IN THE UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF PENNSYLVANIA

TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, Plaintiff, v. ST. JUDE CHILDREN'S RESEARCH HOSPITAL, Defendant.	CIVIL ACTION NO. 13-1502 JURY TRIAL DEMANDED

AMENDED COMPLAINT

Plaintiff Trustees of the University of Pennsylvania (the "University") brings this declaratory judgment action against defendant St. Jude Children's Research Hospital ("St. Jude"), and alleges the following:

PARTIES

1. The University is a non-profit organization devoted to higher education with a principal place of business at 3451 Walnut Street, Philadelphia, Pennsylvania 19104.

2. St. Jude is a non-profit pediatric cancer research hospital with a principal place of business at 262 Danny Thomas Place, Memphis, Tennessee 38105.

3. St. Jude is supported primarily by donations raised by its national fundraising organization, the American Lebanese Syrian Associated Charities ("ALSAC"), which was established expressly for the purpose of funding St. Jude.

JURISDICTION

4. This Court has original jurisdiction over the subject matter of this action pursuant to the provisions of Title 28, United States Code ("U.S.C.") §§ 1331 (Federal Question), 1338(a) (Patents), 2201 & 2202 (Declaratory Relief). Jurisdiction also exists pursuant to § 1332(a).

5. Defendant St. Jude is subject to personal jurisdiction in this district because, *inter alia*, ALSAC, St. Jude's charity, has both an office and a registered representative in the Eastern District of Pennsylvania.

6. Venue is proper in this district pursuant to 28 U.S.C. §§ 1391(b) and (c) and § 1400(b).

BACKGROUND

7. Carl H. June, M.D., the Director of Translational Research Program and the Richard W. Vague Professor of Pathology and Laboratory Medicine at the University's Perelman School of Medicine, has developed a groundbreaking immunotherapy for treatment of cancer (the "Penn Immunotherapy").

8. The Penn Immunotherapy involves use of a CD19 ScFv DNA lentiviral construct that, using proprietary technologies that Dr. June and his colleagues developed while at the University, causes T cells to express chimeric antigen receptors in patients such that their cancer is treated.

A. U.S. Patent No. 8,399,645.

9. Upon information and belief, St. Jude is the owner of U.S. Patent No. 8,399,645 which is entitled "Chimeric Receptors with 4-1BB Stimulatory Signaling Domain" and which issued on March 19, 2013 ("the '645 patent"). *See* the '645 Patent, attached hereto as Exh. A.

10. The '645 patent identifies Dario Campana and Chihaya Imai as the Inventors. *See id.*

11. The '645 patent states that "[t]his invention relates to chimeric cell membrane receptors, particularly chimeric T-cell receptors. This invention further relates to activation and expansion of cells for therapeutic uses, in particular for activation and expansion of NK cells for chimeric receptor-based cell therapy." *Id.,* col. 1, lns. 35-39.

12. The '645 patent states that "[i]n a most preferred embodiment of the invention the extracellular domain comprises a single chain variable domain of an anti-CD19 monoclonal antibody, the transmembrane domain comprises the hinge and transmembrane domain of CD8α, and the cytoplasmic domain comprises the signaling domain of CD3ζ and the signaling domain of 4-1BB." *Id.,* col. 3, lns. 56-62.

13. The '645 patent also states:

> Other aspects of the invention include polynucleotide sequences, vectors and host cells encoding a chimeric receptor that compromises the signaling domain of the 4-1BB. Yet other aspects include methods of enhancing T lymphocyte or natural killer (NK) cell activity in an individual and treating an individual suffering from cancer by introducing into the individual a T lymphocyte or NK cell comprising a chimeric receptor that comprises the signaling domain of 4-1BB. These aspects particularly include the treatment of lung cancer, melanoma, breast cancer, prostate cancer, colon cancer, renal cell carcinoma, ovarian cancer, neuroblastoma, rhabdomyosarcoma, leukemia and lymphoma. Preferred cancer targets for use with the present invention are cancers of B cell origin, particularly including acute lymphoblastic leukemia, B-cell chronic lymphocytic leukemia and B-cell non-Hodgkin's lymphoma.

Id., col. 3, ln. 63 to col. 4, ln. 11.

14. The '645 patent also states:

> Primary T cells expressing chimeric receptors specific for tumor or viral antigens have considerable therapeutic potential as immunotherapy reagents. Unfortunately, their clinical value is limited by their rapid loss of function and failure to expand in vivo, presumably due to the lack of co-stimulator molecules on tumor cells and the inherent limitations of

> signaling exclusively through the chimeric receptor.
>
> The chimeric receptors of the present invention overcome this limitation wherein they have the capacity to provide both the primary effector activity and the co-stimulatory activity upon binding of the receptor to a single ligand. For instance, binding of the anti-CD19-BB-ζ receptor to the CD19 ligand provides not only the primary effector function, but also a proliferative and cytolytic effect.
>
> T cells transduced with anti-CD19 chimeric receptors of the present invention which contain co-stimulatory molecules have remarkable anti-ALL capacity.

Id., col. 7, lns. 45-62.

B. Procedural Posture of Litigation Between the Parties

15. On July 11, 2012, St. Jude filed a complaint against the University in the United States District Court for the Western District of Tennessee (*St. Jude Children's Research Hospital, Inc. v. The Trustees of the University of Pennsylvania*, Civil Action No. 12-2579) ("the Tennessee Action") and alleged that the University breached two Materials Transfer Agreements (the "Agreements") that related to the provision of biological material by St. Jude to the University and Dr. June.

16. The "Material" or "Materials" that are the subject of the Agreements are "biological material" provided by St. Jude to the University and Dr. June, and specifically, "the anti-CD19-BB-ζ chimeric T-cell receptor construct, including any progeny, portions, unmodified derivatives and any accompanying know-how or data." *See* Agreements, attached hereto as Exhibits B and C, ¶ 1.

17. In its Complaint filed in the Tennessee Action, St. Jude states that "one of its researchers, Dr. Dario Campana, MD, Phd ("Dr. Campana) made the anti-CD19-BBζ chimeric T-Cell receptor construct (referred to as the 'Receptor'). The Receptor is a molecule that can be put on the surface of a normal immune T-cell, causing it to recognize and attack B-cells that have

the CD19 molecule on their surface." Complaint in Tennessee Action, attached hereto as Exhibit D ("St. Jude Complaint"), ¶¶ 17-18.

18. St. Jude equates the anti-CD19-BBζ chimeric T-Cell receptor purportedly developed by Dr. Campana with the Materials transferred to the University under the terms of the MTAs. *See id.* ¶ 26.

19. In the Tennessee Action, St. Jude also alleges that the University had discussed the commercialization of the Materials, in violation of the Agreements. *See id.* ¶ 62.

20. In briefing related to the case, St. Jude has stated as follows:

> The Agreements were executed because Penn specifically sought to obtain, and to collaborate with St. Jude on research involving, a biological material proprietary to St. Jude called a "chimeric antigen receptor" ("Receptor"). The Receptor is a molecule that enables a human immune cell to identify and attach a leukemic cancer cell. The Receptor was constructed entirely in a research laboratory at St. Jude in the early 2000s by Dr. Dario Campana and his staff, all of whom were St. Jude employees working at St. Jude in Memphis, Tennessee, where St. Jude's only campus is located. In exchange for the Receptor, Penn agreed that legal title to the Receptor remained with St. Jude and voluntarily assumed strict obligations directed at protecting St. Jude's proprietary and commercial interests in Tennessee: Penn agreed never to transfer the Receptor to anyone else, always to acknowledge the Receptor as St. Jude's in publications, and never to commercialize the Receptor without St. Jude's consent. However, Penn has breached the MTAs by hawking the Receptor as its own in recent scientific and other publications, and by commercializing the Receptor without St. Jude's consent.

St. Jude Opp. to Defendant's Motion to Dismiss, or in the Alternative, for a Change of Venue, attached hereto as Exhibit E, p. 1-2.

21. On July 19, 2012, the University filed a complaint against St. Jude in the United States District Court for the Eastern District of Pennsylvania (*The Trustees of the University of Pennsylvania v. St. Jude Children's Research Hospital, Inc.*, Civil Action No. 12-4122) ("the Pennsylvania Action"), alleging that St. Jude tortiously interfered with the University's

prospective contractual relations and sought a declaratory judgment that the University had not breached the Agreements.

22. In its Complaint, the University states:

> Carl H. June, M.D., a Professor of Pathology and Laboratory Medicine at Perelman, has developed a groundbreaking immunotherapy for treatment of cancer (the "Penn Immunotherapy"). The Penn Immunotherapy involves use of a CD19 ScFv DNA lentiviral construct (the "June Construct") that, using proprietary technologies that Dr. June and his colleagues developed while at the University, causes T cells to express chimeric antigen receptors ("CARs") in patients such that their cancer is treated. The strands of polynucleotide chains that make up DNA are held together by hydrogen bonds between complementary pairs of nitrogenous bases, or "base pairs."

Complaint in the Pennsylvania Action, ("University Complaint"), attached hereto as Exhibit F, ¶ 8.

23. St. Jude moved to dismiss the University's Complaint in the Pennsylvania Action. In its motion to dismiss, St. Jude stated:

> In the early 2000s, one of St. Jude's researchers, Dario Campana, M.D., PhD developed a molecule—called a chimeric antigen receptor ("Receptor")—which can be expressed on the surface of a normal human immune T-cell, and which causes the T-cell to recognize and attack certain leukemia cells. In December 2003, University researcher Dr. Carl June asked Dr. Campana to provide him with the Receptor and suggested a research collaboration involving use of the Receptor.

St. Jude Motion to Dismiss or Stay, attached hereto as Exhibit G, p. 3 (internal citations omitted).

24. St. Jude also alleges in its Motion to Dismiss that it "learned through a venture capitalist that the University was apparently engaging in prohibited commercialization efforts." *See id.* p. 5.

25. St. Jude further alleges that on January 20, 2012, counsel for St. Jude informed University General Counsel that St. Jude was "prepared to sue the University immediately in order to preserve its interests." *See id.*

26. In October 2012, the Tennessee Action was transferred to the United States District Court for the Eastern District of Pennsylvania and consolidated with the Pennsylvania Action. This consolidated proceeding is hereafter referred to as the "Consolidated Pennsylvania Action."

27. The subject matter of the '645 patent directly relates to the same subject matter at issue in the Consolidated Pennsylvania Action.

28. The '645 patent concerns the use of chimeric cell membrane receptors, particularly chimeric T-cell receptors for therapeutic uses. *See* Exh. A, Col. 1, lns. 35-39.

29. St. Jude has already sued the University, claiming, *inter alia*, that it improperly commercialized St. Jude's anti-CD19-BBζ chimeric T-Cell receptor construct, known variously as the Receptor or the Materials. *See* St. Jude Complaint, Exh. D, ¶ 81.

30. Similarly, the University has already brought a declaratory judgment action to determine whether it breached the MTAs based on its use of the University's CD19 ScFv DNA lentiviral construct, which causes T cells to express chimeric antigen receptors. *See* The University Complaint, Exh. F, ¶ 69.

31. St. Jude's statements and actions create a reasonable apprehension and belief on the part of the University that St. Jude will sue the University for infringement of the '645 patent.

32. Based on the foregoing, and in particular the relationship of the '645 patent to the subject matter of the Consolidated Pennsylvania Action, the University and St. Jude have adverse legal interests with respect to the '645 patent, and a substantial controversy exists between the University and St. Jude that is sufficiently immediate to warrant the issuance of a declaratory judgment.

33. By reason of the foregoing, a substantial and continuing controversy exists between the University and St. Jude regarding whether the University is liable for infringing the '645 patent. The University has instituted this Declaratory Judgment action for purposes of adjudicating that controversy.

COUNT I

Non-infringement of United States Patent No. 8,399,645

34. The University hereby incorporates by reference all previously stated allegations as if fully set forth herein.

35. The University has not infringed and is not infringing, either directly or indirectly, any valid claim of the '645 patent by the manufacture, use, sale or offer to sell of the Penn Immunotherapy or June Construct.

36. The University seeks a judicial determination from this Court that it has not willfully or otherwise infringed and is not infringing, either directly or indirectly, any valid claim of the '645 patent.

COUNT II

Invalidity of United States Patent No. 8,399,645

37. The University hereby incorporates by reference all previously stated allegations as if fully set forth herein.

38. The claims of the '645 patent are invalid for failure to meet one or more of the conditions of patentability specified in Title 35 of the United States Code, including but not limited to 35 U.S.C. §§ 102, 103, and/or 112 for at least the following reasons:

(a) All claims of the '645 patent are invalid under 35 U.S.C. §§ 102 and/or 103 in light of at least the following references:

(i) Gideon Gross & Zelig Eshhar, *Endowing T cells with antibody specificity using chimeric T cell receptors*, 6 THE FASEB JOURNAL, Dec. 1992, at 3370;

(ii) Terrence L. Geiger, Phuong Nguyen, David Leitenberg & Richard A. Flavell, *Integrated src kinase and constimulatory activity enhances signal transduction through single-chain chimeric receptors in T lymphocytes*, 98:8 BLOOD, Oct. 15, 2001, at 2364;

(iii) Andreas Homback, Claudia Heuser & Hinrich Abken, *The Recombinant T Cell Receptor Strategy: Insights into Structure and Function of Recombinant Immunoreceptors on the Way Towards an Optimal Receptor Design for Cellular Immunotherapy*, 2:2 CURRENT GENE THERAPY, 2002, at 211;

(iv) D. Moritz & B. Groner, *A spacer region between the single chain antibody- and the CD3 ζ-chain domain of chimeric T cell receptor components is required for efficient ligand binding and signaling activity*, 2:8 GENE THERAPY, at 539;

(v) Ian C. Nicholson, Kelly A. Lenton, Debbie J. Little, Tina DeCoroso, Fook Thean Lee, Andrew M. Scott, Heddy Zola & Arthur W. Hohmann, *Construction and Characterisation of a Function CD19 Specific Single Chain Fv Fragment for Immunotherapy of B Lineage Leukaemia and Lymphoma*, 34:16-17 MOLECULAR IMMUNOLOGY, 1991, at 1157;

(vi) C. Imai, et. al, *Chimeric receptors with 4-1BB signaling capacity provoke potent cytotoxicity against acute lymphoblastic leukemia*, 18 LEUKEMIA, 2004, at 676;

(vii) Michel Sadelain, Isabelle Rivière & Renier Brentjens, *Targeting Tumors with Genetically Enhanced T Lymphocytes*, 3 NATURE REVIEWS: CANCER, Jan. 2003, at 35;

(viii) U.S. Patent App. No. 2005/0113564 A1 (May 26, 2005);

(ix) U.S. Patent App. No. 2004/0043401 A1 (Mar. 4, 2004);

(x) WO publication 00/14257, Michel Sadelain, et. al, "Fusion Receptors Specific for Prostate-Specific Membrane Antigen and Uses Thereof," published Mar. 16, 2000; and

(xi) WO publication 02/33101 A1, Margaret Helene Finney, "Chimeric Cytoplasmic Signaling Molecules Derived from CD137," published Apr. 25, 2002.

(b) The specification of the '645 patent does not provide a written description of at least claims 1-19 of the '645 patent as required by § 112 to the extent that the claims encompass a polynucleotide encoding a chimeric receptor without a hinge region.

(c) The specification of '645 patent does not "enable any person skilled in the art to which it pertains, or with which it is most nearly connected to make and use" at least claims 1-19 of the '645 patent without a hinge region, as required by § 112.

(d) Any other arguments concerning unenforceability, non-infringement, and invalidity under one or more sections of 35 U.S.C. §§ 102, 103, and/or 112, which may arise depending upon facts developed during discovery.

39. The University seeks a judicial determination from this Court that the claims of the '645 patent are invalid.

RELIEF REQUESTED

WHEREFORE, the University requests that the Court enter a judgment in the University's favor and against St. Jude, and provide the University with the following relief:

- Order, adjudge and decree that the University is not infringing any valid claim of the '645 patent;
- Order, adjudge and decree that the '645 patent is invalid;
- Award such other and further relief as the Court may deem just and proper

DEMAND FOR JURY TRIAL

Plaintiff Trustees of the University of Pennsylvania hereby demand a trial by jury for each and every issue so permitted by law and statute.

Dated: June 10, 2013

Respectfully submitted,

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CERTIFICATE OF SERVICE

This is to certify that on the 10th day of June, 2013, the undersigned were served with the foregoing using the Court's Electronic Case Filing System:

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EXHIBIT A

US008399645B2

(12) **United States Patent**
Campana et al.

(10) **Patent No.: US 8,399,645 B2**
(45) **Date of Patent: Mar. 19, 2013**

(54) **CHIMERIC RECEPTORS WITH 4-1BB STIMULATORY SIGNALING DOMAIN**

(75) Inventors: **Dario Campana**, Germantown, TN (US); **Chihaya Imai**, Niigata (JP)

(73) Assignee: **St. Jude Children's Research Hospital, Inc.**, Memphis, TN (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **13/548,148**

(22) Filed: **Jul. 12, 2012**

(65) **Prior Publication Data**

US 2012/0282256 A1 Nov. 8, 2012

Related U.S. Application Data

(63) Continuation of application No. 13/244,981, filed on Sep. 26, 2011, now abandoned, which is a continuation of application No. 12/206,204, filed on Sep. 8, 2008, now Pat. No. 8,026,097, which is a continuation of application No. 11/074,525, filed on Mar. 8, 2005, now Pat. No. 7,435,596, which is a continuation-in-part of application No. 10/981,352, filed on Nov. 4, 2004, now abandoned.

(60) Provisional application No. 60/517,507, filed on Nov. 5, 2003.

(51) **Int. Cl.**
C07H 21/04 (2006.01)
C12N 15/00 (2006.01)

(52) **U.S. Cl.** **536/23.4**; 435/320.1; 435/455

(58) **Field of Classification Search** ..... None
See application file for complete search history.

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(57) **ABSTRACT**

The present invention relates to a chimeric receptor capable of signaling both a primary and a co-stimulatory pathway, thus allowing activation of the co-stimulatory pathway without binding to the natural ligand. The cytoplasmic domain of the receptor contains a portion of the 4-1BB signaling domain. Embodiments of the invention relate to polynucleotides that encode the receptor, vectors and host cells encoding a chimeric receptor, particularly including T cells and natural killer (NK) cells and methods of use.

22 Claims, 2 Drawing Sheets

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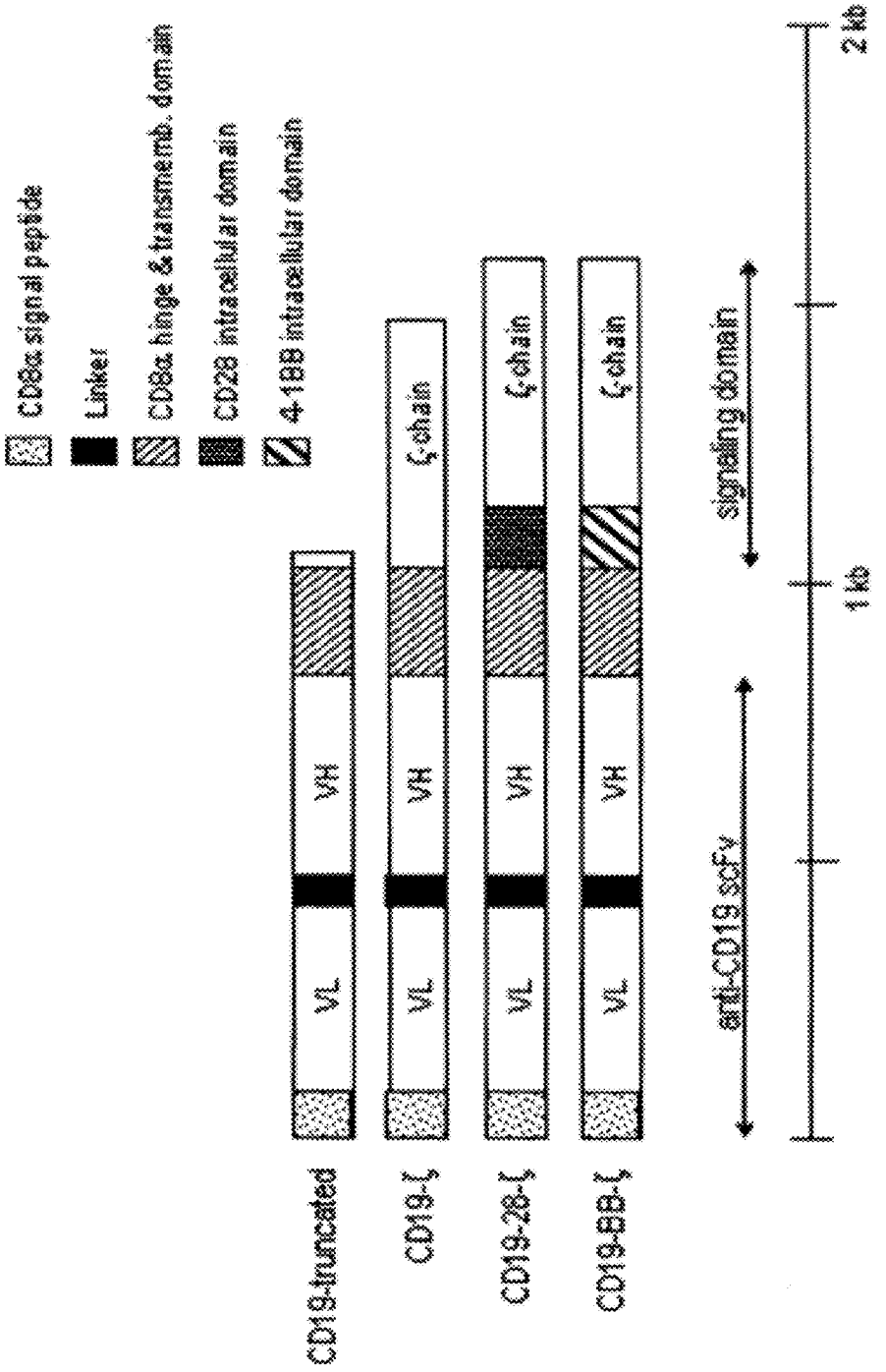
CD8α signal peptide
Linker
CD8α hinge & transmemb. domain
CD28 intracellular domain
4-1BB intracellular domain
VL
VH
ζ-chain
CD19-truncated
CD19-ζ
CD19-28-ζ
CD19-BB-ζ
anti-CD19 scFv
signaling domain
1 kb
2 kb

Figure 1

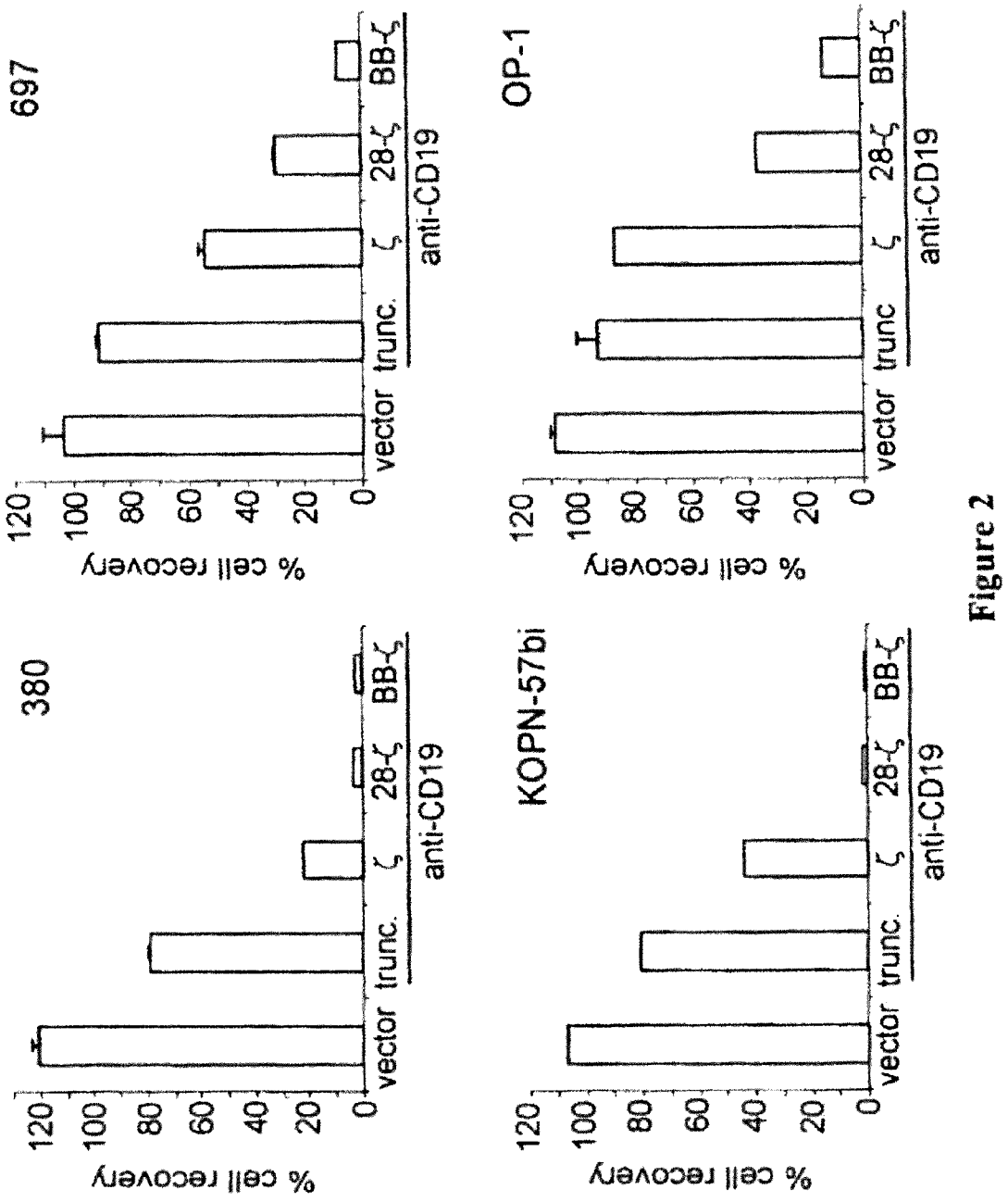
697
OP-1
380
KOPN-57bi
% cell recovery
120
100
80
60
40
20
0
vector
trunc.
ζ
28-ζ
BB-ζ
anti-CD19

Figure 2

CHIMERIC RECEPTORS WITH 4-1BB STIMULATORY SIGNALING DOMAIN

2. CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. application Ser. No. 13/244,981, filed Sep. 26, 2011 now abandoned, which is a continuation of U.S. patent application Ser. No. 12/206,204, filed on Sep. 8, 2008 (granted as U.S. Pat. No. 8,026,097), which is a continuation of U.S. patent application Ser. No. 11/074,525, filed on Mar. 8, 2005 (granted as U.S. Pat. No. 7,435,596), which is a continuation-in-part of U.S. patent application Ser. No. 10/981,352 filed Nov. 4, 2004 (abandoned), which claims the benefit of U.S. Provisional Patent Application Ser. No. 60/517,507 filed on Nov. 5, 2003, each of which is incorporated herein by reference in its entirety.

The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. The Sequence Listing is being concurrently submitted via EFS-Web as an ASCII text file named 13213-005-999_Sequence_Listing.txt, created Jul. 12, 2012, and being 16,298 bytes in size.

1. GOVERNMENT INTEREST

This invention was made in part with U.S. Government support under National Institutes of Health grant No. CA 58297. The U.S. Government may have certain rights in this invention.

3. FIELD OF THE INVENTION

This invention relates to chimeric cell membrane receptors, particularly chimeric T-cell receptors. This invention further relates to activation and expansion of cells for therapeutic uses, in particular for activation and expansion of NK cells for chimeric receptor-based cell therapy.

4. BACKGROUND

Regulation of cell activities is frequently achieved by the binding of a ligand to a surface membrane receptor comprising an extracellular and a cytoplasmic domain. The formation of the complex between the ligand and the extracellular portion of the receptor results in a conformational change in the cytoplasmic portion of the receptor which results in a signal transduced within the cell. In some instances, the change in the cytoplasmic portion results in binding to other proteins, where other proteins are activated and may carry out various functions. In some situations, the cytoplasmic portion is autophosphorylated or phosphorylated, resulting in a change in its activity. These events are frequently coupled with secondary messengers, such as calcium, cyclic adenosine monophosphate, inositol phosphate, diacylglycerol, and the like. The binding of the ligand to the surface membrane receptor results in a particular signal being transduced.

For T-cells, engagement of the T-cell receptor (TCR) alone is not sufficient to induce persistent activation of resting naive or memory T cells. Full, productive T cell activation requires a second co-stimulatory signal from a competent antigen-presenting cell (APC). Co-stimulation is achieved naturally by the interaction of the co-stimulatory cell surface receptor on the T cell with the appropriate counter-receptor on the surface of the APC. An APC is normally a cell of host origin which displays a moiety which will cause the stimulation of

an immune response. APCs include monocyte/macrophages, dendritic cells, B cells, and any number of virally-infected or tumor cells which express a protein on their surface recognized by T cells. To be immunogenic APCs must also express on their surface a co-stimulatory molecule. Such APCs are capable of stimulating T cell proliferation, inducing cytokine production, and acting as targets for cytolytic T cells upon direct interaction with the T cell. See Linsley and Ledbetter, Ann. Rev. Immunol. 4:191-212 (1993); Johnson and Jenkins, Life Sciences 55:1767-1780 (1994); June et al., Immunol. Today 15:321-331 (1994); and Mondino and Jenkins, J. Leuk. Biol. 55:805-815 (1994).

Engagement of the co-stimulatory molecule together with the TCR is necessary for optimal levels of IL-2 production, proliferation and clonal expansion, and generation of effector functions such as the production of immunoregulatory cytokines, induction of antibody responses from B cells, and induction of cytolytic activity. More importantly, engagement of the TCR in the absence of the co-stimulatory signal results in a state of non-responsiveness, called anergy. Anergic cells fail to become activated upon subsequent stimulation through the TCR, even in the presence of co-stimulation, and in some cases may be induced to die by a programmed self-destruct mechanism.

In certain situations, for example where APCs lack the counter-receptor molecules necessary for co-stimulation, it would be beneficial to have the co-stimulatory signal induced by virtue of employing a ligand other than the natural ligand for the co-stimulatory receptor. This might be, for example, the same ligand as that recognized by the TCR (i.e., the same moiety, such that if one signal is received, both signals will be received), or another cell surface molecule known to be present on the target cells (APCs).

Several receptors that have been reported to provide co-stimulation for T-cell activation, including CD28, OX40, CD27, CD2, CD5, ICAM-1, LFA-1 (CD11a/CD18), and 4-1BB. The signaling pathways utilized by these co-stimulatory molecules share the common property of acting in synergy with the primary T cell receptor activation signal.

Previously the signaling domain of CD28 has been combined with the T-cell receptor to form a co-stimulatory chimeric receptor. See U.S. Pat. No. 5,686,281; Geiger, T. L. et al., *Blood* 98: 2364-2371 (2001); Hombach, A. et al., *J Immunol* 167: 6123-6131 (2001); Maher, J. et al. *Nat Biotechnol* 20: 70-75 (2002); Haynes, N. M. et al., *J Immunol* 169: 5780-5786 (2002); Haynes, N. M. et al., *Blood* 100: 3155-3163 (2002). These co-stimulatory receptors provide a signal that is synergistic with the primary effector activation signal, i.e. the TCR signal or the chimeric effector function receptor signal, and can complete the requirements for activation under conditions where stimulation of the TCR or chimeric effector function receptor is suboptimal and might otherwise be detrimental to the function of the cell. These receptors can support immune responses, particularly of T cells, by permitting the use of ligands other than the natural ligand to provide the required co-stimulatory signal.

Chimeric receptors that contain a CD19 specific single chain immunoglobulin extracellular domain have been shown to lyse CD19+ target cells and eradicate CD19+ B cell lymphomas engrafted in mice [Cooper L J, et al., Blood 101:1637-1644 (2003) and Brentjens R J, et al., Nature Medicine 9:279-286 (2003)]. Cooper et al. reported that T-cell clones transduced with chimeric receptors comprising anti-CD19 scFv and CD3ζ produced approximately 80% specific lysis of B-cell leukemia and lymphoma cell lines at a 1:1 effector to target ratio in a 4-hour Cr release assay; at this ratio, percent specific lysis of one primary B-lineage ALL

sample tested was approximately 30%. Brentjens et al. reported that T-cells bearing anti-CD19 scFv and CD3ζ chimeric receptors could be greatly expanded in the presence of exogenous IL-15 and artificial antigen-presenting cells transduced with CD19 and CD80. The authors showed that these T cells significantly improved the survival of immunodeficient mice engrafted with the Raji B-cell lymphoma cell line. Their results also confirmed the importance of co-stimulation in maximizing T-cell-mediated anti-leukemic activity. Only cells expressing the B7 ligands of CD28 elicited effective T-cell responses. This could be a major obstacle in the case of B-lineage ALL because leukemic lymphoblasts typically do not express B7 molecules.

In addition to T cell immune responses, natural killer (NK) cell responses appear to be clinically relevant. While T cells recognize tumor associated peptide antigen expressed on surface HLA class I or class II molecules, antigen nonspecific immune responses are mediated by NK cells that are activated by the failure to recognize cognate "self" HLA class I molecules. The graft-versus-tumor effect of transplants using HLA matched donors is mediated by antigen specific T cells, while transplantation using HLA mismatched donors can also lead to donor NK cells with potent antitumor activity. HLA mismatched haplo-identical transplants can exert a powerful anti-leukemia effect based on expansion of antigen nonspecific donor NK cells.

Immunotherapy with NK cells has been limited by the inability to obtain sufficient numbers of pure NK cells suitable for manipulation and expansion. The established methods for cell expansion favor T cell expansion and even after T cells are depleted, residual T cells typically become prominent after stimulation. Thus there is a need for better methods to expand NK cells from a population without expanding T cells.

5. SUMMARY OF THE INVENTION

The present invention provides a chimeric receptor containing a co-stimulatory signal by incorporation of the signaling domain of the 4-1BB receptor. The chimeric receptor comprises an extracellular ligand binding domain, a transmembrane domain and a cytoplasmic domain wherein the cytoplasmic domain comprises the signaling domain of 4-1BB. In one embodiment of the invention the signaling domain of 4-1BB used in the chimeric receptor is of human origin. In a preferred embodiment, human 4-1BB consists of SEQ ID NO:2. In another embodiment the signaling domain comprises amino acids 214-255 of SEQ ID NO:2.

In another embodiment of the invention the cytoplasmic domain of the chimeric receptor comprises the signaling domain of CD3ζ in addition to the signaling domain of 4-1BB. In another embodiment the extracellular domain comprises a single chain variable domain of an anti-CD19 monoclonal antibody. In another embodiment the transmembrane domain comprises the hinge and transmembrane domains of CD8α. In a most preferred embodiment of the invention the extracellular domain comprises a single chain variable domain of an anti-CD19 monoclonal antibody, the transmembrane domain comprises the hinge and transmembrane domain of CD8α, and the cytoplasmic domain comprises the signaling domain of CD3ζ and the signaling domain of 4-1BB.

Other aspects of the invention include polynucleotide sequences, vectors and host cells encoding a chimeric receptor that comprises the signaling domain of 4-1BB. Yet other aspects include methods of enhancing T lymphocyte or natural killer (NK) cell activity in an individual and treating an individual suffering from cancer by introducing into the individual a T lymphocyte or NK cell comprising a chimeric receptor that comprises the signaling domain of 4-1BB. These aspects particularly include the treatment of lung cancer, melanoma, breast cancer, prostate cancer, colon cancer, renal cell carcinoma, ovarian cancer, neuroblastoma, rhabdomyosarcoma, leukemia and lymphoma. Preferred cancer targets for use with the present invention are cancers of B cell origin, particularly including acute lymphoblastic leukemia, B-cell chronic lymphocytic leukemia and B-cell non-Hodgkin's lymphoma.

A different but related aspect of the present invention provides a method for obtaining an enriched NK cell population suitable for transduction with a chimeric receptor that comprises the signaling domain of 4-1BB. This method comprises the expansion of NK cells within a mixed population of NK cells and T cells by co-culturing the mixed population of cells with a cell line that activates NK cells and not T lymphocytes. This NK activating cell line is composed of cells that activate NK cells, but not T lymphocytes, and which express membrane bound interleukin-15 and a co-stimulatory factor ligand. In a particular embodiment the NK activating cell line is the K562 myeloid leukemia cell line or the Wilms tumor cell line HFWT. In another embodiment of the invention the co-stimulatory factor ligand is CD137L.

Another aspect of the present invention is based on the concept that expression of chimeric receptors on NK cells could overcome HLA-mediated inhibitory signals, thus endowing the cells with cytotoxicity against otherwise NK-resistant cells. The invention provides a method that allows specific and vigorous preferential expansion of NK cells lacking T-cell receptors ($CD56^+$ $CD3^-$ cells) and their highly efficient transduction with chimeric receptors.

6. DESCRIPTION OF THE SEQUENCE LISTING

SEQ ID No. 1 is the nucleotide sequence for human 4-1BB mRNA. The coding sequence for the human 4-1BB protein begins at position 129 and ends at position 893.

SEQ ID No. 2 is the amino acid sequence of human 4-1BB. The signaling domain begins at position 214 and ends at position 255.

SEQ. ID. No. 3 is the nucleotide sequence for murine 4-1BB mRNA. The coding sequence for the murine 4-1BB protein begins at position 146 and ends at position 916.

SEQ ID. No. 4 is the amino acid sequence of murine 4-1BB. The signaling domain begins at position 209 and ends at position 256.

7. DESCRIPTION OF THE FIGURES

FIG. **1** is a schematic representation of the CD19-truncated, CD19-ζ, CD19-28-ζ and CD19-BB-ζ receptor constructs.

FIG. **2** shows the percent of CD19-positive leukemia cell recovery in four different cell lines (380, 697, KOPN-57bi and OP-1) after 24 hours of culture with NK cells with or without a chimeric receptor at a 1:1 ratio relative to cultures with no NK cells. The bars represent each of the 4 cell lines that are co-cultured with NK cells containing either "vector" which is MSCV-IRES GFP only; "trunc." which is vector containing truncated anti-CD19; "ζ" which is vector containing anti-CD19-CD3ζ; "28 ζ" which is vector containing anti-CD19-CD28α-CD3ζ; or "BB-ζ" which is vector containing anti-CD19-4-1BB intracellular domain-CD3ζ. This figure

shows that chimeric receptors confer anti-ALL activity to NK cells which is improved by the addition of the co-stimulatory molecules CD28 or 4-1BB.

8. DETAILED DESCRIPTION OF THE INVENTION

Definitions

4-1BB: The term "4-1BB" refers to a membrane receptor protein also termed CD137, which is a member of the tumor necrosis factor receptor (TNFR) superfamily expressed on the surface of activated T-cells as a type of accessory molecule [Kwon et al., Proc. Natl. Acad. Sci. USA 86:1963 (1989); Pollok et al., J. Immunol. 151:771 (1993)]. 4-1BB has a molecular weight of 55 kDa, and is found as a homodimer. It has been suggested that 4-1BB mediates a signal transduction pathway from outside of the cell to inside [Kim et al., J. Immunol. 151:1255 (1993)].

A human 4-1BB gene (SEQ ID NO:1) was isolated from a cDNA library made from activated human peripheral T-cell mRNA [Goodwin et al., Eur. J. Immunol. 23:2631 (1993);]. The amino acid sequence of human 4-1BB (SEQ ID NO: 2) shows 60% homology to mouse 4-1BB (SEQ ID NO:4) [Kwon et al., Proc. Natl. Acad. Sci. USA 86:1963 (1989); Gen Bank No: NM_011612] which indicates that the sequences are highly conserved. As mentioned supra, 4-1BB belongs to the TNFR superfamily, along with CD40, CD27, TNFR-I, TNFR-II, Fas, and CD30 [Alderson et al., Eur. J. Immunol. 24:2219 (1994)]. When a monoclonal antibody is bound to 4-1BB expressed on the surface of mouse T-cells, anti-CD3 T-cell activation is increased many fold [Pollok et al., J. Immunol. 150:771 (1993)].

4-1BB binds to a high-affinity ligand (4-1BB, also termed CD137L) expressed on several antigen-presenting cells such as macrophages and activated B cells [Pollok et al., J. Immunol. 150:771 (1993) Schwarz et al., Blood 85:1043 (1995)). The interaction of 4-1BB and its ligand provides a co-stimulatory signal leading to T cell activation and growth [Goodwin et al., Eur. J. Immunol. 23:2631 (1993); Alderson et al., Eur. J. Immunol. 24:2219 (1994); Hurtado et al., J. Immunol. 155:3360 (1995); Pollock et al., Eur. J. Immunol. 25:488 (1995); DeBenedette et al., J. Exp. Med. 181:985 (1995)]. These observations suggest an important role for 4-1BB in the regulation of T cell-mediated immune responses [Ignacio et al., Nature Med. 3:682 (1997)].

4-1BB ligand (CD137L) is claimed and described in U.S. Pat. No. 5,674,704.

The term IL-15 (interleukin 15) refers to a cytokine that stimulates NK cells [Fehniger T A, Caligiuri M A. Blood 97(1):14-32 (2001)]. It has become apparent that IL-15 presented through cell-to-cell contact has a higher NK stimulating activity than soluble IL-15 [Dubois S, et al., Immunity 17(5):537-547 (2002); Kobayashi H, et al., Blood (2004) PMID: 15367431; Koka R, et al., J Immunol 173(6):3594-3598 (2004); Burkett P R, et al., J Exp Med 200(7):825-834 (2004)]. To express membrane-bound IL-15 a construct consisting of human IL-15 mature peptide (NM 172174) was fused to the signal peptide and transmembrane domain of human CD8α.

To specifically or preferentially expand NK cells means to culture a mixed population of cells that contains a small number of NK cells so that the NK cells proliferate to numbers greater than other cell types in the population.

To activate T cells and NK cells means to induce a change in their biologic state by which the cells express activation markers, produce cytokines, proliferate and/or become cytotoxic to target cells. All these changes can be produced by primary stimulatory signals. Co-stimulatory signals amplify the magnitude of the primary signals and suppress cell death following initial stimulation resulting in a more durable activation state and thus a higher cytotoxic capacity.

The terms T-cell and T lymphocyte are interchangeable and used synonymously herein.

The term "chimeric receptor" as used herein is defined as a cell-surface receptor comprising an extracellular ligand binding domain, a transmembrane domain and a cytoplasmic co-stimulatory signaling domain in a combination that is not naturally found together on a single protein. This particularly includes receptors wherein the extracellular domain and the cytoplasmic domain are not naturally found together on a single receptor protein. The chimeric receptors of the present invention are intended primarily for use with T cells and natural killer (NK) cells.

The term "host cell" means any cell of any organism that is selected, modified, transformed, grown, used or manipulated in any way, for the production of a substance by the cell, for example the expression by the cell of a gene, a DNA or RNA sequence, a protein or an enzyme. Host cells of the present invention include T cells and NK cells that contain the DNA or RNA sequences encoding the chimeric receptor and express the chimeric receptor on the cell surface. Host cells may be used for enhancing T lymphocyte activity, NK cell activity, treatment of cancer, and treatment of autoimmune diseases.

The terms "express" and "expression" mean allowing or causing the information in a gene or DNA sequence to become manifest, for example producing a protein by activating the cellular functions involved in transcription and translation of a corresponding gene or DNA sequence. A DNA sequence is expressed in or by a cell to form an "expression product" such as a protein. The expression product itself, e.g. the resulting protein, may also be said to be "expressed" by the cell. An expression product can be characterized as intracellular, extracellular or transmembrane. The term "intracellular" means something that is inside a cell. The term "extracellular" means something that is outside a cell. The term transmembrane means something that has an extracellular domain outside the cell, a portion embedded in the cell membrane and an intracellular domain inside the cell.

The term "transfection" means the introduction of a foreign nucleic acid into a cell using recombinant DNA technology. The term "transformation" means the introduction of a "foreign" (i.e. extrinsic or extracellular) gene, DNA or RNA sequence to a host cell, so that the host cell will express the introduced gene or sequence to produce a desired substance, typically a protein or enzyme coded by the introduced gene or sequence. The introduced gene or sequence may also be called a "cloned" or "foreign" gene or sequence, may include regulatory or control sequences, such as start, stop, promoter, signal, secretion, or other sequences used by a cell's genetic machinery. The gene or sequence may include nonfunctional sequences or sequences with no known function. A host cell that receives and expresses introduced DNA or RNA has been "transformed" and is a "transformant" or a "clone." The DNA or RNA introduced to a host cell can come from any source, including cells of the same genus or species as the host cell, or cells of a different genus or species.

The term "transduction" means the introduction of a foreign nucleic acid into a cell using a viral vector.

The terms "vector", "cloning vector" and "expression vector" mean the vehicle by which a DNA or RNA sequence (e.g. a foreign gene) can be introduced into a host cell, so as to transform the host and promote expression (e.g. transcription

and translation) of the introduced sequence. Vectors include plasmids, phages, viruses, etc.

A solid support means any surface capable of having an agent attached thereto and includes, without limitation, metals, glass, plastics, polymers, particles, microparticles, copolymers, colloids, lipids, lipid bilayers, cell surfaces and the like. Essentially any surface that is capable of retaining an agent bound or attached thereto. A prototypical example of a solid support used herein, is a particle such as a bead.

The term "substantially free of" means a population of cells, e.g. NK cells, that is at least 50% free of non-NK cells, or in certain embodiments at least 60, 70, 80, 85, or 90% free of non-NK cells.

A "co-stimulatory signal" refers to a signal, which in combination with a primary signal, such as TCR/CD3 ligation, leads to NK cell proliferation and/or upregulation or downregulation of key molecules.

Description of the Invention

In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook et al, "Molecular Cloning: A Laboratory Manual" (1989); "Current Protocols in Molecular Biology" Volumes [-III [Ausubel, R. M., ed. (1994)]; "Cell Biology: A Laboratory Handbook" Volumes I-III [J. E. Celis, ed. (1994))]; "Current Protocols in Immunology" Volumes I-III [Coligan, J. E., ed. (1994)]; "Oligonucleotide Synthesis" (M. J. Gait ed. 1984); "Nucleic Acid Hybridization" [B. D. Haines & S. J. Higgins eds. (1985)]; "Transcription And Translation" [B. D. Haines & S. J. Higgins, eds. (1984)]; "Animal Cell Culture" [R. I. Freshney, ed. (1986)]; "Immobilized Cells And Enzymes" [IRL Press, (1986)]; B. Perbal, "A Practical Guide To Molecular Cloning" (1984); CURRENT PROTOCOLS IN IMMUNOLOGY Q. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach and W. Strober, eds., 1991); ANNUAL REVIEW OF IMMUNOLOGY; as well as monographs in journals such as ADVANCES IN IMMUNOLOGY. All patents, patent applications, and publications mentioned herein are hereby incorporated herein by reference.

Primary T cells expressing chimeric receptors specific for tumor or viral antigens have considerable therapeutic potential as immunotherapy reagents. Unfortunately, their clinical value is limited by their rapid loss of function and failure to expand in vivo, presumably due to the lack of co-stimulator molecules on tumor cells and the inherent limitations of signaling exclusively through the chimeric receptor.

The chimeric receptors of the present invention overcome this limitation wherein they have the capacity to provide both the primary effector activity and the co-stimulatory activity upon binding of the receptor to a single ligand. For instance, binding of the anti-CD19-BB-ζ receptor to the CD19 ligand provides not only the primary effector function, but also a proliferative and cytolytic effect.

T cells transduced with anti-CD19 chimeric receptors of the present invention which contain co-stimulatory molecules have remarkable anti-ALL capacity. However, the use of allogenic receptor-modified T cells after hematopoietic cell transplantation might carry the risk of severe graft-versus-host disease (GvHD). In this setting, the use of CD3-negative NK cells is attractive because they are not expected to cause GvHD.

Studies suggest an anti-tumor effect of NK cells and Zeis et al., Br J Haematol 96: 757-61 (1997) have shown in mice that NK cells contribute to a graft-versus-leukemia effect, without inducing GvHD.

Expanding NK cells which can then be transfected with chimeric receptors according to this method represents another aspect of the present invention.

The chimeric receptors of the present invention comprise an extracellular domain, a transmembrane domain and a cytoplasmic domain. The extracellular domain and transmembrane domain can be derived from any desired source for such domains.

As described in U.S. Pat. Nos. 5,359,046, 5,686,281 and 6,103,521, the extracellular domain may be obtained from any of the wide variety of extracellular domains or secreted proteins associated with ligand binding and/or signal transduction. The extracellular domain may be part of a protein which is monomeric, homodimeric, heterodimeric, or associated with a larger number of proteins in a non-covalent complex. In particular, the extracellular domain may consist of an Ig heavy chain which may in turn be covalently associated with Ig light chain by virtue of the presence of CH1 and hinge regions, or may become covalently associated with other Ig heavy/light chain complexes by virtue of the presence of hinge, CH2 and CH3 domains. In the latter case, the heavy/light chain complex that becomes joined to the chimeric construct may constitute an antibody with a specificity distinct from the antibody specificity of the chimeric construct. Depending on the function of the antibody, the desired structure and the signal transduction, the entire chain may be used or a truncated chain may be used, where all or a part of the CH1, CH2, or CH3 domains may be removed or all or part of the hinge region may be removed.

Wherein an antitumor chimeric receptor is utilized, the tumor may be of any kind as long as it has a cell surface antigen which may be recognized by the chimeric receptor. In a specific embodiment, the chimeric receptor may be for any cancer for which a specific monoclonal antibody exists or is capable of being generated. In particular, cancers such as neuroblastoma, small cell lung cancer, melanoma, ovarian cancer, renal cell carcinoma, colon cancer, Hodgkin's lymphoma, and childhood acute lymphoblastic leukemia have antigens specific for the chimeric receptors.

The transmembrane domain may be contributed by the protein contributing the multispecific extracellular inducer clustering domain, the protein contributing the effector function signaling domain, the protein contributing the proliferation signaling portion, or by a totally different protein. For the most part it will be convenient to have the transmembrane domain naturally associated with one of the domains. In some cases it will be desirable to employ the transmembrane domain of the .zeta., .eta. or Fc.epsilon.R1.gamma. chains which contain a cysteine residue capable of disulfide bonding, so that the resulting chimeric protein will be able to form disulfide linked dimers with itself, or with unmodified versions of the .zeta., .eta. or Fc.epsilon.R1.gamma. chains or related proteins. In some instances, the transmembrane domain will be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex. In other cases it will be desirable to employ the transmembrane domain of .zeta., .eta., Fc.epsilon. R1-.gamma. and -.beta., MB1 (Ig.alpha.), B29 or CD3-.gamma., .zeta., or .epsilon., in order to retain physical association with other members of the receptor complex.

The cytoplasmic domain of the chimeric receptors of the invention will comprise the 4-1BB signaling domain by itself or combined with any other desired cytoplasmic domain(s) useful in the context of this chimeric receptor type. In a most preferred embodiment of the invention the extracellular domain comprises a single chain variable domain of an anti-CD19 monoclonal antibody, the transmembrane domain comprises the hinge and transmembrane domain of CD8α, and the cytoplasmic domain comprises the signaling domain of CD3ζ and the signaling domain of 4-1BB. The extracellular domain of the preferred embodiment contains the anti-CD19 monoclonal antibody which is described in Nicholson IC, et al., Mol Immunol 34:1157-1165 (1997) plus the 21 amino acid signal peptide of CD8α (translated from 63 nucleotides at positions 26-88 of GenBank Accession No. NM_001768). The CD8α hinge and transmembrane domain consists of 69 amino acids translated from the 207 nucleotides at positions 815-1021 of GenBank Accession No. NM_001768. The CD3ζ signaling domain of the preferred embodiment contains 112 amino acids translated from 339 nucleotides at positions 1022-1360 of GenBank Accession No. NM_000734.

Antigen-specific cells can be expanded in vitro for use in adoptive cellular immunotherapy in which infusions of such cells have been shown to have anti-tumor reactivity in a tumor-bearing host. The compositions and methods of this invention can be used to generate a population of T lymphocyte or NK cells that deliver both primary and co-stimulatory signals for use in immunotherapy in the treatment of cancer, in particular the treatment of lung cancer, melanoma, breast cancer, prostate cancer, colon cancer, renal cell carcinoma, ovarian cancer, neuroblastoma, rhabdomyosarcoma, leukemia and lymphoma. Immunotherapeutics, generally, rely on the use of immune effector cells and molecules to target and destroy cancer cells. The effector may be a lymphocyte carrying a surface molecule that interacts, either directly or indirectly, with a tumor cell target. Various effector cells include cytotoxic T cells and NK cells. The compositions and methods described in the present invention may be utilized in conjunction with other types of therapy for cancer, such as chemotherapy, surgery, radiation, gene therapy, and so forth.

In adoptive immunotherapy, the patient's circulating lymphocytes, or tumor infiltrated lymphocytes, are isolated in vitro, activated by lymphokines such as IL-2 or transduced with genes for tumor necrosis, and readministered [Rosenberg et al., N. Engl. J. Med. 319:1767 (1988)]. To achieve this, one would administer to an animal, or human patient, an immunologically effective amount of activated lymphocytes genetically modified to express a tumor-specific chimeric receptor gene as described herein. The activated lymphocytes will most preferably be the patient's own cells that were earlier isolated from a blood or tumor sample and activated and expanded in vitro. In aspects of the present invention T lymphocytes or NK cells from a patient having a cancer of B cell origin such as lymphoblastic leukemia, B-cell chronic lymphocytic leukemia or B-cell non-Hodgkin's lymphoma would be isolated and transduced with the CD19-BB-ζ polynucleotide so that a chimeric receptor containing 4-1BB in the cytoplasmic domain is express on the cell surface of the T cell or NK cell. The modified cells would then be readministered into the patient to target and kill the tumor cells.

As shown in one Example infra, primary T-cells were transduced with the anti-CD19-BB-ζ receptor of the present invention. One week after transduction the T-cells had expanded 3-4 fold in contrast to cells that were transduced with a chimeric receptor that lacked 4-1BB. After 3 weeks in culture the T-cells had expanded by more than 16-fold.

T-cells that were transduced with the anti-CD19-BB-ζ receptor and cultured in 200 IU/mL of IL-2 for two weeks, then removed from IL-2 and exposed to irradiated OP-1 cells underwent apoptosis. However, cells cultured in 10 IU/mL of IL-2 and exposed to irradiated OP-1 cells for two weeks after transduction remained viable. T-cells that were transduced with CD19 chimeric receptors that lacked 4-1BB underwent apoptosis under these same conditions. These results show that 4-1BB co-stimulation confers a survival advantage on lymphocytes, which overcomes a major obstacle with current chimeric receptors used in immunotherapy.

To determine if T-cells transduced with the anti-CD19-BB-ζ receptor exhibited cytotoxic activity under conditions necessary for immunotherapy, their cytotoxic activity at low effector:target (E:T) ratios were measured. As described in the Example infra, T-cells transduced with the anti-CD19-BB-ζ receptor and control vectors were expanded in vitro for two weeks and mixed with OP-1 cells at various E:T ratios (1:1, 0.1:1, and 0.01:1). Viable leukemic cells were counted after one week of culture. T-cells expressing the anti-CD19-BB-ζ receptor exhibited cytotoxic activity at the 1:1 and 0.1:1 ratios against all $CD19^+$ cell lines tested. The anti-CD19-BB-ζ receptor was not effective at the 0.01:1 ratio. The CD19 chimeric receptor that lacked 4-1BB showed cytotoxic activity at the 1:1 ratio, but at the 0.1:1 ratio the results were inferior to the anti-CD19-BB-ζ receptor.

A surprising result obtained with the anti-CD19-BB-ζ receptor was that the T-cells transduced with the receptor exhibited cytotoxic activity toward $CD19^+$ leukemic cells at a ratio of 0.01:1 when the leukemic cells were co-cultured with bone marrow-derived mesenchymal cells. This result shows that T-cells transduced with the anti-CD19-BB-ζ receptor exhibit cytotoxic activity in an environment critical for B-lineage leukemic cell growth. Another unexpected result was that expression of the anti-CD19-BB-ζ receptor caused higher levels of TRAIL stimulation.

Furthermore, IL-2, which causes $CD8^+$ cells to expand more vigorously, levels in cells expressing the anti-CD19-BB-ζ receptor were higher than in cells expressing the other receptors tested. These results further support the use of the anti-CD19-BB-ζ receptor for immunotherapy.

Construction of the Anti-CD19-BB-ζ Receptor

The present invention provides a chimeric receptor construct which contains the signaling domain of 4-1BB and fragments thereof. In a preferred embodiment of the invention, the genetic fragments used in the chimeric receptor were generated using splicing by overlapping extension by PCR (SOE-PCR), a technique useful for generating hybrid proteins of immunological interest. [Warrens A N, et al. Gene 20; 186: 29-35 (1997)]. Other procedures used to generate the polynucleotides and vector constructs of the present invention are well known in the art.

Transduction of T-Cells

As shown in the Examples, infra, a polynucleotide expressing a chimeric receptor capable of providing both primary effector and co-stimulatory activities was introduced into T-cells and NK cells via retroviral transduction. References describing retroviral transduction of genes are Anderson et al., U.S. Pat. No. 5,399,346; Mann et al., Cell 33:153 (1983); Temin et al., U.S. Pat. No. 4,650,764; Temin et al., U.S. Pat. No. 4,980,289; Markowitz et al., J. Virol. 62:1120 (1988); Temin et al., U.S. Pat. No. 5,124,263; International Patent Publication No. WO 95/07358, published Mar. 16, 1995, by Dougherty et al.; and Kuo et al., Blood 82:845 (1993). International Patent Publication No. WO 95/07358 describes high efficiency transduction of primary B lymphocytes.

Expansion of NK Cells

The present invention shows that human primary NK cells may be expanded in the presence of a myeloid cell line that has been genetically modified to express membrane bound IL-15 and 4-1BB ligand (CD137L). A cell line modified in this way which does not have MHC class I and II molecules is highly susceptible to NK cell lysis and activates NK cells.

For example, K562 myeloid cells can be transduced with a chimeric protein construct consisting of human IL-15 mature peptide fused to the signal peptide and transmembrane domain of human CD8α and GFP. Transduced cells can then be single-cell cloned by limiting dilution and a clone with the highest GFP expression and surface IL-15 selected. This clone can then be transduced with human CD137L, creating a K562-mb15-137L cell line.

To preferentially expand NK cells, peripheral blood mononuclear cell cultures containing NK cells are cultured with a K562-mb15-137L cell line in the presence of 10 IU/mL of IL-2 for a period of time sufficient to activate and enrich for a population of NK cells. This period can range from 2 to 20 days, preferably about 5 days. Expanded NK cells may then be transduced with the anti-CD19-BB-ζ chimeric receptor.

Administration of Activated T Cells and NK Cells

Methods of re-introducing cellular components are known in the art and include procedures such as those exemplified in U.S. Pat. Nos. 4,844,893 and 4,690,915. The amount of activated T cells or NK cells used can vary between in vitro and in vivo uses, as well as with the amount and type of the target cells. The amount administered will also vary depending on the condition of the patient and should be determined by considering all appropriate factors by the practitioner.

Obtaining an enriched population of NK cells for use in therapy has been difficult to achieve. Specific NK cell expansion has been problematic to achieve with established methods, where CD3+ T cells preferentially expand. Even after T cell depletion, residual T cells typically become prominent after stimulation. However, in accordance with the teachings of the present invention NK cells may be preferentially expanded by exposure to cells that lack or poorly express major histocompatibility complex I and/or II molecules and which have been genetically modified to express membrane bound IL-15 and 4-1BB ligand (CDl37L). Such cell lines include, but are not necessarily limited to, K562 [ATCC, CCL 243; Lozzio et al., Blood 45(3): 321-334 (1975); Klein et al., Int. J. Cancer 18: 421-431 (1976)], and the Wilms tumor cell line HFWT. [Fehniger T A, Caligiuri M A. Int Rev Immunol 20(3-4):503-534 (2001); Harada H, et al., Exp Hematol 32(7):614-621 (2004)], the uterine endometrium tumor cell line HHUA, the melanoma cell line HMV-II, the hepatoblastoma cell line HuH-6, the lung small cell carcinoma cell lines Lu-130 and Lu-134-A, the neutoblastoma cell lines NB 19 and N1369, the embryonal carcinoma cell line from testis NEC 14, the cervix carcinoma cell line TCO-2, and the bone marrow-metastated neuroblastoma cell line TNB 1 [Harada H., et al., Jpn. J. Cancer Res 93: 313-319 (2002)]. Preferably the cell line used lacks or poorly expresses both MHC I and II molecules, such as the K562 and HFWT cell lines.

A solid support may be used instead of a cell line. Such supports will have attached on its surface at least one molecule capable of binding to NK cells and inducing a primary activation event and/or a proliferative response or capable of binding a molecule having such an affect thereby acting as a scaffold. The support may have attached to its surface the CD137 ligand protein, a CD137 antibody, the IL-15 protein or an IL-15 receptor antibody. Preferably, the support will have IL-15 receptor antibody and CD137 antibody bound on its surface.

The invention is intended to include the use of fragments, mutants, or variants (e.g., modified forms) of the IL-15 and/or CD137 ligand proteins or antigens that retain the ability to induce stimulation and proliferation of NK cells. A "form of the protein" is intended to mean a protein that shares a significant homology with the IL-15 or CD137 ligand proteins or antigen and is capable of effecting stimulation and proliferation of NK cells. The terms "biologically active" or "biologically active form of the protein," as used herein, are meant to include forms of the proteins or antigens that are capable of effecting enhanced activated NK cell proliferation. One skilled in the art can select such forms based on their ability to enhance NK cell activation and proliferation upon introduction of a nucleic acid encoding said proteins into a cell line. The ability of a specific form of the IL-15 or CD137 ligand protein or antigen to enhance NK cell proliferation can be readily determined, for example, by measuring cell proliferation or effector function by any known assay or method.

Antigen-specific cells can be expanded in vitro for use in adoptive cellular immunotherapy in which infusions of such cells have been shown to have anti-tumor reactivity in a tumor-bearing host. The compositions and methods of this invention can be used to generate a population of NK cells that deliver both primary and co-stimulatory signals for use in immunotherapy in the treatment of cancer, in particular the treatment of lung cancer, melanoma, breast cancer, prostate cancer, colon cancer, renal cell carcinoma, ovarian cancer, neuroblastoma, rhabdomyosarcoma, leukemia and lymphoma. The compositions and methods described in the present invention may be utilized in conjunction with other types of therapy for cancer, such as chemotherapy, surgery, radiation, gene therapy, and so forth.

9. EXAMPLES

9.1 Example 1

Introduction

In approximately 20% of children and 65% of adults with acute lymphoblastic leukemia (ALL), drug-resistant leukemic cells survive intensive chemotherapy and cause disease recurrence. [Pui C H et al, Childhood acute lymphoblastic leukemia—Current status and future perspectives. Lancet Oncology2:597-607 (2001); Verma A, Stock W. Management of adult acute lymphoblastic leukemia: moving toward a risk-adapted approach. Curr Opin Oncol 13:14-20T (2001)] lymphocyte-based cell therapy should bypass cellular mechanisms of drug resistance. Its potential clinical value for leukemia is demonstrated by the association between T-cell-mediated graft-versus-host disease (GvHD) and delay or suppression of leukemia recurrence after allogeneic stem cell transplantation. [Champlin R. T-cell depletion to prevent graft-versus-host disease after bone marrow transplantation. Hematol Oncol Clin North Am 4:687-698 (1990); Porter D L, Antin J H. The graft-versus-leukemia effects of allogeneic cell therapy. Annu Rev Med 50:369-86.:369-386 (1999); Appelbaum F R. Haematopoietic cell transplantation as immunotherapy. Nature 411:385-389 (2001)] Manipulation of GvHD by infusion of donor lymphocytes can produce a measurable anti-leukemic effect. [Porter D L, et al. Induction of graft-versus-host disease as immunotherapy for relapsed chronic myeloid leukemia. N Engl J Med 330:100-106 (1994); Kolb H J, et al. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. Blood 6:2041-2050 (1995); Slavin S, et al. Allogeneic cell therapy with donor peripheral blood cells and recombinant human

interleukin-2 to treat leukemia relapse after allogeneic bone marrow transplantation. Blood 87:2195-2204 (1996); Collins R H, et al. Donor leukocyte infusions in 140 patients with relapsed malignancy after allogeneic bone marrow transplantation. J Clin Oncol 15:433-444 (1997)] However, in patients with ALL this effect is often limited, [Kolb H J, et al. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. Blood 86:2041-2050 (1995); Verdonck L F, et al. Donor leukocyte infusions for recurrent hematologic malignancies after allogeneic bone marrow transplantation: impact of infused and residual donor T cells. Bone Marrow Transplant 22:1057-1063 (1998); Collins R H, Jr., et al. Donor leukocyte infusions in acute lymphocytic leukemia. Bone Marrow Transplant 26:511-516 (2000)] possibly reflecting inadequate T-cell stimulation by leukemic lymphoblasts.

T lymphocyte specificity can be redirected through expression of chimeric immune receptors consisting of an extracellular antibody-derived single-chain variable domain (scFv) and an intracellular signal transduction molecule (e.g., the signaling domain of CD3ζ or FcγRIII). [Geiger T L, Jyothi M D. Development and application of receptor-modified T lymphocytes for adoptive immunotherapy. Transfus Med Rev 15:21-34 (2001); Schumacher T N. T-cell-receptor gene therapy. Nat Rev Immunol. 2:512-519 (2002); Sadelain M, et al. Targeting tumours with genetically enhanced T lymphocytes. Nat Rev Cancer 3:35-45 (2003)] Such T lymphocytes can be activated by cell surface epitopes targeted by the scFv and can kill the epitope-presenting cells. The first requirement to redirect T cells against ALL cells is the identification of target molecules that are selectively expressed by leukemic cells. In B-lineage ALL, CD19 is an attractive target, because it is expressed on virtually all leukemic lymphoblasts in almost all cases. [Campana D, Behm F G. Immunophenotyping of leukemia. J Immunol Methods 243:59-75 (2000)] It is not expressed by normal non-hematopoietic tissues, and among hematopoietic cells, it is expressed only by B-lineage lymphoid cells. [Campana D, Behm F G. Immunophenotyping of leukemia. J Immunol Methods 243:59-75 (2000); Nadler L M, et al. B4, a human B lymphocyte-associated antigen expressed on normal, mitogen-activated, and malignant B lymphocytes. J Immunol 131:244-250 (1983)] Recent studies have shown that T-cells expressing anti-CD19 scFv and CD3ζ signaling domain can proliferate when mixed with $CD19^+$ cells and can lyse $CD19^+$ target cells. [Cooper L J, et al. T-cell clones can be rendered specific for CD19: toward the selective augmentation of the graft-versus-B-lineage leukemia effect. Blood 101:1637-1644 (2003); Brentjens R J, et al. Eradication of systemic B-cell tumors by genetically targeted human T lymphocytes co-stimulated by CD80 and interleukin-15. Nat Med 9:279-286 (2003)]

A prerequisite for the success of T-cell therapy is the capacity of the engineered T lymphocytes to expand and produce a vigorous and durable anti-leukemic response in vivo. The engagement of the TCR, although necessary, is not sufficient to fully activate T cells; a second signal, or co-stimulus, is also required. [Liebowitz D N, et al. Costimulatory approaches to adoptive immunotherapy. Curr Opin Oncol 10:533-541 (1998); Allison J P, Lanier L L. Structure, function, and serology of the T-cell antigen receptor complex. Annu Rev Immunol 5:503-540 (1987); Salomon B, Bluestone J A. Complexities of CD28/B7: CTLA-4 costimulatory pathways in autoimmunity and transplantation. Annu Rev Immunol 19:225-52.:225-252 (2001)] This could be a major obstacle for chimeric receptor-based therapy of B-lineage ALL, because B-lineage leukemic lymphoblasts generally lack B7 molecules that bind to CD28 on T-lymphocytes and trigger the CD28-mediated co-stimulatory pathway. [Cardoso A A, et al. Pre-B acute lymphoblastic leukemia cells may induce T-cell anergy to alloantigen. Blood 88:41-48 (1996)] This limitation might be overcome by incorporating the signal transduction domain of CD28 into chimeric receptors. [Eshhar Z, et al. Functional expression of chimeric receptor genes in human T cells. J Immunol Methods 2001; 248:67-76 (2001); Hombach A, et al. Tumor-specific T cell activation by recombinant immunoreceptors: CD3 zeta signaling and CD28 costimulation are simultaneously required for efficient IL-2 secretion and can be integrated into one combined CD28/CD3 zeta signaling receptor molecule. J Immunol 167: 6123-6131 (2001); Geiger T L, et al. Integrated src kinase and costimulatory activity enhances signal transduction through single-chain chimeric receptors in T lymphocytes. Blood 98:2364-2371 (2001); Maher J, et al. Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRzeta/CD28 receptor. Nat Biotechnol 20:70-75 (2002)] Murine T cells bearing such receptors have shown a greater capacity to inhibit cancer cell growth and metastasis in mice than those with chimeric receptors lacking this domain. [Haynes N M, et al. Rejection of syngeneic colon carcinoma by CTLs expressing single-chain antibody receptors codelivering CD28 costimulation. J Immunol 169:5780-5786 (2002); Haynes N M, et al. Single-chain antigen recognition receptors that costimulate potent rejection of established experimental tumors. Blood 100:3155-3163 (2002)]

A second co-stimulatory pathway in T cells, independent of CD28 signaling, is mediated by 4-1BB (CD137), a member of the tumor necrosis factor (TNF) receptor family. [Sica G, Chen L. Modulation of the immune response through 4-1BB. In: Habib N, ed. Cancer gene therapy: past achievements and future challenges. New York: Kluwer Academic/Plenum Publishers; 355-362 (2000)] 4-1BB stimulation significantly enhances survival and clonal expansion of CD8+ T-lymphocytes, and CD8+ T-cell responses in a variety of settings, including viral infection, allograft rejection, and tumor immunity. [Shuford W W, et al. 4-1BB costimulatory signals preferentially induce CD8+ T cell proliferation and lead to the amplification in vivo of cytotoxic T cell responses. J Exp Med 186:47-55 (1997); Melero I, et al. Monoclonal antibodies against the 4-1BB T-cell activation molecule eradicate established tumors. Nat Med 3:682-685 (1997); Melero I, et al. Amplification of tumor immunity by gene transfer of the co-stimulatory 4-1BB ligand: synergy with the CD28 co-stimulatory pathway. Eur J Immunol 28:1116-1121 (1998); Takahashi C, et al. Cutting edge: 4-1BB is a bona fide CD8 T cell survival signal. J Immunol 162:5037-5040 (1999); Martinet O, et al. T cell activation with systemic agonistic antibody versus local 4-1BB ligand gene delivery combined with interleukin-12 eradicate liver metastases of breast cancer. Gene Ther 9:786-792 (2002); May K F, Jr., et al. Anti-4-1BB monoclonal antibody enhances rejection of large tumor burden by promoting survival but not clonal expansion of tumor-specific CD8+ T cells. Cancer Res 62:3459-3465 (2002)] However, the natural ligand of 4-1BB is weakly and heterogeneously expressed in B-lineage ALL cells (C. Imai, D. Campana, unpublished observations). Therefore, it is likely that this important co-stimulatory signal, like CD28, can become operational only if 4-1BB is added to chimeric receptors. However, it is not known whether such receptors would help deliver effective T-cell responses to cancer cells and, if so, whether these would be equivalent to those elicited by receptors containing CD28.

We constructed a chimeric T-cell receptor specific for CD19 that contains a 4-1BB signaling domain. We determined whether T cells transduced with these receptors could

effectively destroy B-lineage ALL cell lines and primary leukemic cells under culture conditions that approximate the in vivo microenvironment where leukemic cells grow. We compared the properties of T-cells expressing the 4-1BB-containing receptor to those of T-cells expressing an equivalent receptor lacking 4-1BB or containing CD28 instead.

Materials and Methods

Cells

Available in our laboratory were the human B-lineage ALL cell line OP-1, developed from the primary leukemic cells of a patient with newly diagnosed B-lineage ALL with the t(9;22)(q34;q11) karyotype and the BCR-ABL gene fusion; [Manabe A, et al. Interleukin-4 induces programmed cell death (apoptosis) in cases of high-risk acute lymphoblastic leukemia. Blood 83:1731-1737 (1994)] the B-lineage ALL cell lines RS4;11, [Stong R C, et al. Human acute leukemia cell line with the t(4;11) chromosomal rearrangement exhibits B lineage and monocytic characteristics. Blood 1985; 65:21-31 (1985)] and REH [Rosenfeld C, et al. Phenotypic characterisation of a unique non-T, non-B acute lymphoblastic leukaemia cell line. Nature 267:841-843 (1977)]; the T-cell lines Jurkat [Schneider U, et al. Characterization of EBV-genome negative "null" and "T" cell lines derived from children with acute lymphoblastic leukemia and leukemic transformed non-Hodgkin lymphoma. Int J Cancer 1977; 19:621-626 (1977)] and CEM-C7 [Harmon J M, et al. Dexamethasone induces irreversible G1 arrest and death of a human lymphoid cell line. J Cell Physiol 98:267-278 (1979)]; and the myeloid cell lines K562 [Koeffler H P, Golde D W. Acute myelogenous leukemia: a human cell line responsive to colony-stimulating activity. Science 200:1153-1154 (1978)] and U-937. [Sundstrom C, Nilsson K. Establishment and characterization of a human histiocytic lymphoma cell line (U-937). Int J Cancer 1976; 17:565-577 (1976)] Cells were maintained in RPMI-1640 (Gibco, Grand Island, N.Y.) with 10% fetal calf serum (FCS; BioWhittaker, Walkersville, Md.) and antibiotics. Human adenocarcinoma HeLa cells and embryonic kidney fibroblast 293T cells, maintained in DMEM (MediaTech, Herndon, Va.) supplemented with 10% FCS and antibiotics, were also used.

We used primary leukemia cells obtained from 5 patients with newly diagnosed B-lineage ALL with the approval of the St. Jude Children's Research Hospital Institutional Review Board and with appropriate informed consent. The diagnosis of B-lineage ALL was unequivocal by morphologic, cytochemical, and immunophenotypic criteria; in each case, more than 95% of leukemic cells were positive for CD19. Peripheral blood samples were obtained from 7 healthy adult donors. Mononuclear cells were collected from the samples by centrifugation on a Lymphoprep density step (Nycomed, Oslo, Norway) and were washed two times in phosphate-buffered saline (PBS) and once in AIM-V medium (Gibco).

Plasmids

The plasmid encoding anti-CD19 scFv was obtained from Dr. I. Nicholson (Child Health Research Institute, Adelaide, Australia). [Nicholson I C, et al. Construction and characterisation of a functional CD19 specific single chain Fv fragment for immunotherapy of B lineage leukaemia and lymphoma. Mol Immunol 34:1157-1165 (1997)] The pMSCV-IRES-GFP, pEQPAM3(-E), and pRDF were obtained from Dr. E. Vanin at our institution. Signal peptide, hinge and transmembrane domain of CD8α, and intracellular domains of 4-1BB, CD28, CD3ζ and CD19 were subcloned by polymerase chain reaction (PCR) using a human spleen cDNA library (from Dr. G. Neale, St. Jude Children's Research Hospital) as a template. FIG. **1** shows a schematic representation of the anti-CD19-ζ, anti-CD19-BB-ζ anti-CD19-28-sand anti-CD19-truncated (control) constructs. We used splicing by overlapping extension by PCR (SOE-PCR) to assemble several genetic fragments. [Warrens A N, et al. Splicing by overlap extension by PCR using asymmetric amplification: an improved technique for the generation of hybrid proteins of immunological interest. Gene 20; 186:29-35 (1997)] The sequence of each genetic fragment was confirmed by direct sequencing. The resulting expression cassettes were subcloned into EcoRI and XhoI sites of MSCV-IRES-GFP.

To transduce CD19-negative K562 cells with CD19, we constructed a MSCV-IRES-DsRed vector. The IRES and DsRed sequences were subcloned from MSCV-IRES-GFP and pDsRedN1 (Clontech, Palo Alto, Calif.), respectively, and assembled by SOE-PCR. The IRES-DsRed cassette was digested and ligated into XhoI and NotI sites of MSCV-IRES-GFP. The expression cassette for CD19 was subsequently ligated into EcoRI and XhoI sites of MSCV-IRES-DsRed vector.

Virus Production and Gene Transduction

To generate RD114-pseudotyped retrovirus, we used calcium phosphate DNA precipitation to transfect 3×10^6 293T cells, maintained in 10-cm tissue culture dishes (Falcon, Becton Dickinson, Franklin Lakes, N.J.) for 24 hours, with 8 μg of one of the vectors anti-CD19-ζ, anti-CD19-BB-ζ, anti-CD19-28-ζ**0** or anti-CD19-truncated, 8 μg of pEQ-PAM3(-E) and 4 μg of pRDF. After 24 hours, medium was replaced with RPMI-1640 with 10% FCS and antibiotics. Conditioned medium containing retrovirus was harvested 48 hours and 72 hours after transfection, immediately frozen in dry ice, and stored at −80° C. until use. HeLa cells were used to titrate virus concentration.

Peripheral blood mononuclear cells were incubated in a tissue culture dish for 2 hours to remove adherent cells. Non-adherent cells were collected and prestimulated for 48 hours with 7 μg/mL PHA-M (Sigma, St. Louis, Mo.) and 200 IU/mL human IL-2 (National Cancer Institute BRB Preclinical Repository, Rockville, Md.) in RPMI-1640 and 10% FCS. Cells were then transduced as follows. A 14-mL polypropylene centrifuge tube (Falcon) was coated with 0.5 mL of human fibronectin (Sigma) diluted to 100 μg/mL for 2 hours at room temperature and then incubated with 2% bovine serum albumin (Sigma) for 30 minutes. Prestimulated cells (2×10^5) were resuspended in the fibronectin-coated tube in 2-3 mL of virus-conditioned medium with polybrene (4 μg/mL; Sigma) and centrifuged at 2400×g for 2 hours. The multiplicity of infection (4 to 8) was identical in each experiment comparing the activity of different chimeric receptors. After centrifugation, cells were left undisturbed for 24 hours in a humidified incubator at 37° C., 5% CO_2. The transduction procedure was repeated on two successive days. Cells were then washed twice with RPMI-1640 and maintained in RPMI-1640, 10% FCS, and 200 IU/mL of IL-2 until use.

A similar procedure was used to express chimeric receptors in Jurkat cells, except that cells were not prestimulated. K562 cells expressing CD19 were created by resuspending 2×10^5 K562 cells in 3 mL of MSCV-CD19-IRES-DsRed virus medium with 4 μg/mL polybrene in a fibronectin-coated tube; the tube was centrifuged at 2400×g for 2 hours and left undisturbed in an incubator for 24 hours. Control cells were transduced with the vector only. These procedures were repeated on 3 successive days. After confirming CD19 and DsRed expression, cells were subjected to single-cell sorting with a fluorescence-activated cell sorter (MoFlo, Cytomation, Fort Collins, Colo.). The clones that showed the highest expression of DsRed and CD19 and of DsRed alone were selected for further experiments.

Detection of Chimeric Receptor Expression

Transduced Jurkat and peripheral blood cells were stained with goat anti-mouse (Fab)2 polyclonal antibody conjugated with biotin (Jackson Immunoresearch, West Grove, Pa.) followed by streptavidin conjugated to peridinin chlorophyll protein (PerCP; Becton Dickinson, San Jose, Calif.). Patterns of CD4, CD8, and CD28 expression were also analyzed by using anti-CD4 and anti-CD28 conjugated to PE and anti-CD8 conjugated to PerCP (antibodies from Becton Dickinson, and Pharmingen, San Diego, Calif.). Antibody staining was detected with a FACScan flow cytometer (Becton Dickinson).

For Western blotting, 2×10^7 cells were lysed in 1 mL RIPA buffer (PBS, 1% Triton-X100, 0.5% sodium deoxycholate, 0.1% SDS) containing 3 μg/mL of pepstatin, 3 μg/mL of leupeptin, 1 mM of PMSF, 2 mM of EDTA, and 5 μg/mL of aprotinin. Centrifuged lysate supernatants were boiled with an equal volume of loading buffer with or without 0.1 M DTT, then were separated by SDS-PAGE on a precast 12% acrylamide gel (BioRad, Hercules, Calif.). The proteins were transferred to a PVDF membrane, which was incubated with primary mouse anti-human CD3ζ monoclonal antibody (clone 8D3; Pharmingen), 1 μg/mL for 12 hours at 4° C. Membranes were then washed, incubated with a 1:500 dilution of goat anti-mouse IgG horseradish peroxidase-conjugated second antibody for 1 hour, and developed by using the ECP kit (Pharmacia, Piscataway, N.J.).

Changes in Gene Expression and Cytokine Production after Receptor Ligation

Jurkat cells transduced with the chimeric receptors were cocultured with OP-1 leukemic cells fixed with 0.5% paraformaldehyde at an effector:target (E:T) ratio of 1:1. RNA was extracted using Trizol Reagent (Invitrogen, Carlsbad, Calif.). Gene expression of Jurkat cells was analyzed using HG-U133A GeneChip microarrays (Affymetrix, Santa Clara, Calif.) as previously described. [Yeoh E J, et al. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. Cancer Cell 2002; 1:133-143 (2002); Ross M E, et al. Classification of pediatric acute lymphoblastic leukemia by gene expression profiling. Blood. May 2003; 10.1182/blood-2003-01-0338 (2003)] Arrays were scanned using a laser confocal scanner (Agilent, Palo Alto, Calif.) and analyzed with Affymetrix Microarray suite 5.0. We used an arbitrary factor of 2 or higher to define gene overexpression. IL-2, TNF-related apoptosis-inducing ligand (TRAIL), OX40, IL-3 and μ-actin transcripts were detected by semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) using Jurkat cells stimulated as above; primers were designed using the Primer3 software developed by the Whitehead Institute for Biomedical Research.

For cytokine production, Jurkat cells and primary lymphocytes (2×10^5 in 200 μl) expressing chimeric receptors were stimulated with OP-1 cells at a 1:1 E:T ratio for 24 hours. Levels of IL-2 and IFNγ in culture supernatants were determined with a Bio-Plex assay (BioRad). Lymphocytes before and after stimulation were also labeled with anti-TRAIL-PE (Becton Dickinson).

Expansion and Purification of Receptor-Transduced Primary T Cells

Receptor-transduced lymphocytes (3×10^5) were co-cultured with 1.5×10^5 irradiated OP-1 cells in RPMI-1640 with 10% FCS with or without exogenous IL-2. Cells were pulsed weekly with irradiated target cells at an E:T ratio of 2:1. Cells were counted by Trypan-blue dye exclusion and by flow cytometry to confirm the presence of GFP-positive cells and the absence of CD19-positive cells. To prepare pure populations of $CD8^+$ cells expressing chimeric receptors, we labeled cells with a PE-conjugated anti-CD8 antibody (Becton Dickinson) that had been previously dialyzed to remove preservatives and then sterile-filtered. $CD8^+$ GFP+ cells were isolated using a fluorescence-activated cell sorter (MoFlo).

Cytotoxicity Assays

The cytolytic activity of transductants was measured by assays of lactate dehydrogenase (LDH) release using the Cytotoxicity Detection Kit (Roche, Indianapolis, Ind.) according to the manufacturer's instructions. Briefly, 2×10^4 target cells were placed in 96-well V-bottom tissue culture plates (Costar, Cambridge, Mass.) and cocultured in triplicate in RPMI-1640 supplemented with 1% FCS, with primary lymphocytes transduced with chimeric receptors. After 5 hours, cell-free supernatant was harvested and immediately analyzed for LDH activity. Percent specific cytolysis was calculated by using the formula: (Test—effector control—low control/high control—low control)×100, in which "high control" is the value obtained from supernatant of target cells exposed to 1% Triton-X-100, "effector control" is the spontaneous LDH release value of lymphocytes alone, "low control" is the spontaneous LDH release value of target cells alone; background control (the value obtained from medium alone) was subtracted from each value before the calculation.

The anti-leukemic activity of receptor-transduced lymphocytes was also assessed in 7-day cultures using lower E:T ratios. For this purpose, we used bone marrow-derived mesenchymal cells to support the viability of leukemic cells. [Nishigaki H, et al. Prevalence and growth characteristics of malignant stem cells in B-lineage acute lymphoblastic leukemia. Blood 89:3735-3744 (1997); Mihara K, et al. Development and functional characterization of human bone marrow mesenchymal cells immortalized by enforced expression of telomerase. Br J Haematol 120:846-849 (2003)] Briefly, 2×10^4 human mesenchymal cells immortalized by enforced expression of telomerase reverse transcriptase were plated on a 96-well tissue culture plate precoated with 1% gelatin. After 5 days, 1×10^4 CD19+ target cells (in case of cell lines) or 2×10^5 CD19+ target cells (in case of primary ALL cells) were plated on the wells and allowed to rest for 2 hours. After extensive washing to remove residual IL-2-containing medium, receptor-transduced primary T cells were added to the wells at the proportion indicated in Results. Cultures were performed in the absence of exogenous IL-2. Plates were incubated at 37° C. in 5% CO_2 for 5-7 days. Cells were harvested, passed through a 19-gauge needle to disrupt residual mesenchymal-cell aggregates, stained with anti-CD19-PE antibody, and assayed by flow cytometry as previously described. [Ito C, et al. Hyperdiploid acute lymphoblastic leukemia with 51 to 65 chromosomes: A distinct biological entity with a marked propensity to undergo apoptosis. Blood 93:315-320 (1999); Srivannaboon K, et al. Interleukin-4 variant (BAY 36-1677) selectively induces apoptosis in acute lymphoblastic leukemia cells. Blood 97:752-758 (2001)] Expression of DsRed served as a marker of residual K562 cells. Experiments were done in triplicate.

Results

Transduction of Primary Human T Lymphocytes with Anti-CD19-BB-ζ Chimeric Receptors

In preliminary experiments, transduction of lymphocytes stimulated with PHA (7 μg/mL) and IL-2 (200 IU/mL) for 48 hours, followed by centrifugation (at 2400×g) of the activated lymphocytes with retroviral supernatant in tubes coated with fibronectin, consistently yielded a high percentage of chimeric receptor and GFP expression; this method was used in all subsequent experiments. In 75 transduction experiments, 31% to 86% (median, 64%) of mononuclear cells expressed

GFP. In experiments with cells obtained from 6 donors, we tested the immunophenotype of the cells transduced with anti-CD19-BB-ζ receptors. Fourteen days after transduction a mean (±SD) of 89.6%±2.3% (n=6) of GFP cells also expressed CD3; 66.2%±17.9% of CD3 T lymphocytes were transduced. Among GFP^+ cells, 21.1%±8.8% (n=6) were $CD4^+$, 68.1%±8.1% (n=6) were $CD8^+$, 38.1%±16.1% (n=3) were $CD28^+$ and 24.2%±11.6% (n=3) were $CD8^+CD28^+$. These proportions were similar to those obtained with the anti-CD19-ζ receptors lacking 4-1BB. In this case, 85.4%±11.0% (n=6) of GFP^+ cells expressed CD3; 60.8%±10.1% of $CD3^+$ cells were transduced. Among GFP^+ cells, 18.0%±8.7% (n=6) were $CD4^+$, 66.1%±11.7% (n=6) were $CD8^+$, 41.2%±12.2% (n=3) were $CD28^+$ and 20.6%±11.3% (n=3) were $CD8^+CD28^+$. In these experiments, median transduction efficiency was 65% (range, 31% to 86%) for anti-CD19-BB-ζ receptors, and 65% (range, 37% to 83%) for anti-CD19-ζ receptors.

The surface expression of the chimeric receptors on GFP^+ cells was confirmed by staining with a goat anti-mouse antibody that reacted with the scFv portion of anti-CD19. Expression was detectable on most GFP^+ cells and was not detectable on GFP cells and vector-transduced cells. The level of surface expression of anti-CD19-BB-ζ was identical to that of the receptor lacking 4-1BB. Expression was confirmed by Western blot analysis; under non-reducing conditions, peripheral blood mononuclear cells transduced with the chimeric receptors expressed them mostly as monomers, although dimers could be detected.

Signaling Function of Anti-CD19-BB-ζ Chimeric Receptors

To test the functionality of the anti-CD19-BB-ζ chimeric receptor, we used the T-cell line Jurkat and the CD19+ ALL cell line OP-1. After transduction, >95% Jurkat cells were GFP+. Exposure of irradiated OP-1 cells to Jurkat cells transduced with anti-CD19-BB-ζ triggered transcription of IL-2. Notably, in parallel experiments with Jurkat cells transduced with the anti-CD19-ζ receptor lacking 4-1BB, the level of IL-2 transcription was much lower. No IL-2 transcription was detected in Jurkat cells transduced with the anti-CD19-truncated control receptor lacking CD3ζ.

To identify further changes in molecules associated with T-cell activation, survival or cytotoxicity induced by anti-CD19-BB-ζ receptors, Jurkat cells were either transduced with these receptors or with anti-CD19-ζ receptors and then stimulated with paraformadehyde-fixed OP-1 cells. After 12 hours of stimulation, we screened the cells' gene expression using Affymetrix HG-U133A chips. Genes that were overexpressed by a factor of 2 or higher in cells with anti-CD19-BB-ζ included the member of the TNF family TRAIL, the TNF-receptor member OX40, and IL-3. Overexpression of these molecules after stimulation was validated using RT-PCR. In cells bearing the anti-CD19-ζ receptor, there were no overexpressed genes with a known function associated with T-cells. Therefore, anti-CD19-BB-ζ receptors elicit transcriptional responses that are distinct from those triggered by receptors lacking 4-1BB.

Expansion of T Cells Expressing Anti-CD19-BB-ζ Receptors in the Presence of $CD19^+$ Cells

To measure the ability of anti-CD19-BB-ζ transduced lymphocytes to survive and expand in vitro, we first analyzed primary T cells (obtained from 2 donors), 7 days after transduction. Transduction efficiency with the 3 receptors was similar: 72% and 67% for anti-CD19-BB-ζ, 63% and 66% for anti-CD19-ζ and 67% and 68% for the truncated anti-CD19 receptor. When cocultured with irradiated OP-1 ALL cells in the absence of exogenous IL-2, cells transduced with anti-CD19-BB-ζ expanded: after only 1 week of culture, GFP^+ cells recovered were 320% and 413% of input cells. T cells that expressed the anti-CD19-ζ receptor but lacked 4-1BB signaling capacity remained viable but showed little expansion (cell recovery: 111% and 160% of input cells, respectively), whereas those that expressed the truncated anti-CD19 receptor underwent apoptosis (<10% of input cells were viable after 1 week). Lymphocytes transduced with anti-CD19-BB-ζ continued to expand in the presence of irradiated OP-1 cells. After 3 weeks of culture, they had expanded by more than 16-fold, with 98% of the cells at this point being GFP^+. By contrast, cells transduced with only anti-CD19-ζ survived for less than 2 weeks of culture.

We performed the next set of experiments with T cells (obtained from 3 donors) 14 days after transduction with anti-CD19-BB-ζ, anti-CD19-ζ or anti-CD19-truncated, and expanded with high-dose IL-2 (200 IU/mL). Recovery of lymphocytes of each donor with anti-CD19-BB-ζ receptors was significantly higher than that of lymphocytes with anti-CD19-ζ receptors in all 3 comparisons ($P<0.005$). When IL-2 was removed, exposure of the transduced cells to irradiated OP-1 cells induced apoptosis, irrespective of the chimeric receptor expressed. This was in contrast to results with cells 7 days post-transduction, and in accord with the loss of T cell functionality after prolonged culture in IL-2 observed by others. [Brentjens R J, et al. Eradication of systemic B-cell tumors by genetically targeted human T lymphocytes co-stimulated by CD80 and interleukin-15. Nat Med 9:279-286 (2003); Rossig C. et al. Targeting of G(D2)-positive tumor cells by human T lymphocytes engineered to express chimeric T-cell receptor genes. Int J Cancer 94:228-236 (2001)] However, low-dose IL-2 (10 IU/mL) was sufficient to maintain most lymphocytes transduced with anti-CD19-BB-ζ viable after 2 weeks of culture with irradiated OP-1 cells, but did not prevent apoptosis of cells transduced with the other receptors. Taken together, these data indicate that 4-1BB-mediated costimulation confers a survival advantage on lymphocytes.

Cytotoxicity Triggered by Anti-CD19-BB-ζ Chimeric Receptors

Lymphocytes obtained from two donors and transduced with anti-CD19-BB-ζ and anti-CD19-ζ exerted dose-dependent cytotoxicity, as shown by a 5-hour LDH release assay using the OP-1 B-lineage ALL cell line as a target. Transduction efficiencies were 41% and 73% for empty vector, 40% and 67% for anti-CD19-truncated, 43% and 63% for anti-CD19-ζ, and 46% and 72% for anti-CD19-BB-ζ. No differences in cytotoxicities mediated by the two receptors were detectable with this assay. Although no lysis of target cells was apparent at a 1:1 ratio in the 5-hour LDH assay, most leukemic cells were specifically killed by lymphocytes expressing signaling chimeric receptors when the cultures were examined at 16 hours by flow cytometry and inverted microscopy.

To better mimic the application of T-cell therapy, we determined whether T cells expressing the chimeric receptor would exert significant anti-leukemic activity when present at low E:T ratios in prolonged culture. Lymphocytes from various donors were expanded in vitro for 14 days after transduction and were mixed at different ratios with OP-1, RS4;11, or REH B-lineage ALL cells, or with K562 (a CD19-negative myeloid cell line that lacks HLA antigens) transduced with CD19 or with vector alone. Co-cultures were maintained for 7 days, and viable leukemic cells were counted by flow cytometry. As observed in short term cultures, at a 1:1 ratio, T cells expressing signaling chimeric receptors eliminated virtually all leukemic cells from the cultures. At a 0.1:1 ratio, however, T cells transduced with anti-CD19-BB-ζ receptors

were markedly more effective than those lacking 4-1BB signaling. Chimeric receptor-transduced T cells had no effect on cells lacking CD19. The presence of 4-1BB in the chimeric receptor did not increase background, non-CD19-mediated cytotoxicity, in experiments using CEM-C7, U-937 and K-562. As in other experiments, transduction efficiencies with the two chimeric receptors were equivalent, and range from 62% to 73% for anti-CD19-ζ and from 60% to 70% for anti-CD19-BB-ζ.

Cells present in the bone marrow microenvironment may decrease T-cell proliferation in a mixed lymphocyte reaction. [Bartholomew A, et al. Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. Exp Hematol 30:42-48 (2002); Krampera M, et al. Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. Blood 101:3722-3729 (2003); Le Blanc K, et al. Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex. Scand J Immunol 57:11-20 (2003)] To test whether these cells would also affect T-cell-mediated antileukemic activity, we repeated the experiments with OP-1 in the presence of bone marrow-derived mesenchymal cell layers. [Mihara K, et al. Development and functional characterization of human bone marrow mesenchymal cells immortalized by enforced expression of telomerase. Br J Haematol 2003; 120:846-849 (2003)] T-cell cytotoxicity under these conditions was even greater than that observed in cultures without mesenchymal cells. Remarkably, T cells transduced with anti-CD19-BB-ζ were markedly cytotoxic even at a ratio of 0.01:1 in this assay, whereas those transduced with anti-CD19-ζ were not.

Effect of Receptor-Transduced T Cells on Primary Leukemic Cells

We co-cultured primary B-lineage ALL cells with bone marrow-derived mesenchymal cells, which are essential to preserve their viability in vitro. [Nishigaki H, et al. Prevalence and growth characteristics of malignant stem cells in B-lineage acute lymphoblastic leukemia. Blood 1997; 89:3735-3744 (1997); Mihara K, et al. Development and functional characterization of human bone marrow mesenchymal cells immortalized by enforced expression of telomerase. Br J Haematol 120:846-849 (2003)] We tested the effect of T cells expressing anti-CD19-BB-ζ on primary leukemic cells obtained from 5 patients at the time of diagnosis; these patients included 3 who had B-lineage ALL with 11q23 abnormalities, a karyotype associated with drug resistance. [Pui C H, et al. Childhood acute lymphoblastic leukemia—Current status and future perspectives. Lancet Oncology 2:597-607 (2001)] Mesenchymal cells supported ALL cell survival in vitro: in cultures not exposed to exogenous T cells, recovery of leukemic cells from the 5 patients after 5 days of culture ranged from 100.1% to 180.7% of the input cell number. Leukemic cells incubated at a 0.1:1 ratio with lymphocytes expressing anti-CD19-BB-ζ were virtually eliminated in all 5 cultures. Remarkable cytotoxicity was also seen at a 0.01:1 ratio. Importantly, at this ratio, lymphocytes expressing anti-CD19-BB-ζ were consistently more cytotoxic than those expressing the anti-CD19-ζ receptor alone ($P<0.01$ by t test for all comparisons).

Comparisons Between Chimeric Receptors Containing Signaling Domains of 4-1BB and of CD28

We compared responses induced by anti-CD19-BB-ζ to those of an equivalent receptor in which 4-1BB signaling domains were replaced by CD28 signaling domains (FIG. **1**). Expression of the latter was similar to that of anti-CD19-BB-ζ and anti-CD19-ζ receptors: >95% Jurkat cells were consistently GFP+ after transduction with anti-CD19-28-ζ and most of these cells had detectable receptors on the cell surface. In 6 experiments with primary lymphocytes, transduced cells ranged from 42% to 84% (median, 72%).

We tested production of IL-2 in Jurkat cells transduced with the three receptors and stimulated with the CD19+ ALL cell line OP-1. Production of IL-2 was the highest in cells expressing anti-CD19-BB-ζ ($P<0.05$). Production of IL-2 was also tested in primary lymphocytes, which were transduced with the chimeric receptors and then expanded for 5 weeks with pulses of OP-1. The pattern of IL-2 production was similar to that observed in Jurkat cells. Cells expressing anti-CD19-BB-ζ produced higher levels of IL-2 ($P<0.01$). Chimeric receptors containing the co-stimulatory molecules induced a higher IFN-γ production in primary lymphocytes. IFN-γ levels were the highest with the anti-CD19-28-ζ receptor ($P<0.05$). Finally, we tested surface expression of TRAIL protein in primary lymphocytes by staining with a specific antibody. Levels of TRAIL were the highest in cells transduced with the anti-CD19-BB-ζ receptor. These results indicate that anti-CD19-BB-ζ receptors are functionally distinct from those lacking co-stimulatory molecules or containing CD28 instead of 4-1BB.

Next, we compared the cytotoxicity exerted by primary T cells transduced with anti-CD19-BB-ζ receptors to those exerted by T cells bearing receptors lacking 4-1BB. For these experiments, we transduced primary lymphocytes from 2 donors with anti-CD19-BB-ζ anti-CD19-28-ζ, anti-CD19-ζ and anti-CD19-truncated, we expanded them for 2-3 weeks with IL-2, and then purified $CD8^+$, GFP^+ cells by fluorescence activated cell sorting. Confirming our previous results with unsorted cells, $CD8^+$ cells expressing anti-CD19-BB-ζ receptors were significantly more effective than those with anti-CD19-ζ receptors, and were as effective as those with anti-CD19-BB-ζ Finally, we determined the capacity of the purified CD8 cells transduced with the various receptors to expand in the presence of low dose (10 U/mL) IL-2. Cells transduced with anti-CD19-BB-ζ receptor had a significantly higher cell growth under these conditions than those bearing the other receptors ($P<0.001$).

Discussion

Results of this study indicate that anti-CD19-BB-ζ receptors could help achieve effective T-cell immunotherapy of B-lineage ALL. Lymphocytes expressing anti-CD19-BB-ζ survived and expanded better than those with equivalent receptors lacking 4-1BB. These lymphocytes also had higher anti-leukemic activity and could kill B-lineage ALL cells from patients at E:T ratios as low as 0.01:1, suggesting that the infusion of relatively low numbers of transduced T cells could have a measurable anti-leukemic effect in patients. Finally, lymphocytes transduced with anti-CD19-BB-ζ were particularly effective in the presence of bone marrow-derived mesenchymal cells which form the microenvironment critical for B-lineage ALL cell growth, further supporting their potential for immunotherapy.

Two recently reported studies used anti-CD19 scFv as a component of a chimeric receptor for T-cell therapy of B-cell malignancies. Cooper et al. Blood 101:1637-1644 (2003) reported that T-cell clones transduced with chimeric receptors comprising anti-CD19 scFv and CD3ζ produced approximately 80% specific lysis of B-cell leukemia and lymphoma cell lines at a 1:1 E:T ratio in a 4-hour ^{51}Cr release assay; at this ratio, percent specific lysis of one primary B-lineage ALL sample tested was approximately 30%. Brentjens et al. Nat Med 279-286 (2003) reported that T-cells bearing anti-CD19 scFv and CD3ζ chimeric receptors could be greatly expanded in the presence of exogenous IL-15 and artificial antigen-

presenting cells transduced with CD19 and CD80. The authors showed that these T cells significantly improved the survival of immunodeficient mice engrafted with the Raji B-cell lymphoma cell line. Their results demonstrated the requirement for co-stimulation in maximizing T-cell-mediated anti-leukemic activity: only cells expressing the B7 ligands of CD28 elicited effective T-cell responses. However, B-lineage ALL cells typically do not express B7-1(CD80) and only a subset expresses B7-2 (CD86) molecules. [Cardoso A A, et al. Pre-B acute lymphoblastic leukemia cells may induce T-cell anergy to alloantigen. Blood 88:41-48 (1996)]

4-1BB, a tumor necrosis factor-receptor family member, is a co-stimulatory receptor that can act independently from CD28 to prevent activation-induced death of activated T cells. [Kim Y J, et al. Human 4-1BB regulates CD28 co-stimulation to promote Th1 cell responses. Eur J Immunol 28:881-890 (1998); Hurtado J C, et al. Signals through 4-1BB are costimulatory to previously activated splenic T cells and inhibit activation-induced cell death. J Immunol 158:2600-2609 (1997); DeBenedette M A, et al. Costimulation of CD28− T lymphocytes by 4-1BB ligand. J Immunol 1997; 158:551-559 (1997); Bukczynski J, et al. Costimulation of human CD28− T cells by 4-1BB ligand. Eur J Immunol 33:446-454 (2003)] In our study, we found that chimeric receptors containing 4-1BB can elicit vigorous signals in the absence of CD28− mediated co-stimulation. Cytotoxicity against $CD19^+$ cells mediated by these receptors was as good as that mediated by CD28-containing receptors and was clearly superior to that induced by receptors lacking co-stimulatory molecules. It is known that, in contrast to CD28, 4-1BB stimulation results in a much larger proliferation of $CD8^+$ cells than CD4+ cells. [Shuford W W, et al. 4-1BB costimulatory signals preferentially induce $CD8^+$ T cell proliferation and lead to the amplification in vivo of cytotoxic T cell responses. J Exp Med 1997; 186:47-55 (1997)] We found that T cells expressing the anti-CD19-BB-ζ receptor produced more IL-2 upon stimulation, and that $CD8^+$ cells expanded in the presence of low-dose IL-2 more vigorously than those expressing receptors lacking 4-1BB domains, including those containing CD28. Therefore, the presence of 4-1BB in the chimeric receptors may support more durable T cell responses than those induced by other receptors.

Experimental evidence indicates that harnessing 4-1BB signaling could have useful application in antitumor therapy. Melero et al. Nat Med 3:682-685 (1997) found that antibodies to 4-1BB significantly improved long-lasting remission and survival rates in mice inoculated with the immunogenic P815 mastocytoma cell line. Moreover, immunogenic murine tumor cells made to express 4-1BB ligand were readily rejected and induced long term immunity. [Melero I, et al. Chen L. Amplification of tumor immunity by gene transfer of the co-stimulatory 4-1BB ligand: synergy with the CD28 co-stimulatory pathway. Eur J Immunol 28:1116-1121 (1998)] Dramatic results were also observed in vaccination experiments using other tumor cell lines expressing 4-1BB ligands. [Ye Z, et al. Gene therapy for cancer using single-chain Fv fragments specific for 4-1BB. Nat Med 8:343-348 (2002); Mogi S, et al. Tumour rejection by gene transfer of 4-1BB ligand into a CD80(+) murine squamous cell carcinoma and the requirements of co-stimulatory molecules on tumour and host cells. Immunology 101:541-547 (2000); Yoshida H, et al. A novel adenovirus expressing human 4-1BB ligand enhances antitumor immunity. Cancer Immunol Immunother 52:97-106 (2003)] Of note, experiments with the poorly immunogenic Ag104A fibrosarcoma cell line provided some evidence that 4-1BB could be superior to CD28 in eliciting anti-tumor responses: 80% of mice showed tumor regression with 4-1BB stimulation and 50% of mice with widespread metastasis were cured, [Melero I, Shuford W W, Newby S A, et al. Monoclonal antibodies against the 4-1BB T-cell activation molecule eradicate established tumors. Nat Med 3:682-685 (1997)] whereas CD28 costimulation was not effective alone and required simultaneous CD2 stimulation. [Li Y, et al. Costimulation by CD48 and B7-1 induces immunity against poorly immunogenic tumors. J Exp Med 1996; 183:639-644 (1996)] These data, together with our results, indicate that the addition of 4-1BB to the chimeric receptor should significantly increase the probability that transduced T-cells will survive and continue to proliferate when the receptor is engaged in vivo. We think it noteworthy that T cells with chimeric receptors containing 4-1BB expressed the highest levels of TRAIL upon stimulation, given the known tumoricidal activity of this molecule. [Schmaltz C, et al. T cells require TRAIL for optimal graft-versus-tumor activity. Nat Med 8:1433-1437 (2002)]

Clinical precedents, such as administration of T-cell clones that target CMV epitopes [Walter E A, et al. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. N Engl J Med. 333:1038-1044 (1995)] or EBV-specific antigens, [Rooney C M, et al. Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr-virus-related lymphoproliferation. Lancet 345:9-13 (1995)] attest to the clinical feasibility of adoptive T-cell therapy. Transfer of chimeric receptor-modified T cells has the added advantage of permitting immediate generation of tumor-specific T-cell immunity. Subsequently, therapeutic quantities of antigen-specific T cells can be generated quite rapidly by exposure to target cells and/or artificial antigen-presenting cells, in the presence of ligands of co-stimulatory molecules and/or exogenous cytokines such as IL-2, IL-7, and IL-15. [Geiger T L, Jyothi M D. Development and application of receptor-modified T lymphocytes for adoptive immunotherapy. Transfus Med Rev 15:21-34 (2001); Schumacher T N. T-cell-receptor gene therapy. Nat Rev Immunol. 2:512-519 (2002); Sadelain M, et al. Targeting tumours with genetically enhanced T lymphocytes. Nat Rev Cancer 3:35-45 (2003); Brentjens R J, et al. Eradication of systemic B-cell tumors by genetically targeted human T lymphocytes co-stimulated by CD80 and interleukin-15. Nat Med 9:279-286 (2003)] A specific risk of the strategy proposed here relates to the transforming potential of the retrovirus used to transduce chimeric receptors. [Baum C, Dullmann J, Li Z, et al. Side effects of retroviral gene transfer into hematopoietic stem cells. Blood 101:2099-2114 (2003)] We therefore envisage the coexpression of suicide genes as a safety measure for clinical studies. [Marktel S, et al. Immunologic potential of donor lymphocytes expressing a suicide gene for early immune reconstitution after hematopoietic T-cell-depleted stem cell transplantation. Blood 101:1290-1298 (2003)] This approach would also ensure that the elimination of normal $CD19^+$ B-lineage cells is temporary and should therefore have limited clinical consequences.

In view of the limited effectiveness and the high risk of the currently available treatment options for chemotherapy-refractory B-lineage ALL and other B cell malignancies, the results of our study provide compelling justification for clinical trials using T cells expressing anti-CD19-BB-ζ receptors. Donor-derived T cells endowed with chimeric receptors could replace infusion of non-specific lymphocytes post-transplant. To reduce the risk of GvHD mediated by endogenous T-cell receptors, it may be beneficial to use T cells with restricted endogenous specificity, for example, Epstein-Barr-

virus-specific cytotoxic T-lymphocyte lines. [Rossig C, et al. Epstein-Barr virus-specific human T lymphocytes expressing antitumor chimeric T-cell receptors: potential for improved immunotherapy. Blood. 99:2009-2016 (2002)] Therefore, it would be important to test the effects of adding 4-1BB to chimeric receptors transduced in these lines. The reinfusion of autologous T cells collected during clinical remission could also be considered in patients with persistent minimal residual disease. In our experiments, T cells expressing anti-CD19-BB-ζ receptors completely eliminated ALL cells at E:T ratios higher than 1:1, and autologous B lymphocytes became undetectable shortly after transduction of anti-CD19-BB-ζ, suggesting that the potential leukemic cell contamination in the infused products should be greatly reduced or abrogated by the procedure.

9.2 Example 2

T lymphocytes transduced with anti-CD19 chimeric receptors have remarkable anti-ALL capacity in vitro and in vivo, suggesting the clinical testing of receptor-modified autologous T cells in patients with persistent minimal residual disease. However, the use of allogeneic receptor-modified T lymphocytes after hematopoietic cell transplantation (HCT) might carry the risk of severe graft-versus-host disease (GvHD). In this setting, the use of CD3-negative natural killer (NK) cells is attractive because they should not cause GvHD.

Spontaneous cytotoxicity of NK cells against ALL is weak, if measurable at all. To test whether anti-CD19 chimeric receptors could enhance it, we developed methods to specifically expand human primary NK cells and induce high levels of receptor expression. Specific NK cell expansion has been problematic to achieve with established methods which favor CD3+ T cell expansion. Even after T-cell depletion, residual T cells typically become prominent after stimulation.

We overcame this obstacle by generating a genetically-modified K562 myeloid leukemia cell line that expresses membrane-bound interleukin-15 (IL-15) and 4-1BB ligand (CD137L) (K562-mb15-137L). The K562-mb15-137 cell line was generated by retrovirally transducing K562 cells with a chimeric protein construct consisting of human IL-15 mature peptide fused to the signal peptide and transmembrane domain of human CD8 alpha, as well as GFP. Transduced cells were single cell-cloned by limiting dilution and a clone with the highest expression of GFP and membrane-bound (surface) IL-15 was selected. Then, the clone was transduced with human CD137L.

Peripheral blood mononuclear cells from 8 donors were cultured with K562-mb15-137L in the presence of 10 IU/mL IL-2. After 1 week of culture with K562-mb15-137L, NK cells expanded by 16.3±5.9 fold, whereas T cells did not expand. The stimulatory effect of K562-mb15-137L was much higher than that of K562 cells transduced with control vectors, K562 expressing membrane-bound IL-15 or CD137L alone, or K562 expressing wild-type IL-15 instead of membrane-bound IL-15.

NK cells expanded with K562-mb15-137L were transduced with a retroviral vector and the anti-CD19-BB-ζ chimeric receptor. In 27 experiments, mean transduction efficiency (±SD) after 7-14 days was 67.5%±16.7%. Seven to fourteen days after transduction, 92.3% (range 84.7%-99.4%) of cells were CD3-CD56+ NK cells; expression of receptors on the cell surface was high. NK cells expressing anti-CD19-BB-ζ had powerful cytotoxicity against NK-resistant B-lineage ALL cells. NK cells transduced with anti-CD19-BB-ζ had consistently higher cytotoxicity than those transduced with receptors lacking 4-1BB.

Transduction of NK Cells with Chimeric Receptors

Peripheral blood mononuclear cells were stimulated with the K562-mb15-137L cells prior to their exposure to retroviral vectors containing anti-CD19 receptor constructs and GFP. In 10 experiments, median percent of NK cells was 98.4% (93.7-99.4%) 7-11 days after transduction; 77.4% (55.2-90.0%) of these cells were GFP+. We observed high levels of surface expression of the anti-CD19 chimeric receptors.

NK activity against the CD19-negative cells K562 and U937 was not affected by the expression of anti-CD19 receptors. The receptors, however, markedly increased NK activity against $CD19^+$ ALL cells. The following summarizes results obtained with NK cells from 2 donors. At an E:T ratio of 1:1, NK cells from donor 1 lacked cytotoxicity against $CD19^+$ RS4;11 cells and exerted ~50% cytoxicity against $CD19^+$697 cells after 24 hours. NK cells from donor 2 had no cytotoxicity against RS4;11 or 697 cells. Expression of the anti-CD19-CD3ϵ receptor overcame NK resistance. NK cells from donor 1 became cytotoxic to RS4;11 cells and those from donor 2 become cytotoxic to both RS;11 and 697 cells. Moreover, when control cells had some cytotoxicity, this was significantly augmented by expression of signaling anti-CD19 receptor.

Subsequently, we found that addition of the co-stimulatory CD28 or 4-1BB to the anti-CD19 receptor markedly enhanced NK cytotoxicity against NK-resistant ALL cells (FIG. **2**). For example, after 24 hours of culture at 1:1 E:T ratio, the cytotoxicity mediated by the anti-CD19-BB-ζ receptor against the NK-resistant $CD19^+$ ALL cell lines 380, 697, KOPN57bi and OP1 ranged from 86.5% to 99.1%. Therefore, the inclusion of co-stimulatory molecules enhances not only the cytoxicity of T lymphocytes but also that of NK cells.

9.3 Example 3

Artificial Antigen Producing Cells (APCs) Pave the Way for Clinical Application by Potent Primary in Vitro Induction

Materials and Methods

Cells

The CD19 human B-lineage ALL cell lines RS4;11, OP-1, 380, 697, and KOPN57bi; the T-cell line GEM-C7; and the myeloid cell lines K562 and U-937 were available in our laboratory. Cells were maintained in RPMI-1640 (Gibco, Grand Island, N.Y.) supplemented with 10% fetal calf serum (FCS; BioWhittaker, Walkersville, Md.) and antibiotics.

Primary leukemia cells were obtained with appropriate informed consent and Institutional Review Board (M) approval from nine patients with B-lineage ALL; from four of these patients, we also studied (with IRB approval) cryopreserved peripheral blood samples obtained during clinical remission. An unequivocal diagnosis of B-lineage ALL was established by morphologic, cytochemical, and immunophenotypic criteria; in each case, more than 95% of the cells were positive for CD19. Peripheral blood was obtained from eight healthy adult donors. Mononuclear cells collected from the samples by centrifugation on a Lymphoprep density step (Nycomed, Oslo, Norway) were washed twice in phosphate-buffered saline (PBS) and once in AIM-V medium (Gibco).

Plasmids and Retrovirus Production

The anti-CD19-ζ, anti-CD19-BB-i and anti-CD19-truncated (control) plasmids are described in Imai, C, et al., Leukemia 18:676-684 (2004). The pMSCV-IRES-GFP, pEQ-PAM3(-E), and pRDF constructs were obtained from the St.

Jude Vector Development and Production Shared Resource. The intracellular domains of human DAP 10, 4-1BB ligand and interleukin-15 (IL-15) with long signal peptide were subcloned by polymerase chain reaction (PCR) with a human spleen cDNA library (from Dr. G. Neale, St. Jude Children's Research Hospital) used as a template. An antiCD19-DAP 10 plasmid was constructed by replacing the intracellular domain of anti-CD19-ζ with that of DAP 10, using the SOE-PCR (splicing by overlapping extension by PCR) method. The signal peptide of CD8 cc, the mature peptide of IL-15 and the transmembrane domain of CDBα were assembled by SOE-PCR to encode a "membrane-bound" form of IL-15. The resulting expression cassettes were subcloned into EcoRI and XhoI sites of MSCV-IRES-GFP.

The RD114-pseudotyped retrovirus was generated as described in Imai, C, et al., Leukemia 18:676-684 (2004). We used calcium phosphate DNA precipitation to transfect 293T cells with anti-CD19-ζ, anti-CD19-DAP10, anti-CD19-BB-ζ, or anti-CD19-truncated; pEQ-PAM3(-E); and pRDF. Conditioned medium containing retrovirus was harvested at 48 hours and 72 hours after transfection, immediately frozen in dry ice, and stored at −80° C. until use.

Development of K562 Derivatives, Expansion of NK Cells and Gene Transduction

K562 cells were transduced with the construct encoding the "membrane-bound" form of IL-15. Cells were cloned by limiting dilution, and a single-cell clone with high expression of GFP and of surface IL-15 ("K562-mb15") was expanded. This clone was subsequently transduced with human 4-1BB ligand and designated as "K562-mb15-41BBL". K562 cells expressing wild-type IL-15 ("K562-wt15") or 4-IBBL ("K562-41BBL") were produced by a similar procedure. Peripheral blood mononuclear cells (1.5×106) were incubated in a 24-well tissue culture plate with or without 106 K562-derivative stimulator cells in the presence of 10 N/mL human IL-2 (National Cancer Institute BRB Preclinical Repository, Rockville, Md.) in RPMI-1640 and 10% FCS.

Mononuclear cells stimulated with K562-mb15-41BBL were transduced with retroviruses, as previously described for T cells [Melero I, et al., NK1.1 cells express 4-iBB (CDw137) costimulatory molecule and are required for tumor immunity elicited by anti-4-1BB monoclonal antibodies. Cell Immunol 190:167-172 (1998)]. Briefly, 14-mL polypropylene centrifuge tubes (Falcon) were coated with human fibronectin (100 μg/mL; Sigma, St. Louis, Mo.) or RetroNectin (50 μg/mL; TaKaRa, Otsu, Japan). Prestimulated cells (2×10^5) were resuspended in the tubes in 2-3 mL of virus-conditioned medium with polybrene (4 μg/mL; Sigma) and centrifuged at 2400×g for 2 hours (centrifugation was omitted when RetroNectin was used). The multiplicity of infection (4 to 6) was identical in each experiment comparing the activity of different chimeric receptors. After centrifugation, cells were left undisturbed for 24 hours in a humidified incubator at 37° C., 5% CO_2. The transduction procedure was repeated on two successive days. After a second transduction, the cells were re-stimulated with K562-mb 15-4 1BBL in the presence of 10 IU/mL of IL-2. Cells were maintained in RPMI-1640, 10% FCS, and 10 IU/mL IL-2.

Detection of Chimeric Receptor Expression and Immunophenotyping

Transduced NK cells were stained with goat anti-mouse $(Fab)^2$ polyclonal antibody conjugated with biotin (Jackson Immunoresearch, West Grove, Pa.) followed by streptavidin conjugated to peridinin chlorophyll protein (PerCP; Becton Dickinson, San Jose, Calif.). For Western blotting, cells were lysed in RIPA buffer (PBS, 1% Triton-X100, 0.5% sodium deoxycholate, 0.1% SDS) containing 3 μg/mL of pepstatin, 3 μg/mL of leupeptin, 1 mM of PMSF, 2 mM of EDTA, and 5 μg/mL of aprotinin. Centrifuged lysate supernatants were boiled with an equal volume of loading buffer with or without 0.1 M DTT, and then separated by SDS PAGE on a precast 10-20% gradient acrylamide gel (BioRad, Hercules, Calif.). The proteins were transferred to a PVDF membrane, which was incubated with primary mouse anti-human CD3ζ monoclonal antibody (clone 8D3; Pharmingen). Membranes were then washed, incubated with a goat anti-mouse IgG horseradish peroxidase-conjugated second antibody, and developed by using the ECP kit (Pharmacia, Piscataway, N.J.).

The following antibodies were used for immunophenotypic characterization of expanded and transduced cells: anti-CD3 conjugated to fluorescein isothiocyanate (FITC), to peridinin chlorophyll protein (PerCP) or to energy-coupled dye (ECD); anti-CD10 conjugated to phycoerythrin (PE); anti-CD19 PE; anti-CD22 PE; anti-CD56 FITC, PE or allophycocyanin (AFC); anti-CD16 CyChrome (antibodies from Becton Dickinson; Pharmingen, San Diego; or Beckman-Coulter, Miami, Fla.); and anti-CD25 PE (Dako, Carpinteria, Calif.). Surface expression of KIR and NK activation molecules was determined with specific antibodies conjugated to FIX or PE (from Beckman-Coulter or Becton-Dickinson), as previously described [Brentjens R J, Latouche J B, Santos E, et al. Eradication of systemic B-cell tumors by genetically targeted human T lymphocytes co-stimulated by CD80 and interleukin-15. Nat Med 9:279-286 (2003)]. Antibody staining was detected with a FACScan or a LSR II flow cytomete (Becton Dickinson).

Cytotoxicity Assays and Cytokine Production

Target cells (1.5×105) were placed in 96-well U-bottomed tissue culture plates (Costar, Cambridge, Mass.) and incubated with primary NK cells transduced with chimeric receptors at various effector:target (E:T) ratios in RPMI-1640 supplemented with 10% FCS; NK cells were cultured with 1000 U/mL IL-2 for 48 hours before the assay. Cultures were performed in the absence of exogenous IL-2. After 4 hours and 24 hours, cells were harvested, labeled with CD10 PE or CD22 PE and CD56 FITC, and assayed by flow cytometry as previously described. The numbers of target cells recovered from cultures without NK cells were used as a reference.

For cytokine production, primary NK cells (2×10^5 in 200 μl) expressing chimeric receptors were stimulated with various target cells at a 1:1 ratio for 24 hours. The levels of IFN-γ and GM-CSF in cell-free culture supernatants were determined with a Bio-Plex assay (BioRad).

Statistical Analysis

A test of equality of mean NK expansion with various stimuli was performed using analysis of variance for a randomized complete block design with each donor considered a random block. Tukey's honest significant difference procedure was used to compute simultaneous confidence intervals for each pairwise comparison of the differences of treatment means. Differences in cytotoxicities and cytokine production among NK cells bearing different chimeric receptors were analyzed by the paired Student's t test.

Results

Culture Conditions that Favor the Expansion of Primary NK Cells

To transduce chimeric receptors into primary NK cells, we searched for stimuli that would induce specific NK cell proliferation. In preliminary experiments, peripheral blood mononuclear cells of $CD3^+$ T lymphocytes were depleted and the remaining cells were stimulated with IL-2 (1000 U/mL) or IL-15 (10 ng/mL). Under these culture conditions there was no expansion of NK cells, which in fact progressively declined in numbers. With PHA (7 mg/mL) and IL-2 (1000

U/mL) as stimuli, we observed a 2- to 5-fold expansion of $CD56^+$ $CD3^-$ NK cells after 1 week of culture. However, despite the low proportion of contaminating $CD3^+$ cells (<2% in two experiments) at the beginning of the cultures, these cells expanded more than NK cells (>30-fold expansion), and after 1 week of culture represented approximately 35% of the cell population.

NK cells can be stimulated by contact with the human leukemia cell line K562, which lacks HLA-antigen expression, [Robertson M J, Cameron C, Lazo S, Cochran K J, Voss S D, Ritz J. Costimulation of human natural killer cell proliferation: role of accessory cytokines and cell contact-dependent signals. Nat Immun 15:213-226 (1996)] and genetically modified K562 cells have been used to stimulate cytotoxic T lymphocytes [Maus M V, Thomas A K, Leonard D G, et al. Ex vivo expansion of polyclonal and antigen-specific cytotoxic T lymphocytes by artificial APCs expressing ligands for the T-cell receptor, CD28 and 4-1BB. Nat Biotechnol 20:143-148 (2002)]. We tested whether the NK-stimulatory capacity of K562 cells could be increased through enforced expression of additional NK-stimulatory molecules, using two molecules that are not expressed by K562 cells and are known to stimulate NK cells. One molecule, the ligand for 4-1BB (4-1BBL), triggers activation signals after binding to 4-1BB (CD137), a signaling molecule expressed on the surface of NK cells [Melero I, Johnston J V, Shufford W W, Mittler R S, Chen L. NK1.I cells express 4-IBB (CDw137) costimulatory molecule and are required for tumor immunity elicited by anti-4-IBB monoclonal antibodies. Cell Immunol 190:167-172 (1998)]. The other molecule, IL-15, is a cytokine known to promote NK-cell development and the survival of mature NK cells [Carson W E, Fehniger T A, Haldar S, et al. A potential role for interleukin-15 in the regulation of human natural killer cell survival J Clin Invest. 99:937-943 (1997); Cooper M A, Bush J E, Fehniger T A, et al. In vivo evidence for a dependence on interleukin 15 for survival of natural killer cells. Blood 100:3633-3638 (2002); Fehniger T A, Caligiuri M A. Ontogeny and expansion of human natural killer cells: clinical implications. Int Rev Immunol 20:503-534 (2001); Wu J, Lanier L L. Natural killer cells and cancer. Adv Cancer Res 90:127-56.:127-156 (2003)]. Since IL-15 has greater biological activity when presented to NK cells bound to IL-15Rα on the cell membrane of stimulatory cells, rather than in its soluble form, we made a construct containing the human IL-15 gene fused to the gene encoding the human CD8α, transmembrane domain, and used it to transduce K562 cells. Expression of IL-15 on the surface of K562 cells was more than five times higher with the IL-15-CD8α construct than with wild-type IL-15.

To test whether the modified K562 cells expressing both 4-11313L and IL-I5 (K562mb15-41BBL cells) promote NK cell expansion, we cultured peripheral blood mononuclear cells from seven donors in the presence of low-dose (10 U/mL) IL-2 as well as irradiated K562 cells transduced with 4-1BBL and/or IL-15, or with an empty control vector. Expression of either 4-1BBL or IL-15 by K562 cells improved the stimulation of NK-stimulatory capacity of K562 in some cases but not overall, whereas simultaneous expression of both molecules led to a consistent and striking amplification of NK cells (median recovery of $CD56^+$ $CD3^-$ cells at 1 week of culture, 2030% of input cells [range, 1020%-2520%] compared with a median recovery of 250% [range, 150%-640%] for K562 cells lacking 4-1BBL and IL-15; $P<0.0001$). In 24 experiments with cells from 8 donors, NK-cell expansion after 3 weeks of culture with K562 cells expressing both stimulatory molecules ranged from 309-fold to 12,409 fold (median, 1089-fold). Neither the modified nor unmodified K562 cells caused an expansion of T lymphocytes. Among expanded $CD56^+$ $CD3^-$ NK cells, expression of CD56 was higher than that of unstimulated cells; expression of CD16 was similar to that seen on unstimulated NK cells (median CD16+ NK cells in 7 donors: 89% before expansion and 84% after expansion). We also compared the expression of KIR molecules on the expanded NK cells with that on NK cells before culture, using the monoclonal antibodies CD158a (against KIR 2DL1), CD158b (2DL2), NKBI (3DL1) and NKAT2 (2DL3). The prevalence of NK subsets expressing these molecules after expansion resembled that of their counterparts before culture, although the level of expression of KIR molecules was higher after culture. Similar results were obtained for the inhibitory receptor CD94, while expression of the activating receptors NKp30 and NKp44 became detectable on most cells after culture. In sum, the immunophenotype of expanded NK cells reiterated that of activated NK cells, indicating that contact with K562-mb1541BBL cells had stimulated expansion of all subsets of NK cells.

Transduction of NK Cells with Chimeric Receptors

Before transducing peripheral blood mononuclear cells with retroviral vectors containing chimeric receptor constructs and GFP, we stimulated them with K562-mb15-41BBL cells. In 27 experiments, the median percentage of NK cells that were GFP^+ at 7-11 days after transduction was 69% (43%-93%). Chimeric receptors were expressed at high levels on the surface of NK cells and, by Western blotting, were in both monomeric and dimeric configurations.

To identify the specific signals required to stimulate NK cells with chimeric receptors, and overcome inhibitory signals mediated by KIR molecules and other NK inhibitory receptors that bind to HLA class I molecules, we first compared two types of chimeric receptors containing different signaling domains: CD3ζ, a signal-transducing molecule containing three immunoreceptor tyrosine-based activation motifs (ITAMs) and linked to several activating receptors expressed on the surface of NK cells [Farag S S, Fehniger T A, Ruggeri L, Velardi A, Caligiuri M A. Natural killer cell receptors: new biology and insights into the graft-versus-leukemia effect. Blood 100:1935-1947 (2002); Moretta L, Moretta A. Unravelling natural killer cell function: triggering and inhibitory human NK receptors. EMBO J 23:255-259 (2004)], and DAP 10, a signal transducing molecule with no ITAMs linked to the activating receptor NKG2D and previously shown to trigger NK cytotoxicity [Farag S S, Fehniger T A, Ruggeri L, Velardi A, Caligiuri M A. Natural killer cell receptors: new biology and insights into the graft-versus-leukemia effect. Blood 100:1935-1947 (2002); Moretta L, Moretta A. Unravelling natural killer cell function: triggering and inhibitory human NK receptors. EMBO J 23:255-259 (2004); Billadeau D D, Upshaw J L, Schoon R A, Dick C J, Leibson P J. NKG2D-DAPIO triggers human NK cell-mediated killing via a Syk-independent regulatory pathway. Nat ImmuNo. 4:557-564 (2003)]. As a control, we used NK cells transduced with a vector containing an antiCD19 receptor but no signaling molecules or containing GFP alone.

NK cells were challenged with the $CD19^+$ leukemic cell lines 380, 697 and RS4;11, all of which express high levels of HLA-class I molecules by antibody staining By genotyping, RS4;11 is Cw4/Cw3, Bw4 and A3; 380 is Cw4/Cw4, Bw4; and 697 is Cw3/Cw3. Hence, these cell lines were fully capable of inhibiting NK cell cytotoxicity via binding to NK inhibitory receptors.

Expression of receptors without signaling molecules did not increase NK-mediated cytotoxicity over that exerted by NK cells transduced with the vector containing only GFP. By

contrast, expression of anti-CD19-ζ receptors markedly enhanced NK cytotoxicity in all experiments, regardless of the intrinsic ability of donor NK cells to kill leukemic targets. For example, 380 cells were highly resistant to NK cells from donors 2 and 3, but were killed when these donor cells expressed anti-CD19-ζ receptors. Similar observations were made for RS4;11 cells and the NK cells of donor 1 and for 697 cells and NK cells of donor 2. Moreover, the anti-CD19-ζ receptors led to improved killing of target cells even when natural cytotoxicity was present. In all experiments, the cytotoxicity triggered by the anti-CD19-ζ receptor was enhanced over that achieved by replacing CD3ζ with DAP 10 ($P<0.001$).

4-1BB-Mediated Costimulatory Signals Enhance NK Cytotoxicity

Previous studies have shown that the addition of costimulatory molecules to chimeric receptors enhances the proliferation and cytotoxicity of T lymphocytes [Imai C, Mihara K, Andreansky M, Nicholson I C, Pui C H, Campana D. Chimeric receptors with 4-1BB signaling capacity provoke potent cytotoxicity against acute lymphoblastic leukemia. Leukemia 18:676-684 (2004)]. Of the two best known costimulatory molecules in T lymphocytes, CD28 and 4-1BB, only 4-1BB is expressed by NK cells [Melero I, Johnston J V, Shufford W W, Mittler R S, Chen L. NKLI cells express 4-1BB (CDw137) costimulatory molecule and are required for tumor immunity elicited by anti-4-1BB monoclonal antibodies. Cell Immunol 1998; 190:167-172 (1998); Lang S, Vujanovic N L, Wollenberg B, Whiteside T L. Absence of B7.1-CD28/CTLA-4 mediated co-stimulation in human NK cells. Eur J Immunol 28:780-786 (1998); Goodier M R, Londei M. CD28 is not directly involved in the response of human CD3CD56+ natural killer cells to lipopolysaccharide: a role for T cells. Immunology 111:384-390(2004)]. We determined whether the addition of 4-1BB to the anti-CD19-ζ receptor would enhance NK cytotoxicity. In a 4 hour-cytotoxicity assay, cells expressing the 41BB-augmented receptor showed a markedly better ability to kill $CD19^+$ cells than did cells lacking this modification. The superiority of NK cells bearing the anti-CD19-BB-ζ receptor was also evident in 24-hour assays with NK cells from different donors cultured at a 1:1 ratio with the leukemia cell lines 697, KOPN57bi and OP-1.

Next, we determined whether the antileukemic activity of NK cells expressing anti-CD19-BB-ζ receptors extended to primary leukemic samples. In five samples from children with different molecular species of ALL, NK cells expressing the 4-1BB receptors exerted strong cytotoxicity that was evident even at low E:T ratios (e.g., <1:1; FIG. **7**) and uniformly exceeded the activity of NK cells expressing signaling receptors that lacked 4-1BB. Even when donor NK cells had natural cytotoxicity against ALL cells and CD3ζ receptor did not improve it, addition of 4-1BB to the receptor significantly enhanced cytotoxicity. Consistent with their increased cytotoxicity, NK cells expressing anti-CD19-BB-ζ mediated more vigorous activation signals. Forty-six percent of NK cells bearing this receptor expressed the IL2 receptor a chain CD25 after 24 hours of coculture with $CD19^+$ ALL cells, compared with only 17% of cells expressing the anti-CD19-ζ receptor and <1% for cells expressing receptors that lacked stimulatory capacity. Moreover, anti-CD19-BB-C receptors induced a much higher production of IFN-g and GM-CSF upon contact with $CD19^+$ cells than did receptors without 41BB.

We asked whether the expression of signaling chimeric receptors would affect spontaneous NK activity against NK-sensitive cell lines not expressing CD19. Spontaneous cytotoxicity of NK cells from three donors against the $CD19^-$ leukemia cell lines K562, U937 and CEM-C7 was not diminished by expression of chimeric receptors, with or without 4-1BB.

Anti-CD19 Chimeric Receptors Induce NK Cytotoxicity Against Autologous Leukemic Cells

To determine whether the NK cell expansion and transduction system that we developed would be applicable to clinical samples, we studied peripheral blood samples that had been obtained (and cryopreserved) from four patients with childhood B-lineage ALL in clinical remission, 25-56 weeks from diagnosis. NK cell expansion occur in all four samples: recovery of after one week of culture with K562-mb15-41BBL cells, recovery of $CD56^+$ $CD3^-$ NK cells ranged from 1350% to 3680% of the input.

After transduction with chimeric receptors, we tested the cytotoxicity of the NK cells against autologous leukemic lymphoblasts obtained at diagnosis. Expression of anti-CD19-BB-ζ receptors overcame NK cell resistance of autologous cells; NK cells expressing the receptors exerted cytotoxicity which was as powerful as that observed with allogeneic targets.

Discussion

In this study, we demonstrated that the resistance of cancer cells to NK cell activity can be overcome by chimeric receptors expressed on primary NK cells. The stimulatory signals triggered by the receptors upon contact with target cells predominated over inhibitory signals and induced powerful cytotoxicity against NK-resistant leukemic cell lines and primary leukemic cells. We found that the type of stimulatory signal delivered by the chimeric receptor was a key factor in inducing cytotoxicity. Although DAP 10 signaling can elicit NK cytotoxicity, chimeric receptors containing this molecule in our study induced weaker NK cell activity than that generated by CD3ζ-containing receptors, despite identical levels of surface expression. We also found that addition of the costimulatory molecule 4-1BB to the chimeric receptors markedly augmented cytotoxicity, and that receptors containing both CD3ζ and 4-1BB triggered a much more robust NK cell activation and cytokine production than did those containing only CD3ζ.

The important contribution of 4-1BB signals agrees with findings that anti-4-1BB antibodies activate murine NK cells [Pan P Y, et al., Regulation of dendritic cell function by NK cells: mechanisms underlying the synergism in the combination therapy of IL-12 and 4-1BB activation. J Immunol 172: 4779-4789 (2004)], and enhance their anti-tumor activity. Leukemic lymphoid cells usually do not express 4-1BB ligand: only 2 of 284 diagnostic B-lineage ALL samples studied by gene arrays at our institution expressed 4-1BB ligand transcripts [Yeoh E J, et al., Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. Cancer Cell 1:133-143 (2002)]. Hence, 4-1BB signals can be delivered to NK cells only if the molecule is incorporated into the receptor.

Efficient and stable transduction of primary NK cells is notoriously difficult, prompting us to devise a new gene transduction method for the present study. Most investigators have demonstrated efficient gene transfer only in continuously growing NK cell lines [Roberts M R, et al., Antigen-specific cytolysis by neutrophils and NK cells expressing chimeric immune receptors bearing zeta or gamma signaling domains. J Immunol. 161:375-384 (1998); Nagashima S, et al., Stable transduction of the interleukin-2 gene into human natural killer cell lines and their phenotypic and functional characterization in vitro and in vivo. Blood 91:3850-3861(1998)] or

reported methods yielding only transient gene expression [Billadeau D D, et al., NKG2D-DAP 10 triggers human NK cell-mediated killing via a Syk-independent regulatory pathway. Nat ImmuNo. 4:557-564 (2003); Trompeter H I, et al., Rapid and highly efficient gene transfer into natural killer cells by nucleofection. J Immunol Methods 274:245-256 (2003); Schroers R, et al., Gene transfer into human T lymphocytes and natural killer cells by Ad5/F35 chimeric adenoviral vectors. Exp Hematol 32:536-546(2004)]. We achieved stable expression of chimeric receptors in primary $CD56^+$ $CD3^-$ NK cells by using an RD114-pseudotyped retroviral vector and specifically expanding primary $CD56^+$ $CD3^-$ NK cells before they were exposed to the retrovirus, a step that allowed highly efficient gene expression. Although several cytokines such as IL-2, IL-12 and IL-15 have been reported to stimulate NK cells [Carson W E, et al., A potential role for interleukin-15 in the regulation of human natural killer cell survival J Clin Invest. 99:937-943 (1997); Trinchieri G, et al., Response of resting human peripheral blood natural killer cells to interleukin 2 J Exp Med 1984; 160: 1147-1169 (1984); Naume B, et al., A comparative study of IL-12 (cytotoxic lymphocyte maturation factor)-, IL-2-, and IL-7-induced effects on immunomagnetically purified CD56+ NK cells. J Immunol 148:2429-2436 (1992)], their capacity to induce proliferation of resting $CD56^+$ CD3 cells has been poor, unless accessory cells are present in the cultures. Perussia et al. Nat Immun Cell Growth Regul 6:171-188 (1987), found that contact with irradiated B-lymphoblastoid cells induced as high as a 25-fold expansion of NK cells after 2 weeks of stimulation, while Miller et al. Blood; 80:2221-2229 (1992) reported an approximate 30-fold expansion of NK cells after 18 days of culture with 1000 U/mL IL-2 and monocytes. However, these culture conditions are likely to promote the growth of $CD3^+$ T lymphocytes as well as NK cells. Since our ultimate aim is to generate pure preparations for out donor NK cells devoid of $CD3^+$ T lymphocytes, that can be infused into recipients of allogeneic hematopoietic stem cell transplants, we searched for methods that would maximize NK cell expansion without producing T-cell mitogenicity.

Contact with K562 cells (which lack MHC-class I molecule expression and hence do not trigger KIR-mediated inhibitory signals in NK cells) is known to augment NK cell proliferation in response to IL-15. We found that membrane-bound IL-15 and 4-1BBL, coexpressed by K562 cells, acted synergistically to augment K562-specific NK stimulatory capacity, resulting in vigorous expansion of peripheral blood $CD56^+$ $CD3^-$ NK cells without concomitant growth of T lymphocytes. After 2-3 weeks of culture, we observed NK cell expansions of up to 10,000-fold, and virtually pure populations of NK cells could be obtained, even without the need for T-cell depletion in some cases. NK cells expanded in this system retained the immunophenotypic diversity seen among peripheral blood subsets of NK cells, as well as their natural cytotoxicity against sensitive target cells, even after transduction with different chimeric receptors. Hence, this system should help studies of NK cell biology which require specific cell expansion and/or gene transduction, but it should also be adaptable to clinical applications after generating K562mb 15-4 1 BBL cells that comply with current good manufacturing practices for clinical trials. Recently, Harada et al. reported that expansions of $CD56^+$ $CD3^-$ cells (up to 400-fold after 2 weeks) were apparently superior after contact with another HLA class I-negative cell line, the Wilms tumor cell line HFWT [Harada H, Saijo K, Watanabe S, et al. Selective expansion of human natural killer cells from peripheral blood mononuclear cells by the cell line, HFWT. Jpn J Cancer Res 93:313 (2002)]. Future studies should determine whether HFWT cells express 41BBL or whether enforced expression of 4-1BBL together with IL-15 results in a greater specific expansion of NK cells than seen with modified K562 cells.

In the context of allogeneic hematopoietic stem cell transplantation, infusions of activated donor T cells would carry an unacceptably high risk of severe GvHD, particularly in recipients of haploidentical or mismatched transplants. By contrast, infusions of pure CD56 CD3 NK cells should not impose that risk [Ruggeri L, et al., Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science 295:2097-2100 (2002)]. Most clinical studies of the therapeutic effects of NK cells have been performed in an autologous setting and have yielded only moderately promising results [Farag S S, et al., Natural killer cell receptors: new biology and insights into the graft-versus-leukemia effect. Blood 100:1935-1947 (2002); Chiorean E G, Miller J S. The biology of natural killer cells and implications for therapy of human disease. J Hematother Stem Cell Res 10:451-463 (2001)]. This is not surprising because NK cell activity is inhibited by surface receptors that recognize autologous HLA molecules expressed by both normal and neoplastic cells. Allogeneic NK cells may be more effective, but even in an allogeneic setting the capacity of NK cells to kill malignant lymphoid cells is generally modest and often negligible [Caligiuri M A, Velardi A, Scheinberg D A, Borrello I M. Immunotherapeutic approaches for hematologic malignancies. Hematology (Am Soc Hematol Educ Program) 337-353 (2004)]. Leung et al. [J Immunol 172:644-650 (2004)] detected NK cytotoxicity against an ALL cell line expressing particularly low levels of inhibitory HLA molecules, but cytotoxicity was much lower than that observed against the NK-cell target K562: only about 50% of the ALL cells were killed at an effector:target ratio of 40:1. In that study, RS4;11 cells, which express HLA-C alleles that bind the most commonly expressed KIRs, were NK-resistant, whereas these cells, as well as autologous leukemic cells, were highly sensitive to NK cells expressing anti-CD19 signaling receptors in our study. NK cells expressing signaling chimeric receptors have much more powerful antileukemic activity than unmodified NK cells, and can kill target cells irrespective of their HLA profile. An increased understanding of the signals leading to immune cell activation, together with progress in gene cloning and transfer, have made the treatment of cancer with "adoptively acquired immunity" a realistic goal. Clinical precedents, such as administration of T-cell clones that target cytomegalovirus epitopes [Walter E A, et al., Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. N Engl J Med 1995; 333: 1038-1044 (1995)] or EBV-specific antigens [Rooney C M, et al., Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr-virus-related lymphoproliferation. Lancet 345:9-13(1995)], attest to the clinical feasibility of adoptive immune cell therapy. Nonetheless, there are potential limitations that may affect the effectiveness of cell therapy guided by chimeric receptors. One is that the murine scFv portion of the chimeric receptor or the fusion sites of the human regions that compose it may trigger a host immune response leading to elimination of the modified cells [Sadelain M, et al., Targeting tumours with genetically enhanced T lymphocytes. Nat Rev Cancer 3:35-45 (2003)]. Although the impact of such an event in a clinical setting remains to be determined, we anticipate that immune responses against modified NK cells will be limited in immune-suppressed patients after hematopoietic stem cell transplantation. Another potential limitation is that adoptively transferred

cells may have inadequate persistence in vivo, although a recent study showed that NK cells obtained from haploidentical donors and activated ex vivo could expand in patients when infused after administration of high-dose cyclophosphamide and fludarabine, which caused an increased in endogenous IL-15 [Miller J S, et al., Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in cancer patients. Blood; in press (2005)]. We speculate that such expansions would also occur with genetically-modified NK cells, and suggest that further studies to identify signaling molecules that promote NK cell proliferation when incorporated into chimeric receptors are warranted. In patients at a high risk of leukemia or lymphoma relapse, the expected benefits of genetically-modified NK cells will outweigh the risk of insertional oncogenesis posed by the use of retroviruses for chimeric receptor transduction [Baum C, et al., Side effects of retroviral gene transfer into hematopoietic stem cells. Blood 101:2099-2114 (2003)]. We also predict that the coexpression of suicide genes will become a useful safety measure in clinical studies [Marktel S, et al., Immunologic potential of donor lymphocytes expressing a suicide gene for early immune reconstitution after hematopoietic T-cell-depleted stem cell transplantation. Blood 101:1290-1298 (2003)]; this strategy would also ensure that the elimination of normal $CD19^+$ B-lineage cells is only temporary.

Novel therapies that bypass cellular mechanisms of drug resistance are urgently needed for patients with refractory leukemia and lymphoma. NK cell alloreactivity is a powerful new tool for improving the therapeutic potential of allogeneic hematopoietic stem cell transplantation. The results of this study indicate that signaling receptors can enhance the efficacy of NK cell alloreactivity and widen its applicability. We envisage initial clinical trials in which donor NK cells, collected by apheresis, are expanded ex vivo as described here, transduced with chimeric receptors and then infused after transplantation in patients with B-lineage ALL. The target molecule for the chimeric receptors, CD19, was selected because it is one of the most widely expressed surface antigens among B-cell malignancies, including ALL, CLL and NHL. In these malignancies, CD19 is highly expressed on the surface of virtually all cells but has limited or no expression in normal tissues [Campana D, Behm F G. Immunophenotyping of leukemia. J Immunol Methods 243:59-75 (2000)]. However, the NK-cell strategy of immunotherapy we describe would not have to be directed to the CD19 antigen, but could be applied to any of the numerous molecules identified as potential targets for chimeric receptor-based cell therapy in cancer patients.

All of the above U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent publications referred to in this specification, including but not limited to U.S. patent application Ser. No. 09/960,264, filed Sep. 20, 2001; and U.S. application Ser. No. 10/981,352, filed Nov. 4, 2004, are incorporated herein by reference, in their entirety. All of references, patents, patent applications, etc. cited above, are incorporated herein in their entirety.

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Val Leu Asn Phe Glu Arg Thr Arg Ser Leu Gln Asp Pro Cys Ser Asn
15                  20                  25                  30

tgc cca gct ggt aca ttc tgt gat aat aac agg aat cag att tgc agt      266
Cys Pro Ala Gly Thr Phe Cys Asp Asn Asn Arg Asn Gln Ile Cys Ser
                35                  40                  45

ccc tgt cct cca aat agt ttc tcc agc gca ggt gga caa agg acc tgt      314
Pro Cys Pro Pro Asn Ser Phe Ser Ser Ala Gly Gly Gln Arg Thr Cys
            50                  55                  60

gac ata tgc agg cag tgt aaa ggt gtt ttc agg acc agg aag gag tgt      362
Asp Ile Cys Arg Gln Cys Lys Gly Val Phe Arg Thr Arg Lys Glu Cys
        65                  70                  75

tcc tcc acc agc aat gca gag tgt gac tgc act cca ggg ttt cac tgc      410
Ser Ser Thr Ser Asn Ala Glu Cys Asp Cys Thr Pro Gly Phe His Cys
    80                  85                  90
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-continued

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ctg ggg gca gga tgc agc atg tgt gaa cag gat tgt aaa caa ggt caa      458
Leu Gly Ala Gly Cys Ser Met Cys Glu Gln Asp Cys Lys Gln Gly Gln
95                  100                 105                 110

gaa ctg aca aaa aaa ggt tgt aaa gac tgt tgc ttt ggg aca ttt aac      506
Glu Leu Thr Lys Lys Gly Cys Lys Asp Cys Cys Phe Gly Thr Phe Asn
                115                 120                 125

gat cag aaa cgt ggc atc tgt cga ccc tgg aca aac tgt tct ttg gat      554
Asp Gln Lys Arg Gly Ile Cys Arg Pro Trp Thr Asn Cys Ser Leu Asp
            130                 135                 140

gga aag tct gtg ctt gtg aat ggg acg aag gag agg gac gtg gtc tgt      602
Gly Lys Ser Val Leu Val Asn Gly Thr Lys Glu Arg Asp Val Val Cys
        145                 150                 155

gga cca tct cca gcc gac ctc tct ccg gga gca tcc tct gtg acc ccg      650
Gly Pro Ser Pro Ala Asp Leu Ser Pro Gly Ala Ser Ser Val Thr Pro
    160                 165                 170

cct gcc cct gcg aga gag cca gga cac tct ccg cag atc atc tcc ttc      698
Pro Ala Pro Ala Arg Glu Pro Gly His Ser Pro Gln Ile Ile Ser Phe
175                 180                 185                 190

ttt ctt gcg ctg acg tcg act gcg ttg ctc ttc ctg ctg ttc ttc ctc      746
Phe Leu Ala Leu Thr Ser Thr Ala Leu Leu Phe Leu Leu Phe Phe Leu
                195                 200                 205

acg ctc cgt ttc tct gtt gtt aaa cgg ggc aga aag aaa ctc ctg tat      794
Thr Leu Arg Phe Ser Val Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr
            210                 215                 220

ata ttc aaa caa cca ttt atg aga cca gta caa act act caa gag gaa      842
Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu
        225                 230                 235

gat ggc tgt agc tgc cga ttt cca gaa gaa gaa gaa gga gga tgt gaa      890
Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu
    240                 245                 250

ctg tgaaatggaa gtcaataggg ctgttgggac tttcttgaaa agaagcaagg           943
Leu
255

aaatatgagt catccgctat cacagctttc aaaagcaaga acaccatcct acataatacc   1003

caggattccc ccaacacacg ttcttttcta aatgccaatg agttggcctt taaaaatgca   1063

ccacttttt ttttttttg acagggtctc actctgtcac ccaggctgga gtgcagtggc   1123

accaccatgg ctctctgcag ccttgacctc tgggagctca agtgatcctc ctgcctcagt   1183

ctcctgagta gctggaacta caaggaaggg ccaccacacc tgactaactt ttttgttttt   1243

tgtttggtaa agatggcatt tcaccatgtt gtacaggctg gtctcaaact cctaggttca   1303

ctttggcctc ccaaagtgct gggattacag acatgaactg ccaggcccgg ccaaaataat   1363

gcaccacttt taacagaaca gacagatgag gacagagctg gtgataaaaa aaaaaaaaaa   1423

aaagcatttt ctagatacca cttaacaggt ttgagctagt ttttttgaaa tccaaagaaa   1483

attatagttt aaattcaatt acatagtcca gtggtccaac tataattata atcaaaatca   1543

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gtacccgact aaagtttccg tgggttctgt catgtaacac gacatgctcc accgtcaggg   1663

gggagtatga gcagagtgcc tgagtttagg gtcaaggaca aaaaacctca ggcctggagg   1723

aagttttgga aagagttcaa gtgtctgtat atcctatggt cttctccatc ctcacacctt   1783

ctgcctttgt cctgctccct tttaagccag gttacattct aaaaattctt aacttttaac   1843

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aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aa                                  1935
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<210> SEQ ID NO 2

<211> LENGTH: 255
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

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Asn Phe Glu Arg Thr Arg Ser Leu Gln Asp Pro Cys Ser Asn Cys Pro
            20                  25                  30

Ala Gly Thr Phe Cys Asp Asn Asn Arg Asn Gln Ile Cys Ser Pro Cys
        35                  40                  45

Pro Pro Asn Ser Phe Ser Ser Ala Gly Gly Gln Arg Thr Cys Asp Ile
    50                  55                  60

Cys Arg Gln Cys Lys Gly Val Phe Arg Thr Arg Lys Glu Cys Ser Ser
65                  70                  75                  80

Thr Ser Asn Ala Glu Cys Asp Cys Thr Pro Gly Phe His Cys Leu Gly
                85                  90                  95

Ala Gly Cys Ser Met Cys Glu Gln Asp Cys Lys Gln Gly Gln Glu Leu
            100                 105                 110

Thr Lys Lys Gly Cys Lys Asp Cys Cys Phe Gly Thr Phe Asn Asp Gln
        115                 120                 125

Lys Arg Gly Ile Cys Arg Pro Trp Thr Asn Cys Ser Leu Asp Gly Lys
    130                 135                 140

Ser Val Leu Val Asn Gly Thr Lys Glu Arg Asp Val Val Cys Gly Pro
145                 150                 155                 160

Ser Pro Ala Asp Leu Ser Pro Gly Ala Ser Ser Val Thr Pro Pro Ala
                165                 170                 175

Pro Ala Arg Glu Pro Gly His Ser Pro Gln Ile Ile Ser Phe Phe Leu
            180                 185                 190

Ala Leu Thr Ser Thr Ala Leu Leu Phe Leu Leu Phe Phe Leu Thr Leu
        195                 200                 205

Arg Phe Ser Val Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe
    210                 215                 220

Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly
225                 230                 235                 240

Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu
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<210> SEQ ID NO 3
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<212> TYPE: DNA
<213> ORGANISM: Mus musculus
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (146)..(916)
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1253)..(1255)
<223> OTHER INFORMATION: n is a, c, g, or t

<400> SEQUENCE: 3

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ctacaccagg aaaaggacac attcgacaac aggaaaggag cctgtcacag aaaaccacag    120

tgtcctgtgc atgtgacatt tcgcc atg gga aac aac tgt tac aac gtg gtg      172
                               Met Gly Asn Asn Cys Tyr Asn Val Val
                               1               5

gtc att gtg ctg ctg cta gtg ggc tgt gag aag gtg gga gcc gtg cag      220
Val Ile Val Leu Leu Leu Val Gly Cys Glu Lys Val Gly Ala Val Gln
10                  15                  20                  25
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aac tcc tgt gat aac tgt cag cct ggt act ttc tgc aga aaa tac aat      268
Asn Ser Cys Asp Asn Cys Gln Pro Gly Thr Phe Cys Arg Lys Tyr Asn
                30                  35                  40

cca gtc tgc aag agc tgc cct cca agt acc ttc tcc agc ata ggt gga      316
Pro Val Cys Lys Ser Cys Pro Pro Ser Thr Phe Ser Ser Ile Gly Gly
            45                  50                  55

cag ccg aac tgt aac atc tgc aga gtg tgt gca ggc tat ttc agg ttc      364
Gln Pro Asn Cys Asn Ile Cys Arg Val Cys Ala Gly Tyr Phe Arg Phe
        60                  65                  70

aag aag ttt tgc tcc tct acc cac aac gcg gag tgt gag tgc att gaa      412
Lys Lys Phe Cys Ser Ser Thr His Asn Ala Glu Cys Glu Cys Ile Glu
    75                  80                  85

gga ttc cat tgc ttg ggg cca cag tgc acc aga tgt gaa aag gac tgc      460
Gly Phe His Cys Leu Gly Pro Gln Cys Thr Arg Cys Glu Lys Asp Cys
90                  95                  100                 105

agg cct ggc cag gag cta acg aag cag ggt tgc aaa acc tgt agc ttg      508
Arg Pro Gly Gln Glu Leu Thr Lys Gln Gly Cys Lys Thr Cys Ser Leu
                110                 115                 120

gga aca ttt aat gac cag aac ggt act ggc gtc tgt cga ccc tgg acg      556
Gly Thr Phe Asn Asp Gln Asn Gly Thr Gly Val Cys Arg Pro Trp Thr
            125                 130                 135

aac tgc tct cta gac gga agg tct gtg ctt aag acc ggg acc acg gag      604
Asn Cys Ser Leu Asp Gly Arg Ser Val Leu Lys Thr Gly Thr Thr Glu
        140                 145                 150

aag gac gtg gtg tgt gga ccc cct gtg gtg agc ttc tct ccc agt acc      652
Lys Asp Val Val Cys Gly Pro Pro Val Val Ser Phe Ser Pro Ser Thr
    155                 160                 165

acc att tct gtg act cca gag gga gga cca gga ggg cac tcc ttg cag      700
Thr Ile Ser Val Thr Pro Glu Gly Gly Pro Gly Gly His Ser Leu Gln
170                 175                 180                 185

gtc ctt acc ttg ttc ctg gcg ctg aca tcg gct ttg ctg ctg gcc ctg      748
Val Leu Thr Leu Phe Leu Ala Leu Thr Ser Ala Leu Leu Leu Ala Leu
                190                 195                 200

atc ttc att act ctc ctg ttc tct gtg ctc aaa tgg atc agg aaa aaa      796
Ile Phe Ile Thr Leu Leu Phe Ser Val Leu Lys Trp Ile Arg Lys Lys
            205                 210                 215

ttc ccc cac ata ttc aag caa cca ttt aag aag acc act gga gca gct      844
Phe Pro His Ile Phe Lys Gln Pro Phe Lys Lys Thr Thr Gly Ala Ala
        220                 225                 230

caa gag gaa gat gct tgt agc tgc cga tgt cca cag gaa gaa gaa gga      892
Gln Glu Glu Asp Ala Cys Ser Cys Arg Cys Pro Gln Glu Glu Glu Gly
    235                 240                 245

gga gga gga ggc tat gag ctg tga tgtactatcc taggagatgt gtgggccgaa     946
Gly Gly Gly Gly Tyr Glu Leu
250                 255

accgagaagc actaggaccc caccatcctg tggaacagca caagcaaccc caccaccctg   1006

ttcttacaca tcatcctaga tgatgtgtgg gcgcgcacct catccaagtc tcttctaacg   1066

ctaacatatt tgtctttacc ttttttaaat ctttttttaa atttaaattt tatgtgtgtg   1126

agtgttttgc ctgcctgtat gcacacgtgt gtgtgtgtgt gtgtgtgaca ctcctgatgc   1186

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agccggnnng ataggtcggg acggagacct gtcttcttat tttaacgtga ctgtataata   1306

aaaaaaaaat gatatttcgg gaattgtaga gattctcctg acacccttct agttaatgat   1366

ctaagaggaa ttgttgatac gtagtatact gtatatgtgt atgtatatgt atatgtatat   1426

ataagactct tttactgtca aagtcaacct agagtgtctg gttaccaggt caattttatt   1486

ggacatttta cgtcacacac acacacacac acacacacac acgtttatac tacgtactgt   1546
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tatcggtatt ctacgtcata taatgggata gggtaaaagg aaaccaaaga gtgagtgata   1606

ttattgtgga ggtgacagac tacccttct gggtacgtag ggacagacct ccttcggact   1666

gtctaaaact ccccttagaa gtctcgtcaa gttcccggac gaagaggaca gaggagacac   1726

agtccgaaaa gttatttttc cggcaaatcc tttccctgtt tcgtgacact ccacccctg   1786

tggacacttg agtgtcatcc ttgcgccgga aggtcaggtg gtacccgtct gtaggggcgg   1846

ggagacagag ccgcgggggga gctacgagaa tcgactcaca gggcgccccg ggcttcgcaa   1906

atgaaacttt tttaatctca caagtttcgt ccgggctcgg cggacctatg gcgtcgatcc   1966

ttattacctt atcctggcgc caagataaaa caaccaaaag ccttgactcc ggtactaatt   2026

ctccctgccg gcccccgtaa gcataacgcg gcgatctcca ctttaagaac ctggccgcgt   2086

tctgcctggt ctcgctttcg taaacggttc ttacaaaagt aattagttct tgctttcagc   2146

ctccaagctt ctgctagtct atggcagcat caaggctggt atttgctacg gctgaccgct   2206

acgccgccgc aataagggta ctgggcggcc cgtcgaaggc cctttggttt cagaaaccca   2266

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<210> SEQ ID NO 4
<211> LENGTH: 256
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 4

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Gly Cys Glu Lys Val Gly Ala Val Gln Asn Ser Cys Asp Asn Cys Gln
            20                  25                  30

Pro Gly Thr Phe Cys Arg Lys Tyr Asn Pro Val Cys Lys Ser Cys Pro
        35                  40                  45

Pro Ser Thr Phe Ser Ser Ile Gly Gly Gln Pro Asn Cys Asn Ile Cys
    50                  55                  60

Arg Val Cys Ala Gly Tyr Phe Arg Phe Lys Lys Phe Cys Ser Ser Thr
65                  70                  75                  80

His Asn Ala Glu Cys Glu Cys Ile Glu Gly Phe His Cys Leu Gly Pro
                85                  90                  95

Gln Cys Thr Arg Cys Glu Lys Asp Cys Arg Pro Gly Gln Glu Leu Thr
            100                 105                 110

Lys Gln Gly Cys Lys Thr Cys Ser Leu Gly Thr Phe Asn Asp Gln Asn
        115                 120                 125

Gly Thr Gly Val Cys Arg Pro Trp Thr Asn Cys Ser Leu Asp Gly Arg
    130                 135                 140

Ser Val Leu Lys Thr Gly Thr Thr Glu Lys Asp Val Val Cys Gly Pro
145                 150                 155                 160

Pro Val Val Ser Phe Ser Pro Ser Thr Thr Ile Ser Val Thr Pro Glu
                165                 170                 175

Gly Gly Pro Gly Gly His Ser Leu Gln Val Leu Thr Leu Phe Leu Ala
            180                 185                 190

Leu Thr Ser Ala Leu Leu Leu Ala Leu Ile Phe Ile Thr Leu Leu Phe
        195                 200                 205
```

-continued

```
Ser Val Leu Lys Trp Ile Arg Lys Lys Phe Pro His Ile Phe Lys Gln
    210                 215                 220

Pro Phe Lys Lys Thr Thr Gly Ala Ala Gln Glu Glu Asp Ala Cys Ser
225                 230                 235                 240

Cys Arg Cys Pro Gln Glu Glu Glu Gly Gly Gly Gly Gly Tyr Glu Leu
                245                 250                 255
```

What is claimed is:

1. A polynucleotide encoding a chimeric receptor comprising: (a) an extracellular ligand-binding domain comprising an anti-CD19 single chain variable fragment (scFv) domain; (b) a transmembrane domain; and (c) a cytoplasmic domain comprising a 4-1BB signaling domain and a CD3ζ signaling domain.

2. A vector comprising a polynucleotide encoding a chimeric receptor comprising: (a) an extracellular ligand-binding domain comprising an anti-CD19 single chain variable fragment (scFv) domain, (b) a transmembrane domain, and (c) a cytoplasmic domain comprising a 4-1BB signaling domain and a CD3ζ signaling domain, wherein the polynucleotide encoding the chimeric receptor is operatively linked to at least one regulatory element for expression of the chimeric receptor.

3. An isolated host cell comprising a polynucleotide encoding a chimeric receptor comprising: (a) an extracellular ligand-binding domain comprising an anti-CD19 single chain variable fragment (scFv) domain; (b) a transmembrane domain; and (c) a cytoplasmic domain comprising a 4-1BB signaling domain and a CD3ζ signaling domain.

4. The isolated host cell of claim **3** which is a T lymphocyte or an NK cell.

5. The isolated host cell of claim **3** which is a T lymphocyte.

6. The polynucleotide of claim **1** wherein the signaling domain is a human 4-1BB signaling domain.

7. The polynucleotide of claim **6**, Wherein the 4-1BB signaling domain comprises amino acids 214-255 of SEQ ID NO:2.

8. The polynucleotide of claim **7**, wherein the nucleotide sequence encoding the human 4-1BB signaling domain comprises nucleotide residues 129-893 of SEQ ID NO:1.

9. The polynucleotide of claim **1**, wherein the transmembrane domain is the transmembrane domain of CD8α.

10. The polynucleotide of claim **9**, wherein the extracellular ligand-binding domain further comprises a signal peptide of CD8α.

11. The vector of claim **2** which is a viral vector.

12. The vector of claim **11** which is a retroviral vector.

13. The isolated host cell of claim **3** which is an NK cell.

14. The isolated host cell of claim **3** which is an autologous cell isolated from a patient having a cancer of B cell origin.

15. The isolated host cell of claim **14**, wherein the autologous cell is an autologous T lymphocyte.

16. The isolated host cell of claim **15**, wherein the autologous T lymphocyte is derived from a blood or tumor sample of a patient having a cancer of B cell origin and activated and expanded in vitro.

17. The isolated host cell of claim **5**, wherein the T lymphocyte is an activated T lymphocyte.

18. The isolated host cell of claim **5**, wherein the T lymphocyte is isolated from a blood or tumor sample of a patient having a cancer of B cell origin.

19. The isolated host cell of claim **18** wherein the host cell is isolated from a patient having lymphoblastic leukemia, B-lineage acute lymphoblastic leukemia, B-cell chronic lymphocytic leukemia or B-cell non-Hodgkin's lymphoma.

20. The polynucleotide of claim **1**, wherein the chimeric receptor further comprises a hinge domain.

21. The vector of claim **2**, wherein the chimeric receptor further comprises a hinge domain.

22. The isolated cell of claim **3**, wherein the chimeric receptor further comprises a hinge domain.

* * * * *

EXHIBIT B

St. Jude Children's Research Hospital
ALSAC • Danny Thomas, Founder

December 10, 2003

Collaboration and Materials Transfer Agreement

Dr. Carl June
Professor of Pathology and Laboratory Medicine
University of Pennsylvania School of Medicine
Room 554 BRB II/III
421 Curie Boulevard
Philadelphia, PA 19104-6160

Dear Dr. June:

This Agreement, effective upon signing, governs an arrangement whereby Dr. Dario Campana of St. Jude Children's Research Hospital, Inc., ("St. Jude") agrees to provide biological material that is proprietary to St. Jude, for use in a collaborative research study with Dr. Carl June ("Recipient Scientist") of the University of Pennsylvania ("Recipient"), subject to the terms and conditions set forth below. Trustees of the University of Pennsylvania

1. The biological material to be provided to Recipient Scientist is the anti-CD19-BB-ζ chimeric T-cell receptor construct, including any progeny, portions, unmodified derivatives and any accompanying know-how or data ("Material"). Legal title to the Material shall remain with St. Jude. The Recipient acknowledges that the Material is or may be the subject of a patent application. Except as provided in this Agreement, no express or implied licenses or other rights are provided to the Recipient under any patents, patent applications, trade secrets or other proprietary rights of St. Jude, including any altered forms of the Material made by St. Jude.

2. The Material is for use by Recipient Scientist or persons directly supervised by Recipient Scientist. The Material may not be transferred or taken to any other laboratory or made available to any other person or third party, but is to remain under the immediate and direct control of Recipient Scientist.

3. Recipient Scientist agrees that the Material will only be used to create a lentiviral chimeric T-cell receptor construct to be used in pre-clinical studies.

4. The Material may not be used in humans and will be stored, used, and disposed of in accordance with applicable law and regulations. The Material will not be used for any commercial purpose.

5. St. Jude retains the unrestricted right to distribute the Material to other commercial or noncommercial entities.

6. Recipient agrees that any publications that result from the collaborative research study between St. Jude and Recipient Scientist using the Material will be jointly published in accordance with academic standards.

7. The transfer of the Material grants to Recipient no rights in the Material other than those specifically set forth in the Agreement. This agreement may be terminated upon thirty (30) days written notice by either party to the other. Upon termination of

the agreement, Recipient shall destroy all unused Materials.

8. Recipient shall not commercialize any product that contains Material without the prior written approval of St. Jude. The Recipient is free to file patent application(s) claiming inventions made by Recipient through use of the Material but agrees to notify St. Jude within sixty (60) days of filing any patent application which claims subject matter that contains or incorporates the Material or which claims a method of manufacture or use of the Material. Recipient grants to St. Jude a non-exclusive, royalty-free license to use for non-commercial purposes any inventions that arise from the use of the Materials. INVENTORSHIP WILL BE DETERMINED ACCORDING TO US PATENT LAW TJR 12/17/03

9. The Material provided is experimental in nature, and it is provided WITHOUT ANY WARRANTIES, EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR USE. St. Jude MAKES NO REPRESENTATION AND PROVIDES NO WARRANTY THAT THE USE OF THE MATERIAL WILL NOT INFRINGE ANY PATENT OR OTHER PROPRIETARY RIGHT. 12/17/03

10. Except to the extent prohibited by law, the Recipient assumes all liability for damages that may arise from its use, storage or disposal of the Materials. St. Jude and corporate affiliates of St. Jude and their respective Boards of Governors, Directors, officers, staff, representatives and agents will not be liable to the Recipient for any damages, expenses (including without limitation legal expenses), losses, claims, demands, suit or other actions (collectively hereinafter "Claims") made by the Recipient, or made against the Recipient by any other party, due to or arising from the use, storage or disposal of the Materials by the Recipient, except to the extent such Claims are solely caused by the gross negligence or willful misconduct of St. Jude.

If Recipient Scientist and Recipient agree to the above, please sign and have an authorized official of Recipient sign and return a copy of this letter to Esther Allay in the Office of Technology Licensing.

Sincerely,

J. Scott Elmer
Director
Office of Technology Licensing

Agreed and Accepted

Dr. Carl June
12/16/2003
Date

Institution Official

Name: Timothy J. Raynor
Director, Intellectual Property
Center for Technology Transfer
University of Pennsylvania
Title

12/17/03
Date

EXHIBIT C

St. Jude Children's Research Hospital

October 2, 2007

Materials Transfer Agreement

Dr. Carl June
Professor of Pathology and Laboratory Medicine
University of Pennsylvania School of Medicine
Room 554 BRB II/III
421 Curie Boulevard
Philadelphia, PA 19104-6160

Dear Dr. June:

St. Jude Children's Research Hospital ("St. Jude") agrees to provide you and your institute, the Trustees of the University of Pennsylvania (collectively referred to herein as "Recipient"), with materials developed at St. Jude. Before receiving the materials, we ask that you and your institute agree to the following terms and conditions:

1. The biological materials to be provided to Recipient are the anti-CD19-BBζ chimeric receptor construct, including any progeny, portions, unmodified derivatives and any accompanying know-how or data ("Materials"). The Recipient acknowledges that the Materials are or may be the subject of patent(s), pending patent application(s) or other proprietary right of St. Jude. Except as provided in this Agreement, no express or implied licenses or other rights are provided to the Recipient under any patents, patent applications, trade secrets or other proprietary rights of St. Jude, including any altered forms of the Materials made by St. Jude.

2. Recipient accepts sole responsibility for any and all receipt, storage, handling, disposition, transfer and uses of the Materials in compliance with all applicable Federal, State and local laws, rules, regulations and guidelines including, but not limited to Federal and State laws relating to the protection of human research subjects.

3. The Recipient further agrees that the Materials are provided for the sole purpose of allowing Recipient to use Materials to produce a molecular lentiviral vector clone incorporating Materials in compliance with GMP for application in ex vivo autologous cell modification in quantities sufficient to complete a Phase I clinical trial to be conducted at the Recipient's clinical facilities. Without limiting the foregoing, Recipient acknowledges and agrees that (i) the Materials may not be taken or sent to another institution without written permission from St. Jude and (ii) the Materials may not be provided to a commercial entity, and may not be used in research that is subject to consulting or licensing obligations to another party (other than those obligations imposed upon grantee institutions of the U.S. government) without express written consent by St. Jude.

4. Recipient agrees to provide St. Jude with a copy of any publication that contains experimental results obtained from the use of the Materials, and will acknowledge St.

Jude as the source of the Materials.

5. Recipient acknowledges St. Jude's ownership of the Materials and any progeny thereof. If Recipient files a patent application or commercializes a product which contains a portion of the Materials, is derived from the Materials, or which could not have been produced but for the use of the Materials, Recipient agrees to contact St. Jude to determine ownership interests, if any, St. Jude may have in such patent application or commercial product. Ownership shall follow inventorship according to US patent law. Further, Recipient shall not publish or disclose the results of such research using the Materials without submitting the proposed publication or disclosure to St. Jude at least thirty (30) days prior to the submission for publication or disclosure to allow St. Jude to review such publication or disclosure for the disclosure of St. Jude proprietary information.

6. The Materials provided are experimental in nature, and are provided WITHOUT ANY WARRANTIES, EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR USE. ST. JUDE MAKES NO REPRESENTATION AND PROVIDE NO WARRANTY THAT THE USE OF THE MATERIALS WILL NOT INFRINGE ANY PATENT OR OTHER PROPRIETARY RIGHT. IN NO EVENT SHALL ST. JUDE BE LIABLE FOR ANY INDIRECT, INCIDENTAL OR CONSEQUENTIAL DAMAGES, EVEN IF ADVISED OF THE POSSIBILITY OF SUCH DAMAGES.

7. Recipient agrees to indemnify, defend and hold harmless St. Jude and the American Lebanese Syrian Associated Charities (ALSAC) and their respective corporate affiliates and Boards of Governors, trustees, directors, officers, medical and professional staff, employees, representatives and agents and their respective successors, heirs and assigns against any and all liability, claims, demands or legal causes of action of any nature whatsoever (including without limitation legal expenses), arising out of or related to Recipient's acceptance, use and disposal of the Materials and their progeny or derivatives, including but not limited to any and all claims for personal injury or death.

8. Recipient shall maintain liability insurance on an occurrence basis, or if claims made including tail coverage, in the amount of not less than One Million ($1,000,000) dollars per claim and Three Million ($3,000,000) dollars aggregate.

9. Recipient shall provide St. Jude, upon request, with a Certificate of Insurance from its insurer (i) stating that the insurance coverage set forth in section 8 is in full force and effect, and (ii) promising to provide St. Jude with thirty (30) days prior written notice of cancellation or reduction in coverage.

10. St. Jude may terminate this MTA if Recipient breaches any term and fails to cure such breach within thirty (30) days after written notice thereof. Upon such termination, Recipient will immediately cease use of the Materials and all progeny thereof, and return all Materials and progeny to St. Jude. Sections 5, 6 and 7 shall survive termination or expiration of this MTA.

11. This MTA may only be modified in writing signed by an authorized representative of each party. No express or implied waiver by a party of any breach hereunder shall in any way be, or be construed as, a waiver of any subsequent breach. In the event that any provision of this MTA is held by a court of competent jurisdiction to be invalid or unenforceable, such provision will be stricken from this MTA and the remaining provisions shall remain in full force and effect to the extent permitted by law.

If the terms and conditions set forth above are acceptable, please return one copy to Esther Allay in the Office of Technology Licensing after it has been signed by you and by an authorized official of your institution, and retain the other copy for your files. The Materials will be forwarded to you upon receipt of the signed letter.

Regards,

J. Scott Elmer
Director, Office of
Technology Licensing

Edward Pieters, Ph.D.
Associate Director
Research Agreements
Office of Research Services
University of Pennsylvania
P221 Franklin Building
3451 Walnut Street
Philadelphia, Pa. 19104-6205

Accepted and Agreed:

Recipient Scientist

2/5/08
Date

Accepted and Agreed:

Institution Official (The Trustees of the University of Pennsylvania)

Name

Title

8 Feb 08
Date

EXHIBIT D

THE UNITED STATES DISTRICT COURT
FOR THE WESTERN DISTRICT OF TENNESSEE
WESTERN DISTRICT

ST. JUDE CHILDREN'S RESEARCH HOSPITAL, INC. PLAINTIFF

v. Civil Action No. _____

THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA DEFENDANT

COMPLAINT

(Jury Trial Demanded)

Plaintiff St. Jude Children's Research Hospital, Inc. ("St. Jude"), for its Complaint against Defendant The Trustees of The University of Pennsylvania ("Penn"), states as follows:

JURISDICTION AND VENUE

1. This case is a civil action between citizens of different States where the amount in controversy exceeds the sum or value of $75,000, exclusive of interest and costs.

2. St. Jude seeks monetary damages herein in an amount in excess of $75,000, exclusive of interest and costs.

3. Further, the Material Transfer Agreements, explained in more detail below in this Complaint, involve Materials that cost in excess of $75,000 to develop. Accordingly, this Court has subject matter jurisdiction under 28 U.S.C. § 1332.

4. Penn has conducted business in the State of Tennessee, including, but not limited to, entering into approximately 68 Material Transfer Agreements with St. Jude. This Action arises from two such Material Transfer Agreements entered into by Penn and Dr. Carl H. June, M.D. ("Dr. June").

5. Additionally, Penn actively recruits potential students from within the State of

Tennessee, not only with promotional materials and communications purposefully sent into this state, but also with Penn-sponsored events in Tennessee, attended by Penn officials.

6. Penn has purposefully availed itself of the privilege of acting in Tennessee and has caused and is causing consequences within the State of Tennessee.

7. The exercise of personal jurisdiction over Penn satisfies the Due Process Clause of the Fourteenth Amendment to the United States Constitution.

8. This Court has personal jurisdiction over Penn pursuant to Tennessee's long-arm statute, Tenn. Code Ann. § 20-2-214.

9. Venue is proper in this Court pursuant to 28 U.S.C. § 1391 because a substantial part of the events or omissions giving rise to this Action occurred in this judicial district.

THE PARTIES

10. Plaintiff St. Jude is a non-profit corporation duly organized under the laws of the State of Tennessee with its principal place of business in Shelby County, Tennessee. St. Jude is therefore a citizen solely of the State of Tennessee.

11. Penn is a non-profit corporation organized under the laws of the Commonwealth of Pennsylvania with its principal place of business in the Commonwealth of Pennsylvania. Penn is therefore a citizen solely of the Commonwealth of Pennsylvania.

12. According to the records of the Pennsylvania Department of State, Penn may be served with process at its registered office located at 3451 Walnut St., Room 737 the Franklin Building, Office of VP for Finance and Treasurer, Philadelphia PA 19104.

FACTS

13. St. Jude is a world-class research hospital treating children with cancer and other catastrophic diseases. It is the first and only National Cancer Institute-designated Comprehensive Cancer Center devoted solely to children. St. Jude is the only pediatric cancer

research center in the United States where no family ever pays for treatment not covered by insurance. No child is ever denied treatment because of a family's inability to pay.

14. St. Jude attracts world-class researchers and medical personnel to work at its facilities. St. Jude is committed to protecting such individuals' research and scientific reputations, a commitment that comprises a significant component of St. Jude's continuing ability to attract and retain world-class physicians and scientists.

15. St. Jude collaborates with other institutions but, when doing so, takes appropriate steps to protect its proprietary and reputational interests. These steps include, but are not limited to, the policy and practice of entering into Material Transfer Agreements, such as those at issue in this case.

16. B-cell leukemias are cancers of the immune system that result when a particular type of immune cell, the B-cell, starts growing and dividing uncontrollably. There are different types of B-cell leukemias. Almost all B-cell leukemias are caused by cells that have a molecule on their surface called "CD19." Researchers have attempted for many years to attack B-cell leukemia cells by making drugs that recognize the CD19 molecule on the surface of B-cells.

17. In furtherance of St. Jude's research mission, one of its researchers, Dr. Dario Campana, MD, PhD ("Dr. Campana") made the anti-CD19-BBζ chimeric T-Cell receptor construct (referred to as the "Receptor").

18. The Receptor is a molecule that can be put on the surface of a normal immune T-cell, causing it to recognize and attack B-cells that have the CD19 molecule on their surface.

19. The Receptor is an important building block for genetic modulation of cells used for cellular therapies to treat cancer, an alternative to the use of drugs and an area that the scientific community has been working on for years with limited success.

20. The Receptor stimulates T-cells to proliferate so that more cancerous cells can be killed. The Receptor can be modified to target other cancer cells.

21. In December of 2003, Dr. June of Penn requested that Dr. Campana provide him the Receptor and suggested a collaboration involving use of the Receptor.

22. As a result of Dr. June's request to Dr. Campana, St. Jude entered into two agreements with Penn and Dr. June relating to the Receptor: (a) Collaboration and Materials Transfer Agreement dated December 10, 2003 (the "2003 Agreement" attached hereto as Ex. A) and (b) Materials Transfer Agreement dated October 2, 2007 (the "2007 Agreement" attached hereto as Ex. B).

23. Both the 2003 and 2007 Agreements were entered into for the express purpose of ensuring that St. Jude's proprietary and reputational interests in the Receptor were protected. The Receptor was provided to Penn and Dr. June by St. Jude shortly after the 2003 Agreement was fully executed.

24. Under both Agreements, St. Jude agreed to allow Penn and Dr. June to use the Receptor for limited purposes.

25. The Receptor was identified in the 2003 Agreement as the "anti-CD19-BBζ chimeric T-Cell receptor construct" (Ex. A ¶ 1) and in the 2007 Agreement as the "anti-CD19-BBζ chimeric receptor construct." (Ex. B ¶ 1).

26. Both Agreements define "Material" and/or "Materials" (collectively "Materials") as the Receptor together with any progeny, portions, unmodified derivatives and any accompanying know-how or data. (Ex. A ¶1; Ex. B ¶1).

27. The 2003 Agreement was executed by Dr. June on December 16, 2003 and by Timothy J. Raynor, Director of the Intellectual Property Center for Technology Transfer at Penn,

on December 17, 2003.

28. The 2003 Agreement provides that the Materials "may not be transferred or taken to any other laboratory or made available to any other person or third party, but [are] to remain under the immediate and direct control of [Dr. June]." (Ex. A ¶ 2).

29. The 2003 Agreement states that the Materials will only be used in pre-clinical studies and that the Materials may not be used in humans. (Ex. A ¶¶ 3-4).

30. The 2003 Agreement further states that "any publications that result from the collaborative research study between St. Jude and [Dr. June] using the Material will be jointly published in accordance with academic standards." (Ex. A ¶ 6).

31. The 2003 Agreement further states, "the transfer of the Material grants to [Penn] no rights in the Material other than those specifically set forth in the Agreement." (Ex. A ¶ 7).

32. The 2003 Agreement prohibits Penn from using the Materials for any commercial purpose (Ex. A ¶ 4) and further prohibits Penn from commercializing any product that contains the Materials without the prior written approval of St. Jude. (Ex. A ¶ 8).

33. The 2003 Agreement provides that Penn may file patent applications claiming inventions through use of the Materials, but requires Penn "to notify St. Jude within sixty (60) days of filing any patent application which claims subject matter that contains or incorporates the Material or which claims a method of manufacture or use of the Material." (Ex. A ¶ 8).

34. Notwithstanding the clear language of Paragraphs 3 and 4 of the 2003 Agreement expressly limiting use of the Materials to pre-clinical studies and prohibiting the Materials for use with humans, Dr. Campana later learned that Penn, through Dr. June, was recruiting patients for a human clinical trial using the Materials.

35. Even though Dr. June's proposed clinical trial would have constituted a clear

breach of the 2003 Agreement, St. Jude agreed to allow Penn to proceed with the clinical trial subject to conditions outlined in contemporaneous e-mail exchanges between St. Jude and Penn. The execution of the 2007 Agreement was an accommodation to Penn to allow Dr. June to proceed with his proposed clinical trial. (Ex. B ¶ 3). *Inter alia*, the execution of the 2007 Agreement was conditioned explicitly upon Penn's written agreement to abide by the other terms of the 2003 Agreement. (A copy of the February 8, 2007 email from Shawn Hawkins at St. Jude to Dr. Kurt Schwinghammer at Penn is attached hereto as Ex. C).

36. Neither party has exercised its respective right to terminate the 2003 Agreement. (Ex. A ¶ 7).

37. The 2007 Agreement was executed by Dr. June on February 5, 2008 and Dr. Edward Pieters, Associate Director of Research Agreement in the Office of Research Services at Penn, on February 8, 2008. (Ex. B).

38. The 2007 Agreement does not contain an integration clause, nor does the 2007 Agreement in any way refer to superseding, cancelling, terminating, or otherwise affecting the ongoing viability of the 2003 Agreement.

39. Similar to the 2003 Agreement, the 2007 Agreement states that the Materials "may not be taken or sent to another institution without permission from St. Jude." (Ex. B ¶ 3).

40. The 2007 Agreement states that Penn "agrees to provide St. Jude with a copy of any publication that contains experimental results obtained from use of the Materials, and will acknowledge St. Jude as the source of the Materials" and Penn "shall not publish or disclose the results of such research using the Materials without submitting the proposed publication or disclosure to St. Jude at least thirty (30) days prior to the submission for publication or disclosure." (Ex. B ¶¶ 4-5).

41. The 2007 Agreement expressly acknowledges St. Jude's ownership of the Materials. (Ex. B ¶ 5).

42. The 2007 Agreement also prohibits Penn from providing the Materials to a commercial entity or using the Materials in research that is subject to a consulting or licensing obligation to another party without the express written consent of St. Jude. (Ex. B ¶ 3).

43. The 2007 Agreement further provides that if Penn "files a patent application or commercializes a product which contains a portion of the Materials, is derived from the Materials, or which could not have been produced but for the use of the Materials, [Penn] agrees to contact St. Jude to determine ownership interests, if any, St. Jude may have in such patent application or commercial product." (Ex. B ¶ 5).

44. St. Jude may terminate the 2007 Agreement if Penn breaches any term and fails to cure such breach within thirty days after written notice by St. Jude. (Ex. B ¶ 10).

45. If St. Jude terminates the 2007 Agreement, Penn must immediately cease use of the Materials and return all Materials to St. Jude. (Ex. B ¶ 10).

46. No right of termination exists for Penn under the 2007 Agreement.

47. St. Jude does not intend to terminate either the 2003 Agreement or the 2007 Agreement because doing so might delay significant developments in cancer research to the detriment of cancer patients. Penn has putatively terminated the 2007 Agreement even though under the 2007 Agreement Penn possesses no such right of termination.

48. On August 10, 2011, *Science Translational Medicine* published an article by Dr. June and others titled "T Cells With Chimeric Antigen Receptors Have Potent Antitumor Effects and Can Establish Memory in Patients With Advanced Leukemia," Vol. 3 Issue 95 95ra73 ("STM Article") (attached hereto as Ex. D).

49. Contrary to the requirements of the 2003 and 2007 Agreements, the STM Article was not submitted to St. Jude for approval, and Penn and Dr. June failed to acknowledge that St. Jude was the source of the Materials referenced in the STM Article.

50. Such failures and omissions are material breaches of the 2003 and 2007 Agreements.

51. The STM Article states in a footnote that Dr. June and Dr. David L. Porter have filed a patent application, E61/421,470, "Composition and methods for treatment of chronic lymphocytic leukemia," based on the CART19 cell. (Ex. D at n. 42).

52. Despite its obligations in the 2003 Agreement and 2007 Agreement, St. Jude did not receive notice from Penn that it had filed any patent application claiming subject matter that contains or incorporates the Materials. (Ex. A ¶ 8; Ex. B ¶ 5).

53. Such omissions and inactions are material breaches of the 2003 and 2007 Agreements.

54. On August 25, 2011, *The New England Journal of Medicine* published an article by Dr. June and others titled "Chimeric Antigen Receptor–Modified T Cells in Chronic Lymphoid Leukemia," 365:725-733 ("NEJM Article") (attached hereto as Ex. E).

55. Contrary to the requirements of the 2003 and 2007 Agreements, the NEJM Article was not submitted to St. Jude for approval, and Dr. June and Penn failed to acknowledge that St. Jude was the source of the Materials referenced in the NEJM Article.

56. Such omissions and inactions were material breaches of the 2003 and 2007 Agreements.

57. Although Penn has contended that a footnote citation to a previous article co-authored by Dr. Campana is an "acknowledgement" that complies with the 2003 and 2007

Agreements, such alleged "acknowledgement" does not comply with medical and scientific research custom and practice, fails to credit Dr. Campana and St. Jude properly, and in no way mitigates Penn's material breach of the acknowledgement requirements of the Agreements. Neither St. Jude nor Dr. Campana are acknowledged or identified in the acknowledgement at the end of the STM Article or the NEJM Article.

58. The Website for *The New England Journal of Medicine* states that 86 articles have cited to the NEJM Article as of July 11, 2012. (A PDF copy of the website is attached hereto as Ex. F, pursuant to Local Rule 7.2(i)).

59. The STM and NEJM Articles report the first time insertion of the Receptor into a patient's own T-cells and successful use of these genetically modified T-cells to attack and kill chronic lymphoid leukemia cells in the patient's body. The breakthrough research in the STM and NEJM Articles garnered national news coverage in popular non-scientific media outlets. *See, e.g.*, CBS News report at http://www.youtube.com/watch?v=-cR6ZCtYo_s.

60. As set forth above, the 2003 and 2007 Agreements collectively prohibit the commercialization of the Materials without the consent of, and prior written notice to, St. Jude. Without limitation, paragraph 3 of the 2007 Agreement provides in part that "(i) the Materials may not be taken or sent to another institution without written permission from St. Jude and (ii) the Materials may not be provided to a commercial entity, and may not be used in research that is subject to consulting or licensing obligations to another party (other than those obligations imposed upon grantee institutions of the U.S. government) without express written consent by St. Jude."

61. After publication of the STM and NEJM Articles, St. Jude was contacted by Kleiner Perkins, a world-leading venture capital firm, inquiring about St. Jude's proprietary

rights in the Materials.

62. Upon information and belief, St. Jude alleges that Penn is discussing or has discussed with Kleiner Perkins commercialization of the Materials, in violation of the 2003 and 2007 Agreements.

63. Upon information and belief, St. Jude alleges that Penn has distributed the Materials to one or more academic research institutions without obtaining St. Jude's permission.

64. Prior to filing this Action, St. Jude made extensive efforts to have Dr. June and Penn remediate their breaches.

65. Despite St. Jude's written notices and other communications, Dr. June and Penn have refused to remediate their breaches and have refused to provide St. Jude any assurance that Dr. June and Penn will not continue their course of wrongful conduct. Instead, Penn stated that it wished to terminate the 2007 Agreement, despite having no right under the 2007 Agreement to terminate.

COUNT I – BREACH OF CONTRACT

66. St. Jude hereby re-alleges the averments set forth in the preceding paragraphs of this Complaint.

67. St. Jude, Dr. June and Penn entered into two enforceable contracts – the 2003 Agreement and the 2007 Agreement.

68. On information and belief, Penn is vicariously liable for the acts and omissions of Dr. June under the doctrines of respondeat superior, principal and servant, co-adventurer, agency and/or joint venture.

69. The acts and omissions of Dr. June and Penn constitute material uncured breaches of both the 2003 Agreement and 2007 Agreement.

70. Dr. June and Penn have failed to cure their material breaches of the 2003 Agreement and 2007 Agreement.

71. The acts of Dr. June and Penn have caused and will continue to cause St. Jude consequent and proximate injury.

72. St. Jude is suffering immediate and irreparable injury as a result of Dr. June and Penn's breaches of the 2003 Agreement and 2007 Agreement.

73. If Penn's conduct is not preliminarily and permanently enjoined by this Court, St. Jude will continue to be harmed.

74. St. Jude has no adequate remedy at law.

75. There is a substantial likelihood that St. Jude will prevail on the merits of this Action against Penn.

76. There is a substantial threat that St. Jude will suffer irreparable injury if the preliminary injunction is denied.

77. The threatened injury to St. Jude outweighs any putative threatened injury to Penn.

78. Granting the injunctive relief St. Jude seeks will not disserve the public interest.

79. St. Jude is entitled to an Order or Injunction of specific performance, directing Penn to specifically perform the 2003 Agreement and 2007 Agreement.

80. On information and belief formed through communications with Kleiner Perkins, Dr. June and Penn intend to commercially develop or exploit the Materials.

81. If Dr. June and Penn commercially develop or exploit the Materials, their actions and omissions will deprive St. Jude of substantial income to which it is legally and equitably entitled, for the reasons pled herein.

82. On information and belief, Dr. June's and Penn's past and current actions, inactions and omissions, as well as their likely intentions, represent an attempt to obtain legal title to the Materials in violation of their duties to St. Jude and in violation of the 2003 and 2007 Agreements.

83. On information and belief, Dr. June's and Penn's past and current actions, inactions and omissions, as well as their likely intentions, represent an attempt to obtain legal title to the Materials by inequitable and unlawful means.

84. On information and belief, Dr. June's and Penn's past and current actions, inactions and omissions, as well as their likely intentions, represent an attempt to obtain legal title to the Materials with notice of St. Jude's rights of entitlement to the benefits of the Materials.

85. Penn's actions and omissions have been intentional, malicious and/or reckless. Accordingly, St. Jude is entitled to an award of punitive damages against Penn.

PRAYER FOR RELIEF
AND AD DAMNUM

WHEREFORE, St. Jude demands judgment from and against Penn, as follows:

(1) A Preliminary Injunction, as well as a Final Judgment and Permanent Injunction of Specific performance of the 2003 Agreement and 2007 Agreement;

(2) A Preliminary Injunction, as well as a Final Judgment and Permanent Injunction ordering the submission of the following for publication in *The New England Journal of Medicine* by Penn:

> "Dr. Dario Campana and St. Jude Children's Research Hospital designed and provided the chimeric antigen receptor used in the studies described in the manuscript entitled, "*Chimeric Antigen Receptor-Modified T Cells in Chronic Lymphoid Leukemia*" (*N Engl J Med* 8: 725-733 (2011)). We regret the inadvertent omission of an acknowledgement expressing our gratitude to Dr. Campana and St. Jude in the print version of that article[;]"

(3) A Preliminary Injunction, as well as a Final Judgment and Permanent Injunction ordering the submission of the following for publication in *Science Translational*

Medicine by Penn:

> "Dr. Dario Campana and St. Jude Children's Research Hospital designed and provided the chimeric antigen receptor used in the studies described in the manuscript entitled, "*T Cells With Chimeric Antigen Receptors Have Potent Antitumor Effects and Can Establish Memory in Patients With Advanced Leukemia*" (*Science Translational Medicine*, Vol. 3 Issue 95 95ra73 (2011)). We regret the inadvertent omission of an acknowledgement expressing our gratitude to Dr. Campana and St. Jude in that article[;]"

(4) A Preliminary Injunction, as well as a Final Judgment and Permanent Injunction ordering that all future publications and public disclosures that report a study involving the use of the chimeric antigen receptor ("CAR") or CAR coding sequence designed and provided by Dr. Campana, including the plasmid described in *N Engl J Med* 8: 725-733 (2011), as well as any CAR or CAR coding sequence that contains minor changes that do not substantially change the CAR's function, shall include this acknowledgement: "The chimeric antigen receptor used in this study was designed and provided by Dr. Dario Campana and St. Jude Children's Research Hospital[;]"

(5) A Preliminary Injunction, as well as a Final Judgment and Permanent Injunction ordering Penn to utilize a Joint Materials Transfer Agreement ("Joint MTA") covering the distribution of materials that contain the CAR or CAR coding sequence designed and provided by Dr. Campana, including the plasmid described in *N Engl J Med* 8: 725-733 (2011), as well as any CAR or CAR coding sequence that contains minor changes that do not substantially change the CAR's function, to other academics for research purposes;

(6) A Preliminary Injunction, as well as a Final Judgment and Permanent Injunction ordering Penn not to enter into any agreement, without St. Jude's prior written approval, involving the commercialization or exploitation of any Materials that contain the CAR or CAR coding sequence designed and provided by Dr. Campana, including the plasmid described in *N Engl J Med* 8: 725-733 (2011), as well as any CAR or CAR coding sequence that contains minor changes that do not substantially change the CAR's function;

(7) A Preliminary Injunction, as well as a Final Judgment and Permanent Injunction ordering Penn to provide St. Jude a list of everyone to whom Penn or Dr. June have distributed the CAR or CAR coding sequence designed and provided by Dr. Campana, including the plasmid described in *N Engl J Med* 8: 725-733 (2011), as well as any CAR or CAR coding sequence that contains minor changes that do not substantially change the CAR's function, and demand that each distributee execute a Joint MTA with St. Jude or immediately terminate their use of what was distributed to them;

(8) A Preliminary Injunction, as well as a Final Judgment and Permanent Injunction ordering Penn to provide St. Jude a copy of all patent applications for inventions containing the CAR or CAR coding sequence designed and provided by Dr. Campana, including the plasmid described in *N Engl J Med* 8: 725-733 (2011), as

well as any CAR or CAR coding sequence that contains minor changes that do not substantially change the CAR's function and/or that could not have been produced without the use of the Materials;

(9) The award of such actual, compensatory and punitive damages as to which St. Jude may prove its entitlement, but in an amount greater than $75,000, exclusive of interest and costs;

(10) The imposition of a constructive trust and/or legal or equitable lien on the Materials, and any construct, progeny, portions, replications or derivatives of the Materials, so as to ensure St. Jude receives such remuneration in the future to which it is entitled; and

(11) Such further, alternative, different or additional legal and/or equitable relief as may be appropriate under the premises.

Respectfully submitted, this the 11th day of July, 2012.

s/ *Eric E. Hudson*
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EXHIBIT E

THE UNITED STATES DISTRICT COURT
FOR THE WESTERN DISTRICT OF TENNESSEE
WESTERN DIVISION

ST. JUDE CHILDREN'S RESEARCH HOSPITAL, INC. — PLAINTIFF

v. — Civil Action No. 2:12-cv-02579

THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA — DEFENDANT

ST. JUDE'S OPPOSITION TO DEFENDANT'S MOTION TO DISMISS OR, IN THE ALTERNATIVE, FOR CHANGE OF VENUE

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FEDERAL: STATUTES, RULES, REGULATIONS, CONSTITUTIONAL PROVISIONS

INTRODUCTION AND SUMMARY

This case arises directly out of two written material transfer agreements (the "2003 MTA" and the "2007 MTA") between defendant Trustees of the University of Pennsylvania ("Penn") and plaintiff St. Jude Children's Research Hospital, Inc., a Tennessee nonprofit corporation ("St. Jude"). Penn has breached the two agreements and caused untold harm to St. Jude in Tennessee. Over two decades, Penn has continuously and systematically initiated 73 ongoing material transfer agreements with St. Jude under which Penn assumed obligations to St. Jude that continue to this day. *See* Exhibit E (graphic displaying overlapping timelines of agreements). Penn has also entered into dozens of similar agreements with at least two other Tennessee resident organizations. Yet, Penn moves to dismiss this action for lack of personal jurisdiction or, alternatively, to transfer it to Philadelphia, where Penn is based.

Penn's motions should be denied. The reach of this Court's jurisdiction in this diversity case is governed by Tennessee's long-arm statute, which extends "to the full extent permitted by the Due Process Clause of the Constitution." *E.g., Duncan-Williams, Inc. v. Capstone Devel., LLC*, 2010 WL 2710400 at *5 (W.D. Tenn. 2010) (citing *Masada Inv. Corp. v. Allen*, 697 S.W.2d 332, 334 (Tenn. 1985)). Penn's heavy reliance on case law applying Ohio's long-arm statute, which "does not extend to the constitutional limits of the Due Process Clause," corrupts the foundation of its argument. *See Calphalon Corp. v. Rowlette,* 228 F.3d 718, 721 (6th Cir. 2000) (applying Ohio's long-arm statute). Under the appropriate law, jurisdiction exists where either the subject matter of the lawsuit arises out of or is related to the defendant's contact with the forum state, or where a defendant's "continuous and systematic" contacts with the forum state render that defendant amenable to suit. *Brunner v. Hampson*, 441 F.3d 457, 463 (6th Cir. 2006).

The 2003 and 2007 MTAs were executed because Penn specifically sought to obtain, and to collaborate with St. Jude on research involving, a biological material proprietary to St. Jude called a "chimeric antigen receptor" ("Receptor"). The Receptor is a molecule that enables a human immune cell to identify and attack a leukemic cancer cell. The Receptor was constructed

entirely in a research laboratory at St. Jude in the early 2000s by Dr. Dario Campana and his staff, all of whom were St. Jude employees working at St. Jude in Memphis, Tennessee, where St. Jude's only campus is located. In exchange for the Receptor, Penn agreed that legal title to the Receptor remained with St. Jude and voluntarily assumed strict obligations directed at protecting St. Jude's proprietary and commercial interests in Tennessee: Penn agreed never to transfer the Receptor to anyone else, always to acknowledge the Receptor as St. Jude's in publications, and never to commercialize the Receptor without St. Jude's consent. However, Penn has breached the MTAs by hawking the Receptor as its own in recent scientific and other publications, and by commercializing the Receptor without St. Jude's consent. *See* St. Jude's Complaint ("Complaint") ¶¶ 48-57, 60-63. Penn's breaches have injured St. Jude by depriving it not only of justly deserved publication credit and standing for developing the Receptor, but by commercializing St. Jude's Receptor and reaping untold profits without consent or recompense. The Complaint alleges a single claim for damages and injunctive relief which indisputably arises out of the 2003 and 2007 MTAs. *See id*. ¶¶ 66-85.

The Tennessee long-arm statute affords ample basis to hold Penn to answer in this Court for these misdeeds, and for the reasonably foreseeable damages they caused in Tennessee after Penn contacted St. Jude, asked to obtain St. Jude's Receptor and to collaborate with St. Jude, and then breached the collaboration and transfer agreements that allowed Penn to obtain and use the Receptor in the first place. *See* Section II below.

In the alternative to dismissal, Penn asks the Court to transfer this case to the Eastern District of Pennsylvania where Penn is based. However, St. Jude is entitled to a presumption in favor of its selection of this forum "[u]nless the balance is *strongly* in favor of the defendant, the plaintiff's choice of forum should rarely be disturbed." *Reese v. CNH Am. LLC*, 574 F.3d 315, 320 (6th Cir. 2006); *Blane v. Am. Inventors Corp.*, 934 F.Supp. 903, 907 (M.D. Tenn.1996) (citation omitted) (emphasis added). Penn has not begun to carry its heavy burden to show that St. Jude's choice of forum should be undone. Not a single St. Jude percipient witness resides in

Pennsylvania; almost all live and work in the greater Memphis, Tennessee, area. *See* Section III below.

I. STATEMENT OF FACTS

A. St. Jude Constructs The Receptor.

St. Jude, a nonprofit corporation based solely in Tennessee, is a world-renowned hospital and research center dedicated to treating children with cancer and other catastrophic diseases. St. Jude is the first and only National Cancer Institute-designated Comprehensive Cancer Center devoted solely to children. It is also the only pediatric cancer research center in the United States where no family ever pays for treatment not covered by insurance. No child is ever denied treatment because of a family's inability to pay. Complaint ¶¶ 10, 13.

As part of its mission to advance the treatment of devastating childhood illnesses, St. Jude invests significantly in its sophisticated research facilities and technological resources. As a result, St. Jude is able to attract renowned researchers, including Dario Campana, M.D., Ph.D., a highly decorated scientist who specializes in leukemic classification and treatment. *See* Declaration of Dr. Dario Campana ("Campana Decl.") ¶¶ 3-5. Dr. Campana's lab at St. Jude constructed the Receptor. *Id.* ¶ 7-8. The Receptor represents a fundamental building block for research into cellular therapies as alternatives to drugs in the treatment of cancer.

B. Penn Asks To Collaborate With St. Jude In Research Involving the Receptor.

In December 2003, Dr. Carl June contacted Dr. Campana to request access to the Receptor on behalf of himself and Penn, and to propose an inter-institutional collaboration to build on Dr. Campana's research. Campana Decl. ¶ 11 and Ex. 1. In exchange for firm commitments by Penn, Dr. Campana and St. Jude agreed, the parties executed the 2003 MTA, and St. Jude transferred the Receptor to Penn. Ex. A; Complaint ¶ 23; Campana Decl. ¶ 13.

In the ensuing months, Dr. June and his colleagues reaffirmed and expanded their collaboration with St. Jude. They sought and obtained information to help Dr. June's lab conduct research with the Receptor. Campana Decl. ¶¶ 15-16 and Exs. 2-3. Dr. June updated Dr. Campana about his research, and proposed that they collaborate on a research grant related to

the work with the Receptor. *Id.* ¶¶ 15, 17 and Exs. 2, 4. The proposed grant application included a request for $20,000 per year in research supplies, which Dr. June offered to transfer to Dr. Campana's lab in Tennessee. *Id.* ¶ 18 and Ex. 5.

In early 2007, however, Dr. Campana and St. Jude learned that Penn was about to breach its commitments under the 2003 MTA. The 2003 MTA specifically restricted Penn's use of the Receptor to pre-clinical studies, but Penn's website disclosed a clinical trial using the Receptor. *See* Ex. C at 207-08. St. Jude insisted that Penn execute a follow-on agreement that would permit human clinical trials only on stated terms. *Id.* Penn proposed a collaboration for the clinical trial, and St. Jude ultimately agreed to allow Dr. June to perform clinical trials using new therapies that incorporated the Receptor. *See* Ex. C at 203-04; 199-201. The parties executed the 2007 MTA to supplement their rights and obligations. Ex. B.

C. Penn Promises Not To Cause Harm To St. Jude In Tennessee.

In exchange for the right to conduct collaborative research and human clinical trials involving the Receptor, Penn agreed to preserve strictly for St. Jude the benefits of Dr. Campana's research. Penn's obligations are captured in specific contractual terms by which Penn agreed to refrain from causing harm to St. Jude in Tennessee. In the 2003 MTA, Penn agreed: to refrain from transferring the Receptor to any other laboratory or third party (Ex. A ¶ 2); to restrict the scope of its research involving the Receptor (id. ¶ 3); to refrain from using the Receptor for any commercial purpose (id. ¶ 4); to publish jointly any research findings resulting from use of the Receptor (id. ¶ 6); and to obtain prior written approval from St. Jude for the commercialization of any product containing the Receptor (id. ¶ 8).

In addition, Penn promised in the 2007 MTA to refrain from providing the Receptor to another institution or commercial entity without permission (Ex. B ¶ 3); to acknowledge St. Jude as the source of the Receptor in any written publication and to provide St. Jude with a copy of any such publication (id. ¶ 4); to contact St. Jude regarding ownership rights if seeking to patent or commercialize a product related its use of the Receptor (id. ¶ 5); and to indemnify St. Jude against claims or liability arising out of the Penn's use of the Receptor (id. ¶ 7.)

Having agreed to credit St. Jude for the Receptor in publications, Penn knew well that it was depriving St. Jude of justly deserved academic prestige in the scientific community when it failed to do so. Having agreed not to commercialize the Receptor without St. Jude's consent, Penn knew well that it would be profiting handsomely and depriving St. Jude of just recompense for the Receptor when it proceeded to commercialize the Receptor without St. Jude's consent. Penn knew well that all harm it caused by breaking its contractual obligations to St. Jude would inevitably come home to roost in the State of Tennessee, where St. Jude resides.

D. Penn Initiates And Maintains Continuous And Systematic Contractual Relationships With St. Jude And Other Institutions In The State Of Tennessee In Order To Avail Itself Of Their Work.

The 2003 and 2007 MTAs are part of an unbroken stream of contracts under which Penn has bound itself to safeguard and protect St. Jude and its biological materials in order to obtain samples and to collaborate in research. Penn has repeatedly sought and obtained proprietary research materials from St. Jude since at least 1996, and has executed at least 73 agreements with Penn as the recipient of such materials. See Declaration of Esther Allay ("Allay Decl.") ¶ 9; see generally Exs. C, D, E. Penn has repeatedly sought St. Jude's collaboration in research concerning such materials, as in the case of the 2003 and 2007 MTAs.[1] See Ex. C at 45-46; 100-02; 170-72; 203-04; 218-20; 284-86; see also Exs. D and E. With St. Jude alone, Penn has 88 ongoing MTAs dating back to 1996. See Exs. D and E.[2] However, Penn's ties to Tennessee are not limited to its MTAs with St. Jude. Vanderbilt has records of approximately 50

[1] In addition to the collaborative MTAs between Penn and St. Jude, Penn has pursued and maintained collaborative relationships with St. Jude in instances where collaboration is not explicitly contemplated in the MTAs themselves. *See, e.g.*, Ex. C at 73-75; 95-98.

[2] The Complaint alleges that Penn has entered into "approximately 68 Material Transfer Agreements" with St. Jude. Complaint ¶ 4. At this writing, St. Jude has identified a total of 88 MTAs in effect between Penn and St. Jude. *See* Allay Decl. ¶ 9; Ex. D. Review of St. Jude's records is ongoing.

MTAs with Penn, and the University of Tennessee has records of 12 such MTAs. See Declaration of Alan R. Bentley ("Bentley Decl.") ¶ 3; Declaration of Anthony A. Ferrara ("Ferrara Decl.") ¶ 3. Penn cultivates its connections with Tennessee purposefully to avail itself of research conducted here, and now seeks to profit from its ongoing connection with St. Jude by breaching its agreement not to commercialize the Receptor without St. Jude's consent.

II. THIS COURT HAS BOTH SPECIFIC AND GENERAL PERSONAL JURISDICTION OVER PENN.

Rule 4(k)(1)(A) of the Federal Rules of Civil Procedure authorizes a federal district court to exercise personal jurisdiction over a defendant "who is subject to the jurisdiction of a court of general jurisdiction in the state where the district court is located." Jurisdiction may exist "either generally, in cases in which a defendant's 'continuous and systematic' conduct within the forum state renders that defendant amenable to suit in any lawsuit brought against it in the forum state, or specifically, in cases in which the subject matter of the lawsuit arises out of or is related to the defendant's contacts with the forum." *Brunner v. Hampson*, 441 F.3d 457, 463 (6th Cir. 2006) quoting *Nationwide Mut. Ins., Co. v. Tryg Int'l Ins. Co.*, 91 F.3d 790, 793 (6th Cir. 1996) (citation omitted); *Bird v. Parsons*, 289 F.3d 865, 873 (6th Cir. 2002).

Where the jurisdictional challenge is submitted on materials that do not raise factual disputes, a plaintiff's burden of demonstrating personal jurisdiction over a defendant is "relatively slight." *Welsh v. Gibbs,* 631 F.2d 436, 439 (6th Cir. 1980); *Neogen Corp. v. Neo Gen Screening, Inc.,* 282 F.3d 883, 887 (6th Cir. 2002). If disputes arise, the pleadings and affidavits must be viewed in the light most favorable to St. Jude. *Energy Automation Systems Inc. v. Saxton*, 618 F. Supp. 2d 807, 811 (M.D. Tenn. 2009) (citing *Welsh v. Gibbs*, 631 F.2d at 438; 2A Moore's *Federal Practice*, §12.07 (2d Ed. 1985)). St. Jude need only make a *prima facie* showing that jurisdiction exists; all of St. Jude's allegations of jurisdictional facts are presumed true, with all factual disputes decided in St. Jude's favor. *Energy Automation Systems,* 618 F. Supp. 2d at 811 (citing *Nelson Park Industries, Inc.*, 717 F.2d 1120, 1123 (7th Cir. 1983); *Welsh*,

631 F.2d at 439). Facts proffered by Penn should not even be considered if they conflict with St. Jude's facts. *Aristech Chem. Int'l v. Acrylic Fabricators*, 138 F.3d 624, 626 (6th Cir. 1998); *Erwin v. Piscitello*, 627 F. Supp. 2d 855, 858 (E.D. Tenn. 2007) (citing *Neogen,* 282 F. 3d at 888).

In diversity cases like this, federal courts apply the law of the forum state to determine whether personal jurisdiction exists over a nonresident subject to the limitations of the Due Process Clause of the Fourteenth Amendment. *See Youn v. Track, Inc.,* 324 F.3d 409, 417 (6th Cir. 2003). Tennessee's long-arm statute extends to the limits of due process. *Bridgeport Music, Inc. v. Still N The Water Publ'g,* 327 F.3d 472, 477 (6th Cir. 2003). Therefore, the two inquiries are merged, *id.*, and this Court need only determine whether the assertion of personal jurisdiction over Penn violates constitutional due process.

As explained below, Penn is subject to both specific and general personal jurisdiction of this Court consistent with constitutional due process. In an effort to escape this conclusion, Penn mistakenly relies on *Calphalon Corp. v. Rowlette*, 228 F.3d 718 (6th Cir. 2000), which involved the application of the *Ohio* long-arm statute (which does not extend to the limits of due process) to dissimilar facts.[3] This important distinction between Tennessee and Ohio law, which Penn ignores, was specifically remarked upon in *Holley-Adkins v. Holley*, 492 F. Supp. 2d 776, 782 n.5 (S.D. Ohio 2005) (contrasting Ohio and Tennessee law and noting that the Sixth Circuit in *Neal* "stated that ***a single act directed at residents in Tennessee could support the exercise of personal jurisdiction***.") (emphasis added, citation omitted). This Court's determination of

[3] Unlike the contract at issue in *Calphalon* which focused on the non-forum market, the 2003 and 2007 MTAs between Penn and St. Jude began as a collaboration between the parties and were drafted with a specific intent of providing ongoing, multiple protections for a Tennessee resident corporation and the Tennessee property that was the subject of the agreements. On their faces, these agreements are not the one-time, no-strings-attached transfer of materials that Penn tries to depict. Furthermore, unlike this case, the potential breach in *Calphalon* (the forum plaintiff's failure to compensate the non-forum defendant) would not have foreseeable injurious consequences in the forum state, but rather would result in injury (nonpayment) where the non-forum defendant resided.

personal jurisdiction over Penn is controlled by *Southern Mach. Co. v. Mohasco Indus., Inc.*, 401 F.2d 374, 381 (6th Cir. 1968), and *Neal v. Janssen*, 270 F.3d 328, 331 (6th Cir. 2001) (finding personal jurisdiction over Belgian sales agent under Tennessee long-arm statute based on phone calls and faxes to plaintiffs in Tennessee, which formed basis for the claims, even though agent never visited Tennessee), and their progeny. *See* Section II A & B below.

A. This Court Has Specific Jurisdiction Over Penn Because Penn Purposefully Availed Itself Of The Privilege Of Acting In Tennessee By Contracting With St. Jude In Tennessee To Obtain St. Jude's Receptor And Collaboration, And By Expressly Committing To Protect St. Jude's Proprietary Interests.

This Court has specific personal jurisdiction over Penn because this suit arises out of the two MTAs with St. Jude which Penn executed to gain access to the Receptor and St. Jude's research collaboration in Tennessee. Specific jurisdiction occurs where "a State exercises personal jurisdiction over a defendant in a suit arising out of or related to the defendant's contacts with the forum." *Helicopteros Nacionales de Colombia, S.A. v. Hall*, 466 U.S. 408, 414 n.8 (1984). Where a forum seeks to assert specific jurisdiction over an out-of-state defendant, due process is satisfied if the defendant has "purposefully directed his activities at residents of the forum, . . . and the litigation results from alleged injuries that arise out of or relate to those activities . . ." such that it is foreseeable that its activities may subject it to jurisdiction in the forum. *Burger King Corp. v. Rudzewicz,* 471 U.S. 462, 471-72 (1985) (citations omitted). "'[T]he foreseeability that is critical to due process analysis . . . is that the defendant's conduct and connection with the forum State are such that he should reasonably anticipate being haled into court there." *Id.* at 474 (quoting *World-Wide Volkswagen Corp. v. Woodson,* 444 U.S. 286, 295 (1980); *Bridgeport Music, Inc. v. Still N The Water Publ'g*, 327 F.3d 472, 478 (6th Cir. 2003) (focus is on "whether the defendant has engaged in 'some overt actions connecting the defendant with the forum state'"). It is beyond dispute that Penn repeatedly engaged in overt actions that connected it to St. Jude and the State of Tennessee, and that St. Jude's Complaint arises directly out of injuries suffered in this State caused by those actions. This satisfies both

due process and Tennessee law. *See Burger King*, 471 U.S. at 472; *Neal,* 270 F.3d at 331 (citing *Calder v. Jones*, 465 U.S. 783 (1984)).

> A State generally has a "manifest interest" in providing its residents with a convenient forum for redressing injuries inflicted by out-of-state actors. . . . Moreover, where individuals "purposefully derive benefit" from their interstate activities, . . . it may well be unfair to allow them to escape having to account in other States for consequences that arise proximately from such activities; the Due Process Clause may not readily be wielded as a territorial shield to avoid interstate *obligations that have been voluntarily assumed*.

Burger King Corp., 471 U.S. at 473-474 (emphasis added).

The seminal Sixth Circuit case evaluating specific personal jurisdiction under Tennessee's long-arm statute is *Southern Mach. Co. v. Mohasco Indus., Inc.*, 401 F.2d 374, 381 (6th Cir. 1968). *See, e.g., Neal*, 270 F.3d at 332-33. *Southern Mach. Co.* identified a three-part test for specific personal jurisdiction under Tennessee law (the *Mohasco* factors): (1) the defendant must purposefully avail itself of the privilege of acting in Tennessee or causing a consequence in Tennessee; (2) the cause of action must arise from that purposeful availment; and (3) the defendant's actions, or the consequences caused by the defendant, must have a substantial enough connection with Tennessee to make the exercise of jurisdiction over the defendant reasonable. *Southern Mach. Co.,* 401 F.2d at 381. This Court plainly has specific personal jurisdiction over Penn in this lawsuit under *Southern Mach. Co.'s* three-part test, and the more recent Sixth Circuit decision applying Tennessee's long-arm statute in *Neal*, 270 F. 3d at 331.

Under the first *Mohasco* factor, "purposeful availment" is "something akin to a deliberate undertaking," that is, a deliberate effort by the defendant to direct its activities toward, and to make contact with, the forum. *Bridgeport Music, Inc. v. Still N The Water Publ'g*, 327 F.3d 472, 478 (6th Cir. 2003). Purposeful availment exists "when the defendant's contacts with the forum state 'proximately result from actions by the defendant ***himself*** that create a "substantial connection" with the forum State,' and when the defendant's conduct and connection with the forum are such that he 'should reasonably anticipate being haled into court

there.'" *Bridgeport*, 327 F.3d at 478 (emphasis in original). The focus is "whether the defendant has engaged in 'some overt actions connecting the defendant with the forum state.'" *Id.*

Penn voluntarily and deliberately initiated its 2003 MTA with St. Jude, and then agreed to the 2007 MTA so it could use the Receptor in clinical trials. The 2003 MTA has been in place for about eight-and-one-half years, the 2007 MTA for about five years. In these agreements, Penn committed to protecting the interests and property rights of a Tennessee resident, St. Jude, in exchange for receiving St. Jude's Receptor and collaboration. *See* Section I(C) above. Each of these "voluntarily assumed" obligations, *cf. Burger King Corp.,* 471 U.S. at 474, was deliberately directed at protecting a Tennessee entity's (St. Jude's) interests and property rights in material (the Receptor) developed and owned by St. Jude in Tennessee. Penn bargained for the rights to obtain and use St. Jude's Receptor, and knowingly availed itself of the privilege of transacting business with a Tennessee entity and causing consequences to that entity by its actions and omissions under the Agreements. The obligations purposefully undertaken by Penn directed toward the protection of St. Jude, a Tennessee entity, were *designed* to have consequences in Tennessee.

Having dishonored its obligations to protect St. Jude's investment and interests, Penn now resists being haled into the forum where those interests reside. Not only was it reasonably foreseeable that Penn would be haled into court in Tennessee should it breach the 2003 and 2007 MTAs, Tennessee is the *only* forum in which injury would be sustained if Penn breached *any* of its obligations to St. Jude. Thus, Tennessee was the predictable forum for resolution of the types of contract issues that are the subject of St. Jude's lawsuit. Penn finds itself in Tennessee not through "random," "fortuitous," or "attenuated" contacts, *cf.* Defendant's Memorandum Of Law In Support Of Its Motion To Dismiss Or, In The Alternative, For Change Of Venue ("Penn Br.") at. 12-13, but rather because Penn expressly assumed contractual obligations focused solely on the protection of a Tennessee resident's interests and then disregarded those obligations. There can be no question that Penn "purposefully avail[ed] [it]self of the privilege of acting in

[Tennessee] or causing a consequence in [Tennessee.]" *See Functional Pathways of Tenn.*, No. 3:10-cv-409, 2012 U.S. Dist. LEXIS 46030, at *11 (E.D. Tenn. Mar. 30, 2012).

The second *Mohasco* factor, that the cause of action "arise from" Penn's purposeful availment, "is a lenient standard and the cause of action need not formally arise from the defendant's contacts." *Functional Pathways of Tenn.*, 2012 U.S. Dist. LEXIS 46030, at *18. The facts of this case more than satisfy that standard. St. Jude's cause of action does indeed "formally arise" from Penn's contacts with St. Jude in Tennessee. The 2003 and 2007 MTAs are "the very soil from which the action for breach grew." *Functional Pathways of Tenn.*, 2012 U.S. Dist. LEXIS 46030, at *19 (internal quotations omitted). If Penn had not initiated contact with St. Jude, St. Jude would not have sent Dr. Campana's Receptor to Penn, and there would never have been any agreements between the parties concerning that Receptor. St. Jude's cause of action would never have arisen because Penn would have had no contracts to breach concerning the Receptor. Equally important, if Penn had not knowingly and expressly committed to ongoing obligations toward St. Jude specifically designed to protect St. Jude's interests (the breach of which forms the basis of St. Jude's Complaint), there would be no lawsuit before this Court at all.

The third and final element of the *Mohasco* test is that "defendant's actions, or the consequences caused by the defendant, must have a substantial enough connection with Tennessee to make the exercise of jurisdiction over the defendant reasonable." When the first two *Mohasco* factors are met, however, the third factor is *presumed* to be present—"only the unusual case will not meet this third criterion." *Functional Pathways of Tenn.*, 2012 U.S. Dist. LEXIS 46030, at *11 (citing *Morton v. Advance PCS, Inc.*, No. 3:04-DV-278, 2006 U.S. Dist. LEXIS 54423 (E.D. Tenn. Aug. 2, 2006)). "[O]nce the first two questions have been answered affirmatively, resolution of the third involves merely ferreting out the unusual cases where that interest cannot be found." *Functional Pathways of Tenn.*, 2012 U.S. Dist. LEXIS 46030, at *21 (citing *Southern Mach. Co.,* 401 F.2d at 384). Penn has pointed to no facts that mark this as such an "unusual case."

It is not necessary that the actual breach of the contract occur in Tennessee. *Functional Pathways of Tenn.*, 2012 U.S. Dist. LEXIS 46030, at *18 (citation omitted). Even if Penn's breach occurred outside Tennessee, exercising personal jurisdiction is still reasonable when the consequences of that breach have a substantial connection with Tennessee. *Functional Pathways of Tenn.*, 2012 U.S. Dist. LEXIS 46030, at *18, citing *Calphalon*, 228 F.3d at 724. That is certainly true here because St. Jude's damages have been, are, and will continue to be sustained in Tennessee—where St. Jude's scientists invested the time, effort, and money to develop the Receptor; where that Receptor was constructed; and where St. Jude is located. The natural consequence of Penn's failure to meet its voluntarily assumed obligations to protect St. Jude's interests is that those interests here in Tennessee would be compromised. Thus, this Court has specific jurisdiction over Penn.

B. This Court Has General Jurisdiction Over Penn.

General jurisdiction is proper where "'a defendant's contacts with the forum state are of such a continuous and systematic nature that the state may exercise personal jurisdiction over the defendant even if the action is unrelated to the defendant's contacts with the state.'" *Bird v. Parsons*, 289 F.3d 865, 873 (6th Cir. 2002) (quoting *Third Natl. Bank in Nashville v. WEDGE Group, Inc.*, 882 F.2d 1087, 1089 (6th Cir.1989)) (internal quotation marks omitted in *Bird*). General jurisdiction exists when "the defendant's contacts with the forum state are 'substantial' and 'continuous and systematic,' so that the state may exercise jurisdiction over the defendant even if the action does not relate to the defendant's contacts with the state." *Youn v. Track, Inc.*, 324 F.3d 409, 417-18 (6th Cir. 2003). An absence of physical contacts does not defeat personal jurisdiction:

> Although territorial presence frequently will enhance a potential defendant's affiliation with a State and reinforce the reasonable foreseeability of suit there, it is an inescapable fact of modern commercial life that a substantial amount of business is transacted solely by mail and wire communications across state lines, thus obviating the need for physical presence within a State in which business is conducted.

Michigan Nat'l Bank v. Quality Dinette, Inc., 888 F.2d 462 (6th Cir. 1989) (quoting *Burger King Corp. v. Rudzewicz*, 471 U.S. 462 (1985)). Accordingly, where an out-of-state defendant repeatedly and persistently establishes and maintains contact with a state over a course of years, general jurisdiction is appropriate.

1. Penn Has Had Continuous and Systematic Contacts with Tennessee Since at Least 1996.

Penn's contacts with Tennessee have been continuous and systematic for decades. While Penn portrays itself as an insular institution confined within the borders of Pennsylvania, its conduct over the years demonstrates a pattern and practice of purposeful availment of the benefits and protections of Tennessee law. Since 1996, Penn has executed 88 MTAs with St. Jude, at least seven of which are collaborative in nature[4] and contemplate performance by Penn's collaborator, St. Jude, in Tennessee. All of the 88 MTAs remain in effect; none has been terminated.[5] By their terms, the MTAs have no stated durations but continue in effect until terminated. *See generally* Ex. C. Exhibit E attached to this memorandum graphically depicts Penn's continuous and systematic—indeed, unbroken since 1996—contacts with Tennessee by virtue of its 88 MTAs with St. Jude alone. Moreover, 73 of the MTAs involve Penn's usage of St. Jude's materials and resulted from Penn's affirmative efforts to obtain from Tennessee

[4] Penn offers a declaration by Dr. June that he did not collaborate with St. Jude. (Declaration of Carl H. June ¶ 22), which, if true, would be of little consequence for this motion. However, it is contrary to the very title and express terms of the 2003 MTA, to the emails evidencing collaboration, and to Dr. Campana's declaration. Ex. A; Campana Decl. ¶¶ 15-18 and Exs. 2-5. For purposes of determining jurisdiction, Dr. June's denial of collaboration must be disregarded. *See Aristech Chem. Int'l, supra*, 138 F.3d at 626.

[5] On November 22, 2011, Penn purported to terminate the 2007 MTA. As alleged in St. Jude's Complaint, Penn had no right to terminate that agreement. Complaint ¶ 65. Furthermore, contrary to the plain terms of the MTAs, Penn has submitted a declaration stating the bare conclusion, without foundation, that an MTA is "deemed" (impliedly by Penn) to be "complete" when its Office of Research Services receives a signed copy of it. *See* Declaration of Katheryn Steinbugler ¶ 11. This statement must be disregarded as without foundation, irrelevant, and inconsistent with St. Jude's contrary evidence. *See Aristech Chem. Int'l, supra,* 138 F.3d at 626.

proprietary work product developed by St. Jude researchers in Tennessee. The remaining 15 of the MTAs contemplate Penn sending research materials *into* Tennessee. *See* Exs. D and E.

Penn's contacts with Tennessee range well beyond St. Jude; Penn has in recent years also executed some 50 MTAs with Vanderbilt University, Bentley Decl. ¶ 3, and 12 MTAs with the University of Tennessee, Ferrara Decl. ¶ 3. Thus, Penn has availed itself of Tennessee's benefits and protections through at least *150* agreements with Tennessee institutions in recent years. [6]

In addition to its continuous and systematic research collaborations with Tennessee entities through MTAs, Penn has many additional continuous contacts with this state. Penn admits it has periodically made payments to various parties in Tennessee "to support the University's core activities." *See Declaration of Stephen D. Golding*, ¶ 3 It has a designated representative devoted to recruiting prospective students from Tennessee, and it maintains a website through which Tennessee residents may obtain information and pose questions to Penn and its affiliate entities. Penn Admissions: Regional Admissions Officers,

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[6] Of the 73 St. Jude MTAs covering materials requested by Penn, 17 are in the form of a Uniform Biological Material Transfer Agreement ("UBMTA") developed by the National Institutes of Health ("NIH") and the Association of University Technology Managers ("AUTM"). *See* Notice, 60 Fed. Reg. 12771 (Mar. 8, 1995), available at http://www.autm.net/AM/Template.cfm?Section=Technology_Transfer_Resources&Template=/CM/ContentDisplay.cfm&ContentID=2810. A true and correct copy of the Master UBMTA ("Form UBMTA") is attached as Exhibit I. Signatories are not only academic institutions, but private, for-profit pharmaceutical companies as well. Neither the 2003 MTA nor the 2007 MTA is a UBMTA. While Penn tries to downplay the significance of the UBMTAs, Penn's repeated use of 17 of them with St. Jude underscores the fact that Penn's contacts have been *systematic* as well as continuous.

While the UBMTA provides that the agreement may terminate upon the recipient's completion of its research with material transferred, the recipient must then either destroy or return the material at the direction of the transferor. Form UBMTA ¶ 13. Penn has never notified St. Jude that it has completed its research with materials transferred under a UBMTA, and has never asked St. Jude for direction regarding destruction or return of such materials. Allay Decl. ¶ 9. Furthermore, under the UBMTA, Penn remains bound to the terms of the UBMTA as they relate to any modifications of materials supplied by St. Jude which Penn elects to retain. Form UBMTA ¶ 13(iii).

http://www.admissions.upenn.edu/current/regional.php (last visited Sept. 3, 2012) (copy attached as Ex. F; Penn Admissions, http://www.admissions.upenn.edu/inquiry/ (last visited Sept. 3, 2012) (copy attached as Ex. G); Penn Admissions: Join Our Mailing List, http://www.admissions.upenn.edu/request/ (last visited Sept. 3, 2012) (copy attached as Ex. H).

Penn's decades-long, widespread, systematic, and ongoing contacts with Tennessee are more than sufficient to support general jurisdiction. Courts have found much more narrowly directed activities sufficient for general jurisdiction. For instance, in *German Free Bavaria v. Toyobo Co.*, 2007 U.S. Dist. LEXIS 19199 (W.D. Mich. Mar. 19, 2007) the court concluded that general jurisdiction properly could be exercised over a defendant who participated in a collaborative relationship with an in-state entity, where the relationship spanned a period of eleven years, and the defendant's conduct included sharing "synergy and input" with the plaintiff regarding market strategy and the execution of two confidentiality agreements with the plaintiff. Similarly, in *Michigan Nat'l Bank v. Quality Dinette, Inc.*, 888 F.2d 462 (6th Cir. 1989), the court found general jurisdiction where defendants made regular product sales in the forum state for only two years. With the exception of a single independent sales representative, defendants carried on their forum state business by mail order solicitation. *Id.* at 465.

Penn's contacts here far exceed those of the defendants in *German Free Bavaria* and *Quality Dinette.* Over the last two decades, Penn has executed scores of contracts with Tennessee entities, has engaged in multiple collaborative relationships with St. Jude, and has regularly dispatched representatives to Tennessee to further its educational mission. Penn's contacts are continuous and systematic in nature.[7]

[7] *Nationwide Mut. Ins., Co. v. Tryg Int'l Ins. Co.*, 91 F.3d 790 (6th Cir. 1996) and *Third Natl. Bank in Nashville v. WEDGE Group, Inc.*, 882 F.2d 1087 (6th Cir. 1989) (Penn Br., p. 10) are readily distinguishable. The defendant in *Nationwide Mut.* had been a party to only three agreements involving the forum state, and its relationship with the plaintiff lasted only a year. 91 F.3d at 794. In *Third Natl. Bank*, the court relied on the fact that the defendant's contacts with the forum state were "unrelated" to plaintiff's claims. 882 F.2d at 1089. By contrast, St. Jude's claims here arise directly out of two of the MTAs. *See, e.g.*, *Better Bags, Inc. v. Better Bags Mktg., L.L.C.*, 2006 U.S. Dist. LEXIS 1434 (S.D. Tex. Jan. 5, 2006) (finding general jurisdiction

2. Penn's Widespread Contacts With Tennessee Are In Furtherance Of Its Business Purposes And Properly Subject It To General Jurisdiction.

Penn attempts to downplay its continuous and systematic contacts with Tennessee by stressing that it is a not-for-profit organization (Penn Br., p. 2), by repeatedly referring to its research efforts as "non-commercial" in nature (Penn Br., pp. 4, 9, 14), and by asserting that its conduct vis-à-vis Tennessee has been limited to activities merely "typical of other large nationally prominent universities" (Penn Br., p. 9). These arguments are unavailing.

Penn's not-for-profit status, and the ostensibly noncommercial character of its research (notwithstanding its commercialization of St. Jude's Receptor), are beside the point. Courts have consistently held that a nonprofit entity may be subject to jurisdiction in a foreign state so long as it "has purposefully availed itself of the privilege of acting within the state" in carrying out its business purposes. *Bennett v. J.C. Penney*, 603 F. Supp. 1186, 1188 (W.D. Mich. 1985); *see also Mad Hatter, Inc. v. Mad Hatters Night Club Co*., 399 F. Supp. 889, 891 (E.D. Mich. 1975) (defendant's "purposeful" action need not be income-generating); *Benally v. Amon Carter Museum of Western Art*, 858 F.2d 618, 621-623 (10th Cir. 1988) (nonprofits "transact business" within a state by engaging in activities designed to support their core purposes).

A university's research activities, in particular, have been recognized as "commercial" even where the research itself is not directly commercialized:

> [M]ajor research universities, such as [defendant university], often sanction and fund research projects with arguably no commercial application whatsoever. However, these projects unmistakably further the institution's legitimate business objectives, including educating and enlightening students and faculty participating in these projects. These projects also serve, for example, to increase the status of the institution and lure lucrative research grants, students and faculty.

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over a defendant who had maintained business relationships with four entities in the forum state; "it does not escape the court's attention that the products ordered are precisely those in issue in this lawsuit and that the products were purchased from suppliers in Plaintiff's home location.").

Madey v. Duke Univ., 307 F.3d 1351, 1362 (Fed. Cir. 2002) (holding defendant university's research activities commercial, and therefore not protected by the "experimental use" defense in patent infringement action).[8] Penn is subject to general jurisdiction in Tennessee.

III. VENUE SHOULD REMAIN IN TENNESSEE.

Penn lends only cursory argument and minimal substance to its alternative motion to transfer venue. *See* Penn Br. at 19. As Penn undoubtedly recognizes, St. Jude's choice of venue is entitled to great weight under the law. Although the decision whether to grant a change of venue pursuant to 28 U.S.C. § 1404(a) is left to the district court's sound discretion, *see Zomba Enters., Inc. v. Panorama Records, Inc.*, 491 F.3d 574, 588 (6th Cir. 2007), "[u]nless the balance is *strongly* in favor of the defendant, the plaintiff's choice of forum should rarely be disturbed." *Reese v. CNH Am. LLC*, 574 F.3d 315, 320 (6th Cir. 2006); *Blane v. Am. Inventors Corp.*, 934 F.Supp. 903, 907 (M.D. Tenn.1996) (citation omitted) (emphasis added). Thus, Penn's burden to prove a need for transfer is "considerable." *Affinion Benefits Group, LLC v. Econ-O-Check Corp.*, No. 3:09-cv-0273, 2009 U.S. Dist. LEXIS 34326, at *2 (M.D. Tenn. April 20, 2009). In this case Penn simply has not carried its considerable burden to show that any balancing required under 28 U.S.C. § 1404(a) "strongly" favors it. Therefore, the case should not be transferred.[9]

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[8] St. Jude is *not* here urging that Penn is subject to general jurisdiction in Tennessee simply for engaging in activities typical of any large educational institution with limited, incidental relationships. Penn's activities here extend well beyond recruiting students and soliciting donations, and Penn benefits from the protection of Tennessee law under which Tennessee research was conducted.

[9] A week after this action was filed, Penn filed its own action on the same subject matter in the Eastern District of Pennsylvania in violation of the first-filed and compulsory counterclaim rules. *Trustees of the University of Pennsylvania v. St. Jude Children's Research Hospital,* Case No. 12-4122 (E.D. Penn. filed July 19, 2012). Penn's two claims in that action are for a declaration that it did *not* breach the 2003 and 2007 MTAs, and for damages on the theory that St. Jude's filing of this action "tortuously interfered" with Penn's commercialization activities. St. Jude has moved to dismiss or stay the Pennsylvania action in deference to this one. No hearing date has been set.

Penn erroneously argues that St. Jude has significant contacts with Pennsylvania because the American Lebanese Syrian Associated Charities, Inc. ("ALSAC") has an office and registered agent in Pennsylvania. However, ALSAC is a fundraising organization, is not a corporate affiliate of St. Jude, is not a party to this case, had nothing to do with any of the MTAs between Penn and St. Jude, and has no witnesses or sources of proof for this case. Declaration of Michael Canarios ¶¶ 5-7. Rather, the key events giving rise to St. Jude's claims occurred here in Tennessee—where scientists at St. Jude invested the time, work, and money to develop the Receptor; where that Receptor was constructed; where St. Jude is located; where St. Jude executed the MTAs; and where St. Jude has sustained its damages. Penn's actions and omissions in breach of the MTAs had natural and foreseeable consequences in Tennessee. Those consequences will be proven through witnesses and documents almost all located in Tennessee.

Penn argues that Pennsylvania is a more convenient forum for witnesses. However, not a single one of the St. Jude scientists and employees who are percipient witnesses to the development of the Receptor at St. Jude, the formation of the 2003 and 2007 MTAs, and to the harm suffered by St. Jude as a result of Penn's breaches of those agreements, is located in Pennsylvania. *See* Declaration of James R. Downing, M.D. ¶ 3.

Finally, Penn erroneously asserts that some nebulous, unstated public interest favors Pennsylvania over Tennessee because that is where Dr. June worked on his construct using the Receptor. More to the point, Tennessee is where Dr. Campana and his colleagues researched and developed the Receptor that Dr. June and Penn have brazenly hawked and commercialized for their own profit. Having argued unavailingly that Tennessee courts are powerless to call Penn to answer in Tennessee for the damage it caused here by ripping off St. Jude's Receptor, Penn alternatively invites the Court to overturn the strong legal presumption in favor plaintiff's choice of forum for nothing more than Penn's own convenience. Penn's invitation should be firmly declined.

CONCLUSION

For the foregoing reasons, Penn's motion to dismiss this action for lack of personal jurisdiction, and its alternative motion to change venue, should be denied.

Respectfully submitted this the 4th day of September 2012.

s/ *Amy M. Pepke*
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CERTIFICATE OF SERVICE

I hereby certify that on the 4th day of September, 2012, the undersigned electronically filed the foregoing with the Clerk of Court using the CM/ECF system which will send notification of such filing to all counsel of record registered with the CM/ECF system.

/s/ *Amy M. Pepke*

EXHIBIT F

SD

UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF PENNSYLVANIA

TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA, Plaintiff, v. ST. JUDE CHILDREN'S RESEARCH HOSPITAL, Defendant.	CIVIL ACTION NO. 4122 JURY TRIAL DEMANDED

COMPLAINT

Plaintiff Trustees of the University of Pennsylvania ("Plaintiff" or the "University") alleges for its complaint against defendant St. Jude Children's Research Hospital ("St. Jude"), the following:

PARTIES

1. The University is a non-profit organization devoted to higher education with a principal place of business at 3451 Walnut Street, Philadelphia, Pennsylvania 19104.

2. St. Jude is a non-profit pediatric cancer research hospital with a principal place of business at 262 Danny Thomas Place, Memphis, Tennessee 38105.

3. St. Jude is supported primarily by donations raised by its national fundraising organization, the American Lebanese Syrian Associated Charities ("ALSAC"), which was established expressly for the purpose of funding St. Jude.

JURISDICTION AND VENUE

4. The Court has jurisdiction over this action pursuant to 28 U.S.C. § 1332, in that the action is between citizens of different states, and the matter in controversy exceeds $75,000, exclusive of interest and costs.

5. This Court has the authority to issue a declaratory judgment pursuant to 28 U.S.C. §§ 2201 and 2202.

6. Venue in this district is proper pursuant to 28 U.S.C. § 1391 because the University is a citizen and resident in this district, because St. Jude has had significant contacts with this district, because its charity ALSAC has both an office and a registered representative in this district, because a substantial part of the events giving rise to the claims occurred in this district and because a substantial part of the property that is the subject of this action is located in this district.

FACTUAL ALLEGATIONS

Dr. Carl June Of The University Has Developed A Groundbreaking Immunotherapy For Treatment Of Cancer.

7. The University is one of the world's most significant research and teaching institutions, attracting award-winning educators and scholars. The Perelman School of Medicine at the University of Pennsylvania ("Perelman"), a component of the University, is one of the top recipients of National Institutes of Health funding and consistently ranks among the top five in U.S. News and World Report's rankings of research-oriented medical schools.

8. Carl H. June, M.D., a Professor of Pathology and Laboratory Medicine at Perelman, has developed a groundbreaking immunotherapy for treatment of cancer (the "Penn Immunotherapy"). The Penn Immunotherapy involves use of a CD19 ScFv DNA lentiviral construct (the "June Construct") that, using proprietary technologies that Dr. June and his

colleagues developed while at the University, causes T cells to express chimeric antigen receptors ("CARs") in patients such that their cancer is treated. The strands of polynucleotide chains that make up DNA are held together by hydrogen bonds between complementary pairs of nitrogenous bases, or "base pairs."

9. Early clinical trials using the Penn Immunotherapy have been tremendously effective, and have resulted in the eradication of cancer in two patients with terminal leukemia and the partial remission of terminal leukemia in a third patient. In March 2012, a pediatric patient with leukemia, struggling for life, became the first child to receive the Penn Immunotherapy. On May 13, 2012, a Minimal Residual Disease report indicated that this pediatric patient was "cancer-free." See Exhibit A.

10. As a result of the groundbreaking early clinical trials, the Penn Immunotherapy technology has been reported in prominent medical journals, including *The New England Journal of Medicine* and *Science Translational Medicine.* See Exhibit B. Dr. June has been hailed as a "cancer-buster" (*BusinessWeek*), and his "potential breakthrough in cancer research" (*Los Angeles Times*) may signify the "turning point in the long struggle to develop effective gene therapies against cancer." (*The New York Times*). See Exhibit C.

Public Presentations By A St. Jude Researcher Leads To The Parties' Execution Of Two Material Transfer Agreements

11. Dr. June first met Dario Campana, M.D., Ph.D., a Professor of Pediatrics and Member of the Hematology-Oncology and Pathology Departments at St. Jude, while attending an American Society of Hematology ("ASH") conference in San Diego, California in December 2003. At the December 2003 conference, Dr. Campana presented a paper regarding an anti-CD19 BB-ζ chimeric receptor construct (the "Campana Construct").

12. Shortly after the December 2003 ASH conference, Drs. June and Campana communicated with each other about Dr. Campana's presentation and the Campana Construct. In these communications, Dr. June asked Dr. Campana for a sample of the Campana Construct that his laboratory could modify to create a lentiviral vector for pre-clinical, non-human testing for cancer.

13. Thereafter, on December 17, 2003, St. Jude and the University executed a two-page Collaboration and Materials Transfer Agreement, dated December 10, 2003 ("2003 MTA"), and Dr. Campana sent a sample of the Campana Construct to Dr. June in Philadelphia, Pennsylvania on the same day. The 2003 MTA is attached hereto as Exhibit D.

14. Over the next several years, Dr. June and his colleagues conducted a research study involving, among other things, modification of excised segments of the Campana Construct and development of the Penn Immunotherapy. This research led to the invention of a new, different construct to be used for cancer treatment. While Dr. Campana provided the Campana Construct to Dr. June, he was not involved in Dr. June's research, nor did he collaborate with Dr. June or his laboratory in inventing the June Construct.

15. The June Construct and the Campana Construct are different in important ways. While both constructs are viral vectors, the Campana construct is a retroviral vector, whereas the June Construct is a lentiviral construct, created by using a disabled form of HIV-1. Indeed, Dr. June and his colleagues were the first researchers ever to use HIV-1 as the vector in immunotherapy for cancer patients. Furthermore, while both constructs have promoters, i.e., regions of DNA that transcribe a gene, the promoters differ between constructs. Similarly, whereas both constructs express CARs, the CARs have a different sequence of base pairs. The June Construct also contains additional DNA elements, absent in the Campana Construct, that

enhance expression of the CAR. Finally, the June Construct has been shown to perform optimally only when expressed in human T cells using the Penn Immunotherapy technology.

16. In early 2007, Dr. June's research progressed to the point where he wished to conduct human clinical trials using the Penn Immunotherapy technology, including the June Construct. In February 2008, at the request of St. Jude personnel, St. Jude and the University executed a three-page Materials Transfer Agreement dated October 2, 2007 (the "2007 MTA"). The 2007 MTA is attached hereto as Exhibit E.

17. The 2003 MTA and 2007 MTA (collectively, the "Agreements"), have some terms in common. For example, the "Material" or "Materials" that are the subject of both agreements is "biological material" provided by St. Jude to the University and Dr. June, and specifically, "the anti-CD19-BB-ζ chimeric T-cell receptor construct, including any progeny, portions, unmodified derivatives and any accompanying know-how or data." See Exhibits D and E, at ¶ 1.

18. Neither the 2003 MTA nor the 2007 MTA define "Material" or "Materials" to include modified derivatives or modified portions of the anti-CD19-BB-ζ chimeric T-cell receptor construct.

19. Neither the 2003 MTA nor the 2007 MTA provide St. Jude with an ownership or intellectual property interest in any inventions that arise from the use of the Materials. Rather, the only interest provided to St. Jude of such inventions is a non-exclusive, royalty free license to use for non-commercial purposes any inventions that arise from the use of the Materials. See Exhibit D, at ¶ 8.

20. Neither the 2003 MTA nor the 2007 MTA restrict the University's or Dr. June's ability to file patent applications that claim inventions that are made through use of the Material,

that contain a portion of the Materials, that are derived from the Materials, or which could not have been produced but for use of the Materials, but to the contrary expressly allow them to file such patent applications. See Exhibits D and E.

21. In or about August 2011, Dr. June described the results of his study using leukemia patients treated with the Penn Immunotherapy in an article in *The New England Journal of Medicine*, NEW ENG. J. MED. 8: 725-733 (2011).

22. By letter dated November 22, 2011, the University provided St. Jude with written notice that it was terminating the 2003 MTA. See Exhibit F.

The University Seeks A Strategic Partner To Help Make Dr. June's Groundbreaking Immunotherapy Available To All Who Need It To Treat Their Cancer.

23. Given the tremendous potential for the Penn Immunotherapy, the University has actively sought a strategic partner with infrastructure and resources that will fund additional clinical trials, and make Dr. June's immunotherapy available to all those who need it.

24. The University has identified such a partner (hereinafter, the "Strategic Partner").

25. The University has contractually agreed to exclusively negotiate with the Strategic Partner regarding a ground-breaking collaboration that would develop Dr. June's cellular immunotherapy for general cancer patient use.

26. The University has, in fact, actively negotiated with the Strategic Partner a collaboration under which the University would receive funding that would allow it to continue with clinical trials of the Penn Immunotherapy without undue delay.

27. As of July 10, 2012, the University and the Strategic Partner had made substantial progress towards reaching an agreement that would allow continued development of the Penn Immunotherapy Technology.

St. Jude's Suit Is Attempting To Unlawfully Interfere With The University's Prospective Transaction By Filing A Baseless Lawsuit.

28. St. Jude is aware that the University is negotiating an agreement with a strategic partner regarding immunotherapy cancer treatment.

29. St. Jude first asserted an interest in the June Construct in 2011, but delayed litigation until the middle of 2012, when the University was actively involved in negotiation of a major collaboration agreement. On July 11, 2012, St. Jude filed a complaint against the University in the United States District Court for the Western District of Tennessee (the "Tennessee Complaint") (*St. Jude Children's Research Hospital, Inc. v. The Trustees of the University of Pennsylvania*, Civil Action No. 12-2579). A copy of the Tennessee Complaint is attached hereto as Exhibit G.

30. In the Tennessee Complaint, St. Jude alleges that the University has breached the Agreements.

31. The University has not committed any material breach of the Agreements.

32. The United States District Court for the Western District of Tennessee does not have jurisdiction over any dispute regarding the University's alleged breach of the Agreements.

33. In the Tennessee Complaint, St. Jude requests numerous forms of relief, including preliminary and final injunctive relief.

34. Among the requests for injunctive relief that St. Jude has made in the Tennessee Complaint is a request for an injunction ordering the University "not to enter into any agreement, without St. Jude's prior written approval, involving the commercialization or exploitation of any Materials that contain the [chimeric antigen receptor] CAR or CAR coding sequence designed and provided by Dr. Campana, including the plasmid described in *N Engl. J Med* 8: 725-733

(2011), as well as any CAR or CAR coding sequence that contains minor changes that do not substantially change the CAR's function . . . ." See Exhibit G, at 13.

35. Upon information and belief, St. Jude is well aware that the Penn Immunotherapy technology, including the June Construct described in *The New England Journal of Medicine* article, is not "Material" or "Materials" under the 2003 MTA and the 2007 MTA, and that St. Jude is not entitled to the relief it seeks in the Complaint.

36. Upon information and belief, St. Jude is aware that the University has not committed any material breach of the Agreements.

37. Upon information and belief, St. Jude filed the Tennessee Complaint with the intent that the public disclosure of its baseless allegations and requests for injunctive relief therein would disrupt the negotiations between the University and the Strategic Partner.

38. St. Jude's actions have been made with bad motive and with reckless indifference to the interests of the University, the Strategic Partner, and numerous cancer patients seeking treatment using the Penn Immunotherapy.

39. St. Jude's allegations have had a detrimental impact on the University's collaboration with the Strategic Partner, and have increased the likelihood that any availability of the Penn Immunotherapy to cancer patients awaiting treatment – patients who need or will need the immunotherapy for survival – will be delayed.

40. As of July 10, 2012, the University and the Strategic Partner had made substantial progress towards reaching an agreement. Nevertheless, because of St. Jude's wrongful activities, the negotiations between the University and the Strategic Partner have been negatively impacted.

COUNT I

(Tortious Interference with Prospective Contractual Relations)

41. The University repeats and realleges the allegations set forth above as if fully set forth herein.

42. This is a claim for tortious interference with prospective contractual relations.

43. A prospective contract or business relationship is a relationship protected against an intentional interference.

44. St. Jude has purposefully interfered with a prospective contractual relationship between the University and the Strategic Partner.

45. The purposeful actions of St. Jude include the filing of the Tennessee Complaint.

46. St. Jude has taken these actions with the malicious intent to harm the University, to harm the existing relationship between the University and the Strategic Partner, and to prevent further prospective contractual relations from occurring between the University and the Strategic Partner, or any other prospective strategic partner.

47. St. Jude committed the aforementioned actions without privilege, justification, or good faith.

48. St. Jude's conduct has occasioned actual legal damage upon the University, as the University's negotiations with the Strategic Partner have been negatively impacted causing the University to suffer damages, including the deprivation of the benefits of the proposed collaboration with the Strategic Partner, and the ability to advance the development of the Penn Immunotherapy technology so that it is available to the cancer-stricken public.

49. St. Jude's actions have been intentional, malicious, and/or reckless. Accordingly, the University is entitled to an award of punitive damages against St. Jude.

COUNT II

(Declaratory Judgment)

50. The University repeats and realleges the allegations set forth above as if fully set forth herein.

51. This is a claim for a declaratory judgment.

52. There is a real and actual controversy between the parties as to whether the University has materially breached the Agreements as alleged in the Tennessee Complaint.

53. There is a real and actual controversy between the parties as to whether the 2003 MTA has been terminated.

54. The University seeks a judicial determination from this Court of the rights of the parties with respect to such controversies.

55. Whether the University has materially breached the Agreements is of sufficient immediacy and magnitude to justify the issuance of declaratory relief by this Court.

56. The issuance of declaratory relief by this Court will resolve the existing controversies between the parties.

PRAYER FOR RELIEF

WHEREFORE, Plaintiff demands judgment as follows:

a. The award of actual, compensatory and punitive damages to which the University is entitled;

b. A declaration that the University has not materially breached the 2003 MTA;

c. A declaration that the 2003 MTA has been terminated;

d. A declaration that the University has not materially breached the 2007 MTA; and

e. Such other and further relief in favor of the University as this Court deems just

and proper.

JURY TRIAL DEMAND

Plaintiff Trustees of the University of Pennsylvania hereby demands a trial by jury for each and every issue so permitted by law and statute.

Dated: July 19, 2012

MORGAN, LEWIS & BOCKIUS LLP

By: Eric Kraeutler

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Attorneys for Plaintiff Trustees of the University of Pennsylvania

EXHIBIT G

UNITED STATES DISTRICT COURT
EASTERN DISTRICT OF PENNSYLVANIA

TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA,	:	
Plaintiff,	:	**CIVIL ACTION NO. 12-4122**
v.	:	
ST. JUDE CHILDREN'S RESEARCH HOSPITAL,	:	
Defendant.	:	

ORDER

AND NOW this ____ day of _____, 2012, upon consideration of Defendant's Motion to Dismiss or Stay and any responses to it, it is hereby ORDERED that:

1. Defendant's Motion is GRANTED;

2. This action is hereby dismissed without prejudice in deference to the prior action between the same parties pending in the United States District Court for the Western District of Tennessee entitled *St. Jude Children's Research Hospital, Inc. v. The Trustees of the University of Pennsylvania*, Civil Action No. 12-02579 (filed July 11, 2012); and

3. Defendant is awarded its costs in the amount of $_____.

BY THE COURT:

_____
Stewart Dalzell, United States District Judge

UNITED STATES DISTRICT COURT
EASTERN DISTRICT OF PENNSYLVANIA

TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA,	:	
Plaintiff,	:	**CIVIL ACTION NO. 12-4122**
v.	:	
ST. JUDE CHILDREN'S RESEARCH HOSPITAL,	:	
Defendant.	:	

DEFENDANT'S MOTION TO DISMISS OR STAY

For the reasons set forth in the accompanying Memorandum of Law, Defendant St. Jude Children's Research Hospital, Inc. ("St. Jude") hereby moves this Court for an order dismissing this action without prejudice on two grounds: (1) there is a prior action pending in the United States District Court for the Western District of Tennessee entitled *St. Jude Children's Research Hospital, Inc. v. The Trustees of the University of Pennsylvania*, Civil Action No. 12-02579 (filed July 11, 2012) (the "First Action") between the same parties which concerns the same subject matter and which Defendant Trustees of the University of Pennsylvania (the "University") agreed St. Jude would have the opportunity to file first; and (2) the University's claims in this action are compulsory counterclaims in the First Action. In the alternative, St. Jude moves the Court to stay this action pending the district court's ruling on the University's pending motion to dismiss the First Action for want of personal jurisdiction or to change the venue of the First Action. Should the Court elect to retain jurisdiction of this case, St. Jude moves the Court to dismiss Count I of the Amended Complaint (the University's tortious interference claim) with prejudice for failure to state a claim on which relief can be granted.

HANGLEY ARONCHICK SEGAL PUDLIN
& SCHILLER
Daniel Segal

FOLEY & LARDNER LLP
Alan E. Friedman (*pro hac vice*)

By: /s/ Daniel Segal
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Attorneys for Defendant
St. Jude Children's Research Hospital, Inc.

Dated: September 24, 2012

UNITED STATES DISTRICT COURT
EASTERN DISTRICT OF PENNSYLVANIA

TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA,	:	
Plaintiff,	:	
v.	:	**CIVIL ACTION NO. 12-4122**
ST. JUDE CHILDREN'S RESEARCH HOSPITAL,	:	
Defendant.	:	

MEMORANDUM OF LAW IN SUPPORT OF
DEFENDANT'S MOTION TO DISMISS OR STAY

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St. Jude Children's Research Hospital, Inc.

Dated: September 24, 2012

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Plaintiff,	:	
	:	**CIVIL ACTION NO. 12-4122**
v.	:	
ST. JUDE CHILDREN'S RESEARCH HOSPITAL,	:	
Defendant.	:	

MEMORANDUM OF LAW IN SUPPORT OF
DEFENDANT'S MOTION TO DISMISS OR STAY

INTRODUCTION AND SUMMARY

This action, filed by the University against St. Jude on July 19, 2012, is the mirror image of a lawsuit filed by St. Jude against the University eight days earlier in the Western District of Tennessee concerning the same issues and subject matter – *St. Jude Children's Research Hospital, Inc. v. The Trustees of the University of Pennsylvania*, Civil Action No. 12-02579 (July 11, 2012) (the "First Action"). The two actions arise out of the same two written contracts between the same two parties. In the First Action, St. Jude claims the University breached the contracts, and seeks damages and injunctive relief; in this action the University denies it breached the contracts, seeks a declaratory judgment that it did not, and seeks damages for St. Jude's supposed "tortious interference," allegedly committed by the mere act of filing the First Action. The University's claims here thus completely overlap St. Jude's claims in the First Action and depend on the determination of those claims on the merits.

As far back as January 2012, the University agreed that if the parties could not settle their differences, St. Jude would have the opportunity to sue first in Tennessee, with the University reserving the right to challenge jurisdiction. *See* Section I.C. below. (On August 2, 2012, the

University filed a motion to dismiss the First Action for lack of personal jurisdiction or to change its venue, which is now pending in the Tennessee court. *See* Section I.D. below.)

If the University's claims here are tenable at all, they must be asserted as compulsory counterclaims in the First Action. As the court that first had possession of this dispute, the Tennessee district court is bound to decide it under long-established precedent. *See EEOC v. Univ. of Pa.*, 850 F.2d 969, 971 (3d. Cir. 1988); *Crosley Corp. v. Hazeltine Corp.*, 122 F.2d 925, 929 (3d Cir. 1941); *Koresko v. Nationwide Life Ins. Co.*, 403 F. Supp. 2d 394, 399 (E.D. Pa. 2005); *accord Zide Sport Shop of Ohio, Inc. v. Ed Tobergte Assocs., Inc.*, 16 Fed. App'x 433, 437 (6th Cir. 2001). Accordingly, this action should be dismissed without prejudice or stayed in deference to the First Action. *See* Section II below.

In the alternative, the University's tortious interference claim (Count I of its Amended Complaint) should be dismissed on the merits with prejudice. The *Noerr-Pennington* doctrine bars the count, which in any event fails to allege facts sufficient to state a claim for relief under federal pleading standards and state substantive law. *See* Section III below. (Of course, if the Court dismisses this case without prejudice in deference to the First Action, it need not reach the grounds for dismissal on the merits discussed at length in Section III of this memorandum.)

I. STATEMENT OF FACTS

A. The University Asks St. Jude For A Chimeric Antigen Receptor Developed By A St. Jude Researcher in Tennessee

St. Jude, a Tennessee nonprofit corporation, is a world-renowned research hospital that treats children with cancer and other catastrophic diseases. It is the first and only National Cancer Institute-designated Comprehensive Cancer Center devoted solely to children. St. Jude is the only pediatric cancer research center in the United States where no family ever pays for treatment not covered by insurance. No child is ever denied treatment because of a family's inability to pay. *See* St. Jude's Complaint in the First Action ("St. Jude's Complaint" or the

"Tennessee Complaint") ¶¶ 10, 13 (true and correct copy attached as Exhibit A);[1] University's Amended Complaint ¶¶ 2, 3.

St. Jude scientists for decades have pioneered the development of cutting-edge cancer treatments and breakthrough biomedical materials. In the past two decades, in order to avail itself of these materials for research purposes, the University has asked St. Jude to lend it samples, each time under the terms of a Material Transfer Agreement ("MTA") entered into expressly for that purpose. Since 1996, at the University's request, the parties have entered into 73 MTAs covering research materials developed at St. Jude.[2] In several instances, the University has agreed to collaborate actively with St. Jude in joint research pertaining to St. Jude's materials. The contracts that underlie this lawsuit are two such MTAs. University's Amended Complaint ¶¶ 13, 17; St. Jude's Complaint ¶ 22.

In the early 2000's, one of St. Jude's researchers, Dario Campana, M.D., Ph.D. developed a molecule – called a chimeric antigen receptor ("Receptor") – which can be expressed on the surface of a normal human immune T-cell, and which causes the T-cell to recognize and attack certain leukemic cancer cells. University's Amended Complaint ¶¶ 11-12; St. Jude's Complaint ¶¶ 17-20. In December 2003, University researcher Dr. Carl June asked Dr. Campana to provide him with the Receptor and suggested a research collaboration involving use of the Receptor. University's Amended Complaint ¶¶ 11-13; St. Jude's Complaint ¶ 21.

---

[1] The University attached St. Jude's Complaint, filed July 11, 2012, as Exhibit G to its Amended Complaint in this action.

[2] St. Jude's Complaint alleges that the University has entered into "approximately 68 Material Transfer Agreements with St. Jude." St. Jude's Complaint ¶ 4. Since the filing of its Complaint, St. Jude has identified a total of 88 ongoing MTAs with the University – including the two at issue here – dating back to 1996, 73 involving materials transferred from St. Jude to the University and 15 involving materials transferred from the University to St. Jude. *See* Exhibit C to St. Jude's Response to the University's Motion to Dismiss in the First Action, attached hereto as Exhibit D.

B. The Parties Enter Into The 2003 And 2007 MTAs

In response to Dr. June's request, St. Jude entered into two MTAs relating to the Receptor: (1) a Collaboration and Materials Transfer Agreement dated December 10, 2003 (the "2003 MTA"), and (2) a Materials Transfer Agreement dated October 2, 2007 (the "2007 MTA"). University's Amended Complaint, Exs. D, E; St. Jude's Complaint, Exs. A, B The first paragraph of each agreement defined "Material" or "Materials" as the Receptor "including any progeny, portions, unmodified derivatives and any accompanying know-how or data." *Id.*

Both MTAs permitted the University to use the Receptor only for limited purposes. The 2003 MTA provided that: the Material was supplied "for use in a collaborative research study" (2003 MTA preamble); the Material could "not be transferred or taken to any other laboratory or made available to any other person or third party" (*id.* ¶ 2); "any publications that result from the collaborative research study between St. Jude and [Dr. June] will be jointly published in accordance with academic standards" (*id.* ¶ 6); the University was prohibited from using the Materials for any commercial purpose (*id.* ¶ 4) and from commercializing any product containing the Materials without St. Jude's prior written approval (*id.* ¶ 8).

The 2007 MTA provided that: the Materials "may not be taken or sent to another institution without written permission from St. Jude" (2007 MTA ¶ 3); the University had to "provide St. Jude with a copy of any publication that contains experimental results obtained from use of the Materials, and will acknowledge St. Jude as the source of the Materials" (*id.* ¶ 4); the University "shall not publish or disclose the results of such research using the Materials without submitting the proposed publication or disclosure to St. Jude at least thirty (30) days prior to the submission for publication or disclosure" (*id.* ¶ 5); the University is prohibited from providing the Materials to a commercial entity or using the Materials in research that is subject to a consulting or licensing obligation to another party without the express written consent of St. Jude (*id.* ¶ 3); and if the University "files a patent application or commercializes a product which

contains a portion of the Materials, is derived from the Materials, or which could not have been produced but for the use of the Materials, [the University] agrees to contact St. Jude to determine ownership interests, if any, St. Jude may have in such patent application or commercial product" (*id.* ¶ 5). When it entered into the 2007 MTA, the University agreed in writing to abide by the terms of the 2003 MTA (other than those prohibiting human trials).[3] St. Jude's Complaint ¶ 35.

C. The University Agrees To Give St. Jude The Opportunity To File Suit First In Tennessee If The MTA Disputes Are Not Settled

By January 2012, St. Jude had learned that the University had breached both the 2003 MTA and the 2007 MTA by publishing experimental results without the required acknowledgment of St. Jude and without sharing the proposed publication with St. Jude beforehand. St. Jude's Complaint ¶¶ 48-59. St. Jude had also learned through a venture capitalist that the University was apparently engaging in prohibited commercialization efforts. *Id.* ¶¶ 61-62. Accordingly, on January 20, 2012, St. Jude general counsel Clinton Hermes and outside counsel Glenn Krinsky telephoned University general counsel Wendy White. They explained that St. Jude was prepared to sue the University immediately in order to preserve its interests, but would delay suit in favor of negotiations if the parties could agree to a standstill agreement that would give St. Jude the right to sue first should negotiations fail. While the conversation was in progress, Mr. Krinsky sent Ms. White the following confirming email:

> The purpose of this e-mail is to memorialize an understanding reached in a telephone conversation among you, me and St. Jude Children's Research Hospital ("St. Jude") General Counsel Clinton Hermes that is still in progress as this e-mail to you is being drafted. Mr. Hermes and I telephoned you several minutes ago to inform you that St. Jude intended to file suit today against the Trustees of the University of Pennsylvania ("Penn") in connection with disputes arising under that

[3] The 2007 MTA came about because the University was on the verge of breaching the 2003 MTA by conducting clinical trials using the Receptor on humans, which the 2003 MTA prohibited. St. Jude agreed to the 2007 MTA to accommodate the University, but only on condition that the University agree to continue to abide by all other terms of the 2003 MTA (which the University expressly agreed to do). *Id.*

> certain Collaboration and Materials Transfer Agreement between St. Jude and Penn dated December 10, 2003 and that certain Materials Transfer Agreement between St. Jude and Penn dated October 2, 2007 (collectively, the "MTAs"). As an alternative to filing suit, Mr. Hermes offered Penn the opportunity to enter into a "Stand Still Agreement" with St. Jude to enable the parties to discuss the disputes arising under the MTAs with the hopes of resolving those disputes and obviating the need for a lawsuit. You have requested further time to decide whether or not to enter into a Stand Still Agreement. In exchange for this period of time to allow Penn to consider whether it wishes to enter into a proposed Stand Still Agreement, you have agreed on behalf of Penn that Penn will not file a lawsuit or initiate any other type of judicial or administrative proceeding of any sort that in any way relates to or arises out of the MTAs until no earlier than Friday February 3rd, 2012. *On behalf of Penn, you explicitly acknowledge that there are no restrictions on St. Jude's ability to initiate legal proceedings related to the MTAs including, but not limited to, a federal court lawsuit against Penn in the Western District of Tennessee at any time after 3:00pm EST on Tuesday January 31, 2012, in the event that Penn has not executed a Stand Still Agreement and delivered a pdf copy of such executed Stand Still Agreement by e-mail to both Mr. Hermes and me by that time. St. Jude acknowledges that Penn's agreement to the terms of this e-mail does not preclude Penn from contesting jurisdiction in the Western District of Tennessee with respect to any complaint filed in that district by St. Jude in connection with this matter.* PLEASE CONFIRM THAT THIS E-MAIL ACCURATELY DESCRIBES THE UNDERSTANDING REACHED AMONG YOU, ME AND MR. HERMES BY REPLY E-MAIL. YOUR REPLY E-MAIL SHOULD STATE "UNDERSTOOD AND CONFIRMED" AND SHOULD BE SENT TO MR. HERMES AND ME BY "REPLYING TO ALL." You have represented to us during our telephone conversation that you have the authority to bind Penn to the terms set forth in this e-mail. We will keep the telephone line open with you until such time as we receive your requested reply.

Declaration of Glenn L. Krinsky, ¶¶ 2-3 and Ex. 1 (italics added). Ms. White duly responded by email, "Understood and confirmed." *Id.* When the deadline for settlement passed, St. Jude took its agreed-upon opportunity to sue first.

D. Filing Of The Two Actions

St. Jude filed the First Action in the Western District of Tennessee on July 11, 2012, and served the summons and complaint on the University on July 12, 2012.[4] St. Jude's single-count complaint for damages and injunctive relief alleges that the University breached the 2003 and 2007 MTAs by failing to credit St. Jude as the source of the Receptor in publications, by failing to give notice of any patent application involving the Receptor, and by attempting to commercialize the Receptor without St. Jude's consent. St. Jude's Complaint ¶¶ 13-70.

The University filed this action on July 19, 2012, and served the summons and complaint on St. Jude on July 30, 2012. On August 20, 2012, St. Jude moved to dismiss or stay this second-filed action in deference to the First Action as required by law, and to dismiss the University's two claims – one for tortious interference and the other for a declaratory judgment – because they were required to be filed, if at all, as compulsory counterclaims in the First Action. In the alternative, St. Jude moved to dismiss the University's tortious interference count with prejudice for failure to state a claim on which relief could be granted. Rather than oppose these motions, the University elected to file an Amended Complaint on September 6, 2012. It apparently did so in order to bolster its tortious interference count with additional allegations in an effort to save that count from dismissal with prejudice, should the Court reach St. Jude's alternative motion to dismiss on the merits.[5]

The two counts of the University's Amended Complaint overlap entirely with those of St. Jude in the First Action, and arise out of the same 2003 and 2007 MTAs. Count II denies St. Jude's breach-of-contract claims and seeks a declaratory judgment that the University did not

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[4] *See* Affidavit of Service filed in the First Action on July 19, 2012 (copy attached as Exhibit B), of which St. Jude requests judicial notice.

[5] The University's effort to resuscitate its tortious interference claim fails. *See* Section III below.

materially breach the MTAs; it is effectively nothing more than an answer to St. Jude's complaint in the First Action. University's Amended Complaint ¶¶ 69-75.

Count I expressly depends for its very existence on the filing of the First Action. In it, the University incorporates its denials that it is breaching the MTAs by attempting to commercialize the Receptor without St. Jude's consent, and then claims St. Jude has "tortiously interfered" with the University's commercialization negotiations with Novartis[6] by the very filing of the First Action. Count I seeks compensatory and punitive damages based upon this premise. *Id.* ¶¶ 59-68.

On August 2, 2012, the University filed a motion in the Western District of Tennessee to dismiss the First Action for want of personal jurisdiction, and in the alternative to transfer the First Action to this district, attached as Exhibit C. On September 4, 2012, St. Jude filed its opposition to the motion, attached as Exhibit D, and on September 21, 2012, the University filed its reply to that opposition, attached as Exhibit E.[7]

II. THIS ACTION SHOULD BE DISMISSED WITHOUT PREJUDICE, OR STAYED PENDING THE TENNESSEE COURT'S RULING ON THE UNIVERSITY'S MOTION TO DISMISS THE FIRST ACTION

A. In Deference To The First Action, The University's Complaint Should Be Dismissed Without Prejudice Under The Long-Established First-Filed Rule

St. Jude filed and served the First Action in Tennessee a week before the University filed this lawsuit. It is well established that "[i]n all cases of federal concurrent jurisdiction, the court which first has possession of the subject must decide it."[8] *EEOC*, 850 F.2d at 971 (quoting

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[6] The University's original Complaint referred to *Novartis* only as an unnamed "Strategic Partner." Complaint ¶ 25.

[7] St. Jude requests judicial notice of the parties' filings on the motion. *See, e.g., S. Cross Overseas Agencies, Inc. v. Wah Kwong Shipping Grp. Ltd.*, 181 F.3d 410, 426 (3d Cir. 1999) ("To resolve a 12(b)(6) motion, a court may properly look at public records, including judicial proceedings, in addition to the allegations in the complaint.").

[8] Two or more cases have "concurrent jurisdiction" where the cases involve the same parties and the same issues. *Koresko*, 403 F. Supp. 2d at 399.

Crosley, 122 F.2d at 929); *Koresko*, 403 F. Supp. 2d at 399. Exceptions to this rule are "rare" because "due consideration to the orderly administration of justice counsels in favor of ordinarily respecting the first-filed rule." *Colony Nat'l Ins., Co. v. UHS Children Servs.*, No. 09-2916, 2009 WL 3007334, at *2 (E.D. Pa. Sept. 11, 2009) (quoting *Koresko*, 403 F. Supp. 2d at 400); *see also Peregrine Corp. v. Peregrine Indus., Inc.*, 769 F. Supp. 169, 171-72 (E.D. Pa. 1991) (noting that "[i]nvocation of the rule will usually be the norm, not the exception." (quoting *EEOC*, 850 F.2d at 969)); *Servian v. Health Data Sciences Corp.*, No. 92-2693, 1992 WL 174705, at *2 (E.D. Pa. July 21, 1992) ("In the absence of an extraordinary situation . . . this Court is unable to grant any discretionary relief from the 'first filed' rule."); *Colony Nat'l Ins., Co.*, 2009 WL 3007334 at *2 (for a court "to depart from the [first-filed] rule, a showing of 'exceptional circumstances' is generally required") (citing *EEOC*, 850 F.2d at 979)); *Zelenkofske Axelrod Consulting L.L.C. v. Stevenson*, No. 99-3508, 1999 WL 592399, at *2 (E.D. Pa. Aug. 5, 1999) (acknowledging that departures from the first-filed rule "are 'rare' and the second action should proceed only in 'exceptional circumstances.'" (quoting *EEOC*, 850 F.2d at 979).

Strong policies counsel in favor of the first-filed rule:

> The economic waste involved in duplicating litigation is obvious. Equally important is its adverse effect upon the prompt and efficient administration of justice. . . . Courts already heavily burdened with litigation with which they must of necessity deal should therefore not be called upon to duplicate each other's work in cases involving the same issues and the same parties.

Crosley, 122 F.2d at 930. Moreover, it "is of obvious importance to all the litigants to have a single determination of their controversy, rather than several decisions which if they conflict may require separate appeals to different circuit courts of appeals." *Id.*; *see also EEOC*, 850 F.2d at 977 (noting that "the [first-filed] rule's primary purpose is to avoid burdening the federal judiciary and to prevent the judicial embarrassment of conflicting judgments").

The principles underlying the first-filed rule are implicated "where the subject matter of the later filed case *substantially overlaps* with that of the earlier one." *Villari Brandes & Kline, P.C. v. Plainfield Specialty Holdings II, Inc.*, No. 09-2552, 2009 WL 1845236, *6 (E.D. Pa. June 26, 2009) (emphasis added); *QVC v. Patiomats.com, LLC,* No. 12-3168, 2012 WL 3155471, at *3 (E.D. Pa. August 3, 2012) (applying the first-filed rule *sua sponte*). The rule does not require that the claims and issues in both actions overlap entirely, or that the first and second filed actions be "mirror image cases where the parties and the issues perfectly align." *Id.* Rather, "the substantive touchstone of the first-to-file inquiry is [the] subject matter" of the two cases. *Id.* at *3 (citations omitted); *see also Catanese v. Unilever*, 774 F. Supp. 2d 684, 689 (D.N.J. 2011) ("[O]verlapping subject matter is the key; exact identity of claims is not required."); *Freedom Mortg. Corp. v. Irwin Fin. Corp.*, No. 08-146, 2009 WL 763899, at *4 (D. Del. Mar. 23, 2009) ("Complete identity of the parties and issues . . . is not required for the 'first-filed' rule to apply.").

Where, as here, none of the recognized equitable exceptions to the application of the first-filed rule is present, the courts of the Third Circuit rarely decline to apply it. *Sonion Nederland BV v. Asius Techs. LLC*, No. 11-67, 2011 WL 5826047, at *4 (D. Del. Nov. 18, 2011). "The long-standing, general rule in the Third Circuit is that, absent special circumstances, the court which first has possession of the subject matter *must* decide it." *Fun-Damental Too v. Universal Music Group*, No. 97-1595, 1997 WL 181255, at *6 (E.D. Pa. Apr. 10, 1997) (Dalzell, J.) (enjoining a second-filed suit in California, which involved "the same parties and the same issues" already before the court) (*citing Crosley*, 122 F.2d at 929) (emphasis added). *Accord Koresko*, 403 F. Supp. 2d at 399; *Colony Nat'l Ins., Co.*, 2009 WL 3007334 at *2; *Hanover Fire & Cas. Ins. Co. v. Sieron*, No. 06-2758, 2007 WL 120058, at *2 (E.D. Pa. Jan. 9. 2007).

The facts here mandate that the first-filed rule be applied to dismiss the University's Complaint without prejudice. The University agreed that St. Jude would have the opportunity to

file first in Tennessee if the case were not settled. St. Jude did indeed file first. The University's second-filed Complaint completely overlaps the First Action. The two actions arise out of the same two contracts between the same two parties. The University's Count II is nothing more than a denial that it breached the contracts as alleged in St. Jude's Complaint. The University's Count I claims that it did not breach the MTAs by commercializing the Receptor without St. Jude's consent, and that St. Jude's filing of the First Action in and of itself tortiously interfered with the University's commercialization efforts. In addition, as explained in Section II.B below, both counts are compulsory counterclaims in the First Action. Accordingly, this action should be dismissed in deference to the First Action pending in the Western District of Tennessee.

B. The University's Claims Should Be Dismissed Without Prejudice As Compulsory Counterclaims In The First Action In Tennessee

Federal Rule of Civil Procedure 13(a) dictates that a "pleading must state as a counterclaim any claim that – at the time of its service – the pleader has against any opposing party, if the claim arises out of the transaction or occurrence that is the subject matter of the opposing party's claim and does not require adding another party over whom the court cannot acquire jurisdiction." Fed. R. Civ. P. 13(a). The key purpose of the rule is to promote judicial economy. *Transamerica Occidental Life Ins. Co. v. Aviation Office of Am., Inc.*, 292 F.3d 384, 389 (3d Cir. 2002); *see also Great Lakes Rubber Corp. v. Herbert Cooper Co.*, 286 F.2d 631, 633-34 (3d Cir. 1961) (explaining that the compulsory counterclaim is intended to abolish the "evil [of] piecemeal litigation in the federal courts"); *Vukich v. Nationwide Mut. Ins. Co.*, 68 Fed. App'x 317, 319 (3d Cir. 2003) (construing compulsory counterclaims liberally, "in supporting the notions of judicial economy"). The rule effectuates this purpose by "prevent[ing] multiplicity of actions and [] achiev[ing] resolution in a single lawsuit of all disputes arising out of common matters." *Transamerica Occidental Life Ins. Co.*, 292 F.3d at 393 (quoting *Southern Construction Co. v. Pickard*, 371 U.S. 57, 60 (1962)).

Under the rule, a compulsory counterclaim exists where "multiple claims involve many of the same factual issues, or the same factual and legal issues, or where they are offshoots of the same basic controversy between the parties." *Vukich*, 68 Fed. App'x at 319 (citation and internal quotations omitted); *see Great Lakes Rubber Corp.*, 286 F.2d at 633 (defining a compulsory counterclaim as arising "out of the transaction or occurrence that is the subject matter of an opposing party's claim"). To meet this standard, "there need not be precise identity of issues and facts between the claim and the counterclaim; rather, the relevant inquiry is whether the counterclaim bears a logical relationship to an opposing party's claim." *Transamerica Occidental Life Ins. Co.*, 292 F.3d at 389 (internal quotation omitted).

Here, the University's claims involve the same factual and legal issues as St. Jude's claim in the First Action, and arise out of the same dispute between the parties. Indeed, as explained above, the University's claims arise out of the same two MTAs, and are simply the "flip side" of St. Jude's already pending claims in the First Action. In the First Action, St. Jude claims the University breached the MTAs; in this action, the University seeks a declaratory judgment that it did not. In the First Action, St. Jude claims the University breached the MTAs by, among other things, commercializing the subject materials without St. Jude's consent; in this action, the University claims its commercialization efforts did not breach the MTAs, and further claims the First Action is an unlawful interference with its ongoing commercialization effort.

Where claims arise out of contracts that are already the subject of pending litigation initiated by the adverse party, courts routinely hold such claims to be Rule 13(a) compulsory counterclaims. *See, e.g., Transamerica Occidental Life Ins. Co.*, 292 F. 3d at 393 (determining that the compulsory counterclaim rule applied where the same set of reinsurance agreements were at issue in both actions); *Vukich*, 68 Fed. App'x at 319 (applying the compulsory counterclaim rule where two separate actions required interpretation of the same employment agreement between the parties); *Servian*, 1992 WL 174705, at *1 (concluding that counterclaims

were compulsory where both parties sought relief under the same series of prior agreements); *Abbot v. Neal*, No. 90-6619, 1991 WL 42409, *1-2 (E.D. Pa. Mar. 26, 1991) (identifying counterclaims as compulsory where all claims involved a dispute over rights under the same fee agreement).

In particular, breach of contract and declaratory judgment claims based on the same underlying contracts are commonly identified as arising from the same transaction or occurrence. *See, e.g., Transamerica Occidental Life Ins. Co.*, 292 F.3d at 393 ("[T]here is no question that the two actions arise out of the same contracts" where one party sues for breach and the other party seeks a declaratory judgment of no liability under the contracts); *Zelenkofske Axelrod Consulting, L.L.C.*, 1999 WL 592399 at *2 (recognizing claims for breach of contract and declaratory judgment as "clearly offshoots of the same basic controversy between the parties"); *Abbot*, 1991 WL 42409 at *2 (E.D. Pa. Mar. 26, 1991) ("A closer connection between two claims could not be contemplated" where one party sues for breach of contract and the other party sues for a judicial declaration of no liability under the same contract).

Similarly, claims for tortious interference commonly arise out of the same transaction or occurrence as a parallel dispute between the same parties. *See, e.g., Great Lakes Rubber*, 286 F.2d at 634 (determining that a compulsory relationship between the claims is "unquestionable" where a second party claims that the opposing party's claims were brought merely to harass and prevent second party from competing in common market); *GMAC Bank v. HTFC Corp.*, No. 06-5291, 2007 WL 3197153, at *1-2 (E.D. Pa. Nov. 1, 2007) (identifying a proposed counterclaim as compulsory where first party asserted breach of contract, and second party counterclaimed for tortious interference with third-party contracts); *Crutcher v. Aetna Life Ins. Co.*, 746 F.2d 1076, 1079-80 (5th Cir. 1984) (barring a later claim for tortious interference where it was a compulsory counterclaim to an earlier claim based on contract); *Shmuel Shmueli, Basche, Inc. v. Lowenfeld*, 68 F. Supp. 2d 161, 165 (E.D.N.Y. 1999) (identifying tortious interference claims as compulsory

counterclaims in original action, where the claims derived "from a contractual transaction allegedly disrupted by [the original] proceedings").

By these standards, the University's claims here are unquestionably compulsory counterclaims that must be litigated, if at all, in the First Action in Tennessee. Therefore, this action should be dismissed. *See, e.g.*, *Vukich*, 68 Fed. App'x at 319 (affirming dismissal of complaint where claims constituted compulsory counterclaims in separate action); *Transamerica Occidental Life Ins. Co.*, 292 F.3d at 394 (same); *Servian*, 1992 WL 174705 at *2-3 (dismissing complaint where it included compulsory counterclaims to be raised in action pending elsewhere); *Abbot*, 1991 WL 42409 at *3 (same); *Moose Mt. Mktg., Inc. v. Alpha Int'l, Inc.*, No. 03-4035, 2005 WL 3588491 at *1 (D.N.J. Dec. 29, 2005) (dismissing suit under compulsory counterclaim rule; explaining that, "[s]hould a court discover that an action before it is pending in another federal suit, it will stay its own proceedings or dismiss the claim with leave to plead it in the prior action") (internal quotation omitted).

Coupled with the first-filed rule, the compulsory counterclaim rule compels dismissal of this action. *See, e.g., Shire U.S., Inc. v. Johnson Matthey, Inc.*, 543 F. Supp. 2d 404, 411 (E.D. Pa. 2008) (granting motion to dismiss pursuant to first-filed rule where a Texas action "was earlier-filed, the two actions concern the same subject matter, and no exception from the ordinary application of the rule is warranted"); *Colony Nat'l Ins.*, 2009 WL 3007334 (dismissing case where there were no exceptional circumstances justifying departure from the first-filed rule); *Servian*, 1992 WL 174705 (same).

C. At The Very Least, This Court Should Stay These Proceedings Until The Tennessee Court Rules On The University's Motion To Dismiss The First Action For Lack Of Personal Jurisdiction

Should this Court be reluctant to dismiss this action without prejudice, it should, at a minimum, stay all proceedings in this action until the Tennessee court decides in the First Action whether it has jurisdiction over the University. This Court plainly has the power to stay

proceedings before it when doing so will promote judicial economy and orderly resolution of claims. "[T]he power to stay proceedings is incidental to the power inherent in every court to control the disposition of the causes on its docket with the economy of time and effort for itself, for counsel, and for litigants. How this can best be done calls for the exercise of judgment, which must weigh competing interests and maintain an even balance." *Texaco, Inc. v. Borda*, 383 F.2d 607, 608 (3d Cir. 1967) (quoting *Landis v. N. Am. Co.*, 299 U.S. 248, 254-55 (1936)), quoted in *Saunders v. City of Philadelphia*, No. 97-3251, 1997 WL 400034 (E.D. Pa. July 11, 1997) (Dalzell, J.). "In the exercise of its sound discretion, a court may hold one lawsuit in abeyance to abide the outcome of another which may substantially affect it or be dispositive of the issues." *Bechtel Corp. v. Local 215, Laborers' Int'l Union*, 544 F.2d 1207, 1215 (3d Cir. 1976).

The jurisdiction of the Tennessee district court is for that court to determine; after that, there may be little for this Court to decide. *See, e.g.*, *Peregrine*, 769 F. Supp. at 173 (setting aside challenge to first-filed rule based on lack of personal jurisdiction in the court of first filing and noting that "if personal jurisdiction over [first-filed defendant] becomes an issue in the [first-filed action], then that issue should be decided by the district court in the [first-filed venue], not by this court"); *RJF Holdings III, Inc. v. Refractee, Inc.*, No. 03-1600, 2003 WL 22794987 at *4 (E.D. Pa. Nov. 24, 2003) (rejecting argument that first-filed court lacked proper personal jurisdiction, instructing that "[o]bjections to the jurisdiction of the [court of first filing] should be presented to and decided by that court").

* * *

Both the first-filed rule and the compulsory counterclaim rule independently dictate that the University's claims here should be litigated in the First Action in Tennessee. Accordingly, this action should be dismissed without prejudice or, at a minimum, stayed pending the determination by the Tennessee court of its jurisdiction.

III. COUNT I OF THE UNIVERSITY'S AMENDED COMPLAINT FAILS TO STATE A CLAIM ON WHICH RELIEF CAN BE GRANTED

As noted above, Count II of the University's Amended Complaint is nothing more than a sweeping denial of St. Jude's breach-of-contract claims in the guise of a claim for a declaratory judgment. It stands or falls on the merits of St. Jude's First Action in Tennessee. Similarly, the University's Count for "tortious interference" is premised entirely on St. Jude's filing of the First Action; if St. Jude prevails in the First Action, there was no tortious interference. However, Count I of the Amended Complaint, like the original Complaint before it, suffers additional infirmities in that it is barred by the *Noerr-Pennington* doctrine and fails to state a claim for relief under both federal pleading standards and state substantive law. On the facts of this case, these deficiencies are not curable by yet further amendment. Count I of the Amended Complaint should therefore be dismissed with prejudice.

A. The *Noerr-Pennington* Doctrine Bars The University's Purported Claim For Tortious-Interference-By-Lawsuit

1. The *Noerr-Pennington* Doctrine Is, On Its Face, Applicable To The University's Claim

More than fifty years old, the *Noerr-Pennington* doctrine protects those who petition the government for redress from liability that might otherwise arise from that petition. *E.R.R. Presidents Conference v. Noerr Motor Freight, Inc.*, 365 U.S. 127, 137-38 (1961); *United Mine Workers v. Pennington*, 381 U.S. 657, 669-70 (1965). *Noerr-Pennington* immunity extends to all forms of petitions for redress, whether to the legislature, to an administrative agency, or to the courts. *Cal. Motor Transp. Co. v. Trucking Unlimited*, 404 U.S. 508, 510 (1972). The Third Circuit has recognized that the immunity extends to state common law claims of unfair competition, tortious interference, and other commercial torts, not just to federal antitrust claims. *See Cheminor Drugs, Ltd. v. Ethyl Corp.*, 168 F.3d 119, 128-29 (3d Cir. 1999) (affirming dismissal of four state law tort claims on *Noerr-Pennington* grounds); *see also Video Int'l Prod., Inc. v. Warner-Amex Cable Commc'ns, Inc.*, 858 F.2d 1075, 1082 (5th Cir. 1988).

The University's tortious interference claim in Count I is based entirely on St. Jude's filing of the First Action, a petition for redress squarely within the immunity offered by *Noerr-Pennington* and *Cheminor Drugs*. Count I therefore violates St. Jude's First Amendment right of petition and must be dismissed.

2. As A Matter Of Law, The Sham Litigation Exception To *Noerr-Pennington* Immunity Does Not Apply To This Case

There is a limited exception to *Noerr-Pennington* immunity for cases in which the underlying litigation is, in the words of *Noerr*, a "mere sham to cover what is actually nothing more than an attempt to interfere directly with the business relationships of a competitor." *Noerr*, 365 U.S. at 144 (emphasis added). The sham litigation exception has "a very narrow scope." *VIM, Inc. v. Somerset Hotel Ass'n*, 19 F. Supp. 2d 422, 426 (W.D. Pa. 1998) (declining to apply the sham exception); *see also Clipper Exxpress v. Rocky Mountain Motor Tariff Bureau, Inc.*, 690 F.2d 1240, 1251 n.15 (9th Cir. 1982); *Razorback Ready Mix Concrete Co. v. Weaver*, 761 F.2d 484, 486-87 (8th Cir. 1985); *Bright v. Moss Ambulance Serv., Inc.*, 824 F.2d 819, 823 (10th Cir. 1987). "If an objective litigant could conclude that the suit is reasonably calculated to elicit a favorable outcome, the suit is immunized under *Noerr*, and [a claim] premised on the sham exception must fail." *Prof'l Real Estate Investors, Inc. v. Columbia Pictures Indus.*, 508 U.S. 49, 60-61 (1993) ("*PREI*"). "[S]ham litigation must constitute the pursuit of claims so baseless that no reasonable litigant could realistically expect to secure favorable relief." *Id.* at 62. Underlining the narrow scope of the exception, the court in *Cheminor* declined to apply the sham exception to an administrative petition even where it was alleged that the petition contained affirmative misrepresentations. 168 F.3d at 123-24.

Courts routinely dismiss tortious interference claims when they are based on non-sham litigation. *Pennpac Int'l, Inc. v. Rotonics Mfg., Inc.*, No. 99-2890, 2001 WL 569264 (E.D. Pa. May 25, 2001) (granting summary judgment on tortious interference claims where *Noerr-*

Pennington immunity protected a non-sham threat of patent enforcement litigation); *Jeep Eagle 17, Inc. v. Chrysler Fin. Servs. Ams., L.L.C.*, No. 09-23708 (DHS), 2010 WL 4864171 (Bankr. D.N.J. Nov. 23, 2010) (granting summary judgment on tortious interference claims because the underlying litigation was not "objectively baseless" and the action was thus protected under *Noerr-Pennington*); *Pennwalt Corp. v. Zenith Labs., Inc.*, 472 F. Supp. 413 (E.D. Mich. 1979) (dismissing tortious interference claim because the lawsuit forming the basis for the tort claim was not a sham and was therefore protected under *Noerr-Pennington*); *Atico Int'l USA, Inc. v. LUV N' Care, Ltd.*, No. 09-60397-CIV-COHN/SELTZER, 2009 WL 2589148 (S.D. Fla. Aug. 19, 2009) (same).

The sham exception may be evaluated as a matter of law where, as here, there is no factual dispute material to the question of its applicability. *PREI*, 508 U.S. at 63. In order to avoid dismissal at this stage, the University must "allege facts which, if proven, [would] show that [St. Jude] is not entitled to *Noerr-Pennington* immunity under the sham litigation exception." *Rochester Drug Co-op., Inc. v. Braintree Labs.*, 712 F. Supp. 2d 308, 320 (D. Del. 2010).

The Amended Complaint does not and cannot truthfully allege such facts. By their terms, the 2003 and 2007 MTAs apply to "any progeny, portions, [and] unmodified derivatives" of Dr. Campana's chimeric receptor construct, 2003 MTA ¶ 1; 2007 MTA ¶ 1, as well as any product that "contains a portion of the Materials, is derived from the Materials, or which could not have been produced but for the use of the Materials," 2007 MTA ¶ 5. In addition, under the 2003 MTA, the University is prohibited from "us[ing]" the Material "for any commercial purpose," is prohibited from "commercializ[ing] any product that contains Materials without the prior written approval of St. Jude," and is required to notify St. Jude of any patent application "which claims subject matter that contains or incorporates the Material or which claims a method of manufacture or use of the Materials." 2003 MTA ¶ 4, 8. Thus, in order to survive a motion to

dismiss, the Amended Complaint would have to allege facts that, if proven, would show that "no reasonable litigant" could have believed that any portion of Dr. June's research (a) included progeny, portions, or unmodified derivatives of the Receptor, (b) contained the Receptor, (c) incorporated the Receptor, (d) used the Receptor, (e) included a portion of the Receptor, (f) was derived from the Receptor, or (g) could not have been produced but for use of the Receptor. Proof of any of these would entitle St. Jude to "favorable relief" in the First Action.[9]

The Amended Complaint fails to make such allegations. Indeed, to the contrary, it includes a number of allegations specifically supporting St. Jude's position that some or all of the products of Dr. June's research were covered by the MTAs. The University admits that it requested the Receptor so that Dr. June's laboratory "could *modify* [it] to create a lentiviral vector." Amended Complaint ¶ 12 (emphasis added). It also admits that Dr. June's research involved "*modification* of excised segments of the Campana Construct." *Id.* ¶ 15 (emphasis added). And, in its November 22, 2011 letter to St. Jude, attached to the Amended Complaint as Exhibit F, the University expressly states that the construct being used in clinical trials had been "*modified* from that provided by Dr. Campana." Exhibit F at 2 (emphasis added). The University thus admits in its own complaint and correspondence that portions of the so-called "Penn Immunotherapy" were intended to be and actually were derived from St. Jude's Receptor. Moreover, while the University alleges that the Penn Immunotherapy "*could have been* produced without any use of the Materials," Amended Complaint ¶ 22 (emphasis added), it conspicuously fails to allege that the "Penn Immunotherapy" *actually was* produced without any use of the

[9] The Amended Complaint does not contend that, to the extent Dr. June's research is covered by the MTAs, the University has complied with all of the MTAs' requirements. Rather, the University appears to rely entirely for its claims here on the argument that Dr. June's research is not covered by the MTAs and is therefore exempt from its requirements. Thus, if any reasonable litigant could conclude that the MTAs governed any facet of Dr. June's research, *Noerr-Pennington* bars the University's claim.

Materials. The clear implication of the University's allegations is that the "Penn Immunotherapy" was created using the Materials. *See* Amended Complaint ¶¶ 20-22.

The University is free to argue in the First Action that its use and modifications of the Receptor are not encompassed by the MTAs. However, it is preposterous to contend that it is "objectively unreasonable" for St. Jude to have alleged in the First Action that the MTAs' references to "progeny" and "portions" of the Materials, constructs "derived from" the Materials, and "use" of the Materials, bring these modifications within the Agreements' scope. Put simply, since the University repeatedly concedes "modification," how can it possibly be a "sham" for St. Jude to allege "derived from"?

Separately, the University's November 22, 2011 letter details the acknowledgement of Dr. Campana's contribution that the University claims to have been included in Dr. June's published reports and asserts that "the acknowledgement requirement of the MTA has been satisfied." Amended Complaint, Exhibit F at 1. If, as the University now contends, Dr. June's research was not covered by the MTAs at all, *see, e.g.*, Amended Complaint ¶¶ 22, 43, there would have been no "acknowledgement requirement" to satisfy. Thus, less than a year ago, the University understood that Dr. June's research, including the research described in the *New England Journal of Medicine*, was covered by the MTAs. Such an understanding could hardly be further from the University's current litigation position that it is objectively unreasonable to believe that the research is covered by the MTAs at all. The Amended Complaint's allegations cannot un-write the University's past belief. *See ALA, Inc. v. CCAIR, Inc.*, 29 F.3d 855, 859 n.8 (3d Cir. 1994) ("Where there is a disparity between a written instrument annexed to a pleading and an allegation in the pleading based thereon, the written instrument will control.").

The University's allegations do not, as they must to avoid the high barrier of *Noerr-Pennington*, lead to the conclusion that no reasonable litigant in St. Jude's position could believe that Dr. June's research was covered by the MTAs. Only paragraph 16 of the Amended

Complaint alleges any purported factual basis for a conclusion that the "June Construct" is not covered by the MTAs. Paragraph 16 alleges "[t]he June Construct and the Campana Construct are different in important ways." The paragraph goes on to enumerate a handful of alleged differences, but when it comes to the Receptor (or CAR) that is the heart of St. Jude's Materials, it says only that the CAR expressed in the June Construct has "a different sequence of base pairs." *Id.* In fact, as one of the University's own patent applications makes clear, the only difference between the Receptor and the corresponding CAR in the "June Construct" is a single base pair among 1400 base pairs. This change in one base pair was apparently an unintended accident that did not affect the functionality of the Receptor. This so-called "difference" hardly takes the June Construct outside the coverage of the MTAs and, *a fortiori*, certainly does not make it a sham to allege otherwise.

In any event, the alleged differences enumerated in paragraph 16 of the Amended Complaint, even if proven, would do nothing to establish that the University's technology does not include "progeny, portions, [or] unmodified derivatives" of the Receptor, is not "derived from the [Receptor]," and would have been able to be "produced" without use of the Receptor, 2003 MTA ¶ 1; 2007 MTA ¶¶ 1, 5, much less that no reasonable basis exists for concluding that any one of these facts – each of which would bring the technology within the ambit of the MTAs – could be true. The University's conclusory allegation that the "Penn Immunotherapy does not contain a portion of the Materials, is not derived from the Materials, and could have been produced without any use of the Materials," Amended Complaint ¶ 22, does nothing to establish that St. Jude's belief to the contrary is objectively unreasonable.

In an attempt to avoid these key facts, which are fatal to its claim, the University falls back on a series of allegations regarding St. Jude's alleged lack of a proper basis for seeking injunctive relief in the First Action. Amended Complaint ¶¶ 48, 49, 51-53. The allegations lend the University's position no support.

First, straining to argue that St. Jude has no basis for injunctive relief, the University alleges, without support, that St. Jude has not filed a motion for preliminary injunction in the First Action "because St. Jude knew that it had no basis to prevent any commercialization or exploitation of the Penn Immunotherapy." Amended Complaint ¶ 50. However, the University maintains that Tennessee courts lack personal jurisdiction over it. It was entirely reasonable for St. Jude to delay filing a preliminary injunction motion until the jurisdictional issue is resolved.

Second, the University alleges without factual basis in paragraphs 48-53 of the Amended Complaint that injunctive relief is inappropriate. These conclusory allegations are not entitled to the assumption of truth in deciding this motion to dismiss. *Morse v. Lower Merion Sch. Dist.*, 132 F.2d 902, 906 (3d Cir. 1997) ("[A] court need not credit a complaint's 'bald assertions' or 'legal conclusions' when deciding a motion to dismiss." (quoting *In re Burlington Coat Factory Sec. Litig.*, 114 F.3d 1410, 1429-30 (3d Cir. 1997))).

Third, the University's "bald assertions" are directed only to St. Jude's request for injunctive relief. They are insufficient to establish that the entire First Action was a sham. *PREI* requires only that a litigant have an objectively realistic expectation of "favorable relief" or "a favorable outcome." 508 U.S. at 60-62. The First Action seeks money damages in additional to injunctive relief. Tennessee Complaint at page 14. *See Eden Hannon & Co. v. Sumitomo Trust & Banking Co.*, 914 F.2d 556, 565 (4th Cir. 1990) (holding that where plaintiff had succeeded on one of its four claims and won some of the relief it had sought, the lawsuit was "hardly a sham"); *Dentsply Int'l Inc. v. New Tech. Co.*, No. 96-272 MMS, 1996 WL 756766 (D. Del. Dec. 19, 1996) ("[L]itigation will not be considered a 'sham' so long as at least one claim in the lawsuit has objective merit.") (citing *PREI*, 508 U.S. at 60).

Fourth, the University's few allegations purportedly showing that the First Action is "so baseless that no reasonable litigant could realistically expect to secure favorable relief," *PREI*, 508 U.S. at 62, are at best conclusory allegations of bad faith. They do not come close to stating

facts showing that the litigation was a "sham" under the demanding *Noerr-Pennington* standard. *See Raines v. Switch Mfg.*, No. C-96-2648 DLJ, 1997 WL 578547, at *6 (N.D. Cal. July 28, 1997) ("[I]f a bare allegation of bad faith litigation were sufficient to defeat the *Noerr-Pennington* bar, every claimant would be able to avoid the intent of the Supreme Court. . . .").

Paragraph 39 alleges the bare conclusion that the University "has not committed any material breach of the Agreements." This is nothing more than a denial, identical to that made in answer to every breach of contract complaint in which the defendant contests breach. It falls far short of an allegation that the First Action is a "sham." Indeed, even the fact that a lawsuit is ultimately unsuccessful does not make it a sham. In *PREI*, the Supreme Court warned that "a court must resist the understandable temptation to engage in post hoc reasoning by concluding that an ultimately unsuccessful action must have been unreasonable or without foundation." 508 U.S. at 60 n.5 (internal quotation omitted).

Aside from paragraph 39, every other allegation in the Amended Complaint purporting to advance the notion that St. Jude had no basis for filing the First Action is made "upon information and belief." Amended Complaint ¶¶ 43-47, 50. While the Federal Rules permit pleading "upon information and belief," such an allegation must be "'accompanied by a statement of the facts upon which the belief is founded.'" *Navarra v. Marlborough Gallery, Inc.*, 820 F. Supp. 2d 477, 485 (S.D.N.Y. 2011) (quoting *Prince v. Madison Square Garden*, 427 F. Supp. 2d 372, 385 (S.D.N.Y. 2006)). The University's Complaint states no such facts supporting the University's belief of any of these conclusory allegations.

In addition to lacking the required factual support, these allegations focus on St. Jude's purported subjective awareness of the lack of basis for suit. However, this subjective focus is contrary to the clear direction of *PREI* that only where a lawsuit is "objectively baseless in the sense that no reasonable litigant could realistically expect success on the merits" does an inquiry into subjective intent follow. *PREI,* 508 U.S. at 60; *see also Herr v. Pequea Twp.*, 274 F.3d 109,

118 (3d Cir. 2001) (noting that the initial inquiry required by *PREI* is "wholly objective"). Here, because the University has completely failed to allege facts that, if proven, would establish objective baselessness, no inquiry into St. Jude's subjective intent or beliefs is permitted.

* * *

The University has not begun to meet its burden under *Noerr-Pennington*'s "sham litigation" exception to plead objective facts that would establish that "no reasonable litigant could realistically expect to secure favorable relief," *PREI*, 508 U.S. at 62, in the First Action. Accordingly, under the *Noerr-Pennington* doctrine, Count I of the Amended Complaint – the University's claim for tortious interference – should be dismissed.

B. The University's Amended Complaint Fails To State A Claim For Tortious Interference Under Federal Pleading Standards And Tennessee Law

1. Tennessee Law Governs The University's Claim For Tortious Interference

In this diversity case, the choice of law regime of the forum state, Pennsylvania, governs. *Lacey v. Cessna Aircraft Co.*, 932 F.2d 170, 187 (3d Cir. 1991) (citing *Klaxon Co. v. Stentor Elec. Mfg Co.*, 313 U.S. 487 (1941)). In cases alleging the tortious filing of litigation, Pennsylvania courts have generally applied the law of the state in which the allegedly wrongful litigation was filed. *See, e.g., Rosen v. Tesoro Petroleum Corp.*, 582 A.2d 27 (Pa. Super. Ct. 1990). In *Rosen*, which involved an allegedly tortious action filed in Texas, the court relied on "Texas' interest in maintaining liberal access to its court system, in protecting the expectations of Texas litigants and their counsel regarding the circumstances under which they will be held accountable for malicious prosecution, and in 'regulating the use of its process and in determining when its judicial system is maliciously used.'" *Id.* at 31 (quoting *Denenberg v. Am. Family Corp. of Columbus, Ga.*, 566 F. Supp. 1242, 1248 (E.D. Pa. 1983)); *see also Lohman v. Twp. of Oxford,* 816 F. Supp. 1025, 1031 (E.D. Pa. 1993); *Bolanos v. Gulf Oil Corp.*, 502 F. Supp. 689, 692 (W.D. Pa. 1980).

Applying the law of the state in which the allegedly wrongful litigation was filed is consistent with the position of the Restatement of Conflict of Laws concerning the closely related torts of malicious prosecution and abuse of process. The Restatement creates a presumption that "[t]he rights and liabilities of the parties for malicious prosecution or abuse of process are determined by the local law of the state where the proceeding complained of occurred . . . ." Restatement (Second) of Conflict of Laws § 155 (1971); *Wolk v. Teledyne Indus.*, 475 F. Supp. 2d 491, 512 (E.D. Pa. 2007).[10] As the comments explain, "The state where the proceeding complained of occurred has a natural interest in determining the extent to which resort to its legal processes is to be inhibited by the possibility that a person making use of these processes will be held liable for malicious prosecution or abuse of process." *Restatement*, at § 155, cmt. b. This rationale applies equally to a claim for tortious interference based entirely on the filing of a lawsuit. This case presents no special circumstance that warrants deviation from the *Rosen* line of cases or the Restatement's general rule. Therefore, Tennessee law, as the law of the jurisdiction where the allegedly tortious complaint was filed, should be applied.

2. To State A Claim For Tortious Interference Under Tennessee Law, The University Must State Facts Showing Improper Means

In order to make a claim for tortious interference in Tennessee, a plaintiff must plead, *inter alia*, that the defendant has used "improper motive or means."[11] However, two privileges

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[10] Pennsylvania uses a "hybrid approach that 'combines the approaches of both [the Second Restatement] (contacts establishing significant relationships) and "interest analysis" (qualitative appraisal of the relevant States' policies with respect to the controversy).'" *Id.* (quoting *Lacey*, 932 F.2d at 187.)

[11] In *Trau-Med of America, Inc. v. Allstate Insurance Co.*, 71 S.W.3d 691 (Tenn. 2002), the Tennessee Supreme Court set out the general elements of the tort of interference with contractual relations. It held that "liability should be imposed on the interfering party provided that the plaintiff can demonstrate the following: (1) an existing business relationship with specific third parties or a prospective relationship with an identifiable class of third persons; (2) the defendant's knowledge of that relationship and not a mere awareness of the plaintiff's business dealings with others in general; (3) the defendant's intent to cause the breach or termination of the business relationship; (4) the defendant's improper motive or improper means;

applicable to this case – (a) the competitor's privilege, and (b) St. Jude's privilege to assert a legal interest – require that, regardless of St. Jude's *motive*, the Amended Complaint must adequately plead improper *means* in order to survive.

(a) Competitor's Privilege

In *Trau-Med of America, Inc. v. Allstate Ins. Co.*, 71 S.W.3d 691, 700 (Tenn. 2002), the court described a claim for intentional interference as requiring "improper conduct extending beyond the bounds of doing business in a freely competitive economy." The tort "should not be interpreted in such a way as to prohibit or undermine the ability to contract freely and engage in competition." *Watson's Carpet & Floor Coverings, Inc. v. McCormick*, 247 S.W.3d 169, 178 (Tenn. Ct. App. 2007). This concern is in keeping with Tennessee courts' recognition of a broad competitor's privilege. In *Polk & Sullivan, Inc. v. United Cities Gas Co.*, 783 S.W.2d 538, 543 (Tenn. 1989), the Tennessee Supreme Court quoted with approval Section 768(1) of the Restatement (Second) of Torts, which states:

> One who intentionally causes a third person not to enter into a prospective contractual relation with another who is his competitor or not to continue an existing contract terminable at will does not interfere improperly with the other's relation if: (a) the relation concerns a matter involved in the competition between the actor and the other and (b) the actor does not employ wrongful means and (c) his action does not create or continue an unlawful restraint of trade and (d) his purpose is at least in part to advance his interest in competing with the other.

Accordingly, where the parties are competitors, improper motive will not suffice; plaintiff must also plead improper means.

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and finally, (5) damages resulting from the tortious interference." *Id.* at 701 (internal citations and footnotes omitted). The Pennsylvania courts define the fourth element of the *Trau-Med* test in terms of acting "without a privilege to do so." *Thompson Coal Co. v. Pike Coal Co.*, 412 A.2d 466, 470 (Pa. 1979). The effective result is the same: a requirement to demonstrate that the defendant has acted outside the "'rules of the game' which society has adopted." *Glenn v. Point Park Coll.* 272 A.2d 895, 899 (Pa. 1971); *cf. Trau-Med*, 71 S.W.3d at 700 (observing that the tort applies to "improper conduct extending beyond the bounds of doing business in a freely competitive economy").

The comments to the Restatement make clear that competition is to be broadly defined: "The rule stated in this Section applies whether the actor and the person harmed are competing as sellers or buyers or in any other way, and regardless of the plane on which they compete." Restatement (Second) of Torts § 768, cmt. c (1979); *see Assembly Tech. Inc. v. Samsung Techwin Co.,* No. 09-00798, 2009 WL 4430020 (E.D. Pa. Nov. 16, 2009) (noting that Section 768 applies whether the parties are competing for employees, clients, contractual business, or customers and citing cases).

According to the University itself, St. Jude competes with it by soliciting private investment to fund cancer research. Amended Complaint ¶ 25 ("[T]he University has actively sought a strategic partner with infrastructure and resources that will fund additional clinical trials. . . .") *cf.* St. Jude's Complaint ¶ 81 ("If Dr. June and Penn commercially develop or exploit the Materials, their actions and omissions will deprive St. Jude of substantial income to which it is legally and equitably entitled. . . ."). The Amended Complaint's allegations that St. Jude has conducted no clinical trials using the Campana Construct and has not sought to commercialize the Campana Construct, *see* Amended Complaint ¶ 46, do nothing to undermine the fact that the University and St. Jude compete for a finite pool of cancer research funding. Because the University and St. Jude are competitors under the Restatement's broad view, the University must plead improper means, not merely improper motive. As discussed below, *see* pages 29-33 *infra*, the University has failed to do so.[12]

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[12] Ironically, if St. Jude and the University were not competitors, the "baselessness" exception to *Noerr-Pennington* would be foreclosed to the University. The sham litigation exception – the University's only possible avenue to escape *Noerr-Pennington* – comes into play only where "the baseless lawsuit conceals 'an attempt to interfere directly with the business relationships of a competitor.'" *PREI*, 508 U.S. at 60-61 (quoting *Noerr*, 365 U.S. at 144); *see also Noerr*, 365 U.S. at 144 (establishing the sham exception, which applies where a petition or litigation "is a mere sham to cover what is actually nothing more than an attempt to interfere directly with the business relationships of a competitor"). Thus, if St. Jude and the University were not competitors, the sham exception would, for yet another reason, be entirely unavailable.

(b) Assertion Of A Legal Interest

Quite apart from the competitor's privilege, where, as here, plaintiff seeks to state a claim for tortious interference based on defendant's assertion of a legal interest, plaintiff must also plead facts showing improper means, not just improper motive. "One who, by asserting in good faith a legally protected interest of his own or threatening in good faith to protect the interest by appropriate means, intentionally causes a third person not to perform an existing contract or enter into a prospective contractual relation with another does not interfere improperly with the other's relation if the actor believes that his interest may otherwise be impaired or destroyed by the performance of the contract or transaction." Restatement (Second) of Torts § 773 (1979). Thus, where a party acts in good faith to safeguard a legally protected interest by appropriate means, that action cannot form the basis for a tortious interference claim. *Skiff re Bus., Inc. v. Buckingham Ridgeview, LP,* 991 A.2d 956, 967-68 (Pa. Super. Ct. 2010) (citing *Peoples Mortg. Co. v. Federal Nat. Mortg. Ass'n,* 856 F. Supp. 910, 939-43 (E.D. Pa. 1994)). While the Amended Complaint alleges, with no factual support, that St. Jude lacked a good faith basis to seek injunctive relief in the First Action, *see* Amended Complaint ¶¶ 48-55, there are no corresponding allegations regarding St. Jude's claims for monetary and other non-injunctive relief.

Although the Tennessee courts have not explicitly adopted Section 773, this section is strongly aligned with the notion in *Trau-Med* that only "*unfounded* litigation" can form the basis for a tortious interference claim. 71 S.W.3d at 701 n.5 (emphasis added).[13] *Trau-Med* gives every indication that, if faced with the question, the Tennessee Supreme Court would adopt the reasoning of Section 773 of the Restatement. Thus, merely pleading improper motive is

[13] The Pennsylvania Superior Court has expressly adopted Section 773. *See Walnut St. Assocs., Inc. v. Brokerage Concepts, Inc.*, 982 A.2d 94, 101 (Pa. Super. Ct. 2009) *aff'd*, 20 A.3d 468 (Pa. 2011).

insufficient to state a claim for relief in this case. Rather, the University must – but does not – adequately plead that St. Jude has used improper means.

While *Trau-Med* acknowledged that "a precise, all-encompassing definition of the term 'improper' [as in "improper means"] is neither possible nor helpful," it did give examples of improper means including "those means that are illegal or independently tortious, such as violations of statutes, regulations, or recognized common-law rules, violence, threats or intimidation, bribery, unfounded litigation, fraud, misrepresentation or deceit, defamation, duress, undue influence, misuse of inside or confidential information, or breach of a fiduciary relationship." 71 S.W.3d at 701 n.5 (internal citations omitted). Far from alleging anything approaching violence, threats, or bribery, the only means alleged in the University's Complaint was the entirely lawful filing of the First Action. Amended Complaint ¶ 37. Absent such allegations, the University must plead facts to establish that the First Action was "unfounded." It has failed to do so.

3. The University's Complaint Pleads Insufficient Facts To State A Claim For Tortious Interference

Federal Rule of Civil Procedure 8 requires a plaintiff to plead a "short and plain statement of the claim showing that the pleader is entitled to relief." Fed. R. Civ. P. 8(a)(2). As interpreted by our Court of Appeals and the Supreme Court, the rule "requires showing 'more than a sheer possibility that a defendant has acted unlawfully.'" *Burtch v. Milberg Factors, Inc.*, 662 F.3d 212, 221 (3d Cir. 2011) (quoting *Ashcroft v. Iqbal*, 556 U.S. 662, 678 (2009)). Rather, the complaint must allege "enough facts to state a claim to relief that is plausible on its face." *Bell Atl. Corp. v. Twombly*, 550 U.S. 544, 570 (2007). In *Iqbal*, the Supreme Court made clear that, for purposes of deciding a motion to dismiss, allegations that are "no more than conclusions [] are not entitled to the assumption of truth." 556 U.S. at 679. Put another way, "the tenet that a court must accept as true all of the allegations contained in a complaint is inapplicable to legal

conclusions." 556 U.S. at 678; *see also Haines & Kibblehouse, Inc. v. Balfour Beatty Constr., Inc.,* 789 F. Supp. 2d 622, 628 n.6 (E.D. Pa. 2011) (Dalzell, J.) ("[A]lthough a court must accept as true the factual allegations in a complaint, this does not extend to legal conclusions.").

St. Jude's 85-paragraph Complaint in the First Action alleges multiple, specific breaches of the MTAs, e.g., St. Jude's Complaint ¶¶ 49, 55, 62, 63. Nowhere does the University's Complaint allege objective facts demonstrating that St. Jude's allegations are groundless and improper. Instead, the University sweepingly alleges, "[u]pon information and belief, St. Jude is aware that the University has not committed any material breach of the Agreements." Amended Complaint ¶ 44. This is just the kind of conclusory allegation that is not entitled to the "assumption of truth." As our Court of Appeals found in *Howard Hess Dental Laboratories Inc. v. Dentsply International, Inc.,* 602 F.3d 237, 255 (3d Cir. 2010), where, as here, knowledge is an element of plaintiff's cause of action, "to survive dismissal it does not suffice to simply say that the defendants had knowledge; there must be factual allegations to plausibly suggest as much." *See also Hoffman v. L & M Arts*, 774 F. Supp. 2d 826, 846 (N.D. Tex. 2011) ("It is not enough to allege that a defendant had 'knowledge' of a contract or 'intentionally' interfered because this is nothing more than a recital of some of the required elements for a claim of tortious interference with contract."); *Perez-Gonzalez v. Municipality of Anasco*, 769 F. Supp. 2d 52, 61 (D.P.R. 2010) ("The mere allegation that a defendant knew of plaintiff's political affiliation, without providing facts as to the source of that knowledge, is insufficient to satisfy plaintiff's burden."); *United States v. Lloyds TSB Bank PLC,* 639 F. Supp. 2d 326, 343 (S.D.N.Y. 2009); *In re Section 1031 Exch. Litig.,* 716 F. Supp. 2d 415, 423-24 (D.S.C. 2010).

For the same reasons that the University has failed to allege facts sufficient to establish that the First Action is sham litigation exempt from *Noerr-Pennington*, *see* pp. 18-24, *supra*, the allegations of the Amended Complaint fail to establish that St. Jude lacked a proper basis to file the First Action. Nothing in the Amended Complaint addresses or negates a number of detailed

allegations in the First Action of the University's breach of the MTAs. For example, the Amended Complaint is silent on the substance of St. Jude's allegations that: (i) the University failed to seek the approvals and provide the notifications required in the MTAs, *id.* ¶¶ 49, 55, 62, 63; or (ii) the University failed to provide the acknowledgements required by the MTAs, *id.* ¶¶ 49, 57. These allegations of St. Jude's Complaint are objective, specific, and unanswered by the University. The University's threadbare conclusions, which are not entitled to an assumption of truth on this Rule 12 motion, simply do not pass muster as allegations of "improper means" and are insufficient to state a claim to relief that is "plausible on its face." Count I of the University's Complaint should be dismissed.

4. The University's Tortious Interference Claim Is Premature As A Matter Of Law Because The University Has Not Defeated St. Jude's Claims In The First Action

The Tennessee Supreme Court has recognized that the principal way for a plaintiff to establish the "improper means" element of a tortious interference claim is to demonstrate that the means used were "illegal or independently tortious." *Trau-Med,* 71 S.W.3d. at 701 n.5. Among the examples given by the court of means that may be "illegal or independently tortious" is "unfounded litigation." *Id.; see also id.* at 700 (citing with approval the Oregon Supreme Court's holding that "a claim is made when 'interference resulting in injury to another is wrongful by some measure beyond the fact of the interference itself'" (quoting *Top Serv. Body Shop, Inc. v. Allstate Ins. Co.,* 582 P.2d 1365, 1371 (Or. 1978))). The predicate conduct for a tortious interference claim must be *independently* wrongful on some basis beyond the fact of the interference itself.

Both Tennessee and Pennsylvania law require that before a claim for the "independently tortious" bringing of litigation (*i.e.*, a claim for malicious prosecution or abuse of process) is ripe, the underlying claim must have been terminated in favor of the plaintiff. *See, e.g., Roberts v. Fed. Express Corp.,* 842 S.W.2d 246, 247-48 (Tenn. 1992) ("In order to establish the essential

elements of malicious prosecution, a plaintiff must prove that . . . the prior action was finally terminated in plaintiff's favor."); 42 Pa. C.S.A. 8351 (a)(2) (requiring as an element of wrongful use of civil proceedings that "the proceedings have terminated in favor of the person against whom they are brought"). Because the First Action cannot be "illegal or independently tortious" absent a resolution of the claims in the University's favor, any tortious interference claim arising from the First Action is not ripe.

Courts in both Tennessee and Pennsylvania have assumed that resolution of the underlying litigation in favor of the tort plaintiff is a necessary precursor to an action alleging the tortious filing of a complaint. Applying Tennessee law, a Tennessee federal court observed "Mere common sense teaches us that if a frivolous or malicious suit is to be considered tortious, there must be some sort of judgment on the merits disposing of that suit. To hold otherwise might deter plaintiffs from filing meritorious actions." *Nichols v. Merrill Lynch, Pierce, Fenner & Smith,* 706 F. Supp. 1309, 1328 (M.D. Tenn. 1989). This Court has similarly observed that allowing such a claim prior to resolution of the allegedly tortious action would permit an "'end run' around the policy and ripeness considerations mandating that an underlying action terminate before a wrongful use of process claim ensues." *Univ. Patents, Inc. v. Kligman*, No. 89-3525, 1990 WL 29668, at *1 (E.D. Pa. Mar. 16, 1990).

This lawsuit is the University's flawed attempt at such an "end run." Inescapably, the University's charge that St. Jude's filing of the First Action was a tort depends on the University defeating that action and must await its outcome. If St. Jude prevails, the University's claim that the First Action was baseless and malicious will have been decided against it. The University's tortious interference claim should be dismissed.

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Dated: September 24, 2012

CERTIFICATE OF SERVICE

I, Daniel Segal, hereby certify that on September 21, 2012, I caused a true and correct copy of the foregoing Defendant's Motion to Dismiss or Stay to be served via ECF and e-mail upon:

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