus transduction rate. Thus, a POSA would understand that the receptor-modified and receptor-unmodified populations in Mitsuyasu's compositions would show similar rates of CD62L-positivity as their respective overall composition.

(3) A POSA Would Understand That Mitsuyasu's Overall CD8⁺, and Receptor-Modified CD4⁺ and CD8⁺ T-Cell Subpopulations Have A Similar Percentage As Total T Cells For CD62L-Positivity

395. As discussed above, a POSA would have expected that the CD4⁺, CD8⁺, and receptor-modified T-cell populations in Mitsuyasu's compositions would exhibit roughly the same rate of CD62L-positivity as the authors observed in their respective overall compositions. From Mitsuyasu's Table 2, a POSA would have known that seventeen of the tested compositions had greater than 80% CD62L-positivity. *See* Ex. 1004, 788. Therefore, a POSA would have expected that receptor-modified CD4⁺, receptor-modified CD8⁺, and overall CD8⁺ T cells in those seventeen compositions also would have exhibited greater than 80% CD62L-positivity, and therefore would have met the elements of the claims.

396. Even if it is not assumed that CD62L-positivity is substantially similar between CD4⁺ and CD8⁺ T cells, the POSA would understand that most of Mitsuyasu's compositions met the elements of the claims. A POSA viewing the high percentages of CD62L-positivity of the compositions in Table 2 (average of 82% with seventeen compositions >80%) would understand that it is likely that a majority of the compositions would have CD4⁺ and CD8⁺ T cell populations in which at least 50% (or at least 60%, or at least 80%) are CD62L⁺. In fact, mathematically, it would be impossible for less than 8 of Mitsuyasu's compositions to have CD4⁺ and CD8⁺ T cells with less than 50% CD62L positivity. Specifically, a POSA would have

recognized that, in at least compositions H202, Q202, S206, S212, P217, Y254, T232, and Y292, the rate of CD62L-positivity was so high (seven of the compositions had CD62L-positivity that was >90%, seventeen had >80%, and the overall average of all twenty-two compositions was 82%) that the CD4⁺ and CD8⁺ populations must have CD4⁺ and CD8⁺ T cells with more than 50% CD62L positivity. This is the case even if all CD62L⁻ cells (determined by subtracting the percentage of CD62L⁺ cells from 100%) are in either the CD4⁺ or CD8⁺ T-cell populations.

397. Therefore, the percentage CD62L surface positivity limitations of claims 1, 6, and 9-13 are met.

(4) Mitsuyasu's Composition S206 Necessarily Meets All The Claim Limitations

398. One example composition reported by Mitsuyasu—identified as S206—is examined in more detail. A POSA would only need to assume (correctly, as discussed above) that the S206 compositions included both chimeric receptor-modified, CD4⁺ and CD8⁺, T cells to know that this composition must meet the elements of the claims.

399. The S206 composition had an overall CD62L⁺ of 93% for all T cells, meaning only 7% of T cells did not have CD62L. S206 also has 46% of its composition as CD8⁺ T cells, or 46 cells out of 100. S206 also has 52% of its composition as CD4⁺ T cells, or 52 out of 100. Even under the most stringent

scenario that all 7 CD62L⁻ cells were CD8⁺ (which would not be a scientifically correct assumption for a POSA), then 39 of the 46 CD8⁺ T cells would be CD62L⁺, or roughly 85%. Similarly, even under the most stringent scenario that all 7 CD62L⁻ cells were CD4⁺ (which, again, would not be a scientifically correct assumption for a POSA), then 45 of the 52 CD4⁺ T cells would be CD62L⁺, or roughly 87%. As explained more in detail below, the percent CD62L-positivity of receptor-modified CD4⁺ and CD8⁺ T cells could not mathematically have been below 70% and the overall CD8⁺ T cells could not have been below 80%, even if all the CD62L-negative cells were in a claimed subpopulation (which would, in any event, be an incorrect scientific assumption). This satisfies the percentage CD62L⁺ limitations in claims 1, 6, 9-12. It also would have been mathematically necessary for either the receptor-modified CD4⁺, the receptor-modified CD8⁺ T cells, or both to be >80% CD62L⁺. This satisfies the percentage CD62L⁺ limitations in claim 13.

400. In general, engineered T cells produced using the methods of Mitsuyasu will yield receptor-modified populations that are composed of substantially similar numbers of CD8⁺ T cells and CD4⁺ T cells. *See McGuinness et al.*, “Anti-Tumor Activity of Human T Cells Expressing the CC49-ζ Chimeric Immune Receptor,” *Human Gene Therapy* 10: 165-73 (1999) (Ex. 1021) (“McGuinness”), 167-68. McGuinness reported results from transducing peripheral blood mononuclear cells with a CIR called CC49-ζ expressed from a vector backbone similar to the one used

in Mitsuyasu. *See* Ex. 1021, 166 (discussing the “retroviral rkat43.2 vector backbone”); *see also* Ex. 1004, 786 (discussing the “retroviral vector rkat4.2SVGF3e—[a] variant of the previously described rkat43.3F3 vector”). McGuinness showed that the rate of transduction for CD4⁺ and CD8⁺ T cells are similar: “[b]oth CD4⁺ and CD8⁺ T cells were transduced to express CC49-ζ (54 and 56% CC49-ζ positive, respectively, in this representative example).” Ex. 1021, 167. Therefore, a POSA would have expected Mitsuyasu’s CD4⁺ and CD8⁺ T cells to be modified at similar rates.

401. Given this distribution, it would have been impossible for certain compositions not to have more than 50% CD62L-positivity in receptor-modified, CD4⁺ and CD8⁺, T cells. For example, in the composition corresponding to patient S206, the percent CD62L-positivity of receptor-modified CD4⁺ and CD8⁺ T cells could not have been below 70%.

402. I have reproduced the immunophenotype percentages for patient S206 below:

Patient ID	CD4 (%) [*]	CD8 (%) [*]	CD62L (%) [*]	CD28 (%) [*]	CD25 (%) [*]	Culture days [†]	CD4ζ by PCR [‡]
...							
S206	52	46	93	96	74	17	49
...							

403. For ease of calculation, assume that composition S206 includes only 100 T cells. S206 exhibited CD62L-positivity of 93%. A POSA would thus understand that 93 cells out of 100 would be CD62L⁺ and 7 would be CD62L⁻.

404. In the most stringent scenario for this example, all 7 CD62L⁻ T cells would be chimeric receptor-modified. Assigning less than 7 CD62L⁻ T cells to the chimeric receptor-modified subpopulation ultimately increases the percentage of chimeric receptor-modified CD4⁺ and chimeric receptor-modified CD8⁺ T cells that are CD62L⁺, as discussed further below.

405. A POSA would recognize that the right-most column of Figure 2 corresponds to CD4ζ transduction efficiency (here, 49%). *See* Ex. 1004, 788 (Table 2 footnotes) (indicating that the right-most column shows “Transduction efficiency as measured by DNA PCR for CD4ζ”). Therefore, in S206, the number of chimeric receptor-modified T cells would be 49 out of 100 cells.

406. Assuming substantially similar numbers of receptor-modified CD4⁺ and CD8⁺ T cells based on McGuinness, there would be 24 receptor-modified CD4⁺ T cells (roughly half of S206’s 49 chimeric receptor-modified T cells) and 25 receptor-modified CD8⁺ T cells (roughly half of S206’s 49 chimeric receptor-modified T cells):

CAR ⁺ CD4 ⁺	CAR ⁺ CD8 ⁺
24	25

407. With 7 CD62L⁻ T cells being chimeric receptor-modified, there are eight scenarios in which those cells could be distributed differently: at one extreme, all CD62L⁻ cells would be receptor-modified CD4⁺ T cells, and at the other, all CD62L⁻ cells would be receptor-modified CD8⁺ T cells:

CAR⁺ CD4⁺	CAR⁺ CD8⁺	CAR⁺ CD4⁺ that are CD62L⁻	CAR⁺ CD8⁺ that are CD62L⁻
24	25	7	0
24	25	6	1
24	25	5	2
24	25	4	3
24	25	3	4
24	25	2	5
24	25	1	6
24	25	0	7

408. Subtracting the number of receptor-modified CD4⁺ cells that are CD62L⁻ from the overall receptor-modified CD4⁺ population yields the number of receptor-modified CD4⁺ CD62L⁺ T cells. Likewise, subtracting the number of receptor-modified CD8⁺ cells that are CD62L⁻ from the overall receptor-modified CD8⁺ population yields the number of receptor-modified CD8⁺ CD62L⁺ T cells:

CAR⁺ CD4⁺	CAR⁺ CD8⁺	CAR⁺ CD4⁺ that are CD62L⁻	CAR⁺ CD8⁺ that are CD62L⁻	CAR⁺ CD4⁺ CD62L⁺	CAR⁺ CD8⁺ CD62L⁺
24	25	7	0	17	25
24	25	6	1	18	24
24	25	5	2	19	23
24	25	4	3	20	22
24	25	3	4	21	21
24	25	2	5	22	20
24	25	1	6	23	19

CAR⁺ CD4⁺	CAR⁺ CD8⁺	CAR⁺ CD4⁺ that are CD62L⁻	CAR⁺ CD8⁺ that are CD62L⁻	CAR⁺ CD4⁺ CD62L⁺	CAR⁺ CD8⁺ CD62L⁺
24	25	0	7	24	18

409. Dividing the number of receptor-modified CD4⁺ T cells that are CD62L⁺ by the number of receptor-modified CD4⁺ T cells, and multiplying by 100%, yields the percentage of receptor-modified CD4⁺ T cells that are CD62L⁺. Similarly, dividing the number of receptor-modified CD8⁺ T cells that are CD62L⁺ by the number of receptor-modified CD8⁺ T cells, and multiplying by 100%, yields the percentage of receptor-modified CD8⁺ T cells that are CD62L⁺:

7 CD62L⁻ T Cells are Receptor-Modified							
CAR⁺ CD4⁺	CAR⁺ CD8⁺	CAR⁺ CD4⁺ that are CD62L⁻	CAR⁺ CD8⁺ that are CD62L⁻	CAR⁺ CD4⁺ CD62L⁺	CAR⁺ CD8⁺ CD62L⁺	% CAR⁺ CD4⁺ that are CD62L⁺	% CAR⁺ CD8⁺ that are CD62L⁺
24	25	7	0	17	25	71%	100%
24	25	6	1	18	24	75%	96%
24	25	5	2	19	23	79%	92%
24	25	4	3	20	22	83%	88%
24	25	3	4	21	21	88%	84%
24	25	2	5	22	20	92%	80%
24	25	1	6	23	19	96%	76%
24	25	0	7	24	18	100%	72%

410. As shown in the right-most columns, in this most-stringent scenario, even with all 7 CD62L⁻ T cells being receptor-modified, the percentage CD62L-positivity always is above 70% for the receptor-modified CD4⁺ and CD8⁺ subpopulations.

411. If one assumes less stringent scenarios, the percentage CD62L-positivity of both populations necessarily increases, as shown below:

6 CD62L ⁻ T Cells are Receptor-Modified							
CAR ⁺ CD4 ⁺	CAR ⁺ CD8 ⁺	CAR ⁺ CD4 ⁺ that are CD62L ⁻	CAR ⁺ CD8 ⁺ that are CD62L ⁻	CAR ⁺ CD4 ⁺ CD62L ⁺	CAR ⁺ CD8 ⁺ CD62L ⁺	% CAR ⁺ CD4 ⁺ that are CD62L ⁺	% CAR ⁺ CD8 ⁺ that are CD62L ⁺
24	25	6	0	18	25	75%	100%
24	25	5	1	19	24	79%	96%
24	25	4	2	20	23	83%	92%
24	25	3	3	21	22	88%	88%
24	25	2	4	22	21	92%	84%
24	25	1	5	23	20	96%	80%
24	25	0	6	24	19	100%	76%

5 CD62L ⁻ T Cells are Receptor-Modified							
CAR ⁺ CD4 ⁺	CAR ⁺ CD8 ⁺	CAR ⁺ CD4 ⁺ that are CD62L ⁻	CAR ⁺ CD8 ⁺ that are CD62L ⁻	CAR ⁺ CD4 ⁺ CD62L ⁺	CAR ⁺ CD8 ⁺ CD62L ⁺	% CAR ⁺ CD4 ⁺ that are CD62L ⁺	% CAR ⁺ CD8 ⁺ that are CD62L ⁺
24	25	5	0	19	25	79%	100%
24	25	4	1	20	24	83%	96%
24	25	3	2	21	23	88%	92%
24	25	2	3	22	22	92%	88%
24	25	1	4	23	21	96%	84%
24	25	0	5	24	20	100%	80%

4 CD62L ⁻ T Cells are Receptor-Modified							
CAR ⁺ CD4 ⁺	CAR ⁺ CD8 ⁺	CAR ⁺ CD4 ⁺ that are CD62L ⁻	CAR ⁺ CD8 ⁺ that are CD62L ⁻	CAR ⁺ CD4 ⁺ CD62L ⁺	CAR ⁺ CD8 ⁺ CD62L ⁺	% CAR ⁺ CD4 ⁺ that are CD62L ⁺	% CAR ⁺ CD8 ⁺ that are CD62L ⁺
24	25	4	0	20	25	83%	100%
24	25	3	1	21	24	88%	96%
24	25	2	2	22	23	92%	92%
24	25	1	3	23	22	96%	88%
24	25	0	4	24	21	100%	84%

3 CD62L ⁻ T Cells are Receptor-Modified							
CAR ⁺ CD4 ⁺	CAR ⁺ CD8 ⁺	CAR ⁺ CD4 ⁺ that are CD62L ⁻	CAR ⁺ CD8 ⁺ that are CD62L ⁻	CAR ⁺ CD4 ⁺ CD62L ⁺	CAR ⁺ CD8 ⁺ CD62L ⁺	% CAR ⁺ CD4 ⁺ that are CD62L ⁺	% CAR ⁺ CD8 ⁺ that are CD62L ⁺
24	25	3	0	21	25	88%	100%
24	25	2	1	22	24	92%	96%
24	25	1	2	23	23	96%	92%
24	25	0	3	24	22	100%	88%

2 CD62L ⁻ T Cells are Receptor-Modified							
CAR ⁺ CD4 ⁺	CAR ⁺ CD8 ⁺	CAR ⁺ CD4 ⁺ that are CD62L ⁻	CAR ⁺ CD8 ⁺ that are CD62L ⁻	CAR ⁺ CD4 ⁺ CD62L ⁺	CAR ⁺ CD8 ⁺ CD62L ⁺	% CAR ⁺ CD4 ⁺ that are CD62L ⁺	% CAR ⁺ CD8 ⁺ that are CD62L ⁺
24	25	2	0	22	25	92%	100%
24	25	1	1	23	24	96%	96%
24	25	0	2	24	23	100%	92%

1 CD62L ⁻ T Cell is Receptor-Modified							
CAR ⁺ CD4 ⁺	CAR ⁺ CD8 ⁺	CAR ⁺ CD4 ⁺ that are CD62L ⁻	CAR ⁺ CD8 ⁺ that are CD62L ⁻	CAR ⁺ CD4 ⁺ CD62L ⁺	CAR ⁺ CD8 ⁺ CD62L ⁺	% CAR ⁺ CD4 ⁺ that are CD62L ⁺	% CAR ⁺ CD8 ⁺ that are CD62L ⁺
24	25	1	0	23	25	96%	100%
24	25	0	1	24	24	100%	96%

0 CD62L ⁻ T Cells are Receptor-Modified							
CAR ⁺ CD4 ⁺	CAR ⁺ CD8 ⁺	CAR ⁺ CD4 ⁺ that are CD62L ⁻	CAR ⁺ CD8 ⁺ that are CD62L ⁻	CAR ⁺ CD4 ⁺ CD62L ⁺	CAR ⁺ CD8 ⁺ CD62L ⁺	% CAR ⁺ CD4 ⁺ that are CD62L ⁺	% CAR ⁺ CD8 ⁺ that are CD62L ⁺
24	25	0	0	24	25	100%	100%

412. In all thirty-six scenarios listed above, the percentage of chimeric receptor-modified CD4⁺, chimeric receptor-modified CD8⁺, and overall CD8⁺¹⁹ T cells that were CD62L⁺ was above 50% (relevant to claim 1). It thus was mathematically impossible for Mitsuyasu's composition not to meet the surface positivity limitations of claim 1. The percentage of chimeric receptor-modified CD4⁺ and chimeric receptor-modified CD8⁺ T cells that were CD62L⁺ is always above 70%. In addition, when one population's percentage CD62L-positivity is below 80%, the other population's is at least 80% (relevant for dependent claim 13 discussed below).

¹⁹ As discussed, in S206, the percentage CD62L-positivity in overall CD8⁺ T cells must be above 85%. As mentioned, for ease of explanation, assume there are 100 T cells in S206. 93 of them would be CD62L⁺ (93%). 7 cells (7%) thus would be CD62L⁻. In the most stringent scenario, assume that all 7 CD62L⁻ cells are CD8⁺. There are 46 CD8⁺ T cells (46%) in S206. Subtracting the 7 CD62L⁻ cells from the 46 CD8⁺ cells yields 39 CD8⁺ CD62L⁺ T cells. These 39 CD8⁺ CD62L⁺ cells are roughly 85% of the 46 CD8⁺ T cells in S206, which meets the CD62L-positivity requirements for CD8⁺ T cells recited in claim 1.

b. Dependent Claims 6 and 9-13

413. Claims 6 and 9-13 include additional limitations concerning the percentage of modified T cells that are surface positive for various surface markers:

6. The adoptive cellular immunotherapy composition according to claim 1, wherein:

(a) at least 60% of the chimeric antigen receptor-modified CD4⁺ T lymphocytes are surface positive for CD62L and/or CD45RA;

(b) at least 60% of the chimeric antigen receptor-modified CD8⁺ cytotoxic T lymphocytes are surface positive for CD62L and/or CD45RO;

(c) at least 80% of the chimeric antigen receptor-modified CD4⁺ T lymphocytes are surface positive for CD62L and/or CD45RA; and/or

(d) at least 80% of the chimeric antigen receptor-modified CD8⁺ cytotoxic T lymphocytes are surface positive for CD62L and/or CD45RO.

9. The adoptive cellular immunotherapy of claim 1, wherein at least 50% of the chimeric antigen receptor-modified CD4⁺ helper T lymphocytes in the composition are surface positive for CD62L.

10. The adoptive cellular immunotherapy composition of claim 1, wherein at least 50% of the chimeric antigen receptor-modified CD8⁺ cytotoxic T lymphocytes in the composition are surface positive for CD62L.

11. The adoptive cellular immunotherapy composition of claim 1, wherein at least 50% of the chimeric antigen receptor-modified CD4⁺ helper T lymphocytes in the composition are surface positive for CD62L and at least 50% of the chimeric antigen receptor-modified CD8⁺ cytotoxic T lymphocytes in the composition are surface positive for CD62L.

12. The adoptive cellular immunotherapy composition of claim 1, wherein at least 60% of the chimeric antigen receptor-modified CD4⁺ helper T lymphocytes in the composition are surface positive for CD62L and/or at least 60% of the chimeric antigen receptor-modified CD8⁺ cytotoxic T lymphocytes in the composition are surface positive for CD62L.

13. The adoptive cellular immunotherapy composition of claim 1, wherein at least 80% of the chimeric antigen receptor-modified CD4⁺ helper T lymphocytes in the composition are surface positive for CD62L and/or at least 80% of the chimeric antigen receptor-modified CD8⁺ cytotoxic T lymphocytes in the composition are surface positive for CD62L.

414. For the reasons discussed above in paragraphs 388 and 395, a POSA would have expected that the total CD8⁺ T cells, and the receptor-modified CD4⁺ and CD8⁺ T cells, in seventeen of Mitsuyasu's compositions would have exhibited greater than 80% CD62L-positivity in each of these subpopulations, and therefore would have met the elements of these claims.

415. Furthermore, for the reasons discussed above in paragraph 396, a POSA would have recognized that it would be impossible for less than 8 out of Mitsuyasu's compositions to have CD4⁺ and CD8⁺ T cells with less than 50% CD62L positivity. *See, e.g.*, Ex. 1001, cls. 1 and 9-11.

416. Additionally, for the reasons discussed above in paragraphs 398-412, Mitsuyasu disclosed at least one composition, S206, that mathematically must have met the percentage cutoffs of these claims. As shown in the above tables, the percentage CD62L-positivity in S206's CD8⁺ T cells must be higher than 85% and

the CD62L-positivity in CD4⁺ T cells must be higher than 87%. In all thirty-six scenarios listed in the above tables, the percentage of chimeric receptor-modified CD4⁺ and chimeric receptor-modified CD8⁺ T cells that were CD62L⁺ was always above 70%. This satisfies the percentage CD62L⁺ limitations in claims 1, 6, 9-12. In addition, when one population's percentage CD62L-positivity is below 80%, the other population's is at least 80%. This satisfies the percentage CD62L⁺ limitations in claim 13.

417. For these reasons, a POSA would understand that Mitsuyasu in view of Singh and Cooper renders dependent claims 6 and 9-13 obvious.

B. Dependent Claims Directed to Ratios

418. Claims 14 and 15 recite additional composition ratio limitations. Mitsuyasu and Singh teach all elements of these claims. *See* Sections X.E.1, XII.D.

419. Assuming a relatively equivalent rate of CIR transduction, a POSA would understand that Mitsuyasu's Table 2 shows at least two compositions, S206 and P217, that meet the elements of these claims.

420. S206 exhibited a ratio of receptor-modified CD4⁺ T cells to receptor-modified CD8⁺ T cells of about 1:1: 52% CD4⁺, and 46% CD8⁺, T cells. Fifty-two percent divided by forty-six percent equals 1.13 (about 1:1).

421. P217 had a ratio of receptor-modified CD4⁺ T cells to receptor-modified CD8⁺ T cells of about 1:2: 31% CD4⁺, and 68% CD8⁺, T cells. Thirty-one percent divided by sixty-eight percent equals 0.46 (about 1:2).

422. For these reasons, Mitsuyasu in view of Singh and Cooper render obvious an adoptive cellular immunotherapy wherein the CAR-modified CD4⁺ helper T lymphocytes and the CAR-modified CD8⁺ cytotoxic T lymphocytes in the composition are present at a ratio of between about 2:1 to about 1:2, and specifically in ratios of about 1:1 and about 1:2.

C. Dependent Claim Directed to CAR Components

423. Claim 16 specifies “wherein the chimeric antigen receptor contained by the CD8⁺ T lymphocytes is the same as the chimeric antigen receptor contained by the CD4⁺ T lymphocytes.”

424. Mitsuyasu and Singh used their chimeric receptors in both CD4⁺ T cells and the CD8⁺ T cells. *See* Sections X.A.1.b, X.A.1.c, XIII.A.1.b, XIII.A.1.c.

425. For these reasons, a POSA would understand that Mitsuyasu in view of Singh and Cooper teaches an adoptive cellular immunotherapy composition wherein the CAR contained by the CD8⁺ T lymphocytes is the same as the CAR contained by the CD4⁺ T lymphocytes.

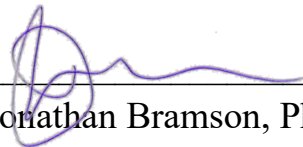
XIV. CONCLUSION

426. For at least the reasons I discussed above, it is my opinion that the Challenged Claims are anticipated and obvious, and therefore unpatentable.

Declaration of Jonathan Bramson, Ph.D.

In signing this declaration, I understand that this declaration will be filed as evidence in a contested case before the Patent Trial and Appeal Board of the USPTO. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true.

Executed on: March 22, 2023



Jonathan Bramson, Ph.D.

APPENDIX A

CURRICULUM VITAE

NAME: Jonathan Lorne Bramson
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EDUCATION: Jan. 1991 - Dec. 1994 Ph.D. Degree, Experimental Medicine, McGill University.
Sept. 1987 - Dec. 1990 B.Sc. Degree, Biochemistry, McGill University.

CURRENT STATUS: Vice Dean, Research
Professor, Department of Medicine
Associate member, Department of Biochemistry
Associate member, Department of Oncology
Associate member, Escarpment Cancer Research Institute

EMPLOYMENT HISTORY:

07/2017 – present Vice Dean Research, Faculty of Health Sciences
10/2014 – 06/2017 Assistant Dean, Research Infrastructure, Faculty of Health Sciences
10/2014 – 06/2017 Director, Fraunhofer-McMaster Project Centre for Biomedical Engineering and Advanced Manufacturing
07/2009 – present Professor (tenured), Dept. Path. & Mol. Med., McMaster University
07/2009 – 12/2014 Director, McMaster Immunology Research Centre, McMaster University
05/2007 – 06/2009 Associate Professor (tenured), Dept. Path. & Mol. Med., McMaster University
07/2004 – 04/2007 Associate Professor (CAWAR), Dept. Path. & Mol. Med., McMaster University
06/1999 – 06/2004 Assistant Professor, Department of Pathology & Molecular Medicine, McMaster University
07/1997 – 06/1999 Research Scientist/Project Leader, Inex Pharmaceuticals, Corp.
10/1994 – 07/1997 Post-doctoral position in the labs of Drs. J. Gauldie and F. Graham, McMaster University.
01/1991 – 09/1994 Ph. D. Thesis project in the lab of Dr. L. Panasci, McGill University.

CURRENT SCHOLARLY AND PROFESSIONAL ACTIVITIES

2022 – present Consultant, Orrick, Herrington & Sutcliffe LLP
2021 - present Consultant, LeasonEllis LLP
2021 – present Chair, Triumvira Immunologics Scientific Advisory Board
2020 – present Member, NIH Immuno-Oncology Research (ZRG1-OTC-M-08) grant review panel
2020 – present Chair, Board of Directors, Chronic Pain Centre of Excellence
2022 – present Chair, CIHR Operating grant review panel Cancer Biology-Therapeutics 2
2017 – present Member, Board of Directors, McMaster Innovation Park
2017 – present Member, Council of Ontario Faculties of Medicine Research Committee
2015 – present Member, Association of Faculties of Medicine of Canada Research Council
2008 – present Steering Committee, Canadian Cancer Immunotherapy Consortium

PREVIOUS SCHOLARLY AND PROFESSIONAL ACTIVITIES

2021 Consultant, Durie Tangri LLP

2020 – 2022	Scientific Officer/Chair, CIHR Operating grant review panel Cancer Biology-Therapeutics 2
2020	External Review Committee member, Ste. Justine Hospital Research Centre (Montreal)
2020	Consultant, Wolf Greenfield LLP
2019 – 2020	Consultant, Wilmer Hale LLP
2019	Scientific Officer, CIHR Operating grant review panel Cancer Biology-Therapeutics
2019	Reviewer, European Commission Horizon 2020
2019	Reviewer, CCSRI Innovation and Innovation to Impact Panel I3
2017 – 2018	Reviewer, Terry Fox Foundation Research Institute New Investigator awards
2017	Scientific Officer, CIHR Operating grant review panel Immunology and Transplantation (Fall competition)
2017	Consultant/KOL, Realist Pharma
2017	Consultant/KOL, EMD-Serono
2017	Consultant/KOL, FACIT Immuno-Oncology asset evaluation
2016	Chair, Canadian Breast Cancer Foundation National Grant Competition – Panel I
2015 – 2021	Founding scientist and CSO, Triumvira Immunologics, Inc.
2015	Chair, Canadian Breast Cancer Foundation grant review – Panel I
2014 – 2016	Member, CBCF-Ontario Region Research and Health Care Advisory Committee (RHCAC)
2014	Consultant/KOL, Transgene Corporation (Lyon, France)
2014	Member, CIHR-III Human Immunology Steering Committee
2012	Review panel member, NIH special emphasis panel ZAI1-QV-I-C1
2012 – 2015	Member, Department of Pathology Finance Committee
2012	Consultant/KOL, Transgene Corporation (Lyon, France)
2011 – 2015	Member, Department of Pathology Executive Committee
2011 – 2014	Research Advisory Board, NIAID Modeling Immunity for Biodefense (MIB) program
2011	Chair, Terry Fox New Frontiers Program in Cancer review panel
2011	Member, CIHR Operating grant review panel – Cancer Biology-Therapeutics
2011	Member, 7 th Framework Research Programme (EU) grant review panel
2010 – 2015	Member, Hamilton Health Sciences Executive Committee on Research
2009	Research Advisory Board, Hopital Maisonneuve-Rosemont Research Centre (Montreal)
2009 – 2017	Member, Faculty of Health Sciences Research Council
2007 – 2009	Member, NCIC operating grants (Panel A) review panel
2007 – 2009	Member, Israel Cancer Research Fund operating grants review panel
2006 – 2009	Scientific Officer, CIHR Operating grant review panel Cancer Biology-Therapeutics
2006	Member, NCIC operating grants (Panel A) review panel
2006	Member (invited), NIH IDM-G90(S) Study Section
2005 – 2011	Member, Faculty of Health Sciences Finance Committee
2004 – 2009	Co-organizer, Southern Ontario Gene Therapy Conference at Langdon Hall
2004 – 2005	Member, CIHR operating grant review panel – Cancer Progression and Therapeutics
2004 – 2005	CIHR-III New Investigators' Forum 2005 organizing committee
2004	Review panel member (invited), CIHR University/Industry grants
2004	Member, Biochemistry Department Undergraduate Curriculum Renewal Committee
2003 – 2009	Member, Medical Sciences Graduate Program Curriculum Committee
2003	Review panel member, NIH P01 grant
2002 – 2009	Member, McMaster Biosafety Committee
2002 – 2004	Member, Israel Cancer Research Fund operating grants review panel
2002	Member (invited), CIHR operating grants review panel – Virology and Viral Pathogenesis
2002	Member, Lakehead University CIHR Development grant review panel
2001 – 2014	Coordinator, McMaster Infection and Immunity Program seminar
2001 – 2007	Review panel member, Cancer Research Society operating grants
2001 – 2004	Consultant, Altea Genomics, Inc.
2001 – 2004	Review panel member, CIHR doctoral awards "A"

JOURNAL REVIEWER (*ad-hoc*)

Bone Marrow Transplantation, Cancer Immunology Research, Cancer Research, Cancer Immunology Immunotherapy, European Journal of Immunology, Expert Opinion on Biological Therapy, Gene Therapy, Human Gene Therapy, Journal of Immunology, Journal of Leukocyte Biology, Journal of Virology, Molecular Cancer, Molecular Therapy family, Oncotarget, Viral Immunology, Virology

POST-GRADUATE AWARDS:

2013	Tier I Canada Research Chair in Translational Cancer Immunology
2012	John Bienenstock Chair in Molecular Medicine
2001	CIHR/Rx & D Health Research Foundation Career Scientist Award Canadian Foundation for Innovation New Opportunities Award Premier's Research Excellence Award
1996	Medical Research Council of Canada Post-Doctoral Fellowship. Travel Award for American Association of Cancer Research meeting in Washington, D.C.
1991	Fonds pour la Formation de Chercheurs et L'Aide à la Recherche (FCAR) Studentship

GRADUATE STUDENTS

Name	Position	Dates	Current Position
Gregory Harder	M.Sc. student	Jan. 2000 – Oct. 2002	Senior Marketing Manager, Baxter International
Samir Vattompadam	M.Sc. student	Sept. 2000 – Aug. 2002	Executive Director, Seattle Genetics
Laura-Lee Tannis	M.Sc. student	Sept. 2001 – Sept. 2003	Technician, U of T
Tony Yang	Ph.D. student	Sept. 2001 – June 2007	General Surgeon, Huntsville
James Millar	Ph.D. student	Sept. 2002 – Sept. 2009	Medical Science Liaison, Bristol Meyers Squibb
Natalie Grinshtein	Ph.D. student	Jan. 2003 – Oct. 2008	Manager, Research Programs, Fusion Pharmaceuticals
Jennifer Bassett	Ph.D. student	Sept. 2004 – Apr. 2011	Project Manager, Drug Development, Tetra Bio-Pharma
Korosh Kianizad	M.Sc. student	Sept. 2004 – Sept. 2006	Manager, Applied Immunology Programs, University of Toronto

Oula Akawi	M.Sc. student	Sept. 2005 – Aug. 2007	IB Chemistry Teacher, Modern Knowledge School
Dannie Bernard	Ph.D. student	Sept. 2005 – Sept. 2011	Scientist, SGS Life Sciences Services
Alina Lelic	Ph.D. student	Sept. 2006 – Sept. 2013	Application Scientist, Beckman-Coulter
Michael Ventresca	M.Sc. student	Sept. 2006 – Aug. 2008	Lawyer, Blake, Cassels & Graydon LLP
Kristy Jenkins	M.Sc. student	Sept. 2007 – Sept. 2009	unknown
Lesley Latham	M.Sc. student	Sept. 2007 – Sept. 2009	unknown
Bob McGray	Ph.D. student	Jan. 2008 – Dec. 2012	Assistant Professor, SUNY Buffalo
Heather VanSeggelen	Ph.D. student	Sept. 2008 – Dec. 2014	Medical Affairs Manager, Glaxo Smith Kline
Joanne Hammill	Ph.D. student	Sept. 2011 – Jan. 2018	Program Manager, Bramson lab
Daniela Tantalo	M.Sc. student	Sept. 2012- Aug. 2014	Senior Research Assistant, Peter MacCallum Cancer Centre
Arya Afsahi	M.Sc. student	Sept. 2013 – Sept. 2015	
	Ph.D. student	Jan. 2017 – present	
Ken Mwawasi	Ph.D. student	Sept. 2014 – April 2020	Medical Science Liaison, GSK Pharma
Ksenia Bezverbnaya	Ph.D. student	Sept. 2014 – August 2021	Analyst, Centre for Commercialization of Regenerative Medicines
Lisa Newhook	M.Sc. student	Sept. 2015 – Aug. 2017	Research Associate, Zymeworks
Vivian Lau	M.Sc. student	Sept. 2016 – Aug. 2018	PhD student, Oxford University
Rebecca Burchett	M.Sc. student	Sept. 2017 – Aug. 2019	
	Ph.D. student	Aug. 2019 - present	

Sarah Asbury	M.Sc. student	Sept. 2019 – Aug. 2021	Technician, St. Joseph's Healthcare Hamilton
Kaylyn Bacchiocchi	M.Sc. student	Sept. 2019 – Aug. 2021	Unknown
Nickolas Serniuck	M.Sc. student	Sept. 2020 – June 2022	
	Ph.D. student	July 2022 - present	
Christopher Silvestri	M.Sc. student	Sept. 2020 – June 2022	
	Ph.D. student	July 2022 - present	
Allyson Moore	M.Sc. student	Sept. 2021 – present	
Princeton Luong	M.Sc. student	Sept. 2021 – present	
Rex Huang	M.Sc. student	Sept. 2022 - present	
Claire Morris	M.Sc. student	Sept. 2022 - present	
Zoya Adeel	M.Sc. student	Sept. 2022 – present	
Arthur Srayeddin	M.Sc. student	Sept. 2022 - present	

Post-doctoral fellows

Name	Dates	Current Position
Wenzhong Zhou, MD	Oct. 2002 – Sept. 2003	unknown
Timothy Groves, PhD	Sept. 2003 – Aug. 2004	School principal
Galina Denisova	Dec. 2004 – Dec. 2010	deceased
Jonathan Finn, PhD	Apr. 2005 – Aug. 2007	VP Discovery Research, Codiak Sciences
Raluca Eftimie, PhD	July 2008 – Sept. 2011	Professor, University of Dundee
Stephanie Smith, PhD	Jan. 2009 – Oct. 2013	Freelance scientific writer
Jennifer Bassett, PhD	May 2011 – May 2012	Project Manager, Drug Development, Tetra Bio-Pharma
Farzad Habibagahi, PhD	July 2011 – Mar. 2013	Asst. Prof., University of Shiraz
Christopher Helsen, PhD	June 2012 – Aug. 2017	Executive Director, Triumvira

		Immunologics, Inc.
Rajanish Giri, PhD	July 2014 – Jan. 2015	Assoc. Prof., Indian Biotechnology Institute
Seung Mi Yoo, PhD	Sept. 2015 – July 2020	Research scientist, Triumvira Immunologics, Inc.
Kimberley Braz Gomez	Sept. 2017 – Dec. 2019	Medical Science Liaison, Merck
Carly Graham	Jan. 2021 – present	

RESEARCH SUPPORT (Bramson is PI unless otherwise indicated):

Period	Type	Agency	Project Title
04/2023 – 03/2025	Operating	Ontario Centre for Innovation	Deep interrogation of clinical grade TAC T cells through transcriptional profiling
09/2022 – 08/2024	Operating	Cancer Research Society	Programming universal T cells using covalent chemistry
01/2021 – 12/2023	Operating	Samuel Family Foundation	Development of engineered T cells for cancer immunotherapy
07/2020-06/2023	Operating	BioCanRx	GLP support for Human Immune Testing Suite (HITS)
04/2020 – 03/2023	Operating	BioCanRx	Phase II CYTOSHRINK trial (PI: Aly-Khan Lalani)
06/2020 – 05/2023	Operating	Triumvira Immunologics	Development and optimization of TAC receptors
09/2021 – 08/2022	Operating	NRC	Digital droplet PCR assay development for single cell analytics
05/2020-10/2022	Operating	Ontario Centres of Excellence	Development of next generation T cell antigen coupler (TAC) receptor
01/2019 – 12/2020	Operating	Samuel Family Foundation	Development of engineered T cells for cancer immunotherapy
10/2018-09/2020	Operating	Ontario Centres of Excellence	Developing an off-the-shelf TAC T cell

01/2018- 12/2019	Operating	BioCanRx	GLP support for Human Immune Testing Suite (HITS)
06/2017 – 05/2020	Operating	Genome Canada	Validation of TAC receptors for use against...tumors.
07/2017 – 03/2020	Operating	BioCanRx	A Phase 1/2...trial of oncolytic MG1...with Adenovirus vaccine...in patients with HPV positive...tumors (PI: Marcus Butler)
07/2017 – 06/2024	Operating	Terry Fox Foundation	Universal boosting system for oncolytic virus-based adoptive cell therapy
01/2017 – 03/2018	Operating	BioCanRx	Validation and manufacturing of anti-BCMA-TACT cells for the treatment of multiple myeloma (PI: Jean Sebastien Delisle)
01/2016 – 12/2017	Operating	BioCanRx	Establishment of GLP in Human Immune Testing Suite
03/2016 – 12/2018	Operating	Juravinski Foundation	Development of engineered T cells for lung cancer immunotherapy
01/2016 – 12/2018	Operating	Samuel Family Foundation	Development of engineered T cells for cancer immunotherapy
07/2015 – 06/2019	Operating	BioCanRx	A phase Ib study of MG1 MARABA/MAGE-A3 with adenovirus vaccine in combination... in patients with incurable...solid tumors (PI: Derek Jonker)
10/2015 – 12/2016	Operating	BioCanRx	Development of hollow fiber membrane bioreactors for automated GMP T cell manufacturing
10/2015 – 09/2016	Operating	Ontario Centres of Excellence	Optimization of T-Cell chimeric receptor platform for treatment of cancer
2015	Equipment	ORI	Infrastructure for developing novel cell therapies and manufacturing methods
2015	Equipment	CFI	Infrastructure for developing novel cell therapies and manufacturing

			methods
07/2015 – 07/2017	Operating	Adiga Life Sciences	Immune analysis of RES-010
06/2015 – 06/2020	Operating	Triumvira Immunologics	Development and optimization of TAC receptors
07/2014 – 06/2017	Operating	Canadian Breast Cancer Foundation	CAR-T cells for breast cancer therapy
04/2014 – 03/2018	Operating	Ontario Institute for Cancer Resesarch	Immune monitoring of patients undergoing cancer immunotherapy
01/2014 – 12/2015	Operating	Samuel Family Foundation	Development of engineered T cells for cancer immunotherapy
11/2013 – 10/2014	Operating	Pan-Canadian Influenza Research Network	Analysis of T cell immunity in patients from SOS cohort
03/2013 – 02/2014	KT	Canadian Institutes for Health Research	Canadian Cancer Immunotherapy Consortium Symposium 2013
07/2012 – 06/2017	Operating	Terry Fox Foundation	Dual-specific T cells for cancer immunotherapy
09/2011 – 0/2014	Network catalyst	Canadian Institutes for Health Research	Canadian Human Immunology Network (PI: Madrenas; 6 co-PIs)
06/2011 – 08/2012	Operating	Merck	Immune biomarkers of responsiveness to Zostavax
04/2011 – 03/2014	Operating	Candian Institutes for Health Research	CAR-engineered T cells for cancer immunotherapy
10/2010 – 09/2013	Operating	Candian Institutes for Health Research	Identifying biomarkers that predict influenza infection in the nursing home aged (PI: Loeb; 6 co-PIs)
01/2009 – 03/2014	Operating	Ontario Institute for Cancer Resesarch	Immune monitoring of patients undergoing cancer immunotherapy
11/2008	Equipment	Ontario Institute for Cancer Research	Ontario Regional Biotherapeutics Program
10/2008 – 09/2013	Group	Canadian Institutes for Health Research	CIHR Team in Mucosal Innate Immunity (PI: J. Gaudie; 11 co-PIs)

07/2008 – 06/2011	Operating	Ontario Cancer Research Network	Optimizing targeted cancer immunotherapy by segregating antitumor function from autoimmune pathology (Co-PI: Y. Wan)
01/2008	Equipment	Ontario Institute for Cancer Research	Ontario Regional Biotherapeutics Program
01/2008 – 12/2008	Operating	Ontario Institute for Cancer Research	Ontario Regional Biotherapeutics Program
07/2007 – 06/2010	Group	National Cancer Institute of Canada	Cancer Immunotherapy Program (PI: J. Bramson; 17 co-PIs)
04/2007 – 03/2008	Operating	Canadian Breast Cancer Research Alliance	Developing vaccines based on antibody mimotopes for breast cancer treatment
10/2006 – 09/2011	Operating (2 nd renewal)	Canadian Institutes for Health Research	The immunobiology of recombinant adenovirus vaccines
06/2006-05/2007	Operating	Congressionally Directed Medical Research Program	Identification of Novel Mimotopes for the Development of Multivalent Breast Cancer Vaccines
05/2005 – 04/2008	Operating	Ontario Cancer Research Network	Interactions between conventional cancer therapy and gene-based cancer vaccines
09/2004 – 09/2009	Operating	National Institutes of Health	Population genomics of West Nile virus infection (PI: Mark Loeb)
10/2004 – 09/2007	Operating (1 st renewal)	Canadian Institutes for Health Research	Adenovirus vectors for gene therapy of muscle (Co-PI: Robin Parks)
09/2003 – 08/2005	Operating	Cancer Research Society	Influence of lymphopenia on genetic vaccination against cancer
09/2003 – 08/2006	Operating (1 st renewal)	Canadian Institutes for Health Research	The immunobiology of genetic vaccines
07/2003 – 06/2007	Operating	Canadian Breast Cancer Research Alliance	Immune monitoring of patients following genetic vaccination with an adenovirus vector expressing rat HER-2 (Co-PI: Rafick Sekaly)
05/2003 –	Research	Altea Therapeutics, Inc.	Transdermal immunization: Evaluation of immunogenicity and

04/2004	Contract		efficacy of genetic vaccines delivered following microporation in mice
2003	Multi-user equipment	Canadian Institutes for Health Research	Maintenance Support And Cytometer Purchase For Mc Master Flow Cytometer Facility (PI: Denis Snider)
04/2003 – 03/2004	Research Contract	Microscience, Ltd.	Salmonella mediated delivery of cancer antigens
10/2002 – 09/2007	Group	Canadian Institutes for Health Research	CIHR Group in Mucosal Immunity (Co-PIs: Collins, Jordana, Gauldie, Rosenthal and Xing)
05/2002-04/2003	Operating	Canadian Institutes for Health Research	Delivery of genetic vaccines through Microporated Skin.
05/2002 – 04/2003	Research Contract	Altea Genomics, Inc.	Delivery of DNA using plasmids and viral vectors through Microporated Skin
04/2002 – 03/2003	Operating	CANVAC	Development of novel delivery systems for adenoviral vectors.
07/2001 – 06/2006	Salary	Rx & D Health Research Foundation	Understanding the communication between gene-modified cells...
10/2001 – 09/2004	Operating	Canadian Institutes for Health Research	Adenovirus vectors for gene therapy of muscle (Co-PI: Robin Parks)
01/2001 – 12/2005	Operating	Premier's Research Excellence Award	Understanding the communication between virus-infected cells and the immune system...
10/2000 – 09/2003	Operating	Canadian Institutes for Health Research	Influence of Type I Interferons on T cell Activation In Vivo.
05/2001 – 04/2002	Research Contract	Altea Genomics, Inc.	Delivery of DNA using plasmids and viral vectors through Microporated Skin
04/2001 – 09/2001	Operating	Canadian Institutes for Health Research	Adenovirus vectors for gene therapy of muscle (Co-PI: Robin Parks)
2001	Equipment	Ontario Innovation Trust	Understanding the communication between virus-infected cells and

			the immune system...
2001	Equipment	Canadian Foundation for Innovation	Understanding the communication between virus-infected cells and the immune system...

PEER-REVIEWED RESEARCH PAPERS: (underlined denotes lab members)

136. Afsahi, A., Silvestri, C.M., Moore, A.E., Graham, C.F., Bacchiochi, K., Baker, C.L., Beug, S., Korneluk, R., LaCasse, E., and **Bramson, J.L.** The SMAC mimetic, LCL161, provides costimulatory signals to engineered T cells through a CD30 and FAIM3-dependent mechanism that is opposed by Fas signaling. Submitted to Frontier Immunology.
135. Afsahi, A., Baker, C.L., Burchett, R., and **Bramson, J.L.** Constitutive expression of IL-27 diminishes proinflammatory cytokine production without impairing effector function of engineered T cells. Cytotherapy, in revision.
134. Bezverbnaya, K., Hammill, J.A., Cummings, D., Bojovic, B., Groisman, B., Baker, C.L., Aarts, C.A., Helsen, C.W., and **Bramson, J.L.** Development of a fully humanized BCMA-specific T cell antigen coupler (TAC) receptor. Cytotherapy, in press.
133. Breznik, J.A., Huynh, A., Zhang, A., Bilaver, L., Bhakta, H., Stacey, H.D., Ang J.C., **Bramson, J.L.**, Nazy, I., Miller, M.S., Denburg, J., Costa, A.P., and Bowdish, D.M.E.; other members of the COVID-in-LTC Investigator Group. Cytomegalovirus Seropositivity in Older Adults Changes the T Cell Repertoire but Does Not Prevent Antibody or Cellular Responses to SARS-CoV-2 Vaccination. J. Immunol. 209:1892-1905, 2022.
132. Broadfield, L.A., Saigal, A., Szamosi, J.C., Hammill, J.A., Bezverbnaya, K., Wang, D., Gautam, J., Tsakiridis, E., Di Pastena, F., McNicol, J., Wu, J., Syed, S., Lally, J.S.V., Raphenya, A.R., Blouin, M.J., Pollak, M., Sacconi, M., Blandino, G., McArthur, A.G., Schertzer, J.D., Surette, M., Collins, S.M., **Bramson, J.L.**, Muti, P., Tsakiridis, T., and Steinberg, G.R. Metformin-induced reductions in tumor growth involves modulation of the gut microbiome. Mol. Metabol. 61:101498, 2022
131. Begum, H., Swaminath, A., Fahim, C., **Bramson, J.**, Naqvi, A., Shargall, Y., Finley, C., Hanna, W., and Agzarian, J. The histologic effects of neoadjuvant stereotactic body radiation therapy (SBRT) followed by pulmonary metastasectomy— rationale and protocol design for the Post SBRT Pulmonary Metastasectomy (PSPM) trial. Transl. Cancer Res. 11(4):918-927, 2022
130. Kennedy, A.E., Cook, L., Breznik, J.A., Cowbrough, B., Wallace, J.G., Huynh, A., Smith, J.W., Son, K., Stacey, H., Ang, J., McGeer, A., Coleman, B.L., Larché, M., Larché, M., Hambly, N., Nair, P., Ask, K., Miller, M.S., **Bramson, J.**, Levings, M.K., Nazy, I., Svenningsen, S., Mukherjee, M., Bowdish, D.M.E. Lasting Changes to Circulating Leukocytes in People with Mild SARS-CoV-2 Infections. Viruses. 2021 Nov 8;13(11):2239.
129. Yoo, S.M., Lau, V., Hammill, J.A., Aarts, C., Bojovic, B., Dvorkin-Gheva, A., Ghosh, R., and **Bramson, J.** A novel operating mode of cell culture in hollow fibre membrane bioreactors changes T cell programming and enhances the potency of T cell therapies. Oncoimmunology 10:1995168, 2021.
128. Bezverbnaya, K., Moogk, D., Cummings, D., Baker, C.L., Aarts, C., Denisova, G., Sun, M., Foley, S.R. and **Bramson, J.L.** Evolution of a BCMA-specific T cell antigen coupler (TAC) receptor for multiple myeloma. Cytotherapy 2021 Jun 30:S1465-3249(21)00687-3. doi: 10.1016/j.jcyt.2021.05.007..
127. Chu, D.K., Baumert, J.L., Taylor, S.L., Nordlee, J.A., Nham, T., **Bramson, J.**, La Vieille, S., Abbott, M.A., Spill, P., Marrin, A., Jordana, M., and Waserman, S. Peanut allergen reaction thresholds during controlled food challenges in 2 Canadian randomized studies (Canada-ARM1 and PISCES). J Allergy Clin Immunol Pract. 16:S2213-2198(21)00189-6, 2021.
126. Workenhe, S.T., Nguyen, A., Bakhshinyan, D., Wei, J., Hare, D.N., MacNeill, K.L., Wan, Y., Oberst, A., **Bramson, J.L.**, Nasir, J.A., Vito, A., El-Sayes, N., Singh, S.K., McArthur, A.G., and Mossman, K.L. De novo

necroptosis creates an inflammatory environment mediating tumor susceptibility to immune checkpoint inhibitors. *Commun. Biol.* 3: 645, 2020.

125. Vora, P., Venugopal, C., Salim, S.K., Tatari, N., Bakhshinyan, D., Singh, M., Seyfrid, M., Upreti, D., Rentas, S., Wong, N., Williams, R., Qazi, M.A., Chokshi, C., Ding, A., Subapanditha, M., Savage, N., Mahendram, S., Ford, E., Adile, A.A., McKenna D., McFarlane, N., Huynh, V., Wylie, R.G., Pan, J., **Bramson, J.**, Hope, K., Moffat, J., Singh, S. The rational development of CD133-targeting immunotherapies for glioblastoma. *Cell Stem Cell* 26: 832-844.e6, 2020.

124. Hammill, J.A., Kwiecien, J.M., Dvorkin-Gheva, A., Lau, V., Bezverbnaya, K., Aarts, C., Heslen, C.W., Denisova, G.F., Derocher, H., Milne, K., Nelson, B.H. and **Bramson, J.L.** A cross-reactive small protein binding domain provides a model to study off-tumor CAR-T cell toxicity. *Mol. Ther. Oncol.* 17: 278-292, 2020.

123. Walsh, S.R., Simovic, B., Chen, L., Bastin, D., Nguyen, A., Stephenson, K., Mandur, T.S., **Bramson, J.L.**, Lichty, B.D., and Wan, Y. Endogenous T cells prevent tumor immune escape following adoptive T cell therapy. *J. Clin. Invest.* 129: 5400 – 5410, 2019.

122. Dahmani, A., Janelle, V., Carli, C., Richaud, M., Lamarch, C., Khalili, M., Goupil, Bezverbnaya, K., **Bramson, J.**, and Delisle, J.S. TGF- β programs central-memory differentiation in ex vivo stimulated human T cells. *Cancer Imm. Res.* 7: 1426-1439, 2019.

121. Walsh, S.R., Bastin, D., Chen, L., Nguyen, A., Storbeck, C.J., Lefebvre, C., Stojdl, D., **Bramson, J.L.**, Bell, J.C., and Wan Y. Type I IFN blockade uncouples immunotherapy-induced antitumor immunity and autoimmune toxicity. *J. Clin. Invest.* 129: 518 – 530, 2019.

120. Pol, J., Acuna, S., Yadollahi, B., Tang, N., Stephenson, K., Atherton, M.J., Hanwell, D., El-Warrak, A., Goldstein, A., Moloo, B., Turner, P., Lopez, R., LaFrance, S., Eveleigh, C., Denisova, G., Parsons, R., Millar, J., Stoll, G., Martin, C., Pomoransky, J., Breitbach, C., **Bramson, J.**, Bell, J., Wan, Y., Stojdl, D., McCart, A., and Lichty, B. Preclinical evaluation of a MAGE-A3 vaccination utilizing the oncolytic Maraba virus currently in first-in-human trials. *OncoImmunology* 8: e1512329, 2018.

119. Nguyen, A., Ho, L., Workenhe, S.T., Chen, L., Samson, J., Walsh, S.R., Pol, J., **Bramson, J.L.**, and Wan, Y. HDACi delivery reprograms tumor-infiltrating myeloid cells to eliminate antigen-loss variants. *Cell Rep.* 24: 642 - 654, 2018.

118. Poznanski, S., Nham, T., Chew, M., Lee, A., Hammill, J., Fan, I., Butcher, M., **Bramson, J.**, Lee, D., Hirte, H., and Ashkar, A. Expanded CD56bright/brightCD16+ NK cells from ovarian cancer patients are highly cytotoxic against autologous tumor in a patient-derived xenograft model. *Cancer Immunol. Res.* 6: 1174 - 1185, 2018.

117. Helsen, C.W., Hammill, J.A., Mwawasi, K.A., Lau, V.W.C., Afsahi, A., Bezverbnaya, K., Newhook, L., Hayes, D.L., Aarts, C., Bojovic, B., Denisova, G.F., Kwiecien, J.M., Brain, I., Derocher, H., Milne, K., Nelson, B.H. and **Bramson, J.L.** The chimeric TAC receptor co-opts the T cell receptor yielding robust anti-tumor activity without toxicity. *Nat. Comm.* 9: 3049, 2018.

116. Verschoor, C.P., Lelic, A., Parsons, R., Eveleigh, C., **Bramson, J.L.**, Johnstone J, Loeb MB, Bowdish DME. Serum C-Reactive Protein and Congestive Heart Failure as Significant Predictors of Herpes Zoster Vaccine Response in Elderly Nursing Home Residents. *J Infect Dis.* 216:191-197, 2017.

115. Bassey-Archibong, B.I., Rayner, L.G.A., Hercules, S.M., Aarts, C.W., Dvorkin-Gheva, A., **Bramson, J.L.**, Hassell, J.A. and Daniel, J.M. Kaiso depletion attenuates the growth and survival of triple negative breast cancer cells. *Cell Death Dis.* 8:e2689, 2017.

114. Johnstone, J., Parsons, R., Botelho, F., Millar, J., McNeil, S., Fulop, T., McElhaney, J., Andrew, M.K., Walter, S.D., Devereaux, P.J., Malek, M., Brinkman, R., **Bramson, J.**, and Loeb, M. T-cell Phenotypes Predictive of Frailty and Mortality in Elderly Nursing Home Residents. *J. Am. Geriatr. Soc.* 65: 153 – 159, 2017.

113. Jeyanathan, M., Damjanovic, D., Yao, Y., **Bramson, J.**, Smail, F., and Xing Z. Induction of an immune protective T cell repertoire with diverse genetic coverage by a novel viral-vectored tuberculosis vaccine in man. *J. Infect. Dis.* 214: 1996 – 2005, 2016.

112. Lelic, A., Verschoor, C.P., Lau, V.W.C., Parsons, R., Eveleigh, C., Bowdish, D.M.E., **Bramson, J.L.** and Loeb, M. Immunogenicity of Varicella Zoster Vaccine and Immunologic Predictors of Response in a Cohort of Elderly Nursing Home Residents. *J. Infect. Dis.* 214: 1905 – 1910, 2016.
111. Lhoták, Š., Gyulay, G., Cutz, J.C., Al-Hashimi, A., Trigatti, B.L., Richards, C.D., Igdoura, S.A., Steinberg, G.R., **Bramson, J.**, Ask, K., and Austin, R.C. Characterization of Proliferating Lesion-Resident Cells During All Stages of Atherosclerotic Growth. *J. Am. Heart Assoc.* 5: e003945, 2016.
110. Provine, N.M., Larocca, R.A., Penalzoza-MacMaster, P., Badamchi-Zadeh, A., Borducchi, E.N., Yates, K.B., Abbink, P., Kirilova, M., Ng'ang'a D., **Bramson, J.**, Haining, W.N., Barouch, D.H. Immediate dysfunction of vaccine-elicited CD8+ T cells primed in the absence of CD4+ T cells. *J. Immunol.* 197: 1809 – 1822, 2016.
109. Bridle, B.W., Nguyen, A., Salem, O., Zhang, L., Koshy, S., Clouthier, D., Chen, L., Pol, J., Swift, S., Bowdish, D.M.E., Lichty, B.D., **Bramson, J.L.**, and Wan, Y. Privileged Antigen Presentation in Splenic B Cell Follicles Maximizes T Cell Responses in Prime-Boost Vaccination. *J. Immunol.* 106: 4587 – 45955, 2016.
108. Ananth, A.A., Tai, L.H., Lansdell, C., Alkayyal, A.A., Baxter, K.E., Angka, L., Zhang, J., Tanese de Souza, C., Stephenson, K.B., Parato, K., **Bramson, J.L.**, Bell, J.C., Lichty, B.D., Auer, R.C. Surgical Stress Abrogates Pre-Existing Protective T Cell Mediated Anti-Tumor Immunity Leading to Postoperative Cancer Recurrence. *PLoS One* 19:e0155947, 2016.
107. Anipindi, V.C., Bagri, P., Roth, K., Dizzell, S.E., Nguyen, P.V., Shaler, C.R., Chu, D.K., Jiménez-Saiz, R., Liang, H., Swift, S., Nazli, A., Kafka, J.K., **Bramson, J.**, Xing, Z., Jordana, M., Wan, Y., Snider, D.P., Stampfli, M.R., Kaushic, C. Estradiol Enhances CD4+ T-Cell Anti-Viral Immunity by Priming Vaginal DCs to Induce Th17 Responses via an IL-1-Dependent Pathway. *PLoS Pathog.* 12:e1005589, 2016.
106. Hammill, J.A., VanSeggelen, H., Helsen, C.W., Denisova, G.F., Eveleigh, C., Tantalo, D., Bassett, J.D., and **Bramson, J.L.** Designed ankyrin repeat proteins are effective targeting elements for chimeric antigen receptors. *J. Immunother. Cancer.* 3: 55, 2015.
105. VanSeggelen, H., Tantalo, D., Afsahi, A., Hammill, J., and **Bramson, J.L.** Chimeric antigen receptor-engineered T cells as oncolytic virus carriers. *Mol. Ther. Oncol.* 2: 15014, 2015.
104. VanSeggelen, H., Hammill, J.A., Tantalo, D., Dvorkin-Gheva, A., Kwiecien, J.M., Denisova, G., Rabinovich, B., and **Bramson, J.L.** Systemic toxicities associated with chimeric antigen receptor-engineered T cells that target NKG2D ligands. *Mol. Ther.* 23: 1600 – 1611, 2015.
103. Verschoor, C.P., Loukov, D., Naidoo, A., Johnstone, J., Millar, J., Lelic, A., Novakowski, K.E., Dorrington, M.G., Loeb, M., **Bramson, J.L.**, and Bowdish, D.M.E. Circulating TNF and mitochondrial DNA are major determinants of neutrophil phenotype in the advanced-age, frail elderly. *Mol. Immunol.* 65: 148 – 156, 2015.
102. Karimi, K., Chan, J., Boudreau, J., Bassett, J., Chew, M., Reid, S., **Bramson, J.**, Wan, Y., Ashkar, A. Type I IFN signaling on dendritic cells are required for NK cell-mediated anti-tumor immunity. *Innate Immun.* 21: 626 – 634, 2015.
101. Verschoor, C.P., Naidoo, A., Wallace, J.G., Johnstone, J., Loeb, M., **Bramson, J.L.**, Bowdish, D.M. Circulating muramyl dipeptide is negatively associated with interleukin-10 in the frail elderly. *Inflammation* 38: 272 – 277, 2015.
100. Verschoor, C.P., Johnstone, J., Loeb, M., **Bramson, J.L.**, and Bowdish, D. Anti-pneumococcal deficits of monocyte-derived macrophages from the advanced-age, frail elderly and related impairments in PI3K-AKT signaling. *Hum. Immunol.* 75: 1192 – 1196, 2014.
99. Alqahtani, A., Heesom, K., **Bramson, J.**, Curiel, D., Ugai, H., and Matthews, D. Analysis of purified wild type and mutant adenovirus particles by SILAC based quantitative proteomics. *J. Gen. Virol.* 95: 2504 – 2511, 2014.
98. Chu, D., Zahraa, M.-A., Walker, T., Goncharova, S., Llop-Guevara, A., Barra, N., Gillgrass, A., Jimenez-Saiz, R., Ashkar, A., **Bramson, J.**, Humbles, A., Kolbeck, R., and Jordana, M. T helper cell-intrinsic IL-4 drives intestinal Th2 priming to oral peanut antigen, under the control of OX40L and independent of innate-like lymphocytes. *Mucosal Immunol.* 7: 1395 – 1404, 2014.

97. Johnstone, J., Parsons, R., Botelho, F., Millar, J., Fulop, T., McElhaney, J., Andrew, M., Walter, S., Devereaux, P.J., Malekesmaeili, M., Brinkman, R., Mahony, J., **Bramson, J.**, and Loeb, M. Immune biomarkers predictive of respiratory viral infection in elderly nursing home residents. *PLoS ONE* 9: e108481, 2014.
96. Verschoor, C.P., Johnstone, J., Millar, J., Parsons, R., Lelic, A., Loeb, M., **Bramson, J.L.** and Bowdish, D.M.E. Alterations to the frequency and function of peripheral blood monocytes and associations with chronic disease in the advanced-age, frail elderly. *PLoS ONE* 9:e104522, 2014.
95. Chabierski, S., Barzon, L., Papa, A., Niedrig, M., **Bramson, J.L.**, Richner, J.M, Palù, G., Diamond, M.S., and Ulbert, S. Distinguishing West Nile virus infection using a recombinant envelope protein with mutations in the conserved fusion-loop. *BMC Infect. Dis.* 14: 246, 2014.
94. Johnstone, J., Millar, J., Lelic, A., Verschoor, C., Walter, S., Devereaux, P., **Bramson, J.**, and Loeb, M. Immunosenescence in the Nursing Home Elderly. *BMC Geriatrics* 14: 50, 2014.
93. Pol, J., Zhang, L., Bridle, B., Resseui, J., Stephenson, K., Hanson, S., Kazhdan, N., **Bramson, J.**, Stojdl, D., Wan, Y. and Lichty, B. Maraba virus as a novel oncolytic vaccine vector. *Mol. Ther.* 22: 420 – 429, 2014.
92. McGray, A.J.R., Hallett, R., Bernard, D., Swift, S., Zhu, Z., Teodorescu, F., VanSeggelen, H., Hassell, J.A., Wan, Y., Hurwitz, A.A., and **Bramson, J.L.** Immunotherapy-induced CD8+ T cells instigate immune suppression in the tumor. *Mol. Ther.* 22: 206 – 218, 2014.
91. Bridle, B., Clouthier, D., Zhang, L., Pol, J., Chen, L., Lichty, B., **Bramson, J.**, and Wan, Y. Oncolytic Vesicular Stomatitis Virus Vaccines Amplify Primary CD8+ T Cell Responses and Improve CD8+ T Cell Functionality. *Oncoimmunology* 2: e2601, 2013.
90. Smaill, F., Smieja, M., Medina, M.F., Jeyanathan, M., Thantrige-Don, N., Zganiacz, A., Yin, C., Heriazon, A., Puri, L., Hamid, J., Xie, F., Foley, S.R., **Bramson, J.**, Gaudie, J, and Xing, Z. Potent T cell immunogenicity of a novel human type 5 adenovirus-based tuberculosis vaccine in humans despite pre-existing anti-adenovirus immunity. *Sci. Trans. Med.* 5: 205ra134, 2013.
89. Zhang, L., Bridle, B.W., Chen, L., Pol, J., Spaner, D., Boudreau, J.E., Rosen, A., Bassett, J.D., Lichty, B.D., **Bramson, J.L.**, and Wan, Y. Delivery of viral vectored vaccines by B cells represents a novel strategy to accelerate CD8+ T cell recall responses. *Blood* 121: 2432 – 2439, 2013.
88. Verschoor, C.P., Millar, J., Habibagahi, M., Lelic, A., Johnstone, J., Loeb, M., **Bramson, J.L.**, and Bowdish, D. Blood CD33(+)HLA-DR(-) myeloid-derived suppressor cells are increased with age and a history of cancer. *J. Leuk. Biol.* 93: 633 – 637, 2013.
87. Bridle, B.W., Chen, L., Lemay, C.G., Diallo, J.-S., Pol, J., Nguyen, A., Capretta, A., He, R., **Bramson, J.L.**, Bell, J.C., Lichty, B.D., and Wan, Y. HDAC inhibition suppresses primary immune responses, enhances secondary immune responses and abrogates autoimmunity during tumor immunotherapy. *Mol. Ther.* 21: 887 - 894, 2013
86. Aghaeepour, N. (97 co-authors, including **J.L. Bramson**) and Scheuermann, R.H. Critical assessment of automated flow cytometry data analysis techniques. *Nat. Methods* 10: 228 – 238, 2013.
85. Lelic, A., Verschoor, C.P., Ventresca, M., Parsons, R., Eveleigh, C., Bowdish, D., Betts, M.R., Loeb, M.B. and **Bramson, J.L.** The polyfunctionality of memory CD8+ T cells elicited by chronic and acute virus infections is not influenced by age. *PLoS Pathogens* 8: e1003076, 2012.
84. Kulkarni, Y.M., Chambers, E., McGray, A.J.R., Ware, J.S., **Bramson, J.L.**, and Klinke, D.J. B16 melanoma inhibits cellular response to Interleukin-12 via multiple mechanisms including paracrine action of Wnt-inducible Signaling Protein-1. *Integrative Biology* 4: 925 – 936, 2012.
83. Chenoweth, M.J., Mian, M.F., Barra, N.G., Alain, T., Sonenberg, N., **Bramson, J.**, Lichty, B.D., Ma, A. and Ashkar, A.A. Interleukin-15 can signal via IL-15R α , JNK and NF- κ B to drive RANTES production by myeloid cells. *J. Immunol.* 188: 4149 – 4157, 2012.
82. McGray, A.J., Bernard, D., Kelly, R., Hallett, R., Jha, M., Gregory, C., Bassett, J.D., Hassell, J.A., Wan, Y., and **Bramson, J.L.** Combined vaccination and immunostimulatory antibodies provides durable cure of murine

melanoma and reveals an immune signature associated with good outcome in human melanoma patients. *OncoImmunology* 1: 4, 1 – 13, 2012.

81. Bassett, J.D., Swift, S.L., VanSeggelen, H., Hammill, J., McGray, A.J., Eveleigh, C., and **Bramson, J.L.** Combined mTOR inhibition and OX40 agonism enhances CD8+ T cell memory and protective immunity produced by recombinant adenovirus vaccines. *Mol. Ther.* 20: 860 – 869, 2012.
80. Botelho, F.M., Bauer, C.M., Finch, D., Nikota, J.K., Zavitz, C.C., Kelly, A., Lambert, K.N., Piper, S., Foster, M.L., Goldring, J.J., Wedzicha, J.A., Bassett, J., **Bramson, J.**, Iwakura, Y., Sleeman, M., Kolbeck, R., Coyle, A.J., Humbles, A.A., and Stampfli, M.R. Cigarette smoke elicits airway inflammation and disease exacerbation via IL-1a/IL-1R1 crosstalk between hematopoietic and airway structural cells. *PLoS ONE* 6: e28457, 2011.
79. Lanteri, M.C., Kaidarova, Z., Peterson, T., Cate, S., Custer, B., Wu, S., Agapova, M., Law, J.P., Bielawny, T., Plummer, F., Tobler, L.H., Loeb, M., Busch, M.P., **Bramson, J.**, Luo, M. and Norris, P.J. Association between HLA class I and class II alleles and the outcome of West Nile virus infection: An exploratory study. *PLoS ONE* 6: e22948, 2011.
78. Loeb, M., Eskandarian, S., Rupp, M., Fishman, N., Gasnik, L., Patterson, J., **Bramson, J.**, Hudson, T.J., and Lemire M. Genetic variants and susceptibility to neurological complications following West Nile virus infection. *J. Infect. Dis.* 204: 1031 – 1037, 2011..
77. Denisova, G., Jenkins, K.M., Eveleigh, C., **Bramson, J.L.** Novel method for differentiation between Trastuzumab and host adaptive response. *Mol. Immunol.* 48: 1882 – 1885, 2011.
76. Felizardo, T.C., Wang, J.C.M., McGray, A.J.R., Eveleigh, C., Spaner, D.E., **Bramson, J.L.**, *JA Medin.* Differential immune responses mediated by adenovirus- and lentivirus- transduced DCs in a HER-2/neu over-expressing tumor model. *Gene Ther.* 18: 986 – 995, 2011.
75. Eftimie, R., Dushoff, J., **Bramson, J.L.**, and Earn, D.J.D. Multi-stability and multi-instability phenomena in a mathematical model of tumor-immune-virus interactions. *Bull. Math. Biol.* 73: 2932 - 2961, 2011.
74. Boudreau, J.E., Stephenson, K.B., Wan, F., Ashkar, A.A., Mossman, K.L., Lenz, L.L., **Bramson, J.L.**, Lichty, B.D., Wan, Y. VSV-infected dendritic cells are potent activators of tumoricidal NK cells in tumor-bearing animals. *Cancer Res.* 71: 2497 – 2506, 2011.
73. Wang, A.Y., Crome, S., Jenkins, K., Medin, J., **Bramson, J.** and Levings, M.K. Adenoviral transduced dendritic cells promote the conversion of T regulatory cells into Th17 cells. *Cancer Immunol. Immunother.* 60: 381 – 388, 2011.
72. Bassett, J., Yang, T.C., Bernard, D., Millar, J.B., Swift, S., Parsons, R., Eveleigh, C., Finn, J.D., Wan, Y., and **Bramson, J.L.** Expansion and maintenance of the CD8+ T cell population produced by recombinant adenovirus relies upon the combined activity of hematopoietic and non-hematopoietic antigen presenting cells. *Blood* 117: 1146 – 1155, 2011.
71. McMurtrey, C.P., Piazza, P., Lelic, A., Cook, R.L., Hess, R., Yablonsky, E., Borowski, L., Loeb, M.B., **Bramson, J.L.**, Hildebrand, W.H., and Rinaldo, C.R. Surface phenotype and functionality of WNV specific T cells differ with age and disease severity. *PLoS ONE* 5: e15343, 2010.
70. Romieu-Mourez, R., François, M., Abate, A., Boivin, M.-N., Birman, E., Bailey, D., **Bramson, J.**, Forner, K., Young, Y.-K., Medin, J.A., and Galipeau, J. Mesenchymal stromal cells expressing ErbB-2/neu elicit protective anti-breast tumor immunity in vivo, which is paradoxically suppressed by IFN- γ and TNF- α priming. *Cancer Res.* 70: 7742 – 7747, 2010.
69. Larsen, M.V., Lelic, A., Parsons, R., Nielsen, M., Hoof, I., Lamberth, K., Loeb, M.B., Buus, S., **Bramson, J.L.**, Lund, O. Identification of CD8+ T Cell Epitopes in the West Nile Virus Polyprotein by Reverse-Immunology Using NetCTL. *PLoS ONE* 5: e12697, 2010.
68. Bridle, B., Stephenson, K.B., Boudreau, J.E., Koshy, S., Kazdhan, N., Brunellière, J., **Bramson, J.L.**, Lichty, B.D., and Wan, Y. Potentiating cancer immunotherapy using an oncolytic virus. *Mol. Ther.* 18: 1430 – 1439, 2010.

67. Eftimie, R., **Bramson, J.L.**, and Earn, D.J. Modeling of anti-tumor Th1 and Th2 immunity in the rejection of melanoma. *J. Theor. Biol.* 265: 467 – 480, 2010.
66. Bridle, B.W., Jiang, S., Chang, R., Lichty, B.D., **Bramson, J.L.**, and Wan, Y. Immunotherapy can reject intracranial tumor cells without damaging the brain despite sharing the target antigen. *J. Immunol.* 184: 4269 – 4275, 2010.
65. Bernard, D., Ventresca, M., Eveleigh, C., Wan, Y., and **Bramson, J.L.** Manipulation of tumor antigen differentially impacts the development of effector CD4+ T cell and helper CD4+ T cell responses. *Mol. Ther.* 18: 1224 – 1232, 2010
64. Finn, J.F., Millar, J.B., Bassett, J., Grinshtein, N., Eveleigh, C., Parks, R.J., and **Bramson, J.L.** Persistent transgene expression is required for maximal expansion and maintenance of the CD8+ T cell population generated by recombinant adenovirus. *J. Virol.* 83: 12027 - 12036, 2009.
63. Francois, M., Romieu, R., Martineau, S. Boivin, M, **Bramson, J.**, and Galipeau, J. Mesenchymal stromal cells cross-present soluble exogenous antigens as part of their antigen presenting cell properties. *Blood*: 2632-2638, 2009.
62. Bridle, B.W., Boudreau, J.E., Lichty, B.D., Brunellière, J., Stephenson, K., **Bramson, J.L.** and Wan, Y. Vesicular stomatitis virus as a novel cancer vaccine vector to prime anti-tumor immunity amenable to rapid boosting with adenovirus. *Mol. Ther.* 17: 1814-1821, 2009.
61. Boudreau, J.E., Bridle, B.W., Stephenson, K.B., Jenkins, K.M., Brunellière, J., **Bramson, J.L.**, Lichty, B.D. and Wan, Y. Transduction of dendritic cells with recombinant vesicular stomatitis viral vector enhances their ability to activate both innate and adaptive antitumor immunity. *Mol. Ther.* 17:1465-72, 2009.
60. Denisova, G., Denisov, D., Eveleigh, C., Weissgram, M., Beck, J., Foley, S.R, and **Bramson, J.L.** Characterizing complex polysera produced by antigen-specific immunization through the use of affinity-selected mimotopes. *PLoS ONE* 4(4): e5309, 2009.
59. Grinshtein, N., Bridle, B.W., Wan, Y., and **Bramson, J.L.** Neo-adjuvant vaccination provides superior protection against tumor relapse following surgery compared to adjuvant vaccination. *Cancer Res.* 69: 3979 - 3985, 2009.
58. Zhang, S., Bernard, D., Khan, W., Kaplan, M.H., **Bramson, J.L.** and Wan, Y. CD4+ T cell-mediated anti-tumor immunity can be uncoupled from auto-immunity via the STAT-4/STAT-6 signaling axis. *Eur. J. Immunol.* 39: 1252 – 1259, 2009.
57. Denisov, D.A., Denisova, G.F., Lelic, A., Loeb, M., and **Bramson, J.L.** Deciphering epitope specificities within polyserum using affinity selection of random peptides and a novel algorithm based on pattern recognition theory. *Mol Immunol.* 46: 429 - 436, 2009.
56. Grinshtein, N., Ventresca, M., Margl, R., Bernard, D., Yang, T.C., Millar, J.B., Hummel, J., Beermann, F., Wan, Y., and **Bramson J.L.** High-dose chemotherapy augments the efficacy of recombinant adenovirus vaccines and improves the therapeutic outcome. *Cancer Gene Ther.* 16: 338 - 350, 2009.
55. Denisova, G.F., Denisov, D.A., Yeung, J., Loeb, M., Diamond, M.S., and **Bramson, J.L.** A novel computer algorithm improves antibody epitope prediction using affinity-selected mimotopes: A case study using monoclonal antibodies against the West Nile virus E protein. *Mol. Immunol.* 46: 125 – 134, 2008.
54. Parsons, R., Lelic, A., Hayes, L., Carter, A., Marshall, L., Eveleigh, C., McMurtrey, C., Hildebrand, W., Loeb, M. and **Bramson, J.L.** The Memory T cell Response to West Nile Virus in Symptomatic Humans Following Natural Infection is not Influenced by Age and is Dominated by a Restricted Set of CD8+ T Cell Epitopes. *J. Immunol.* 181: 1563-1572, 2008.
53. McMurtrey, C., Lelic, A., Piazza, P., Chakrabarti, A.K., Yablonski, E.J., Wahl, A., Bardet, W., Eckerd, A., Cook, R.I., Buchli, R., Loeb, M., Rinaldo, C.R., **Bramson, J.**, and Hildebrand, W.H. Epitope discovery in West Nile virus infection: Identification and immune recognition of viral epitopes. *PNAS* 105:2981-2986. 2008.

52. Mossoba, M., Walia, J.S., Rasaiah, V.I., Buxhoeveden, N., Head, R., Ying, C., Foley, J.E., **Bramson, J.L.**, Fowler, D.H., and Medin, J. Tumor protection following vaccination with low doses of lentivirally transduced DCs expressing the self-antigen erbB-2. *Mol Ther.* 16: 607-617, 2008.
51. Karimi, K., Boudreau, J., Fraser, K., Liu, H., Delanghe, J., Gauldie, J., Xing, Z., **Bramson, J.**, and Wan, Y. Robust antitumor immunity elicited by dendritic cell vaccines is a result of their ability to engage both CTL and IFN γ -producing NK Cells. *Mol. Ther.* 16: 411-418, 2008.
50. Millar, J., Dissanayakke, D., Yang, T., Grinshtein, N., Eveleigh, C., Wan, Y. and **Bramson, J.** The magnitude of the CD8+ T cell response produced by recombinant virus vectors is a function of both the antigen and the vector. *Cell. Immunol.* 250: 55 – 67, 2007.
49. Oliphant, T., Nybakken, G.E., Austin, K., Xu, Q., **Bramson, J.**, Loeb, M., Throsby, M., Fremont, D.H., Pierson, T.C., and Diamond, M.S. The Induction of Epitope-Specific Neutralizing Antibodies against West Nile virus. *J. Virol.*, 81: 11828-11833, 2007.
48. Luketic, L., Delanghe, J., Frotten, E., Yang, P., **Bramson, J.** and Wan, Y. Antigen presentation by exosomes released from peptide-pulsed dendritic cells is not suppressed by the presence of active CTL. *J. Immunol.* 179: 5024-5032, 2007.
47. Divangahi, M., Yang, T., Kugathasan, K., McCormick, S., Takenaka, S., Gaschler, G., Ashkar, A., Stampfli, M., Gauldie, J., **Bramson, J.**, Takai, T., Brown, E., Yokoyama, W., Aoki, N., and Xing, Z. Critical Negative Regulation of Type 1 T Cell Immunity and Immunopathology by Signaling Adaptor DAP12 during Intracellular Infection. *J. Immunol.* 179: 4015 – 4026, 2007.
45. Kianizad, K., Marshall, L.A., Grinshtein, N., Bernard, D., Beermann, F., Zhang, S., Wan, Y., and **Bramson, J.L.** Elevated frequencies of self-reactive CD8+ T cells following immunization with a xenoantigen is due to the presence of heteroclitic CD4+ T cell epitopes. *Cancer Res.* 67: 6459-6467, 2007.
44. Yang, T.C., Millar, J., Groves, T., Zhou, W., Grinshtein, N., Xing, Z., Wan, Y. and **Bramson, J.L.** On the role of CD4+ T cells in the CD8+ T cell response elicited by recombinant adenovirus vaccines. *Mol. Ther.* 15: 997-1006, 2007.
43. Zhang, X., Divanghi, M., Ngai, P., Santosuosso, M., Millar, J., Zganiacz, A., Wang, J., **Bramson, J.** And Xing, Z. Intramuscular immunization with a monogenic plasmid DNA tuberculosis vaccine: Enhanced immunogenicity by electroporation and co-expression of GM-CSF transgene. *Vaccine* 25: 1342-52, 2007.
42. Malowany, J.I., McCormick, S., Santosuosso, M., Zhang, X., Aoki, N., Ngai, P., Wang, J., Leitch, J., **Bramson, J.**, Wan, Y. and Xing, Z. Development of cell-based tuberculosis vaccines: genetically modified dendritic cell vaccine is a much more potent activator of CD4 and CD8 T cells than peptide- or protein-loaded counterparts. *Mol. Ther.* 13: 766-775, 2006.
41. Alvarez, D., Swirski, F.K., Yang, T.C., Fattouh, R., Croitoru, K., **Bramson, J.L.**, Stampfli, M.R., Jordana, M. Inhalation Tolerance Is Induced Selectively in Thoracic Lymph Nodes But Executed Pervasively at Distant Mucosal and Non-Mucosal Tissues. *J. Immunol.* 176: 2568 – 2580, 2006.
40. Yang, T.C., Millar, J., Groves, T., Grinshtein, N., Parsons, R., Wan, Y., and **Bramson, J.L.** The CD8+ T cell population elicited by recombinant adenovirus displays a novel partially-exhausted phenotype associated with prolonged antigen presentation that nonetheless provides long-term immunity. *J. Immunol.*, 176: 200-210, 2006.
39. Grinshtein, N., Yang, T.C., Millar, J., Parsons, R., Denisova, G., Leitch, J., Dissanayake, D., Wan, Y. and **Bramson, J.L.** Recombinant adenovirus vaccines can successfully elicit CD8+ T cell immunity under conditions of extreme leukopenia. *Mol. Ther.* 13: 270 – 279, 2006.
38. Tannis, L.L., Gauthier, A., Eveleigh, C., Parsons, R., Nyholt, D., Khromykh, A., and **Bramson, J.L.** Semliki Forest virus and Kunjin virus RNA replicons elicit comparable cellular immunity but distinct humoral immunity. *Vaccine* 23: 4189-4194, 2005.

37. Alvarez, D., Harder, G., Fattouh, R., Sun, J., Goncharova, S., Stampfli, M.R., Coyle, A.J., **Bramson, J.L.** and Jordana, M. Cutaneous antigen priming via Gene Gun leads to skin-selective Th2 immune-inflammatory responses. *J. Immunol.* 174: 1664-1674, 2005.
36. Leitch, J., Lane, C., Fraser, K., Dayball, K., **Bramson, J.L.**, and Wan, Y. CTL-dependent and -independent antitumor immunity is determined by the tumor not the vaccine. *J. Immunol.* 172: 5200-5205, 2004.
35. Miller, M., Rekas, G., Dayball, K., Wan, Y., and **Bramson, J.L.** The efficacy of electroporated plasmid vaccines correlates with cumulative antigen production not short-term expression levels. *Vaccine* 22: 2517 – 2523, 2004.
34. Lane, C., Leitch, J., Tan, X., Hadjati, J., **Bramson, J.L.** and Wan, Y. Vaccination-induced autoimmune vitiligo is a consequence of secondary trauma to the skin. *Cancer Res.* 64: 1509-1514, 2004.
33. Zganiacz, A., Santosuosso, M., Wang, J., Yang, T., Chen, L., Anzulovic, M., Alexander, S., Gicquel, B., Wan, Y., **Bramson, J.**, Inman, M., and Xing, Z. TNF- α is a critical negative regulator of type 1 immune activation during intracellular bacterial infection. *J. Clin. Invest.* 113: 401-413, 2004.
32. **Bramson, J.L.**, Grinshtein, N., Meulenbroek, R.A., Lunde, J., Kottachchi, D., Lorimer, I.A., Jasmin, B.J. and Parks, R.J. Helper-dependent adenoviral vectors containing modified fibre for improved transduction of mature muscle. *Hum. Gene Ther.* 15: 179 – 188, 2004.
31. Bertram, E.M., Dawicki, W., **Bramson, J.L.**, Lynch, D.H. and Watts, T.H. The secondary CD8 T cell response in vivo is limited by factors extrinsic to the T cell and involves a switch in costimulatory signal from CD28 to 4-1BB. *J. Immunol.* 172:981-8, 2004.
30. Alexander, S., **Bramson, J.**, Foley, R., and Xing, Z. Protection from septic shock by adenoviral-mediated transgene expression of human bactericidal/permeability-increasing protein. *Blood* 103: 93-99, 2004.
29. Yang, T.C., Dayball, K., Wan, Y. and **Bramson, J.L.** Detailed analysis of the CD8+ T cell response following adenovirus vaccination. *J. Virol.* 77: 13407-13411, 2003.
28. Dayball, K., Millar, J.B., Miller, M., Wan, Y., and **Bramson, J.L.** In vivo electroporation enables plasmid vaccines to activate CD8+ T cells in the absence of CD4+ T cells. *J. Immunol.* 171: 3379-3384, 2003.
27. **Bramson, J.L.**, Dayball, K., Hall, J.R., Millar, J.B., Miller, M., Wan, Y.-H., Lin, R., and Hiscott, J. Super-activated interferon-regulatory factors can enhance plasmid vaccination. *Vaccine* 21: 1363-1370, 2003.
26. **Bramson, J.L.**, Dayball, K., Eveleigh, C., Wan, Y.-H., Page, D., and Smith, A. Enabling topical immunization via microporation: a novel method for pain-free and needle-free delivery of adenovirus-based vaccines. *Gene Ther.* 10: 251 - 260, 2003.
25. Duguay, D., Mercier, F., Stagg, J.G., Martineau, D., **Bramson, J.**, Servant, M., Lin, R., Galipeau, J., and Hiscott, J. In Vivo IRF-3 Tumor Suppressor Activity in B16 Melanoma Tumors. *Cancer Research* 62: 5148 – 5152, 2002.
24. Wan, Y.-H., Lu, L., **Bramson, J.L.**, Baral, S., Zhu, Q., Pilon, A., and Dayball, K. Dendritic Cell-Derived IL-12 is not Required for the Generation of Cytotoxic, IFN- γ -secreting, CD8+ CTL In Vivo. *J. Immunol.* 167: 5027 – 5033, 2001.
23. Gorczynski, R.M., **Bramson, J.**, Cattral, M., Huang, X., Lei, J., Min, W., Wan, Y., and Gauldie, J. Dendritic cells expressing TGF β /IL-10 and CHO cells with OX-2 increase graft survival. *Transplant. Proc.* 33: 1565 – 1566, 2001.
22. **Bramson, J.L.**, Bodner, C.A., Johnson, J., Semple, S. and Hope, M.H. Intravenous administration of Stabilized Antisense Lipid Particles (SALP) leads to activation and expansion of liver NK cells. *Antisense Nuc. Acid Drug Dev.* 10: 217 – 224, 2000.
21. Gorczynski, R.M., **Bramson, J.**, Cattral, M., Huang, X., Lei, J., Xiaorong, L., Min, W.P., Wan, Y., and Gauldie, J. Synergy in induction of increased renal allograft survival after portal vein infusion of dendritic cells transduced

to express TGFbeta and IL-10, along with administration of CHO cells expressing the regulatory molecule OX-2. Clin Immunol 95:182-189, 2000.

20. Wan, Y.-H., **Bramson, J.L.**, Pilon, A., Zhu, Q., and Gauldie, J. Genetically modified dendritic cells prime auto-reactive T cells through a pathway independent of IL-12 and CD40: Implications for cancer vaccines. Cancer Res. 60: 3247-3253, 2000

19. **Bramson, J.L.**, Bodner, C.A., and Graham, R. Activation of host anti-tumor responses by cationic lipid/DNA complexes. Cancer Gene Ther. 7: 353-359, 2000.

18. Parks, R.J., **Bramson, J.L.**, Wan, Y.-H., Addison, C.L., and Graham, F.L. Effect of stuffer DNA on transgene expression from helper-dependent adenoviral vectors. J. Virol. 73: 8027 – 8034, 1999.

17. Addison, C.L., **Bramson, J.L.**, Hitt, M., Muller, W.J., Gauldie, J., and Graham, F.L. Intratumoral coinjection of adenoviral vectors expressing IL-2 and IL-12 results in enhanced frequency of regression of injected and untreated distal tumors. Gene Ther. 5: 1400 – 1409, 1998.

16. Pützer, B.M., **Bramson, J.L.**, Addison, C.L., Hitt, M., Siegel, P.M., Muller, W.J., and Graham, F.L. Combination therapy with IL-2 and wild-type p53 expressed by Ad-vectors induces tumor regression without cytokine related toxicity. Hum. Gene Ther. 9: 70 – 718, 1998.

15. Emtage, P.C.R., Wan, Y.-H., **Bramson, J.L.**, Graham, F.L., and Gauldie, J. A double recombinant adenovirus expressing the co-stimulatory molecule B7-1 (murine) and human interleukin-2 induces complete tumor regression in a murine breast adenocarcinoma model. J. Immunol. 160: 2531-2538, 1998.

14. **Bramson, J.L.**, Hitt, M., Gauldie, J., and Graham, F.L. Pre-existing immunity to adenovirus does not prevent tumor regression following intratumoral administration of a vector expressing IL-12 but inhibits virus dissemination. Gene Ther. 4: 1069-1076, 1997.

13. Zisman DA, Kunkel SL, Strieter RM, Gauldie J, Tsai WC, **Bramson J**, Wilkowski JM, Bucknell KA, Standiford TJ. Anti-interleukin-12 therapy protects mice in lethal endotoxemia but impairs bacterial clearance in murine Escherichia coli peritoneal sepsis. Shock: 8, 349-356, 1997

12. Wan, Y.-H., **Bramson, J.**, Carter, R., Graham, F.L., and Gauldie, J. Dendritic cells transduced with an adenovirus vector encoding a model tumor-associated antigen for tumor vaccination. Hum. Gene Ther.: 8, 1355-1363, 1997.

11. **Bramson, J.**, Hitt, M., Addison, C.L., Muller, W.J., Gauldie, J., and Graham, F.L. Direct intratumoral injection of an adenovirus expressing IL-12 induces regression and long-lasting immunity associated with highly localized expression of IL-12. Hum. Gene Ther. 7: 1995-2002, 1996.

10. Greenberger, M.J., Kunkel, S.L., Streiter, R.M., Lukacs, N.W., **Bramson, J.**, Gauldie, J., Graham, F.L., Hitt, M., Danforth, J.M., and Standiford, T.J. IL-12 gene therapy protects mice in lethal Klebsiella pneumonia. J. Immunol. 157: 3006 - 301, 1996.

9. **Bramson, J.**, Hitt, M., Gallichan, W.S., Rosenthal, K.L., Gauldie, J. and Graham, F.L. Construction of a double recombinant adenovirus vector expressing a heterodimeric cytokine: *In vitro* and *in vivo* production of biologically active interleukin 12. Hum. Gene Ther. 7: 333-342, 1996.

8. **Bramson, J.**, McQuillan, A., Aubin, R., Alaoui-Jamali, M., Batist, G., Christodoulopoulos, G. and Panasci, L. Nitrogen mustard resistance in chronic lymphocytic leukemia as an *in vivo* model for crosslinking agent resistance. Mutat. Res. 336: 269-278, 1995.

7. **Bramson, J.**, O'Connor, T., and Panasci, L. Effect of alkyl-N-purine DNA glycosylase overexpression on cellular sensitivity to bifunctional alkylating agents. Biochem. Pharmacol. 15: 39-44, 1995

6. **Bramson, J.**, McQuillan, A., and Panasci, L. DNA repair enzyme expression in chronic lymphocytic leukemia vis-à-vis nitrogen mustard drug resistance. Cancer Lett. 90: 139-148, 1995.

5. **Bramson, J.** and Panasci, L. Potentiation of chlorambucil toxicity in B-cell chronic lymphocytic leukemia lymphocytes using DNA synthesis inhibitors. Biochem. Pharmacol. 15: 131-135, 1995.

4. **Bramson, J.**, Prévost, J., Malapetsa, A., Noë, A.J., Poirier, G.G., DesNoyers, S., Alaoui-Jamali, M., and Panasci, L. Poly (ADP-Ribose) Polymerase can bind to melphalan damaged DNA. *Cancer Res.* 53: 5370-5373, 1993.
3. **Bramson, J.** and Panasci, L. Effect of ERCC-1 overexpression on sensitivity of chinese hamster ovary cells to DNA damaging agents. *Cancer Res.* 53: 3237-3240, 1993.
2. Malapetsa, A., **Bramson, J.**, Noë, A.J., and Panasci, L. Lack of evidence for a high affinity sarcosinamide carrier in Calu-1 lung cancer cells, HT-29 colon cancer cells, and DHF fibroblasts. *Cancer Chemo. Pharmacol.* 31: 146-151, 1992.
1. Geleziunas, R., McQuillan, A., Malapetsa, A., Hutchinson, M., Kopriva, D., Wainberg, M.A., Hiscott, J., **Bramson, J.**, and Panasci, L. Increased DNA synthesis and repair enzyme expression in lymphocytes from chronic lymphocytic leukemia patients resistant to the nitrogen mustards. *J. Natl. Cancer Inst.* 83: 557-564, 1991.

PATENTS (ISSUED, only USPTO serial numbers provided):

Vaccination methods. Inventors: Brian Lichty, Byram Bridle, Yonghong Wan, **Jonathan Bramson**. PCT/CA2010/000379, US 9707285 B2

Trifunctional T cell antigen-coupler and methods and uses thereof. Inventors: **Jonathan Bramson**, Christopher Helsen, Galina Denisova, Ken Mwawasi and Rajanish Giri. PCT/CA2015/000068, US 10435453 B2.

T cell-antigen coupler with various construct optimizations. Inventors: **Jonathan Bramson**, Christopher Helsen, Joanne Hammill, Ken Mwawasi. PCT/US2019/042297, US 10640562 B2, US 11111298 B2, US 11110123 B2.

T cell-antigen coupler with Y182T mutation and methods and uses thereof. Inventors: Christopher Helsen, **Jonathan Bramson**, Anna Dvorkin-Gheva, Galina Denisova, Ksenia Bezverbnaya. PCT/CA2018/051290, US 11198737 B2.

BCMA T Cell-Antigen Couplers and Uses Thereof. Inventors: **Jonathan Bramson**, Joanne Hammill, Christopher Helsen. US 11453723 B2.

PATENTS (PENDING):

Chimeric Costimulatory Receptors and Methods and Uses Thereof. Inventors: Phillip Marvyn, **Jonathan Bramson**. US Provisional Application No. 62/900,911

Covalent immune recruiter compounds for immune cell recognition and associated uses. Inventors: Anthony Rullo, **Jonathan Bramson**, Yonghong Wan, Benjamin Lake, Nicholas Serniuck. US Provisional Application No. 62/981,767.

Cells Comprising T Cell-Antigen Couplers and Uses Thereof. Inventors: **Jonathan Bramson**, Seung Mi Yoo, Sarah Asbury, Allyson Moore. US Provisional Application No. 63/110,902.

Membrane-Associated T cell-Tumor Cell Hitch Receptors and Uses Thereof. Inventors: **Jonathan Bramson**, Christopher Helsen, Andreas Bader, Bojana Bojovic, Joanne Hammill, Philbert Ip, Duane Moogk, Kenneth Mwawasi. US Provisional Application No. 63/363,803.

Covalent Immune Recruits for Synthetic Antigen Receptors and Methods and Uses Thereof. Inventors: Nickolas Serniuck, Anthony Rullo, **Jonathan Bramson**, Benjamin Lake, Allyson Moore. US Provisional Application No. 63/348,744.

REVIEWS:

Burchett, R., Walsh, S., Wan, Y., and **Bramson, J.L.** A Rational Relationship: Oncolytic virus vaccines as functional partners for adoptive T cell therapy. *Cytokine and Growth Factor Reviews* 56: 149-159, 2020.

McGray, A.J.R., and **Bramson, J.L.** Adaptive Resistance to Cancer Immunotherapy. *Adv Exp Med Biol.* 1036: 213-227, 2017.

Bezverbnaya, K., Matthews, A., Sidhu, J., Helsen, C.W., and **Bramson, J.L.** Tumor targeting domains for CAR T cells. *Immunotherapy* 9: 33 – 46, 2017.

Hammill, J.A., Afsahi, A., **Bramson, J.L.** and Helsen C.W. Viral engineering of chimeric antigen receptor expression in murine and human T lymphocytes. *Methods Mol. Biol.* 1458: 137 – 157, 2016.

Fox, B.A. (84 co-authors, including **J.L. Bramson**) and Disis, M.L. Defining the critical hurdles in cancer immunotherapy. *J. Translational Med.* 9: 214, 2011.

Bassett, J.D., Swift, S.L. and **Bramson, J.L.** Optimizing vaccine-induced CD8+ T cell immunity: focus on recombinant adenovirus vectors. *Expert Rev. Vaccines* 10: 1307 – 1319, 2011.

Grinshtein, N. and **Bramson, J.L.** Combining cancer vaccines with conventional therapies. *Experimental and Applied Immunotherapy [Medin and Fowler (Eds)]*: 323 - 338, 2011.

Eftimie, R., **Bramson, J.L.**, Earn, D.J. Interactions between the immune system and cancer: A brief review of non-spatial mathematical models. *Bull. Math. Biol.* 73: 2 - 32, 2011.

Denisova, G., Denisov, D.A., and **Bramson, J.L.** Applying bioinformatics for antibody epitope prediction using affinity-selected mimotopes – relevance for vaccine design. *Immunome Research.* 6 Suppl 2: S6, 2010.

Spaner, D.E., Foley, R., Galipeau, J. and **Bramson, J.** Obstacles to effective Toll-Like Receptor agonist therapy for hematologic malignancies. *Oncogene* 27: 208-217, 2008.

Yang, T.C., Millar, J., Grinshtein, N., Bassett, J., Finn, J., and **Bramson, J.** T cell immunity generated by recombinant adenovirus vaccines. *Expert Rev. Vaccines* 6: 347-356, 2007

Xing, Z., Santosuosso, M., McCormick, S., Yang, T.C., Millar, J., Hitt, M., Wan, Y., **Bramson, J.** and Vordermeier, H.M. Recent advances in the development of adenovirus- and poxvirus-vectored tuberculosis vaccines. *Curr. Gene Ther.* 5: 485-492, 2005.

Bramson, J.L. and Parks, R.J. Adenoviral Vectors for Gene Therapy. In: A. Rolland and S. Sullivan, eds. *Pharmaceutical Gene Delivery Systems.* Marcel Dekker, Inc., New York, Chpt. 7, pp. 149-182, 2003.

Bramson, J.L. and Wan, Y.-H. The efficacy of genetic vaccination is dependent upon the nature of the antigen and vector system. *Expert Opin. Biol. Ther.* 2: 75 - 86, 2002.

Wan, Y.-H. and **Bramson, J.L.** Role of dendritic cell-derived cytokines in immune regulation. *Curr. Pharm. Des.* 7: 977 – 992, 2001.

Parks, R.J., and **Bramson, J.L.** Adenoviral vectors: prospects for gene delivery to the central nervous system. *Gene Ther.* 6: 1349 – 1350, 1999.

Bramson, J., Graham, F.L., and Gauldie, J. The use of adenoviral vectors for gene therapy and gene transfer *in vivo*. *Curr. Op. Biotech.* 6: 590 - 595, 1995.

ORAL PRESENTATIONS:

"Adventures in Cell Therapy with Jonathan Bramson". Cole Foundation Research Celebration Day. Montreal, QC., April 2023.

"Cancer Cell Therapy in Canada: Current State of Affairs". Canada Cancer Trials Group IND Committee meeting. On-line. April 2023.

"Triggering the magic bullet : How synthetic antigen receptors influence T cell therapy". 4th Annual Immuno-Oncology Symposium, Western University, London, ON., Mar. 2023.

"Cell Therapy in Hamilton". 13th Annual Myeloma Canada Scientific Roundtable, Montreal, PQ. Nov. 2022.

"Engineering the T cell response against multiple myeloma." Janssen Immuno-Oncology Consortium meeting. Toronto, ON. Oct. 2022

"Engineered T cell Therapy: CAR, TAC, SAR – a rose by any other name?". Cell Therapy Transplant Canada annual meeting. Niagara-on-the-lake, ON. June 2022.

"Engineered T cell Therapy: CAR, TAC, SAR – a rose by any other name?". Keynote Presentation. Translation Regional Comprehensive Cancer Centres annual meeting, Seven Springs, PA, June 2022.

"Employing covalent chemistry to drive T cell therapies." OICR Mondays, Ontario Institute for Cancer Research. On-line, March 2022.

"An introduction to cancer immunotherapy". Western Cancer Awareness Society. On-line, March 2022.

"Engineered T cell Therapy: CAR, TAC, SAR – a rose by any other name?". Department of Medicine Grand Rounds, Roswell Park Cancer Institute. On-line, February 2022.

"Contemporary Immuno-Oncology". Future of Oncology - Universities Allied for Essential Medicines oncology panel. On-line, October 2021.

"Biology and efficacy of a TCR-centric synthetic antigen receptor". Cellicon Valley 2021, On-line, May 2021.

"Engineering the fight against cancer". Oncology Research Association, University of Toronto, On-line, Oct. 2020.

"Approaches to deal with the challenges of longitudinal studies" Beckman Coulter Global CytoFLEX User Group Meeting. On-line. Oct. 2020.

"Biology and Efficacy of Synthetic Antigen Receptors that Signal via the TCR" The Scientist Webinars: Adoptive Cell Therapy Summit, Sept. 2020

"T Cell Therapy for Multiple Myeloma: The Canadian Context" Myeloma Canada Scientific Roundtable, Sept. 2020.

"Cells as drugs – the next frontier in pharmaceuticals". Third Age Learning, Burlington, ON, Jan. 2020

"Engineering the fight against cancer". Department of Immunology, Roswell Park Cancer Institute, Buffalo, NY, Jan. 2020.

"Combining engineered T cells and oncolytic viruses". Department of Immunology, Mayo Clinic, Rochester, MN. Nov. 2019.

"Cancer Vaccines have no place in the era of Cell Therapy: A Debate". BioCanRx Annual Meeting, Summit for Cancer Immunotherapy, Victoria, BC, Oct. 2019.

"Engineering the fight against cancer". Department of Immunology, University of Sherbrooke, Sherbrooke, QC, Oct. 2019.

"Innovating T cell therapies at Triumvira Immunologics". McMaster Therapeutics Symposium. Hamilton, ON, Oct. 2019.

"Tri-functional T-cell Antigen Coupler (Tri-TAC) and Adaptive T-cell Strategies for Multiple Myeloma". 10th Annual Myeloma Canada Scientific Roundtable, Montreal, QC, Oct. 2019.

"A novel chimeric receptor that re-directs the endogenous T cell receptor". Next Generation CAR & T Cell Therapies, San Francisco, CA, June 2019.

"The evolution of a T Cell Antigen Coupler (TAC) receptor directed against BCMA". Fifth Annual Immunotherapy in Myeloma Scientific Workshop, Denver, CO, May 2019.

"An Immuno-Oncology primer". Celgene Canada sales meeting, Toronto, Apr. 2019.

"The T cell antigen coupler (TAC) receptor is a novel chimeric receptor that re-directs the endogenous TCR". Translation Regional Comprehensive Cancer Centres annual meeting, Seven Springs, PA, Feb. 2019.

"Immunology 101". Celgene Canada, Toronto, Feb. 2019.

"Engineering white blood cells to fight cancer". Terry Fox Research Institute Ontario Node Symposium, Toronto, ON, Dec. 2018.

"Engineering the fight against cancer". The Hamilton Association for the Advancement of Literature, Science and Art, Hamilton, ON, Nov. 2018.

"Engineering T cells to fight cancer". Beatrice Hunter Cancer Research Institute Cancer (BHCRI) Conference, Halifax, NS, Nov. 2018.

"The T cell antigen coupler (TAC) receptor is a novel chimeric receptor that re-directs the endogenous TCR". BioCanRx Summit for Cancer Immunotherapy, Banff, AB, Oct. 2018

"The T cell antigen coupler (TAC) receptor is a novel chimeric receptor that re-directs the endogenous TCR". Canadian Cancer Immunotherapy Consortium meeting, Vancouver, BC, June 2018.

"An update on the T cell antigen coupler (TAC) receptor". iTNT ACT Workshop, Montreal, QC, Dec. 2017.

"A novel chimeric receptor for engineered T cells". Cancer Immunotherapy TRCCC-CIM Symposium, Havana, Cuba, July 2017.

"atTACKing tumors: Better living through engineering". McMaster Infection and Immunity Program. Hamilton, ON, May 2017.

*"Everything you always wanted to know about Immuno-Oncology (*but were afraid to ask)"* Canadian Association of Medical Oncologists, Toronto, ON, Apr. 2017.

"TAC receptors: Taking a new CAR for a spin". Pan-Canadian Strategic Forum, Cell & Gene Therapy, Montreal, QC, Mar. 2017.

"A new way to atTACK cancer". Karmanos Cancer Institute, Detroit, MI, Dec. 2016.

"Engineered T cell therapies for cancer". McCarthy Tetrault, ON, Sept. 2016.

"Engineering and manufacturing T cells to Fight Cancer" Immunotherapy: The Ontario Landscape, Toronto, ON, Nov. 2015.

"TACsing T cells to beat cancer". World Congress on Regenerative Medicine, Leipzig, Germany, Oct. 2015.

"TACsing T cells to beat cancer". Institut de Recherche en Immunologie et Cancer, Montreal, PQ, Sept. 2015.

"Strange Love or: How I Learned to Stop Worrying and Love Translational Research". Department of Oncology Student Research Day, McMaster University, Hamilton, ON, June 2015.

"TAC'sing T cells for cancer immunotherapy". Association de Therapy Genie du Quebec annual meeting. Quebec City, PQ, June 2015.

"TACsing T cells to beat cancer" Centre for Drug Research and Development, Vancouver, BC, April 2015.

"TACsing T cells to beat cancer" Child & Family Research Institute, Vancouver, BC, April 2015.

"Engineering white blood cells to fight cancer". McMaster Integrated Science Symposium – Synthesis. Hamilton, ON, April 2015.

"A 5 minute primer on Immuno-Oncology". Immuno-Oncology Day, EMD-Serono, Mississauga, ON, Feb. 2015

"Hamilton, Ontario: A place for cancer immunotherapy". Immuno-Oncology Day, EMD-Serono, Mississauga, ON, Feb. 2015

"Engineering immune cells to fight cancer" Department of Chemical Engineering, McMaster University, Hamilton, ON, Nov. 2014.

"Transcriptional profiling of tumors for immune monitoring" Next Generation Vaccine Biomarker Workshop, Ottawa, ON, October 2014.

"Engineering immune cells to fight cancer" Fraunhofer Life Science Symposium, Leipzig, Germany, October 2014.

"Engineering T Cells in the Bramson Lab" Terry Fox Research Institute Immunotherapy Workshop, June 2014.

"Development of recombinant virus vaccines at the McMaster Immunology Research Centre" Transgene Corp., Lyon, France, March 2014.

"The status of cancer immunotherapy clinical trials in Canada" Terry Fox Research Institute Annual Scientific Meeting, Ottawa, May 2013.

"T cells instigate immune suppression in the tumor" Chinese Academy of Medical Sciences, Beijing, China, July 2012.

"Adaptive resistance to cancer immunotherapy is natural – So, what do we do about it?" Taiyuan Cancer Hospital, Taiyuan, China, July 2012.

"Maintaining CD8+ T cell memory the adenovirus way" Transgene Corp., Lyon, France, May 2012.

"Adaptive resistance to cancer immunotherapy is natural – So, what do we do about it?" Juravinski Cancer Centre Regional Oncology Rounds, Hamilton, ON, May 2012.

"Cancer vaccines instigate immune suppression within the tumor microenvironment" Tumor Immunology and Immunotherapy Series, Campbell Family Research Institute, Toronto, ON, Apr. 2012.

"T cells instigate immune suppression in the tumor" Translational Research Cancer Centers Consortium annual meeting, 7 Springs, PA, Feb. 2012.

"Plasticity of the tumor environment: T cells instigate immune suppression within the tumor" University of Oklahoma College of Medicine, Oklahoma City, OK, Oct. 2011.

"Maintaining CD8+ T cell memory the adenovirus way". NRC Institute for Biological Sciences, Ottawa, ON, Sept. 2011

"Immune evasion by tumors". CIHR Immunomodulation of Cancer workshop, Vancouver, BC, Mar. 2011.

"Cancer vaccines are limited by adaptive changes within the tumor microenvironment" The First International Student Congress on Cell and Molecular Medicine, Shiraz, Iran, Feb. 2011

"Cancer Biotherapeutics at McMaster University" Roswell Park Cancer Institute, Buffalo, NY, Sept. 2010.

"Enhancing cancer vaccines with conventional and immunological adjuvant therapies" Queen's University, Kingston, ON, Apr. 2010.

"Implementing and interrogating immunotherapies in ORBiT" OICR Annual Scientific Meeting, Alliston, ON, Mar. 2010.

"Maintaining T cell memory the adenovirus way" University of Toronto, Toronto, ON, Oct. 2009.

"T cell immunity to West Nile virus in naturally-infected humans" NIAID flavivirus epitope workshop, Bethesda, MD, Sept. 2009

"Adenovirus-transduced dendritic cells for cancer immunotherapy" American Society for Gene Therapy, San Diego, CA, May 2009.

"Terry Fox Foundation Cancer Immunotherapy Program" Terry Fox Foundation Research Institute Annual Scientific Meeting, Vancouver, BC, May 2009.

"T cell immunity to West Nile virus in naturally-infected humans" Advances in West Nile virus Research, Washington, DC, Mar. 2009

"The road less traveled: CD4+ T cell-mediated anti-tumor immunity" Cancer Immunotherapy Symposium, Toronto, ON, Nov. 2008.

"Engaging the immune system to enhance conventional cancer therapies" Juravinski Cancer Center, Hamilton, ON, April 2008.

"The road less traveled: CD4+ T cell-mediated anti-tumor immunity" CHUM Research Center, Montreal, QC, March 2008.

"Cancer Immunotherapy: The translational experience at McMaster". CPCRI Canadian Experiences and Opportunities Workshop: *A Roadmap for Translation of Discovery to the Clinic*. Vancouver, BC, January 2008.

"Reconsidering the importance of antigen persistence in the development of T cell memory". University of Guelph, November 2007.

"The road less traveled: CD4+ T cell-mediated anti-tumor immunity" Deeley Research Center, Victoria, BC, November 2007.

"Investigation of West Nile virus infection in humans: Genetic, Functional and Immunological studies". West Nile: Research Advances in Immunology, Virology, and Genetics, Rockville, MD, June 2007.

"If the vaccine is so good, why can't it cure established tumors?" Immunotherapy of Cancer Workshop, University of Toronto, May 2006.

"Is the development of vaccines for cancer a quixotic endeavor?" Henderson Research Centre, Hamilton, ON, Nov. 2005.

"Is the development of vaccines for cancer a quixotic endeavor?" Montreal Center for Experimental Therapeutics in Cancer, May 2005.

"A vector is not just a vector" Southern Ontario Gene Therapy Conference, April 2005.

"Using viruses to elicit immunity against cancer". 3rd International Meeting on Replicating Oncolytic Virus Therapeutics, March 2005.

"Development of genetic vaccines for cancer and infectious diseases" 43rd Annual Symposium of the Canadian Association for Laboratory Animal Science, June 2004.

"Nothing is ever simple: The immune biology of recombinant adenovirus vaccines" Southern Ontario Gene Therapy Conference, May 2004.

"The Immunobiology of Genetic Vaccination" Robarts Research Institute, Jan. 2004

"A Center for Innovation: The McMaster University Center for Gene Therapeutics" The Golden Horseshoe Venture Forum, Jan. 2004.

"Gene Therapy and Genetic Vaccination: A rose by any other name..." Invited presentation, Southern Ontario Gene Therapy Conference, May 2003.

"Deconstructing vaccination: Lessons learned from gene-based immunization" Invited presentation, Canadian Society for Immunology, March 2003.

"Adventures in Gene Therapy: A View from the Abyss". Invited presentation, Southern Ontario Gene Therapy Conference, April 2002.

"Novel Technologies for Vaccine Delivery". Invited presentation, Center for Business Intelligence conference on drug delivery systems, April 2002.

"The Immunology of Gene Therapy: Applied approaches to basic research" Invited presentation at the Ottawa Regional Cancer Center, March 2001.

APPENDIX B

Prior Testimony

In the last four years, I served as an expert in, and was deposed and/or testified in connection with the following matter:

- *Cabaret Biotech Ltd. v. Kite Pharma, Inc., et al.*, C.A. No. 19-1732
(LPS) (D. Del.) (on behalf of Cabaret Biotech Ltd.)