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(71) **Applicant** *(for all designated States except US)*: **THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES** [US/US]; Office of Technology Transfer, National Institutes of Health, 6011 Executive Boulevard, Suite 325, MSC 7660, Bethesda, Maryland 20892-7660 (US).

(72) **Inventors; and**
(75) **Inventors/Applicants** *(for US only)*: **PALMER, Douglas C.** [US/US]; 210 Market Street, Brookeville, Maryland 20833 (US). **RESTIFO, Nicholas P.** [US/US]; 6808 Florida Street, Chevy Chase, Maryland 20815 (US).

(74) **Agents**: **JAY, Jeremy M.** et al.; LEYDIG, VOIT & MAYER, Two Prudential Plaza, Suite 4900, 180 N. Stetson Avenue, Chicago, Illinois 60601 (US).

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(54) **Title**: MODULATING IMMUNE CELL ACTIVITY USING CYTOKINE-INDUCED SRC HOMOLOGY 2 AND/OR HIGH TEMPERATURE REQUIREMENT A-1

(57) **Abstract**: The invention provides a peripheral blood cell (PBC) comprising a high temperature requirement serine peptidase 1 (HTRA1)/*htra1* activator, a host cell comprising a cytokine-induced Src homology 2 protein (CIS)/*cish* inhibitor and a HTRA1/*htra1* activator, a host cell comprising an anti-cish shMIR comprising SEQ ID NO: 3 or 4, and related populations of cells, pharmaceutical compositions, methods of treating or preventing cancer or a chronic infectious disease in a mammal, and methods of increasing T cell activity in a mammal. The invention also provides a host cell comprising a HTRA1/*htra1* inhibitor and a CIS/*cish* activator, a PBC comprising a HTRA1/*htra1* inhibitor, and related populations of cells, pharmaceutical compositions, methods of treating or preventing an auto- and/or allo-immune disease in a mammal, and methods of suppressing T cell activity in a mammal.

MODULATING IMMUNE CELL ACTIVITY USING CYTOKINE-INDUCED SRC HOMOLOGY 2 AND/OR HIGH TEMPERATURE REQUIREMENT A-1

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This patent application claims the benefit of U.S. Provisional Patent Application No. 61/420,825, filed December 8, 2010, which is incorporated herein by reference in its entirety.

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0002] Incorporated by reference in its entirety herein is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: One 20,489 Byte ASCII (Text) file named "709100_ST25.TXT" dated October 25, 2011.

BACKGROUND OF THE INVENTION

[0003] Adoptive cell therapy can be an effective treatment for cancer in some patients. However, obstacles to the overall success of adoptive cell therapy still exist. For example, the adoptive transfer of T-cells specific for tumor antigens or self-antigens can, in some cases, result in toxicities (Palmer et al., *PNAS* 105(23):8061-66 (2008); Johnson et al., *Blood* 114(3):535-46 (2009)). In addition, the *in vivo* persistence, survival, and anti-tumor activity of T cells can, in some cases, decrease following adoptive transfer (Heemskerk et al. *Hum. Gene Ther.* 19(5):496-510 (2008)).

[0004] In spite of considerable research into treatments for cancer, chronic infectious diseases, autoimmune diseases, and allo-immune diseases, there still exists a need for improved compositions and methods for treating and/or preventing cancer, chronic infectious diseases, autoimmune diseases, and allo-immune diseases.

BRIEF SUMMARY OF THE INVENTION

[0005] An embodiment of the invention provides a host cell comprising an high temperature requirement serine peptidase 1 (HTRA1)/*htra1* inhibitor and a cytokine-induced Src homology 2 protein (CIS)/*cish* activator.

[0006] Another embodiment of the invention provides a peripheral blood cell (PBC) comprising a HTRA1/*htra1* inhibitor.

[0007] Still another embodiment of the invention provides a host cell comprising an anti-*cish* shMIR comprising SEQ ID NO: 3 or 4 or a nucleotide sequence that is at least 95% identical to SEQ ID NO: 3 or 4.

[0008] Another embodiment of the invention provides a PBC comprising a HTRA1/*htra1* activator.

[0009] Still another embodiment of the invention provides a host cell comprising a CIS/*cish* inhibitor and a HTRA1/*htra1* activator.

[0010] An embodiment of the invention provides a composition comprising nanoparticles and PBCs, wherein the nanoparticles comprise an HTRA1/*htra1* inhibitor and/or a CIS/*cish* activator.

[0011] An embodiment of the invention provides a composition comprising nanoparticles and PBCs, wherein the nanoparticles comprise an HTRA1/*htra1* activator and/or a CIS/*cish* inhibitor.

[0012] The invention further provides embodiments including populations of cells and pharmaceutical compositions relating to the host cells (e.g., PBCs and T cells) and populations of cells of the invention.

[0013] Embodiments of methods of treating or preventing cancer or a chronic infectious disease in a mammal, methods of treating or preventing an auto- and/or allo-immune disease in a mammal, methods of suppressing T cell activity in a mammal, methods of increasing T cell activity in a mammal, and methods of treating or preventing a disease selected from the group consisting of age-related macular degeneration, Alzheimer's disease, and familial ischemic cerebral small-vessel disease in a mammal are further provided by the invention.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

[0014] Figure 1A is a graph showing tumor size (mm^2) in tumor-bearing mice treated with interleukin (IL)-2 and *cish*$^{-/-}$ ($P_{cish-/-}$) (closed square (■)), wild-type (P_{WT}) (open square (□)), or T cells that underwent no treatment (NT) (∗) at days post-treatment.

[0015] Figure 1B is a graph showing tumor size (mm^2) in tumor-bearing mice treated with *cish*$^{-/-}$ ($P_{cish-/-}$) T cells with (closed circle (●)) or without interleukin (IL)-2 (closed triangle (▲)), WT (P_{WT}) T cells with (open circle (○)) or without IL-2 (open triangle (Δ)), or NT (∗) T cells at days post-treatment.

[0016] Figure 1C is a graph showing survival of tumor-bearing mice treated with a retroviral vector encoding human gp100 (V), exogenous IL-2 (I), and *cish*$^{-/-}$ T cells (closed square (■)), WT T cells (open square (□)), or NT (∗) T cells at days post-adoptive cell transfer (ACT).

[0017] Figure 1D is a graph showing tumor size (mm^2) in tumor-bearing mice treated with control shMIR-treated (open squares (□)) or anti-*cish* shMIR-treated (closed squares (■)) WT $CD8^+$ pmel-1 T cells, control shMIR-treated *cish*$^{-/-}$ T cells (circles (●)), or NT (∗) T cells at days post-treatment.

[0018] Figure 2A is a graph showing Tbet expression of *cish*$^{-/-}$ (squares (■)) or WT (circles (○)) T cells relative to β-actin (ACTb) expression at hours post stimulation.

[0019] Figure 2B is a graph showing cMyc expression of *cish*$^{-/-}$ (squares (■)) or WT (circles (○)) T cells relative to β-actin (ACTb) expression at hours post stimulation.

[0020] Figure 2C is a graph showing cMyc activity in arbitrary units (AU) of *cish*$^{-/-}$ T cells untransduced (▼) or transduced with a *Myc* (c-Myc) luciferase reporter (squares (■)) or WT T cells T cells untransduced (▲) or transduced with a *Myc* (c-Myc) luciferase reporter (circles (○)) relative to β-actin (ACTb) expression at hours post stimulation.

[0021] Figure 2D is a graph showing *Bcl2l1* (Bcl-xL) expression of *cish*$^{-/-}$ (squares (■)) or WT (circles (○)) T cells relative to β-actin (ACTb) expression at hours post stimulation.

[0022] Figure 2E is a graph showing *ccnd2* (cyclin D2) expression of *cish*$^{-/-}$ (squares (■)) or WT (circles (○)) T cells relative to β-actin (ACTb) expression at hours post stimulation.

[0023] Figure 3A is a graph showing ID1 expression of *cish*$^{-/-}$ (squares (■)) or WT (circles (○)) T cells relative to β-actin (ACTb) expression at hours post stimulation.

[0024] Figure 3B is a graph showing Stra13 expression of *cish*$^{-/-}$ (squares (■)) or WT (circles (○)) T cells relative to β-actin (ACTb) expression at hours post stimulation.

[0025] Figure 3C is a graph showing SMAD activity in arbitrary units (AU) of *cish*$^{-/-}$ T cells untransduced (⊠) or transduced with a SMAD luciferase reporter (squares (■)) or WT T cells untransduced (⊗) or transduced with a SMAD luciferase reporter (circles (○)) relative to β-actin (ACTb) expression at hours post stimulation.

[0026] Figure 4A is a graph showing Tbet expression relative to β-actin (ACTb) expression of *cish*$^{-/-}$ T cells treated with control (closed circles (●)) or anti-*htra1* shMIR (open squares (□)) or WT T cells treated with control (grey circles (○)) or anti-*htra1* shMIR (grey squares (□)) at hours post stimulation.

[0027] Figure 4B is an enlargement of Figure 4A, and is a graph showing Tbet expression relative to β-actin (ACTb) expression of *cish*$^{-/-}$ T cells treated with control (black circles) or anti-*htra1* shMIR (white squares) or WT T cells treated with control (grey circles) or anti-*htra1* shMIR (grey squares) at hours post stimulation.

[0028] Figure 4C is a graph showing *myc* (c-Myc) expression relative to β-actin (ACTb) expression of *cish*$^{-/-}$ T cells treated with control (black circles) or anti-*htra1* shMIR (white squares) or WT T cells treated with control (grey circles) or anti-*htra1* shMIR (grey squares) at hours post stimulation.

[0029] Figure 4D is a graph showing *Bcl2l1* (Bcl-xL) expression relative to β-actin (ACTb) expression of *cish*$^{-/-}$ T cells treated with control (black circles) or anti-*htra1* shMIR (white squares) or WT T cells treated with control (grey circles) or anti-*htra1* shMIR (grey squares) at hours post stimulation.

[0030] Figure 4E is a graph showing cyclin-E1 expression relative to β-actin (ACTb) expression of *cish*$^{-/-}$ T cells treated with control (black circles) or anti-*htra1* shMIR (white squares) or WT T cells treated with control (grey circles) or anti-*htra1* shMIR (grey squares) at hours post stimulation.

[0031] Figure 4F is a graph showing CDC2a expression relative to β-actin (ACTb) expression of *cish*$^{-/-}$ T cells treated with control (black circles) or anti-*htra1* shMIR (white squares) or WT T cells treated with control (grey circles) or anti-*htra1* shMIR (grey squares) at hours post stimulation.

[0032] Figure 4G is a graph showing *Ccnd2* (cyclin D2) expression relative to β-actin (ACTb) expression of *cish*$^{-/-}$ T cells treated with control (black circles) or anti-*htra1* shMIR (white squares) or WT T cells treated with control (grey circles) or anti-*htra1* shMIR (grey squares) at hours post stimulation.

[0033] Figure 5A is a graph showing relative luminescence (RLU) of *SBE-luc* WT or *cish-/-* pmel-1 T cells after CD3 stimulation in the presence of exogenous HTRA1 or inactive HTRA1 (S328A) (50 μg/mL). WT + HTRA1 (S328A) (open squares (□)); WT + HTRA1 (diamonds (◇)); *cish-/-* + HTRA1 (S328A) (closed squares (■)); *cish-/-* + HTRA1 (circles (●)).

[0034] Figure 5B is a graph showing IFN-γ secretion (ng/mL^{-1}) after an overnight co-culture of WT or *cish-/-* pmel-1 T cells with peptide-pulsed splenocytes in the presence of exogenous HTRA1 or inactive HTRA1 (S328A) (50 μg/mL). WT + HTRA1 (S328A) (open squares (□)); WT + HTRA1 (diamonds (◇)); *cish-/-* + HTRA1 (S328A) (closed squares (■)); *cish-/-* + HTRA1 (circles (●)).

[0035] Figure 5C is a graph showing relative luminescence (RLU) of *SBE-luc* WT or *cish-/-* pmel-1 T cells after CD3 stimulation in the presence or absence of inactive HTRA1 (S328A) (50 μg/mL). WT + HTRA1 (S328A) (diamonds (◆)); WT alone (open squares (□)); *cish-/-* + HTRA1 (S328A) (closed squares (■)); *cish-/-* alone (circles (●)).

[0036] Figures 6A-6C are graphs showing expression of *gata3* (A), *IL-4* (B), or *IL-5* (C) (x 10^{-3}) relative to genes housekeeping (HKG) genes (*gusb, hprt1, hsp90ab1, gapdh,* and *actb*) in WT (circles (○)) or *cish-/-* p-mel-1 (squares (■)) T cells after CD3 stimulation.

[0037] Figures 7A-7H are graphs showing expression of *cd27* (A), *cd28* (B), *icos* (C), *IL-1a* (D), *IFN-γ* (E), *gzmb* (F), *prcd1* (G), or *tnfrsf9* (H) (x 10^{-3}) relative to genes housekeeping (HKG) genes (*gusb, hprt1, hsp90ab1, gapdh,* and *actb*) in WT (circles (○)) or *cish-/-* p-mel-1 (squares (■)) T cells after CD3 stimulation.

[0038] Figure 8 is a graph showing tumor size (mm^2) in tumor-bearing *rag1-/-* mice days after treatment with nontransduced (NT) pmel-1 T cells (X), wild-type T cells (open circle (○)), or *cish* -/- T cells (closed circle (●)).

[0039] Figure 9 is a graph showing tumor size (mm^2) in tumor-bearing *rag1-/-* mice days after treatment with nontransduced (NT) pmel-1 T cells (X), *cish-/-* T cells and antiCD8 antibody on day 38, (grey square), or *cish* -/- T cells and anti-IgG antibody on day 38 (black square).

DETAILED DESCRIPTION OF THE INVENTION

[0040] An embodiment of the invention provides a host cell comprising a cytokine-induced Src homology 2 protein (CIS)/*cish* inhibitor and a high temperature requirement serine peptidase 1 (HTRA1)/*htra1* activator.

[0041] CIS (also known as G18, SOCS, CIS-1, and CISH) is a member of the suppressor of cytokine signaling (SOCS) family of proteins. In addition to CIS, the SOCS family also includes SOCS1, SOCS2, SOCS3, SOCS4, SOCS5, SOCS6, and SOCS 7. Without being bound to a particular theory, it is believed that SOCS proteins inhibit the Janus kinase (JAK)-signal transducers and activators of transcription (STAT) signaling pathway.

[0042] The CIS/*cish* inhibitor may be any suitable agent that inhibits the expression of a *cish* mRNA or CIS protein. The CIS/*cish* inhibitor can be a nucleic acid at least about 10 nucleotides in length that specifically binds to and is complementary to a target nucleic acid encoding CIS/*cish* or a complement thereof. The CIS/*cish* inhibitor may be introduced into a host cell, wherein the cell is capable of expressing CIS/*cish*, in an effective amount for a time

and under conditions sufficient to interfere with expression of the CIS/*cish*. In some embodiments, RNA interference (RNAi) is employed. In this regard, the CIS/*cish* inhibitor may comprise an RNAi agent. In an embodiment, the RNAi agent may comprise a small interfering RNA (siRNA), a microRNA (miRNA), or an antisense nucleic acid. The RNAi agent, e.g., siRNA, miRNA, and/or antisense nucleic acid can comprise overhangs. That is, not all nucleotides need bind to the target sequence. RNA interference nucleic acids employed can be at least about 19, at least about 40, at least about 60, at least about 80, at least about 100, at least about 120, at least about 140, at least about 160, at least about 180, at least about 200, at least about 220, at least about 240, from about 19 to about 250, from about 40 to about 240, from about 60 to about 220, from about 80 to about 200, from about 60 to about 180, from about 80 to about 160, and/or from about 100 to about 140 nucleotides in length.

[0043] The RNAi agent, e.g., siRNA or shRNA, can be encoded by a nucleotide sequence included in a cassette, e.g., a larger nucleic acid construct such as an appropriate vector. Examples of such vectors include lentiviral and adenoviral vectors, as well as other vectors described herein with respect to other aspects of the invention. An example of a suitable vector is described in Aagaard et al. *Mol. Ther.*, 15(5): 938–45 (2007). When present as part of a larger nucleic acid construct, the resulting nucleic acid can be longer than the comprised RNAi nucleic acid, e.g., greater than about 70 nucleotides in length. In some embodiments, the RNAi agent employed cleaves the target mRNA. In other embodiments, the RNAi agent employed does not cleave the target mRNA.

[0044] Any type of suitable siRNA, miRNA, and/or antisense nucleic acid can be employed. In an embodiment, the antisense nucleic acid comprises a nucleotide sequence complementary to at least about 8, at least about 15, at least about 19, or from about 19 to about 22 nucleotides of a nucleic acid encoding CIS or a complement thereof. In an embodiment, the siRNA may comprise, e.g., trans-acting siRNAs (tasiRNAs) and/or repeat-associated siRNAs (rasiRNAs). In another embodiment, the miRNA may comprise, e.g., a short hairpin miRNA (shMIR).

[0045] The CIS/*cish* inhibitor may inhibit or downregulate to some degree the expression of the protein encoded by a *cish* gene, e.g., at the DNA, RNA, or other level of regulation. In this regard, a host cell comprising a CIS/*cish* inhibitor expresses no CIS/*cish* or lower levels of CIS/*cish* as compared to a host cell that lacks a CIS/*cish* inhibitor. In accordance with an embodiment of the invention, the CIS/*cish* inhibitor, such as an RNAi agent, such as a

shMIR, can target a nucleotide sequence of a *cish* gene or mRNA encoded by the same. In an embodiment, the *cish* sequence is a human sequence. For example, human *cish* is assigned Gene NCBI Entrez Gene ID No. 1154, and an Online Mendelian Inheritance in Man (OMIM) No. 602441. The human *cish* gene is found on chromosome 3 at 3p21.3. Two transcriptional variants include mRNAs: NM_013324 and NM_145071, with corresponding protein sequences NP_037456 and NP_659508, respectively. Accordingly, NM_013324 is provided as SEQ ID NO: 1, and NM_145071 is provided as SEQ ID NO: 2. Human genomic *cish* sequences include AC096920, AF035946, CH471055, and Z77852. Human *cish* mRNA sequences also include AF035947, AF132297, AK313850, BC031590, BC064354, BF511692, CA435538, CR594144, CR602225, D83532, and DA957793. Human CIS amino acid sequences include AAQ13420, EAW65127, EAW65128, EAW65129, EAW65130, AAF97410, AAD28471, BAG36578, AAH31590, AAH64354, and BAA92328. Other human sequences, as well as other CIS/*cish* species can be employed in accordance with the invention.

[0046] In accordance with an embodiment of the invention, the CIS/*cish* inhibitor, such as an RNAi agent, such as a shMIR, can target a nucleotide sequence selected from the group consisting of the 5' untranslated region (5' UTR), the 3' untranslated region (3' UTR), and the coding sequence of *cish*, complements thereof, and any combination thereof. Any suitable *cish* target sequence can be employed. The sequences of the CIS/*cish* inhibitor can be designed against a human *cish* with Accession No. NM_013324 (SEQ ID NO: 1) but also recognize NM_145071 (SEQ ID NO: 2). In an embodiment, the shMIR comprises SEQ ID NO: 3, encoded by nucleotide sequence SEQ ID NO: 12 and designed against the *cish* gene target GAGCCTGTTTCTGGGAGAA (SEQ ID NO: 9). In another embodiment, the shMIR comprises SEQ ID NO: 4, encoded by the nucleotide sequence SEQ ID NO: 13 and designed against the *cish* gene target GTCAACGCCTCTAGGTACA (SEQ ID NO: 10). RNAi agents can be designed against any appropriate *cish* mRNA sequence. In this regard, an embodiment of the invention provides a host cell comprising an anti-*cish* shMIR comprising SEQ ID NO: 3 or 4.

[0047] The host cell may or may not further comprise a high temperature requirement serine peptidase 1 (HTRA1)/*htra1* activator. HTRA1 (also known as L56, ARMD7, ORF480, and PRSS11) is a serine protease that is a member of the trypsin family of serine proteases. Without being bound to a particular theory, it is believed that HTRA1 inhibits the transforming growth factor (TGF)-β family of proteins.

[0048] The high temperature requirement serine peptidase 1 (HTRA1)/*htra1* activator may comprise any suitable agent that provides the over-expression of a *htra1* mRNA or HTRA1 protein. In this regard, a host cell comprising a HTRA1/*htra1* activator expresses higher levels of HTRA1/*htra1* than host cells that lack a HTRA1/*htra1* activator. The HTRA1/*htra1* activator may be introduced into a host cell, wherein the cell is capable of expressing HTRA1/*htra1*, in an effective amount for a time and under conditions sufficient to cause the over-expression of HTRA1/*htra1*. In this regard, the HTRA1/*htra1* activator may comprise a recombinant nucleic acid that encodes HTRA1/*htra1*. In an embodiment of the invention, the recombinant polynucleotide that encodes HTRA1/*htra1* may comprise a nucleotide sequence comprising SEQ ID NO: 5 (human *htra1*) or SEQ ID NO: 11 (mouse *htra1*).

[0049] In another embodiment, the invention provides a PBC comprising a HTRA1/*htra1* activator. Suitable HTRA1/*htra1* activators are as described herein with respect to other aspects of the invention. The PBC may or may not further comprise a CIS/*cish* inhibitor. Suitable CIS/*cish* inhibitors are as described herein with respect to other aspects of the invention.

[0050] In an embodiment of the invention, a host cell comprising a HTRA1/*htra1* activator and/or a CIS/*cish* inhibitor may further comprise and express a nucleic acid comprising a nucleotide sequence encoding a T-cell receptor (TCR) having antigenic specificity for a cancer antigen or a chronic infectious disease antigen. The phrase "antigenic specificity" as used herein means that the TCR can specifically bind to, immunologically recognize, and/or mediate an immune response against a cancer antigen or a chronic infectious disease antigen. An immune response may be characterized by an increased production of cytokines such as, e.g., interferon gamma (IFNγ), the stimulation of a cell-mediated immune response such as, e.g., the activation of T-cells and/or macrophages, and/or the destruction of cells expressing the cancer antigen or chronic infectious disease antigen. Exemplary TCRs having antigenic specificity for a cancer antigen include those disclosed in U.S. Patent Application Publication Nos. 2009/0053184 (disclosing TCRs having antigenic specificity for MART-1, NY-ESO-1, or gp100); 2010/0015113 (disclosing TCRs having antigenic specificity for gp100); 2010/0034834 (disclosing TCRs having antigenic specificity for NY-ESO-1, MART-1, TRP-1, TRP-2, gp100, p53, or tyrosinase tumor antigen); and 2009/0304657 (disclosing TCRs having antigenic specificity for MART-1, NY-ESO-1, p53, or gp100); U.S. Patents 7,820,174 (disclosing TCRs having antigenic specificity for TRAIL

presented by TRAIL-R1) and 5,830,755 (disclosing TCRs having antigenic specificity for MART-1 or gp100); and International Publications WO 2009/042570 (disclosing TCRs having antigenic specificity for NY-ESO-1, MART-1, TRP-1, TRP-2, gp100, p53, or tyrosinase tumor antigen) and WO 2010/088160 (disclosing TCRs having antigenic specificity for tyrosinase tumor antigen), each of which are incorporated herein by reference.

[0051] In an embodiment, any of the HTRA1/*htra1* activators and/or CIS/*cish* inhibitors described herein can be loaded onto nanoparticles. The loaded nanoparticles can be combined with T cells (hereinafter referred to as "nanoparticle composition") and adoptively transferred into patients. In this regard, the invention provides a composition comprising nanoparticles and PBCs, wherein the nanoparticles comprise an HTRA1/*htra1* activator and/or a CIS/*cish* inhibitor. For example, an HTRA1/*htra1* activator can be loaded on nanoparticles and combined with T cells. After adoptive transfer of the T cells and loaded nanoparticles, the T cells would then traffic to the tumor, and the HTRA1/*htra1* activator would enhance T cell-mediated tumor killing. Preferably, the HTRA1/*htra1* activator is a HTRA1 protein. The HTRA1 protein may comprise any of the human HTRA1 amino acid sequences described herein. Preferably, the HTRA1 protein comprises SEQ ID NO: 7. The PBC may be any PBC. Preferably, the PBC is a T-cell.

[0052] A T cell comprising a HTRA1/*htra1* activator and/or a CIS/*cish* inhibitor advantageously demonstrates increased T cell activity. A composition comprising nanoparticles and PBCs, wherein the nanoparticles comprise an HTRA1/*htra1* activator and/or a CIS/*cish* inhibitor, also advantageously demonstrates increased T cell activity. T cell activity is increased in accordance with the invention if T cell activity is greater, quantitatively or qualitatively, after administration of a HTRA1/*htra1* activator and/or a CIS/*cish* inhibitor as compared to the immune response in the absence of the administration of a HTRA1/*htra1* activator and/or a CIS/*cish* inhibitor. A quantitative increase in T cell activity encompasses an increase in the magnitude or degree of the T cell activity. The magnitude or degree of T cell activity can be measured on the basis of any number of known parameters, such as an increase in the level of antigen-specific cytokine production (cytokine concentration), an increase in the number of T cells activated (e.g., proliferation of antigen-specific T cells) or recruited, an increase in the persistence and/or survival of T cells, and/or a decrease in T-cell death (e.g., apoptosis). Methods of detecting and measuring an increase in T cell activity are known in the art. For example, measuring the types and levels of cytokines produced can detect and measure an increase in T cell activity. An increase in T cell activity

may be characterized by an increase in the production of, e.g., IFNγ, TNF-α, GM-CSF, and/or interleukin (IL)-2, an increase in the production of Th1 immunoglobulins, an increase in tumor destruction *in vivo*, a decrease in TGFβ1, and/or an increase in the differentiation of Th1 cells. A qualitative increase in T cell activity encompasses any change in the nature of the T cell activity that renders the T cell more effective at combating a given antigen or disease. Qualitative and quantitative increases in T cell activity can occur simultaneously, and are not mutually exclusive.

[0053] The inhibition of CIS/*cish* also increases the expression of signal transducer and activator of transcription 5 (STAT5) target genes such as, for example, v-myc, myelocytomatosis viral oncogene homolog (avian) (cMYC), Bcl-2-associated death promoter (*bcl2l1* (Bcl-xL)), granzyme B, T-cell-specific T-box transcription factor (Tbet)/*tbx21* and cell cycle genes (e.g., *ccnd2* (cyclin D2), *ccne1* (cyclin E1), and/or *cdk1* (cyclin CDC2a)), but does not increase STAT5 phosphorlyation and does not alter the expression of T cell receptor (TCR) signaling genes such as, for example, tumor necrosis factor receptor superfamily, member 9 (4-1BB) and programmed cell death 1 (PD1).

[0054] The inhibition of CIS/*cish* decreases TGF-β protein levels, decreases TGF-β signaling (e.g., decreases activation of the TGF-β signaling molecule, SMAD2), and decreases the expression of TGF-β target genes such as, for example, cyclin-dependent kinase inhibitor 2B (Cdkn2b) (believed to inhibit the cell cycle), deleted in esophageal cancer 1 (DEC1) (believed to inhibit cell function), inhibitor of DNA binding 1 (ID1) (believed to induce apoptosis), Stra13, and CD103. Without being bound to a particular theory, it is believed that CIS interacts with HTRA1, a protein believed to degrade TGF-β family members. It is believed that the improved functionality of T cells or nanoparticle compositions comprising a CIS/*cish* inhibitor may be the result of decreased degradation of HTRA1.

[0055] In still another embodiment, the invention provides a host cell comprising a HTRA1/*htra1* inhibitor and a CIS/*cish* activator.

[0056] The HTRA1/*htra1* inhibitor may be any suitable agent that inhibits the expression of a *htra1* mRNA or protein. The HTRA1/*htra1* inhibitor can be a nucleic acid that specifically binds to and is complementary to a target nucleic acid encoding HTRA1/*htra1* or a complement thereof. The HTRA1/*htra1* inhibitor may be introduced into a host cell, wherein the cell is capable of expressing HTRA1, in an effective amount for a time and under conditions sufficient to interfere with expression of HTRA1. In some embodiments, RNA

interference (RNAi) is employed. In this regard, the HTRA1/*htra1* inhibitor may comprise an RNAi agent.

[0057] Any type of suitable siRNA, miRNA, and/or antisense nucleic acid can be employed. In an embodiment, the antisense nucleic acid comprises a nucleotide sequence complementary to at least about 8, at least about 15, at least about 19, or from about 19 to about 22 nucleotides of a nucleic acid encoding HTRA1 or a complement thereof. In an embodiment, the siRNA may comprise, e.g., trans-acting siRNAs (tasiRNAs) and/or repeat-associated siRNAs (rasiRNAs). In another embodiment, the miRNA may comprise, e.g., a short hairpin miRNA (shMIR).

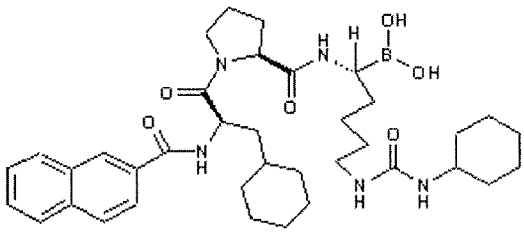
[0058] The HTRA1/*htra1* inhibitor may inhibit or downregulate to some degree the expression of the protein encoded by a *htra1* gene, e.g., at the DNA, RNA, or other level of regulation. In this regard, a host cell comprising a HTRA1/*htra1* inhibitor expresses no HTRA1/*htra1* or lower levels of HTRA1/*htra1* as compared to a host cell that lacks a HTRA1/*htra1* inhibitor. In accordance with the invention, the HTRA1/*htra1* inhibitor, such as an RNAi agent, such as a shMIR, can target a nucleotide sequence of a *htra1* gene or mRNA encoded by the same. In an embodiment, the *htra1* sequence is a human sequence. For example, human *htra1* is assigned Gene NCBI Entrez Gene ID No. 5654, and an Online Mendelian Inheritance in Man (OMIM) No. 602194. The human *htra1* gene is found on chromosome 10 at 10q26.3. The *htra1* mRNA sequence is NM_002775, with corresponding protein sequence NP_002766. Accordingly, NM_002775 is provided as SEQ ID NO: 5. Human genomic *htra1* sequences include AF157623, BX842242, and CH471066. Human *htra1* mRNA sequences include AF070555, AF097709, AI423369, AK091944, AK092476, AK290089, BC011352, BC031082, BM993893, CR590731, CR593682, CR596858, CR597732, CR603877, CR605013, CR611805, CR613636, CR623030, CR624458, CR624921, D87258, DA633502, and Y07921. Mouse *htra1* mRNA sequences include NM_019564. Human HTRA1 amino acid sequences include AAD41525, EAW49312, EAW49313, AAC97211, BAG52446, BAG52557, BAF82778, and AAH11352. Other human sequences, as well as other HTRA1/*htra1* species can be employed in accordance with the invention.

[0059] In accordance with an embodiment of the invention, the HTRA1/*htra1* inhibitor, such as an RNAi agent, such as a shMIR, can target a nucleotide sequence selected from the group consisting of 5' untranslated region (5' UTR), the 3' untranslated region (3' UTR), and the coding sequence of *htra1*, complements thereof, and any combination thereof. Any

suitable *htra1* target sequence can be employed. The sequences of the HTRA1/*htra1* inhibitor can be designed against a human *htra1* with Accession No. NM_002775 (SEQ ID NO: 5). In an embodiment, the shMIR comprises SEQ ID NO: 6, designed against the *htra1* gene target GCTATCATCAATTATGGAA (SEQ ID NO: 15). RNAi agents can be designed against any appropriate *htra1* mRNA sequence.

[0060] Other aspects of the HTRA1/*htra1* inhibitor comprising an RNAi agent are as described for the CIS/*cish* inhibitor.

[0061] In an embodiment of the invention, the HTRA1/*htra1* inhibitor comprises NVP-LBP976. NVP-LBP976 has the chemical structure:



[0062] The CIS/*cish* activator may comprise any suitable agent that provides the over-expression of a *cish* mRNA or protein. A host cell comprising a CIS/*cish* activator expresses higher levels of CIS/*cish* than host cells that lack a CIS/*cish* activator. The CIS/*cish* activator may be introduced into a host cell, wherein the cell is capable of expressing CIS/*cish*, in an effective amount for a time and under conditions sufficient to cause the over-expression of CIS/*cish*. In this regard, the CIS/*cish* activator may comprise a recombinant nucleic acid that encodes CIS/*cish*. In an embodiment of the invention, the recombinant polynucleotide that encodes CIS/*cish* may comprise a nucleotide sequence comprising SEQ ID NO: 1 or 2.

[0063] In another embodiment, the invention provides a PBC comprising a HTRA1/*htra1* inhibitor. Suitable HTRA1/*htra1* inhibitors are as described herein with respect to other aspects of the invention. The PBC may or may not further comprise a CIS/*cish* activator. Suitable CIS/*cish* activators are as described herein with respect to other aspects of the invention.

[0064] In yet another embodiment, the invention provides a T cell comprising a CIS/*cish* activator, wherein the T cell has suppressed T cell activity. Suitable CIS/*cish* activators are as described herein with respect to other aspects of the invention. The T cell may or may not

further comprise a HTRA1/*htra1* inhibitor. Suitable HTRA1/*htra1* inhibitors are as described herein with respect to other aspects of the invention.

[0065] In an embodiment, any of the HTRA1/*htra1* inhibitors and/or CIS/*cish* activators described herein can be loaded onto nanoparticles. The loaded nanoparticles can be combined with T cells (hereinafter referred to as "nanoparticle composition") and adoptively transferred into patients. In this regard, the invention provides a composition comprising nanoparticles and PBCs, wherein the nanoparticles comprise an HTRA1/*htra1* inhibitor and/or a CIS/*cish* activator. Preferably, the CIS/*cish* activator is a CIS protein. The CIS protein may comprise any of the human CIS amino acid sequences described herein. The CIS protein may comprise SEQ ID NO: 8. The PBC may be any PBC. Preferably, the PBC is a T-cell.

[0066] A nanoparticle composition or T cell comprising a HTRA1/*htra1* inhibitor and/or a CIS/*cish* activator advantageously demonstrates suppressed T cell activity. T cell activity is suppressed in accordance with the invention if the T cell activity is diminished, quantitatively or qualitatively, after administration of a HTRA1/*htra1* inhibitor and/or a CIS/*cish* activator as compared to the immune response in the absence of the administration of a HTRA1/*htra1* inhibitor and/or a CIS/*cish* activator. A quantitative decrease in T cell activity encompasses a decrease in the magnitude or degree of the activity. The magnitude or degree of T cell activity can be measured on the basis of any number of known parameters, such as a decrease in the level of cytokine (e.g., antigen-specific cytokine) production (cytokine concentration), a decrease in the number of T cells activated (e.g., proliferation of T cells (e.g., antigen-specific T-cells)) or recruited, etc., an decrease in the persistence and/or survival of T cells, and/or an increase in T -cell death (e.g., apoptosis). Methods of detecting and measuring suppression of T cell activity are known in the art. For example, measuring the types and levels of cytokines produced can detect and measure suppression of T cell activity. Suppression of T cell activity may be characterized by a decrease in the production of, e.g., IFNγ, TNF-α, GM-CSF, and/or IL-2; a decrease in the production of Th1 immunoglobulins, a decrease in tumor destruction *in vivo*, an increase in TGFβ1, and/or a decrease in the differentiation of Th1 cells. A qualitative decrease in T cell activity encompasses any change in the nature of the T cell activity that renders it less effective at mediating the destruction of a given antigen. Qualitative and quantitative suppression of T cell activity can occur simultaneously, and are not mutually exclusive.

[0067] Without being bound to a particular theory, it is believed that the inhibition of HTRA1 increases the expression of TGF-β target genes such as, for example, plasminogen activator inhibitor-1 (PAI-1); increases TGF-β levels; and decreases the expression of STAT5 target genes such as, for example, cMYC, *bcl2l1* (Bcl-xL), granzyme B, *tbx21* (Tbet), and cell cycle genes (e.g., *ccnd2* (cyclin D2), *ccne1* (cyclin E1), and/or *cdk1* (cyclin CDC2a)).

[0068] By "nucleic acid" as used herein includes "polynucleotide," "oligonucleotide," and "nucleic acid molecule," and generally means a polymer of DNA or RNA, which can be single-stranded or double-stranded, synthesized or obtained (e.g., isolated and/or purified) from natural sources, which can contain natural, non-natural or altered nucleotides (e.g., ribonucleic acid nucleotides as well as deoxyribonucleic acid nucleotides), and which can contain a natural, non-natural or altered internucleotide linkage, such as a phosphoroamidate linkage or a phosphorothioate linkage, instead of the phosphodiester found between the nucleotides of an unmodified oligonucleotide. When a nucleic acid is recited it refers generically to nucleic acids and includes DNA and RNA unless the recitation explicitly states that the nucleic acid is a specific one, e.g., DNA or RNA. If a nucleic acid refers to a sequence that contains thymine (t), that does not necessarily indicate that the nucleic acid is DNA; in some embodiments the nucleic acid is RNA and/or DNA. Similarly, if a nucleic acid refers to a sequence that contains uracil (u) that does not necessarily indicate that the nucleic acid is RNA; in some embodiments the nucleic acid is DNA and/or RNA. It is generally preferred that the nucleic acid does not comprise any insertions, deletions, inversions, and/or substitutions. However, it may be suitable in some instances, as discussed herein, for the nucleic acid to comprise one or more insertions, deletions, inversions, and/or substitutions.

[0069] The nucleic acid molecules relevant to the invention can readily be obtained in a variety of ways, including, without limitation, chemical synthesis, cDNA or genomic library screening, expression library screening, and/or PCR amplification of cDNA. These methods and others useful for isolating such DNA are set forth, for example, by Sambrook et al., "Molecular Cloning: A Laboratory Manual," Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989), by Ausubel, et al., eds., "Current Protocols In Molecular Biology," Current Protocols Press (1994), and by Berger and Kimmel, "Methods In Enzymology: Guide To Molecular Cloning Techniques," vol. 152, Academic Press, Inc., San Diego, Calif. (1987).

[0070] Chemical synthesis of a nucleic acid molecule can be accomplished using methods well known in the art, such as those set forth by Engels et al., Angew. Chem. Intl. Ed., 28:716-734 (1989). These methods include, *inter alia*, the phosphotriester, phosphoramidite and H-phosphonate methods of nucleic acid synthesis. Nucleic acids larger than about 100 nucleotides in length can be synthesized as several fragments, each fragment being up to about 100 nucleotides in length. The fragments can then be ligated together to form a full length nucleic acid encoding the polypeptide. One method is polymer-supported synthesis using standard phosphoramidite chemistry.

[0071] Alternatively, the nucleic acid can be obtained by screening an appropriate cDNA library prepared from one or more tissue source(s) that express the polypeptide, or a genomic library from any subspecies. The source of the genomic library may be any tissue or tissues from a mammalian or other species believed to harbor a gene encoding a protein relevant to the invention (e.g., CIS and/or HTRA1). The library can be screened for the presence of a cDNA/gene using one or more nucleic acid probes (oligonucleotides, cDNA or genomic DNA fragments that possess an acceptable level of homology to the gene or gene homologue cDNA or gene to be cloned) that will hybridize selectively with the gene or gene homologue cDNA(s) or gene(s) that is(are) present in the library. The probes preferably are complementary to or encode a small region of the DNA sequence from the same or a similar species as the species from which the library was prepared. Alternatively, the probes can be degenerate. After hybridization, the blot containing the library is washed at a suitable stringency, depending on several factors such as probe size, expected homology of probe to clone, type of library being screened, number of clones being screened, and the like. Stringent washing solutions can be low in ionic strength and are used at relatively high temperatures.

[0072] Another suitable method for obtaining a nucleic acid for use in accordance with the invention is the polymerase chain reaction (PCR). In this method, poly(A)+RNA or total RNA is extracted from a tissue that expresses the gene product. cDNA is then prepared from the RNA using the enzyme reverse transcriptase. Two primers typically complementary to two separate regions of the cDNA (oligonucleotides) are then added to the cDNA along with a polymerase such as Taq polymerase, and the polymerase amplifies the cDNA region between the two primers.

[0073] The invention provides for the use of isolated, purified or enriched nucleic acid sequences of, for example, about 15 to about 500 nucleotides in length, about 15 to about 100

nucleotides in length, about 15 to about 50 nucleotides in length, and about 15 to about 30 nucleotides in length, which have sequence that corresponds to a portion of one of the nucleotides described herein. The nucleic acid can be at least about 17, about 20, about 22, or about 25 nucleotides in length. The nucleic acid sequence can be about 30 to about 300 nucleotides in length, or about 45 to about 200 nucleotides in length, or about 45 to about 100 nucleotides in length. The nucleic acid can be at least about 5, about 6, about 7, about 8, about 9, about 10, about 12, about 15, about 17, about 20, about 22, about 25, about 30, about 35, about 40, about 50, about 100, about 150, about 200, about 250, about 300, about 350, about 400, about 450, about 500, about 1000, about 10,000, about 50,000, about 100,000 or more nucleotides in length, or about 100,000, about 75,000, about 50,000, about 10,000, about 5,000, about 1000, about 750, about 500, about 250, about 200, about 100, about 50, about 40, about 30, about 25, about 22, about 20, about 17, about 15, about 12, about 10, about 9, about 8, about 7, about 6, about 5, or fewer nucleotides in length. The nucleic acid can have a length in a range from any one of the above lengths to any other of the above lengths including endpoints.

[0074] A nucleic acid or protein in accordance with the invention can be at least, e.g., 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 82%, 84%, 86%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and 100% identical to reference sequences provided herein. A nucleotide that hybridizes under stringent conditions to a nucleotide described herein can be employed. Unless otherwise specified, percent identities for nucleic acids and amino acid sequences are determined as follows. Percent identity of two nucleic acid sequences or two amino acid sequences is determined using the algorithm of Karlin and Altschul (*Proc. Natl. Acad. Sci. USA*, 87:2264-2268 (2002), modified as in Karlin and Altschul et al., *Proc. Nat. Acad. Sci. USA*, 90:5873-5877 (1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al., *J. Mol. Biol.* 215:403-410 (1990). BLAST nucleotide searches are performed with the NBLAST program, score=100, wordlength=1, to obtain nucleotide sequences with a percent identity to a nucleic acid employed in the invention. BLAST protein searches are performed with the XBLAST program, score=50, wordlength=3, to obtain amino acid sequences with a percent identity to a reference polypeptide. To obtain gapped alignments for comparison purposes, Gapped BLAST is utilized as described in Altschul et al., *Nucleic Acids Res.*, 25:3389-3402 (1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) are used. See <www.ncbi.nih.gov>.

[0075] In connection with nucleic acid hybridization, the term "specifically hybridizes" indicates that the probe hybridizes to a sufficiently greater degree to the target sequence than to a non-target sequence, e.g., at a level which allows ready identification of probe/target sequence hybridization under selective hybridization conditions. "Selective hybridization conditions" refer to conditions that allow such differential binding. Similarly, the terms "specifically binds" and "selective binding conditions" refer to such differential binding of any type of probe, and to the conditions that allow such differential binding.

[0076] The nucleotide sequence encoding CIS and/or HTRA1 encodes any suitable CIS and/or HTRA1, respectively, including functional portions or functional variants thereof. The term "functional portion" refers to any part or fragment of the CIS or HTRA1, which part or fragment retains the biological activity of the CIS or HTRA1, respectively, of which it is a part (the parent CIS and/or HTRA1, respectively). In reference to the parent CIS or HTRA1, the functional portion can comprise, for instance, about 10%, 25%, 30%, 50%, 68%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or more, of the parent CIS or HTRA1, respectively. The term "functional variant" as used herein refers to CIS or HTRA1 having substantial or significant sequence identity or similarity to a parent CIS or HTRA1, respectively, which functional variant retains the biological activity of the CIS and/or HTRA1, respectively, of which it is a variant. In reference to the parent CIS or HTRA1, the functional variant can, for instance, be at least about 30%, 50%, 75%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, or more identical in amino acid sequence to the parent CIS or HTRA1, respectively. Functional portions and functional variants of HTRA1 encompass, for example, those parts and variants, respectively, of HTRA1 that retain the ability to induce the production of effector cytokines (e.g., IFN-γ, TNF-α, and/or GM-CSF), enhance the production of Th1 immunoglobulins, increase tumor destruction *in vivo*, decrease TGFβ1 levels, induce the differentiation of Th1 cells, or treat or prevent cancer and/or a chronic infectious disease, to a similar extent, the same extent, or to a higher extent, as the parent HTRA1. Functional portions and functional variants of CIS encompass, for example, those parts and variants, respectively, of CIS that retain the ability to reduce the production of effector cytokines (e.g., IFN-γ, TNF-α, and/or GM-CSF), reduce the production of Th1 immunoglobulins, decrease tumor destruction *in vivo*, increase TGFβ1 levels, reduce the differentiation of Th1 cells, or treat or prevent auto and/or allo-immune diseases, to a similar extent, the same extent, or to a higher extent, as the parent CIS.

[0077] Preferably, the nucleic acids described herein are recombinant. As used herein, the term "recombinant" refers to (i) molecules that are constructed outside living cells by joining natural or synthetic nucleic acid segments to nucleic acid molecules that can replicate in a living cell, or (ii) molecules that result from the replication of those described in (i) above. For purposes herein, the replication can be *in vitro* replication or *in vivo* replication.

[0078] The nucleic acids of the invention can be incorporated into a recombinant expression vector. For purposes herein, the term "recombinant expression vector" means a genetically-modified oligonucleotide or polynucleotide construct that permits the expression of an mRNA, protein, polypeptide, or peptide by a host cell, when the construct comprises a nucleotide sequence encoding the mRNA, protein, polypeptide, or peptide, and the vector is contacted with the cell under conditions sufficient to have the mRNA, protein, polypeptide, or peptide expressed within the cell. The vectors of the invention are not naturally-occurring as a whole. However, parts of the vectors can be naturally-occurring. The recombinant expression vectors can comprise any type of nucleotides, including, but not limited to DNA and RNA, which can be single-stranded or double-stranded, synthesized or obtained in part from natural sources, and which can contain natural, non-natural or altered nucleotides. The recombinant expression vectors can comprise naturally-occurring, non-naturally-occurring internucleotide linkages, or both types of linkages. Preferably, the non-naturally occurring or altered nucleotides or internucleotide linkages do not hinder the transcription or replication of the vector.

[0079] The recombinant expression vector of the invention can be any suitable recombinant expression vector, and can be used to transform or transduce any suitable host cell. Suitable vectors include those designed for propagation and expansion or for expression or both, such as plasmids and viruses. The vector can be selected from the group consisting of the pUC series (Fermentas Life Sciences), the pBluescript series (Stratagene, LaJolla, CA), the pET series (Novagen, Madison, WI), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), and the pEX series (Clontech, Palo Alto, CA). Bacteriophage vectors, such as λGT10, λGT11, λZapII (Stratagene), λEMBL4, and λNM1149, also can be used. Examples of plant expression vectors include pBI01, pBI101.2, pBI101.3, pBI121 and pBIN19 (Clontech). Examples of animal expression vectors include pEUK-Cl, pMAM and pMAMneo (Clontech). Preferably, the recombinant expression vector is a viral vector (e.g., adenoviral vector, adeno-associated viral (AAV) vector, herpes viral vector, retroviral vector, or lentiviral vector) or a transposon vector.

[0080] The recombinant expression vectors described herein can be prepared using standard recombinant DNA techniques described in, for example, Sambrook et al., *supra*, and Ausubel et al., *supra*. Constructs of expression vectors, which are circular or linear, can be prepared to contain a replication system functional in a prokaryotic or eukaryotic host cell. Replication systems can be derived, e.g., from ColEl, 2 μ plasmid, λ, SV40, bovine papilloma virus, and the like.

[0081] Desirably, the recombinant expression vector comprises regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type of host cell (e.g., bacterium, fungus, plant, or animal) into which the vector is to be introduced, as appropriate and taking into consideration whether the vector is DNA- or RNA-based.

[0082] The recombinant expression vector can include one or more marker genes, which allow for selection of transformed or transduced host cells. Marker genes include biocide resistance, e.g., resistance to antibiotics, heavy metals, etc., complementation in an auxotrophic host cell to provide prototrophy, and the like. Suitable marker genes for the expression vectors include, for instance, neomycin/G418 resistance genes, hygromycin resistance genes, histidinol resistance genes, tetracycline resistance genes, and ampicillin resistance genes.

[0083] As used herein, the term "host cell" refers to any type of cell that can contain the recombinant expression vector(s) described herein. The host cell can be a eukaryotic cell, e.g., plant, animal, fungi, or algae, or can be a prokaryotic cell, e.g., bacteria or protozoa. The host cell can be a cultured cell or a primary cell, i.e., isolated directly from an organism, e.g., a human. The host cell can be an adherent cell or a suspended cell, i.e., a cell that grows in suspension. Suitable host cells are known in the art and include, for instance, DH5α E. coli cells, Chinese hamster ovarian cells, monkey VERO cells, COS cells, HEK293 cells, and the like. For purposes of amplifying or replicating the recombinant expression vector, the host cell is preferably a prokaryotic cell, e.g., a DH5α cell. For purposes of producing cells for adoptive cell therapy, the host cell is preferably a mammalian cell. Most preferably, the host cell is a human cell. While the host cell can be of any cell type, can originate from any type of tissue, and can be of any developmental stage, the host cell preferably is a peripheral blood cell (PBC), peripheral blood leukocyte (PBL), or a peripheral blood mononuclear cell (PBMC). More preferably, the host cell is a T cell.

[0084] For purposes herein, the T cell can be any T cell, such as a cultured T cell, e.g., a primary T cell, or a T cell from a cultured T cell line, e.g., Jurkat, SupT1, etc., or a T cell obtained from a mammal. If obtained from a mammal, the T cell can be obtained from numerous sources, including but not limited to blood, bone marrow, lymph node, the thymus, or other tissues or fluids. T cells can also be enriched for or purified. Preferably, the T cell is a human T cell. More preferably, the T cell is a T cell isolated from a human. The T cell can be any type of T cell and can be of any developmental stage, including but not limited to, $CD4^+/CD8^+$ double positive T cells, $CD4^+$ helper T cells, e.g., Th_1 and Th_2 cells, $CD8^+$ T cells (e.g., cytotoxic T cells), tumor infiltrating cells (TILs), memory T cells, naïve T cells, and the like. Preferably, the T cell is a CD8+ T cell or a CD4+ T cell.

[0085] The host cells can be transduced with the nucleic acids using any suitable method. For example, host cells can be transduced with viral vectors using viruses (e.g., retrovirus or lentivirus) and host cells can be transduced with transposon vectors using electroporation.

[0086] Also provided by an embodiment of the invention is a population of cells comprising at least one host cell (e.g., a PBC or a T cell) described herein. The population of cells can be a heterogeneous population comprising the host cell comprising any of the recombinant expression vectors described herein, in addition to at least one other cell, e.g., a host cell (e.g., a T cell), which does not comprise any of the recombinant expression vectors, or a cell other than a T cell, e.g., a B cell, a macrophage, a neutrophil, an erythrocyte, a hepatocyte, an endothelial cell, an epithelial cells, a muscle cell, a brain cell, etc. Alternatively, the population of cells can be a substantially homogeneous population, in which the population comprises mainly of host cells (e.g., consisting essentially of) comprising the recombinant expression vector. The population also can be a clonal population of cells, in which all cells of the population are clones of a single host cell comprising a recombinant expression vector, such that all cells of the population comprise the recombinant expression vector. In one embodiment of the invention, the population of cells is a clonal population comprising host cells comprising a recombinant expression vector as described herein.

[0087] The inventive host cells (including populations thereof) can be isolated and/or purified. The term "isolated" as used herein means having been removed from its natural environment. The term "purified" as used herein means having been increased in purity, wherein "purity" is a relative term, and not to be necessarily construed as absolute purity. For

example, the purity can be at least about 50%, can be greater than 60%, 70% or 80%, or can be 100%.

[0088] The inventive host cells (including populations thereof) and nanoparticle compositions can be formulated into a composition, such as a pharmaceutical composition. In this regard, an embodiment of the invention provides a pharmaceutical composition comprising any of the host cells (including populations thereof) and/or nanoparticle compositions described herein, and a pharmaceutically acceptable carrier. The pharmaceutical composition can comprise an inventive host cell or population thereof and/or nanoparticle composition in combination with other pharmaceutically active agents or drugs, such as a chemotherapeutic agents, e.g., asparaginase, busulfan, carboplatin, cisplatin, daunorubicin, doxorubicin, fluorouracil, gemcitabine, hydroxyurea, methotrexate, paclitaxel, rituximab, vinblastine, vincristine, etc.

[0089] Preferably, the carrier is a pharmaceutically acceptable carrier. With respect to pharmaceutical compositions, the carrier can be any of those conventionally used and is limited only by chemico-physical considerations, such as solubility and lack of reactivity with the active compound(s), and by the route of administration. The pharmaceutically acceptable carriers described herein, for example, vehicles, adjuvants, excipients, and diluents, are well-known to those skilled in the art and are readily available to the public. It is preferred that the pharmaceutically acceptable carrier be one which is chemically inert to the active agent(s) and one which has no detrimental side effects or toxicity under the conditions of use.

[0090] The choice of carrier will be determined in part by the particular host cell, population thereof, or nanoparticle composition, as well as by the particular method used to administer the inventive host cell, population thereof, or nanoparticle composition. Accordingly, there are a variety of suitable formulations of the pharmaceutical composition of the invention. The following formulations for parenteral, intratumoral, subcutaneous, intravenous, intramuscular, intraarterial, intrathecal, and interperitoneal administration are exemplary and are in no way limiting. More than one route can be used to administer the inventive host cell, population thereof, or nanoparticle composition, and in certain instances, a particular route can provide a more immediate and more effective response than another route.

[0091] Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain anti-oxidants, buffers,

bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. The inventive host cell, population thereof, or nanoparticle composition can be administered in a physiologically acceptable diluent in a pharmaceutical carrier, such as a sterile liquid or mixture of liquids, including water, saline, aqueous dextrose and related sugar solutions, an alcohol, such as ethanol or hexadecyl alcohol, a glycol, such as propylene glycol or polyethylene glycol, dimethylsulfoxide, glycerol, ketals such as 2,2-dimethyl-1,3-dioxolane-4-methanol, ethers, poly(ethyleneglycol) 400, oils, fatty acids, fatty acid esters or glycerides, or acetylated fatty acid glycerides with or without the addition of a pharmaceutically acceptable surfactant, such as a soap or a detergent, suspending agent, such as pectin, carbomers, methylcellulose, hydroxypropylmethylcellulose, or carboxymethylcellulose, or emulsifying agents and other pharmaceutical adjuvants.

[0092] Oils, which can be used in parenteral formulations include petroleum, animal, vegetable, or synthetic oils. Specific examples of oils include peanut, soybean, sesame, cottonseed, corn, olive, petrolatum, and mineral. Suitable fatty acids for use in parenteral formulations include oleic acid, stearic acid, and isostearic acid. Ethyl oleate and isopropyl myristate are examples of suitable fatty acid esters.

[0093] Suitable soaps for use in parenteral formulations include fatty alkali metal, ammonium, and triethanolamine salts, and suitable detergents include (a) cationic detergents such as, for example, dimethyl dialkyl ammonium halides, and alkyl pyridinium halides, (b) anionic detergents such as, for example, alkyl, aryl, and olefin sulfonates, alkyl, olefin, ether, and monoglyceride sulfates, and sulfosuccinates, (c) nonionic detergents such as, for example, fatty amine oxides, fatty acid alkanolamides, and polyoxyethylenepolypropylene copolymers, (d) amphoteric detergents such as, for example, alkyl-β-aminopropionates, and 2-alkyl-imidazoline quaternary ammonium salts, and (e) mixtures thereof.

[0094] The parenteral formulations will typically contain from about 0.05% to about 50% by weight of the host cell, population thereof, or nanoparticle composition, in solution. Preservatives and buffers may be used. In order to minimize or eliminate irritation at the site of injection, such compositions may contain one or more nonionic surfactants having a hydrophile-lipophile balance (HLB) of from about 12 to about 17. The quantity of surfactant in such formulations will typically range from about 5% to about 15% by weight. Suitable surfactants include polyethylene glycol sorbitan fatty acid esters, such as sorbitan monooleate

and the high molecular weight adducts of ethylene oxide with a hydrophobic base, formed by the condensation of propylene oxide with propylene glycol. The parenteral formulations can be presented in unit-dose or multi-dose sealed containers, such as ampoules and vials, and can be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid excipient, for example, water, for injections, immediately prior to use. Extemporaneous injection solutions and suspensions can be prepared from sterile powders, granules, and tablets.

[0095] Injectable formulations are in accordance with the invention. The requirements for effective pharmaceutical carriers for injectable compositions are well-known to those of ordinary skill in the art (see, e.g., Pharmaceutics and Pharmacy Practice, J.B. Lippincott Company, Philadelphia, PA, Banker and Chalmers, eds., pages 238-250 (1982), and ASHP Handbook on Injectable Drugs, Toissel, 4th ed., pages 622-630 (1986)). Preferably, when administering cells, e.g., T cells, the cells are administered via injection.

[0096] It will be appreciated by one of skill in the art that, in addition to the above-described pharmaceutical compositions, the inventive host cell or population thereof can be formulated as inclusion complexes, such as cyclodextrin inclusion complexes, nanoparticles, or liposomes.

[0097] Many assays for determining an administered dose are known in the art. For purposes of the invention, an assay, which comprises comparing the extent to which target cells are lysed by or IFN-γ secretion is increased or decreased in T cells comprising the nucleic acid or recombinant expression vectors described herein upon administration of a given dose of such T cells to a mammal among a set of mammals of which each is given a different dose of the T cells, could be used to determine a starting dose to be administered to a mammal. The extent to which IFN-γ is secreted upon administration of a certain dose can be assayed by methods known in the art, including, for instance, the methods described herein in the Examples (e.g., ELISA).

[0098] The dose of the inventive host cell, populations thereof, or nanoparticle composition also will be determined by the existence, nature and extent of any adverse side effects that might accompany the administration of a particular inventive host cell, populations thereof, or nanoparticle composition. Typically, the attending physician will decide the dosage of the inventive host cell, populations thereof, or nanoparticle composition, with which to treat each individual patient, taking into consideration a variety of factors, such as age, body weight, general health, diet, sex, inventive host cell, populations thereof, or

nanoparticle composition to be administered, route of administration, and the severity of the condition being treated. By way of example and not intending to limit the invention, the dose of the inventive host cell, populations thereof, or nanoparticle composition can be about 0.001 to about 1000 mg/kg body weight of the subject being treated/day, from about 0.01 to about 10 mg/kg body weight/day, about 0.01 mg to about 1 mg/kg body weight/day.

[0099] It is contemplated that the inventive host cell, population of cells, or nanoparticle composition, comprising a CIS/*cish* inhibitor, HTRA1/*htra1* activator, and/or pharmaceutical compositions comprising the same, can be used in methods of increasing T cell activity in a mammal or methods of treating or preventing cancer or a chronic infectious disease in a mammal. Without being bound to a particular theory, it is believed that nanoparticle compositions or cells comprising a CIS/*cish* inhibitor and/or a HTRA1/*htra1* activator advantageously increase the production of effector cytokines (e.g., IFN-γ, IL-2); increase T cell proliferation, persistence, survival, and cytolytic activity; decrease T cell apoptosis; and/or decrease tumor size *in vivo* as compared to nanoparticle compositions or cells that lack any one or more of a CIS/*cish* inhibitor and/or a HTRA1/*htra1* activator. In this regard, an embodiment of the invention provides a method of treating or preventing cancer or a chronic infectious disease in a mammal, comprising administering to the mammal a host cell, population of cells, or nanoparticle composition, comprising a CIS/*cish* inhibitor, a HTRA1/*htra1* activator, and/or pharmaceutical compositions comprising the same, in an amount effective to treat or prevent cancer or the infectious disease in the mammal.

[0100] Another embodiment of the invention comprises a method of increasing T cell activity in a mammal, comprising administering to the mammal a host cell, population of cells, or nanoparticle composition, comprising a CIS/*cish* inhibitor, a HTRA1/*htra1* activator, and/or pharmaceutical compositions comprising the same, in an amount effective to increase T cell activity in the mammal.

[0101] It is also contemplated that the inventive host cell, population of cells, or nanoparticle composition, comprising a CIS/*cish* activator, a HTRA1/*htra1* inhibitor, and/or pharmaceutical compositions comprising the same, can be used in methods of suppressing T cell activity in a mammal or methods of treating or preventing an auto- and/or allo-immune disease in a mammal. Without being bound to a particular theory, it is believed that cells or nanoparticle compositions comprising a CIS/*cish* activator and/or a HTRA1/*htra1* inhibitor advantageously decrease the production of effector cytokines (e.g., IFN-γ, IL-2); decrease T cell proliferation, persistence, survival, and cytolytic activity; and/or increase T cell apoptosis

as compared to nanoparticle compositions or cells that lack any one or more of a CIS/*cish* activator and/or a HTRA1/*htra1* inhibitor. In this regard, an embodiment of the invention provides a method of treating or preventing an auto- and/or allo-immune disease in a mammal comprising administering to the mammal a host cell, population of cells, or nanoparticle composition, comprising any one or more of a CIS/*cish* activator, a HTRA1/*htra1* inhibitor, and/or pharmaceutical compositions comprising the same, in an amount effective to treat or prevent an auto- and/or allo-immune disease in the mammal.

[0102] Another embodiment of the invention comprises a method of suppressing T cell activity in a mammal, comprising administering to the mammal a host cell, population of cells, or nanoparticle composition comprising a CIS/*cish* activator, a HTRA1/*htra1* inhibitor, and/or pharmaceutical compositions comprising the same, in an amount effective to suppress T cell activity in the mammal.

[0103] Another embodiment of the invention comprises a method of treating or preventing a disease selected from the group consisting of age-related macular degeneration, Alzheimer's disease, and familial ischemic cerebral small-vessel disease in a mammal, comprising administering to the mammal a PBC, a host cell, population of cells, or nanoparticle composition, comprising a CIS/*cish* inhibitor, a HTRA1/*htra1* activator, a CIS/*cish* activator, a HTRA1/*htra1* inhibitor, and/or pharmaceutical compositions comprising the same, in an amount effective to treat or prevent the disease in the mammal. Preferably, the method comprises treating or preventing Alzheimer's disease or familial ischemic cerebral small-vessel disease comprising administering to the mammal a PBC, a host cell, population of cells, or nanoparticle composition, comprising a CIS/*cish* inhibitor or a HTRA1/*htra1* activator, and/or pharmaceutical compositions comprising the same, in an amount effective to treat or prevent the disease in the mammal. Preferably, the method comprises treating or preventing age-related macular degeneration comprising administering to the mammal a PBC, a host cell, population of cells, or nanoparticle composition, comprising a CIS/*cish* activator or a HTRA1/*htra1* inhibitor, and/or pharmaceutical compositions comprising the same, in an amount effective to treat or prevent the disease in the mammal.

[0104] The terms "treat," and "prevent" as well as words stemming therefrom, as used herein, do not necessarily imply 100% or complete treatment or prevention. Rather, there are varying degrees of treatment or prevention of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect, which could include 100% or complete

treatment or prevention. In this respect, the inventive methods can provide any amount of any level of treatment or prevention of cancer, a chronic infectious disease, an allo-immune disease, and/or an autoimmune disease in a mammal. Furthermore, the treatment or prevention provided by the inventive method can include treatment or prevention of one or more conditions or symptoms of the disease, e.g., cancer, a chronic infectious disease, an allo-immune disease, and/or an autoimmune disease, being treated or prevented. Also, for purposes herein, "prevention" can encompass delaying the onset of the disease, or a symptom or condition thereof.

[0105] For purposes of the inventive methods, wherein host cells or populations of cells are administered, the cells can be cells that are allogeneic or autologous to the mammal. Preferably, the cells are autologous to the mammal.

[0106] With respect to the inventive methods, the cancer can be any cancer, including any of acute lymphocytic cancer, acute myeloid leukemia, alveolar rhabdomyosarcoma, bone cancer, brain cancer, breast cancer, cancer of the anus, anal canal, or anorectum, cancer of the eye, cancer of the intrahepatic bile duct, cancer of the joints, cancer of the neck, gallbladder, or pleura, cancer of the nose, nasal cavity, or middle ear, cancer of the oral cavity, cancer of the vulva, chronic lymphocytic leukemia, chronic myeloid cancer, colon cancer, esophageal cancer, cervical cancer, gastrointestinal carcinoid tumor, Hodgkin lymphoma, hypopharynx cancer, kidney cancer, larynx cancer, liver cancer, lung cancer, malignant mesothelioma, melanoma, multiple myeloma, nasopharynx cancer, non-Hodgkin lymphoma, ovarian cancer, pancreatic cancer, peritoneum, omentum, and mesentery cancer, pharynx cancer, prostate cancer, rectal cancer, renal cancer, skin cancer, small intestine cancer, soft tissue cancer, stomach cancer, testicular cancer, thyroid cancer, ureter cancer, and urinary bladder cancer. Preferably, the cancer is skin cancer. More preferably, the cancer is melanoma.

[0107] With respect to the inventive methods, the chronic infectious disease can be any chronic infectious disease, including any of HIV, influenza, herpes, and malaria.

[0108] The auto- or allo-immune disease that may be treated by the inventive methods may be any auto- or allo-immune disease. The autoimmune diseases that may be treated by the inventive methods may be generally characterized by an inappropriate immune reaction against self cells, tissues, and/or organs. Exemplary autoimmune diseases include, but are not limited to: vitiligo, alopecia, autoimmune kidney disease, celiac disease, inflammatory bowel disease, hepatitis, Addison's disease, Hashimoto's disease, Graves disease, atrophic gastritis/pernicious anemia, acquired hypogonadism/infertility, hypoparathyroidism, multiple

sclerosis, Myasthenia gravis, Coombs positive hemolytic anemia, systemic lupus erythematosis, allergic diseases (such as asthma, hay fever, or allergic rhinitis), Sjogren's syndrome, rheumatoid arthritis, auto-immune thyroiditis, Crohn's disease, ulcerative colitis, cardiovascular disease (e.g., atherosclerosis), and immune mediated (type-1) diabetes mellitus. The allo-immune diseases that may be treated by the inventive methods may be generally characterized by an immune reaction against foreign or transplanted organs, tissues, and/or cells. Exemplary allo-immune diseases include, but are not limited to: acute and chromic graft-versus-host disease (GVHD) and/or graft rejection that can occur, e.g., in the setting of solid organ transplantation (e.g., pancreatic, renal, cardiac, stem cell, or liver transplantation) and graft-versus-host disease that can occur in the setting of allogeneic hematopoietic stem cell transplantation (e.g., bone marrow, peripheral blood, or cord blood transplantation).

[0109] The mammal referred to in the inventive methods can be any mammal. As used herein, the term "mammal" refers to any mammal, including, but not limited to, mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Logomorpha, such as rabbits. It is preferred that the mammals are from the order Carnivora, including Felines (cats) and Canines (dogs). It is more preferred that the mammals are from the order Artiodactyla, including Bovines (cows) and Swines (pigs) or of the order Perssodactyla, including Equines (horses). It is most preferred that the mammals are of the order Primates, Ceboids, or Simoids (monkeys) or of the order Anthropoids (humans and apes). An especially preferred mammal is the human.

[0110] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLES

Mice and Cell Lines

[0111] Pmel-1 Thy1.1, Pmel-1 Ly5.1, pmel-1 *cish*-/-, RAG1-/-, C57BL/6 mice (NCI-Frederick and The Jackson Laboratory) were bred, genotyped and housed according to the guidelines of the Animal Care and Use Committee at the National Institutes of Health. B16 melanoma was obtained from the NCI Tumor Repository and grown in 10% FCS containing culture media. Where indicated, $CD8^+$ T cells were isolated from splenocytes by magnetic

bead negative selection (Miltenyi Biotec or Stemcell Technologies). Primary stimulation was accomplished using plate-bound anti-CD3 (1 μg/mL) and soluble anti-CD28 (1 μg/mL) (BD Biosciences). To generate effector cells, Pmel-1 splenocytes were stimulated with 1 μM $hgp100_{25–33}$ peptide (Anaspec) in culture medium containing 2 ng ml^{-1} IL-2 (Chiron Corporation) and cultured in 10% FCS containing RPMI for 1 week.

Adoptive Immunotherapy

[0112] Mice were implanted with subcutaneous B16 melanoma (1×10^5-5×10^5). At the time of ACT, 10-14 days post implantation, mice ($n \geq 5$ for all groups unless otherwise indicated) were injected intravenously (i.v.) with CD8-enriched naive or *in vitro* activated pmel-1 splenocytes (0.25×10^6–1×10^6 CD8+ Vβ13+ T cells), and received 0.5×10^7-2×10^7 plaque-forming units of rVV or fowlpox encoding hgp100 (Bernard Moss, National Institutes of Health). Where indicated, mice received interperitoneal (i.p.) injections of hIL-2 (Palmer et al. *PNAS* 105:8061-66 (2008)) or 100 μg of CD8-depleting antibody (53.6.7, BD PharMingen) was injected i.p. Mice were randomized, and tumors were blindly measured by using digital calipers. The products of the perpendicular diameters are presented as mean ± SEM. At times indicated after ACT spleens were harvested, ACK-lysed, enumerated, stained, and evaluated by flow cytometry. For time course experiments, splenocytes were enriched for the congenic marker using Miltenyi or Cell-Sep protocols, cyro-preserved, thawed simultaneously, enumerated and stimulated as indicated.

Flow Cytometry, ELISA, Western Blotting and Immunoprecipitation

[0113] For flow cytometry cells were stained with antibodies acquired from BD Pharmigen or eBioscience and processed as previously described (Palmer et al. *PNAS* 105:8061-66 (2008)). Intracellular staining was performed per manufacturer protocol (BD Biosciences). Carboxyfluorescein succinimidyl ester (CFSE) labeling was per manufacturer protocol (Invitrogen). Cytokine quantities were determined by ELISA (R&D Systems). Real-time PCR was conducted using commercially available primer/probe sets (Applied Biosystems) and a Prism® 7900HT (Applied Biosystems). Gene expression levels were calculated relative to the housekeeping gene encoding beta-actin (Actb). For apoptosis evaluation, following stimulation with aCD3/aCD28, cells were stained with annexin and 7AAD as per the manufacturer's instructions (BD Pharmigen). After surface staining, intracellular staining was accomplished by using Ctyofix/cytoperm, then Phosphobuffer II or

III, and antibody as per the manufacturer's instructions (BD Pharmigen). For the co-immunoprecipitation protocol, cells were lysed, cleared, and normalized using a bicinchoninic acid (BCA) assay (Pierce). Cleared supernatants were applied to antibody-bonded immunoprecipitation (IP) direct columns and treated as per the manufacturer's instructions (Pierce). Western blotting was performed using Bio-Rad reagents and protocols on nitrocellulose paper, stained with aV5, aCIS, aHtrA1, (Santa Cruz), SMAD2, pSMAD2, pSTAT5 (Cell Signal Technologies). Blots were developed using chemiluminescence (Pierce) and acquired using Chemidoc™ system (Bio-Rad).

Transfections and Transductions

[0114] For transient transfection, 293T cells were transfected with Lipofectamine™ 2000 as per the manufacturer's instructions (Invitrogen). iCIS and iRFP cell lines were developed by transducing 293T cells with a lentivirus containing CIS or RFP driven by a tetracycline promoter, and phoshoglycerate kinase promoter driven TetR and puromycin resistance genes. Cells were then selected using puromycin (3 μg/mL). Retroviral production and transduction were accomplished as previously described (Abad et al. *J. Immunother.* 31:1-6 (2008)). The shorthairpin microRNA sequences used are shown in Table 1. Knockdown was evaluated by taqman or western blotting.

TABLE 1

cish (D2)	ACGAGCCUGUUUCUGGGAGAAUUAGUGAAGCCACAGAUGUAAUUCUCCCAG AAACAGGCUCGC (SEQ ID NO: 3)
cish (F2)	CGGUCAACGCCUCUAGGUACAAUAGUGAAGCCACAGAUGUAUUGUACCUAGA GGCGUUGACCU (SEQ ID NO: 4)
htra1 (A8)	CCGCUAUCAUCAAUUAUGGAAAUAGUGAAGCCACAGAUGUAUUUCCAUAAUU GAUGAUAGCGU (SEQ ID NO: 6)

Yeast two hybrid

[0115] Yeast two-hybrid screen was performed by ProteinLinks as previously described (Xu et al. *PNAS* 94:12473-78 (1997)). Briefly, CIS-bait was cloned into TetR or LexA

vectors and screened against a murine spleen-derived cDNA library (5×10^6 or 1×10^7 clones). All positive interactions were confirmed using co-immunoprecipitation.

EXAMPLE 1

[0116] This example demonstrates that the lymphopoeisis, memory state, proliferation, and cell surface marker expression of *cish*$^{-/-}$ CD8$^+$ T cells is similar to that of wild-type cells. This example also demonstrates that *cish* mRNA expression increases following stimulation of wild-type T cells but not *cish*$^{-/-}$ T cells.

[0117] To evaluate the role of CIS in CD8$^+$ T cell function, a *cish* knockout mouse was developed. *cish*$^{-/-}$ mice were then backcrossed with C57BL/6 mice and bred with the melanoma/melanocyte-specific T cell receptor (TCR) α transgenic mouse, termed pmel-1. CIS has been shown to be induced by IL-2 and TCR stimulation (Aman et al. *J. Biol. Chem.* 274:30266-72 (1999)). *cish* mRNA expression was found to increase in both naive and primed CD8$^+$ pmel-1 T cells (WT) upon stimulation (or re-stimulation), but not *cish* knockouts (*cish*$^{-/-}$).

[0118] To further characterize *cish*$^{-/-}$ CD8$^+$ T cells, extensive phenotyping using flow cytometric analysis was performed. Here, lymphopoeisis in *cish*$^{-/-}$ pmel-1 mice was found to be similar to that of wild-type (WT) littermates in splenic CD8$^+$ and CD4$^+$ ratios. A comparable memory state as indicated by CD44 and CD62L expression was also found in CD8$^+$ T cells from WT and *cish*$^{-/-}$ pmel-1 mice. Expression analysis of CD8, clonotypic Vβ13, tetramer-binding, and IL-2 receptor (CD25, CD122 and CD132) yielded no discernable differences between CD8$^+$ WT and *cish*$^{-/-}$ pmel-1 T cells. Analysis of other markers such as CD27, CD28, CD69, CD80, CD86, LFA-1, Sca-1, CD127, KLRG1 and many others also yielded no significant differences. Furthermore, *cish*$^{-/-}$ CD8$^+$ pmel-1 T cells acquired the memory marker CD44, diluted out carboxyfluorescein (CFSE), and proliferated similarly to pmel-1 WT CD8$^+$ T cells after stimulation. In addition, the onset of skin depigmentation, vitiligo, which is typically observed in pmel-1 transgenic mice, was not altered in any of the genotypes pmel-1 WT, *cish*$^{+/-}$, and *cish*$^{-/-}$.

EXAMPLE 2

[0119] This example demonstrates that *cish*-deficient T cells secrete higher levels of IFN-γ and IL-2 upon antigen encounter as compared to WT cells.

[0120] To assess if there were any functional differences in *cish*$^{-/-}$ CD8^{+} T cells, CD8^{+} enriched naïve WT or *cish*$^{-/-}$ pmel-1 T cells were co-cultured with pulsed targets ($gp100_{25-33}$). IFN-γ secretion was evaluated by ELISA after the overnight co-culture. Interestingly, *cish*-deficient pmel-1 T cells released significantly more IFN-γ upon antigen encounter (Table 2A). IFN-γ secretion was also evaluated by ELISA after an overnight co-culture of primed WT or *cish*$^{-/-}$ pmel-1 T cells. Upon antigen re-encounter, previously primed *cish*$^{-/-}$ pmel-1 T cells released dramatically more IFN-γ than wild-type (WT) T cells (Table 2A). IL-2 secretion was also evaluated by ELISA after an overnight co-culture of primed WT or *cish*-/- pmel-1 T cells. When assayed for IL-2 or TNF-α after a co-culture of primed CD8^{+} T cells, it was observed that *cish*$^{-/-}$ CD8^{+} T cells also released more IL-2 or TNF-α (Table 2A).

TABLE 2A

log $gp100_{25-33}$ [M]	**IFN-γ (pg mL^{-1}) (naïve)**		**IFN-γ (ng mL^{-1}) (primed)**		**IL-2 (pg mL^{-1}) (primed)**		**TNF-α (ng/ml^{-1})**	
	WT	***cish*$^{-/-}$**	**WT**	***cish*$^{-/-}$**	**WT**	***cish*$^{-/-}$**	**WT**	***Cish-/-***
-6	250	1750	25	175	-	-	-	-
-7	0	750	40	150	1500	4100	2	3.5
-8	0	0	20	80	1000	4000	1.7	3.0
-9	0	0	0	0	<100	1100	0.7	1.8
-10	-	-	-	-	0	<100	0.4	0.8

[0121] PBL transduced with a T cell receptor also secrete greater levels of IFN-γ in the absence of *cish*. PBL that were mock-transduced or transduced with T cell receptor 1G4 and shRNA retrovirus targeting *cish* (or scramble as a control) were co-cultured with 624mel, 526mel, or 888mel overnight and selected for 4 days with puromycin (5×10^4: 5×10^4). The results are shown in Table 2B.

TABLE 2B

	IFN-γ (pg/ml)		
	624mel	526mel	888mel
Mock	<100	<100	<100
Scramble + 1G4	1,000	<100	<100
Cish shRNA + 1G4	>2,500	<100	<100

EXAMPLE 3

[0122] This example demonstrates that *cish*-deficient T cells mediate tumor regression *in vivo*, and that T cells treated with anti-*cish* shMIR increases the *in vitro* and *in vivo* functionality of T cells as compared to WT cells.

[0123] To evaluate the *in vivo* functionality of *cish*$^{-/-}$ CD8^{+} T cells, the pmel-1 tumor model (Palmer et al. *PNAS* 105:8061-66 (2008)) was employed. C57BL/6 mice were implanted with non-immunogenic B16 melanoma cells. After the tumor was established and vascularized, the mice were treated with naïve CD8^{+} pmel-1 T cells (*cish*$^{-/-}$ or WT) and recombinant vaccine encoding gp100 with or without exogenous IL-2. Here, a strong and durable regression of melanoma was observed after the adoptive transfer of *cish*$^{-/-}$ pmel-1 T cells that was not observed after the adoptive transfer of WT pmel-1 T cells (Figure 1A). Adoptive transfer of *cish*$^{-/-}$ pmel-1 T cells enhanced the survival of tumor-bearing mice as compared to the adoptive transfer of WT pmel-1 T cells (Figure 1C). Exogenous IL-2 significantly augmented the *in vivo* functionality of *cish*$^{-/-}$ pmel-1 T cells (Figure 1B).

[0124] To obviate any potential confounding factors associated with the *cish*$^{-/-}$ mice, *cish* was knocked down in WT CD8^{+} pmel-1 T cells using a retrovirus shMIR targeting *cish* (*cish* shMIR D2 SEQ ID NO: 3) and knockdown was confirmed. Here, it was confirmed that the *cish* depletion resulted in increased *in vitro* (Table 3) and *in vivo* (Figure 1D) functionality of CD8^{+} T cells. Similar results were also obtained using *cish* shMIR F2 SEQ ID NO: 4.

TABLE 3

log $gp100_{25-33}$ [M]	IFN-γ (ng mL^{-1})	
	P_{WT} & anti-*cish* shMIR	P_{WT} & control shMIR
-6	17	6
-7	8	2
-8	7	1
-9	3	<1
-10	<1	0

EXAMPLE 4

[0125] This example demonstrates that CIS associates with HTRA1.

[0126] CIS has been implicated in the suppression of STAT5 signaling, presumably by blocking STAT5 docking to the activated cytokine receptor (Aman et al. *J. Biol. Chem.* 274:30266-72 (1999); Matsumoto et al. *Blood* 89:3148-54 (1997); Yoshimura et al. *EMBO J.* 14:2816-26 (1995)). These findings have been observed in ectopic expression models, but have not been evaluated in the complete absence of *cish*. Based on the increased *in vitro* and *in vivo* functionality observed in the $CD8^+$ $cish^{-/-}$ pmel-1 T cells, it was hypothesized that *cish* depletion would result in enhanced STAT5 phosphorylation following exposure to common gamma chain cytokines (γc).

[0127] STAT5 phosphorylation was measured by flow cytometric analysis in naïve $CD8^+$ WT or $cish^{-/-}$ pmel-1 T cells after the addition of 150 IU of IL-2. However, after careful analysis with a myriad of doses of cytokines, including IL-2, IL-7, IL-15, or IL-21, and time points, no discernable differences in STAT5 phosphorylation were observed in the absence of *cish*. Because increased functionality after TCR stimulation was observed, it was hypothesized that perhaps STAT5 phosphorylation was enhanced in this setting. However, after TCR stimulation and *cish* upregulation, STAT5 phosphorylation appeared similar in both primed $cish^{-/-}$ and primed WT $CD8^+$ T cells after CD3 stimulation. Phosphorylation of several other signal transduction pathways, including pERK1/2, pSTAT1, pSTAT4, pSTAT4 and p38, was measured by flow cytometric analysis after CD3 stimulation of primed WT or $cish^{-/-}$ pmel-1 T cells, but failed to reveal insights into the observed enhanced functionality of the $cish^{-/-}$ $CD8^+$ T cells.

[0128] In order to investigate what accounted for the functional differences in *cish*$^{-/-}$ CD8^{+} T cells, a yeast-two hybrid screen was performed using CIS as bait and a mouse spleen cDNA library as prey. Here, it was found that CIS interacted with the ATP-independent serine protease HTRA1. In order to confirm this interaction in mammalian cells, a model was developed in which 293 cells were stably transduced with red fluorescent protein (RFP) or CIS tet-inducible lentiviruses termed iRFP and iCIS, respectively. After the addition of doxycyline, iRFP and iCIS-transduced cells expressed their respective products, as confirmed by flow cytometric and immunoblot analyses. These lines were then transfected with Chloromphenicol^{R}-V5 or *htra1*-V5 containing plasmids and confirmed for protein expression by immunoblot analysis. Upon immunoprecipation of CIS and immunoblotting for V5, it was found that CIS directly associated with HTRA1 and not the control. Immunoprecipation of V5 and immunoblotting of CIS yielded a similar result. Co-immunoprecipitation of endogenous HtrA1 and CIS in primed WT CD8+ pmel-1 T cells (and *vice-versa*), but not in the absence of *cish,* also suggested a direct association of CIS and HtrA1.

EXAMPLE 5

[0129] This example demonstrates that the absence of *cish* results in increased HTRA1 protein expression and decreased TGF-β signaling. This example also demonstrates that pharmacological blocking of HTRA1 may reverse this decrease in TGF-β signaling.

[0130] Real-time polymerase chain reaction (PCR) revealed similar expression of *htra1* mRNA in both primed WT and *cish*$^{-/-}$ pmel-1 T cells after CD3 stimulation. Interestingly, immunoblotting revealed elevated HTRA1 protein in the *cish*$^{-/-}$ CD8^{+} T cells. Transduction of *htra1* resulted in 2-3 logs higher levels of *htra1* mRNA but not HTRA1 protein in *cish*-replete T cells. HTRA1 is a serine protease and has been implicated in the degradation of TGF-β family members (Launay et al. *Cell Death Differ.* 15:1408-16 (2008); Tocharus et al. *Dev. Growth Differ.* 46:257-74 (2004); Tsuchiya et al. *Bone*: 37: 323-36 (2005)). TGF-β1 evaluation by ELISA in an overnight co-culture with $gp100_{25\text{-}33}$ revealed decreased TGF-β1 protein in *cish*$^{-/-}$ (80 pg mL^{-1} TGF-β1) as compared to WT (225 pg mL^{-1} TGF-β1) pmel-1 T cells, which was not attributable to decreased TGF-β1 mRNA levels.

[0131] Phosphorylation of SMAD2 (s465/467) (and control beta-actin) after CD3 stimulation of primed WT or *cish*$^{-/-}$ pmel-1 T cells was measured by immunoblot analysis. Markedly enhanced TGF-β signaling, as assessed by SMAD2 phosphorylation, in CD8+ T

cells was found in WT mice but not in *cish*-/- mice. To more accurately quantify TGF-β/SMAD activity, transgenic mice containing the firefly luciferase gene under the control of 12 SMAD-binding element repeats, called SBE-luc (Lin et al., *J. Immunol.*, 175: 547-54 (2005)) were used, which were crossed with *cish*-/- mice. Significantly more SMAD reporter activity was observed in *cish*-replete versus *cish*-deficient CD8+ T cells. These results were consistent with the hypothesis that CIS mediates the destruction of the TGF-β protease, HTRA1. This notion was further confirmed when it was observed that this SMAD2 phosphorylation was inhibited by a cell-permeable small molecule, 2-(3-(6-methylpyridin-2-yl)-1H-pyrazol-4-yl)-1,5-naphthyridine ("compound 19") (Gellibert et al., *J. Med. Chem.*, 47: 4494-506 (2004)) capable of potent, selective, reversible, and ATP-competitive inhibition of TGF-β receptor phosphorylation.

[0132] In addition, TGF-β1 secretion was evaluated by ELISA in an overnight culture of primed WT or *cish*$^{-/-}$ pmel-1 T cells in the presence or absence of NVP-LBP976. Inhibition of HTRA1 proteolytic activity by NVP-LBP976 resulted in increased TGF-β1 protein levels in the absence of *cish* (Table 4).

TABLE 4

	TGF-β1 (pg/mL^{-1})
P_{WT}	1060
P_{WT} + HTRA1/*htra1* inhibitor	1130
$P_{cish-/-}$	500
$P_{cish-/-}$ + HTRA1/*htra1* inhibitor	1060

EXAMPLE 6

[0133] This example demonstrates that the absence of *cish* results in decreased TGF-β signaling, including reduced repression of *tbx21* (T-bet), *myc* (c-Myc), and survival genes such as *bcl2l1* (Bcl-xL) and cyclins.

[0134] TGF-β signaling has been implicated in the suppression of many pro-functional/survival genes such as *tbx21* (T-bet), *myc* (c-Myc), Bcl-2/xL, and cyclins in CD8^{+} T cells (Li et al. *Cell* 134: 392-04 (2008); Sanjabi et al. *Immunity* 31:131-44 (2009); Wan et al. *Immunol. Rev.* 220:199-13 (2007); Wan et al. *J. Clin. Immunol.* 2: 647-59 (2008)). Using

real-time PCR analysis, it was observed that *tbx21* (T-bet) and *myc* (c-Myc) expression increased in *cish*$^{-/-}$ pmel-1 T cells after TCR stimulation (Figure 2A-B). Increased *myc* (c-Myc) expression correlated to elevated *myc* (c-Myc) reporter activity in lentiviral transduced *cish*$^{-/-}$ pmel-1 T cells (Figure 2C). In addition, real-time PCR analysis revealed increased expression of *bcl2l1* (Bcl-xL) and cyclin in *cish*$^{-/-}$ CD8^{+} T cells (Figure 2D-E).

[0135] TGF-β has been implicated in the induction of the gut-homing receptor CD103 in CD8^{+} T cells (El-Asady et al. *J. Exp. Med.* 201:1647-57 (2005); Uss et al. *J. Immunol.* 177: 2775-83 (2006)). In accordance with decreased TGF-β signaling, flow cytometric analysis revealed reduced CD103 expression in primed *cish*$^{-/-}$ pmel-1 T cells relative to WT pmel-1 T cells. CD103 was highly upregulated in the presence of cish after stimulation. Conversely, TGF-β signaling can induce the expression of genes such as ID1 and Stra13 that were found to be upregulated in WT pmel-1 T cells after stimulation (Figure 3A-B). These findings correlated with increased SMAD reporter activity in lentiviral transduced WT pmel-1 T cells (Figure 3C).

EXAMPLE 7

[0136] This example demonstrates that in the absence of *cish*, there is decreased TGF-β signaling, increased *tbx21* (T-bet), m*yc* (c-Myc), *bcl2l1* (Bcl-xL), and Bcl-2 expression, and increased *in vivo* survival and function of T cells, as measured by flow cytometric analysis.

[0137] Recent work has implicated TGF-β in CD8^{+} T cell apoptosis (Sanjabi et al. *Immunity* 31:131-44 (2009); Tinoco et al. *Immunity* 31:145-57 (2009)). After an acute stimulation of primed CD8^{+} pmel-1 T cells, *cish*$^{-/-}$ T cells accumulated about half as much annexin V as WT T cells.

[0138] Gene-marked CD8+ T cells were adoptively transferred into unmanipulated mice. The *in vivo* survival of WT or *cish*$^{-/-}$ pmel-1 T cells was measured after the adoptive transfer of 5 x 10^{5} WT or *cish*$^{-/-}$ cells. The results are shown in Table 5. Increased numbers of CD8+ T cells in the absence of *cish* compared to *cish*-intact littermates were observed.

TABLE 5

Days Post Transfer	Total pmel-1 cells (1 x 10^6)	
	P_{WT}	$P_{cish-/-}$
0	0.5	0.5
5	0.5	1.4
6	0.2	1.2
7	0	0.1

[0139] To evaluate if these increased numbers of *cish*-/- CD8+ T cells were attributable to enhanced proliferation, bromodeoxyuridine (BrdU) was injected. *In vivo* BrdU incorporation or annexin V staining on congenically marked WT or *cish*$^{-/-}$ pmel-1 T cells was measured five days after adoptive transfer. *cish*$^{-/-}$ pmel-1 T cells demonstrated decreased *ex vivo* annexin V uptake as compared to WT cells. However, the proliferation of *cish*-/- pmel-1 T cells, as measured by BrdU incorporation, was similar to that of WT cells.

[0140] *Ex vivo* staining of T cells with annexin V, which binds an epitope of phosphatidylserine normally expressed only on the cytoplasmic side of the cell membrane except in T cells experiencing apoptosis, revealed that the presence of *cish* was associated with increased staining and thus more apoptotic cell death. The percentage of annexin V positive WT or *cish*$^{-/-}$ pmel-1 T cells was measured on days 5, 6, and 7 following adoptive transfer. The results are shown in Table 6.

TABLE 6

Days Post Transfer	Annexin V (%)	
	P_{WT}	$P_{cish-/-}$
5	50	25
6	45	21
7	48	28

[0141] Accordingly, the reduced contraction of *cish*$^{-/-}$ pmel-1 T cells after vaccination (Table 5) appeared to be attributable to reduced apoptosis (Table 6) and not proliferation of the cells.

[0142] Expression of Bcl-2, an anti-apoptotic factor suppressed by TGF-β, was measured by mean fluorescence intensity (MFI) and found to be higher in *cish*$^{-/-}$ (900 MFI) than in WT

cells (550 MFI). Thus, Bcl-2 was highly expressed in *cish*$^{-/-}$ pmel-1 T cells during the peak of the response, perhaps accounting for the delayed *in vivo* contraction.

[0143] This example demonstrated that the absence of CIS is associated with decreased TGF-β signaling and an increase in expression of *tbx21* (T-bet), *myc* (c-Myc), *bcl2l1* (Bcl-xL), and Bcl-2, as well as reduced apoptosis of T cells.

EXAMPLE 8

[0144] This example demonstrates that the knockdown of *htra1* mitigates the augmented function of *cish*-deficient CD8+ T cells.

[0145] To evaluate the role of HTRA1 in *cish*$^{-/-}$ T cell function, WT and *cish*$^{-/-}$ CD8^{+} pmel-1 T cells were transduced with retroviruses encoding shMIR targeting *htra1* (SEQ ID NO: 6) or a scramble sequence (control) and knockdown of *htra1* was confirmed. Similar to previous observations, control-transduced *cish*$^{-/-}$ CD8^{+} pmel-1 T cells experienced enhanced up-regulation of *tbx21* (T-bet), *myc* (c-Myc), *bcl2l1* (Bcl-xL), *ccne1* (cyclin E1), *cdk1* (cyclin CDC2a) and *ccnd2* (cyclin D2) (Figure 4A-F). In contrast to control-transduced *cish*$^{-/-}$ CD8^{+} pmel-1 T cells, the knockdown of *htra1* in *cish*$^{-/-}$ CD8^{+} pmel-1 T cells significantly abrogated the enhanced expression of these target genes (Figure 4A-F). As previously shown, WT CD8^{+} pmel-1 T cells experienced a significantly lower induction of these target genes and expressed low levels of HTRA1. Nonetheless, the knockdown of *htra1* in WT CD8^{+} pmel-1 T cells appeared to result in a significant ($p < 0.0001$) albeit modest, inhibition of several of these targets compared to control WT T cells.

[0146] Five days after control or *htra1* (SEQ ID NO: 6) shMIR transduction of WT or *cish*$^{-/-}$ pmel-1 T cells, CD103 expression was measured by flow cytometric evaluation. In addition, annexin V staining was measured four hours after restimulation five days after *htra1* or control shMIR transduction of WT or *cish*$^{-/-}$ pmel-1 T cells. The knockdown of *htra1* in *cish*$^{-/-}$ CD8^{+} pmel-1 T cells resulted in the selective induction of CD103 and increased annexin V staining that resembled control and *htra1* shMIR transduced WT CD8^{+} pmel-1 T cells.

[0147] To assess the functional significance of *htra1* knockdown in *cish*$^{-/-}$ T cells, an overnight co-culture with peptide-pulsed target cells was performed and IFN-γ levels were determined by ELISA. The results are set forth in Table 7.

TABLE 7

log $gp100_{25-33}$ [M]	IFN-γ (ng/mL^{-1})			
	P_{WT} shControl	P_{WT} sh*htra1*	$P_{cish-/-}$ shControl	$P_{cish-/-}$ sh*htra1*
-8	125	200	550	340
-9	110	95	375	200
-10	25	25	225	145
-11	<25	<25	25	<25
0	<25	25	25	<25

[0148] Here, it was found that the knockdown of *htra1* in *cish*$^{-/-}$ $CD8^+$ pmel-1 T cells significantly ($p < 0.04$) abrogated IFN-γ production (Table 7). No significant changes in IFN-γ production were observed in control or *htra1* shMIR (SEQ ID NO: 6) transduced WT $CD8^+$ pmel-1 T cells (Table 7).

[0149] *cish* expression in control or *htra1* (SEQ ID NO: 6) shMIR transduced WT or *cish*$^{-/-}$ pmel-1 T cells was also measured by real-time PCR. There was no significant difference in the induction of *cish* expression with the knockdown of *htra1* in WT $CD8^+$ pmel-1 T cells. Knockdown of *htra1* in *cish*$^{-/-}$ T cells abrogated the enhanced expression of known targets of TGF-β suppression and induced expression of TGF-β promoted factors. Thus, it appears that there exists a causal relationship between the enhanced functionality of *cish*$^{-/-}$ $CD8^+$ T cells and the presence of *htra1*. It appears that the presence of CIS might be involved in the removal or degradation of HTRA1.

[0150] This example demonstrated that the knockdown of *htra1* decreases the enhanced function of *cish*-deficient CD8+ T cells.

EXAMPLE 9

[0151] This example demonstrates that CIS degrades HTRA1, and proteasomal blockage of CIS results in the accumulation of HTRA1 in the presence of CIS.

[0152] In order to evaluate if CIS is involved in HTRA1 degradation, the model described in Example 4 was used. HtrA1 V5 or Control-V5 was transfected into iCIS or iRFP cells. After induction of CIS or RFP by doxycycline, V5 levels were evaluated by immunoblotting. A densitometric evaluation of HtrA1 V5 or control V5 staining relative to β-actin was also performed following the administration of doxycycline. The results are shown in Table 8.

TABLE 8

Post-doxycycline (hours)	HtrA1V5:β-actin		ControlV5:β-actin	
	iCIS	iRFP	iCIS	iRFP
0	3.25	3.75	0	0
24	1.25	4.0	0.3	0.25
48	1.0	3.3	0.75	0

[0153] Here, a selective decrease in HTRA1 was found only in the presence of CIS and not RFP or CIS transfected with control (Table 8).

[0154] To determine if the presence of CIS in T cells results in HTRA1 degradation, titrated amounts (0, 0.1, 1, or 10 μM) of proteasome inhibitor I (Calbiochem) were added after stimulation of primed WT or *cish*$^{-/-}$ CD8^{+} pmel-1 T cells. Here, a selective and titratable increase in HTRA1 protein was observed as the concentration of proteasome inhibitor increased in the presence (WT) but not in the absence of *cish*. To determine if increased HTRA1 levels correlated with decreased TGF-β signaling, titrated levels (0, 0.1, 1, or 10 μM) of proteasome inhibitor I were added and immunoblot analysis for SMAD2 phosphorylation was performed. Here, it was found that with increased protreasome inhibitor and subsequently increased HTRA1, decreased SMAD2 phosphorylation was observed that was similar to *cish*$^{-/-}$ T cells after stimulation. Thus, it appears that the presence of CIS results in proteasome-mediated destruction of HTRA1.

[0155] Based on these findings, without being bound to a particular theory, it is believed that γc cytokines such as IL-2, presumably through TCR stimulation, drive the expression of STAT5 target genes including *cish*. It is believed that TGF-β is degraded by HTRA1, but in the presence of CIS, TGF-β is preserved while HTRA1 is degraded. It is also believed that TGF-β then acts in a negative feed-back loop, inhibiting STAT5 target gene expression, and that TGF-β does not appear to negatively regulate CIS expression.

EXAMPLE 10

[0156] This example demonstrates that recombinant HtrA degrades TGFβ1 *in vitro*.

[0157] Recombinant HtrA and control HtrA S328A (deleteriously mutated) were produced. Micro-scale purification screening of HtrA and HtrA S328A from insect and mammalian cell culture was performed using 20 ml of transiently transfected HEK293E

expression culture or 50 ml of Baculovirus Expression Vector System (BEVS) expression culture as source material. Gels are stained with Coomassie® blue dye, which confirmed that HtrA and HtrA S328A were purified.

[0158] HTRA1 or HTRA1 (S328A) (1 μg) was cultured overnight with hTGFβ1 (2 μg) with or without 50mM Tris. The samples were run on a gel and stained with Coomassie® blue dye. The gels showed that HTRA1 modestly degraded TGFβ1, but HTRA1 (S328A) did not degrade TGFβ1.

[0159] The relative density of the TGFβ1 bands in the gel were measured and quantified. The results are shown in Table 9.

TABLE 9

	Relative TGFβ1 Density
None	1.0
HTRA1 (-Tris)	0.5
HTRA1 (S328A) (-Tris)	0.98
HTRA1 (+Tris)	0.6
HTRA1 (S328A)(+Tris)	1.05

[0160] The addition of exogenous HTRA1 significantly reduced TGF-β/SMAD signaling in WT CD8+ T cells after stimulation as compared to the mutant HTRA1 (Figure 5A). As a control in the same experiment, the impact of the presence or absence of mutant HTRA1 was measured and no change was found in TGF-β/SMAD signaling, indicating that the mutant HTRA1 protein lacked enzymatic activity (Figure 5C).

EXAMPLE 11

[0161] This example demonstrates that the function of wild-type T cells is enhanced in the presence of recombinant HTRA1.

[0162] WT or *cish-/-* pmel-1 T cells were cultured overnight with peptide-pulsed splenocytes in the presence of exogenous HTRA1 or inactive HTRA1 (S328A) (50 μg/mL). IFN-γ production was evaluated by ELISA.

[0163] The addition of HTRA1 to an overnight co-culture of pmel-1 T cells and peptide-pulsed targets significantly enhanced the production of IFN-γ in WT CD8+ T cells compared to that of inactive HTRA1 (Figure 5B). There was no significant difference in *cish-/-* pmel-1 T cell production of IFN-γ in the presence of either intact or mutant *htra1*, perhaps indicating saturation

of endogenous HTRA1 in the absence of *cish*. Taken together, these data revealed that the removal of *cish* dramatically enhanced CD8+ T cell functionality and tumor killing.

EXAMPLE 12

[0164] This example demonstrates increased NFκB transcriptional activity in the absence of *cish*.

[0165] Pmel-1 T cells were transduced with reporter-lentivirus after CD3 stimulation and the relative luminescence (RLU) was measured. An increase in NFκB expression was observed in *cish* deficient T cells as compared to WT cells. No significant difference in MAPK/ERK was observed in *cish* deficient T cells as compared to WT cells.

EXAMPLE 13

[0166] This example demonstrates increased GATA3 target expression in the absence of *cish*.

[0167] Real-time PCR analysis of *gata3, IL-4,* or *IL-5* normalized to five housekeeping (HKG) genes (*gusb, hprt1, hsp90ab1, gapdh,* and *actb*) was performed with respect to primed WT or *cish*-/- pmel-1 T cells after CD3 stimulation. An increase in expression of each of *gata3, IL-4,* and *IL-5* was observed in *cish* deficient T cells as compared to WT cells (Figures 6A-6C).

EXAMPLE 14

[0168] This example demonstrates increased pro-stimulatory/pro-inflammatory target expression in the absence of *cish*.

[0169] Real-time PCR analysis of *cd27, cd28, icos, IL-1a, IFN-γ, gzmb, prcd1,* and *tnfrsf9* normalized to five housekeeping (HKG) genes (*gusb, hprt1, hsp90ab1, gapdh,* and *actb*) was performed with respect to primed WT or *cish*-/- pmel-1 T cells after CD3 stimulation. An increase in expression of each of *cd27, cd28, icos, IL-1a, IFN-γ, gzmb,* and *tnfrsf9* was observed in *cish* deficient T cells as compared to WT cells (Figures 7A-7H).

EXAMPLE 15

[0170] This example demonstrates that the removal of *cish* prolongs tumor therapy in the absence of host T and B cells.

[0171] P-mel-1 T cells (2.5×10^5) (nontransduced (NT), WT or *cish-/-*) were adoptively transferred into tumor-bearing *rag1-/-* mice (8×10^6 rVVgp100, IL-2 (12 μg) BID x 3 days). Tumor size was measured. The removal of *cish* prolonged tumor therapy in the absence of host T cells (Figure 8).

[0172] P-mel-1 T cells (2.5×10^5) (nontransduced (NT), *cish-/-* with anti-CD8 on day 38, or *cish-/-* with anti-IgG on day 38) were adoptively transferred into tumor-bearing *rag1-/-* mice (8×10^6 rVVgp100, IL-2 (12 μg) BID x 3 days). Tumor size was measured. The removal of *cish* prolonged tumor therapy in the absence of host T cells or host B cells (Figure 9).

[0173] Four to seven days after adoptive transfer, naïve WT or $cish^{-/-}$ pmel-1 T cells were co-cultured with pulsed targets ($gp100_{25\text{-}33}$). IFN-γ secretion was evaluated by ELISA after overnight co-culture. It was observed that removal of *cish* resulted in increased *ex vivo* function as measured by IFN-γ secretion (Table 10).

TABLE 10

Log gp100 [M]	Pwt				P*cish-/-*			
	IFN-γ (pg/mL) at Days Post Adoptive Cell Transfer							
	4	5	6	7	4	5	6	7
-6	60000	22000	11000	11000	100000	58000	25000	38000
-7	35000	19000	10000	5000	60000	50000	23000	36000
-8	20000	10000	5000	3000	40000	35000	15000	20000
-9	5000	9000	3000	2000	20000	25000	6000	10000
-10	0	3000	1000	0	0	3000	0	5000
-11	0	3000	3000	1000	0	1000	0	1000

[0174] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0175] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0176] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

CLAIM(S):

1. A peripheral blood cell (PBC) comprising a high temperature requirement serine peptidase 1 (HTRA1)/*htra1* activator.

2. The PBC according to claim 1, wherein the HTRA1/*htra1* activator comprises a recombinant nucleic acid that encodes HTRA1.

3. The PBC according to claim 2, wherein the HTRA1/*htra1* activator that comprises a recombinant nucleic acid that encodes HTRA1 comprises a nucleotide sequence comprising SEQ ID NO: 5 or 11 or a nucleotide sequence that is at least 95% identical to SEQ ID NO: 5 or 11.

4. The PBC according to claims 1-3, further comprising a cytokine-induced Src homology 2 protein (CIS)/*cish* inhibitor.

5. The PBC according to claim 4, wherein the CIS/*cish* inhibitor comprises a small interfering RNA (siRNA), a microRNA, or an antisense nucleic acid.

6. The PBC according to claim 5, wherein the CIS/*cish* inhibitor comprises siRNA complementary to a nucleotide sequence encoding *cish*.

7. The PBC according to claim 6, wherein the siRNA is complementary to a nucleotide sequence comprising SEQ ID NO: 1 or 2.

8. The PBC according to claim 5, wherein the CIS/*cish* inhibitor comprises an antisense nucleic acid complementary to a nucleotide sequence encoding *cish*.

9. The PBC according to claim 8, wherein the antisense nucleic acid is complementary to a nucleotide sequence comprising SEQ ID NO: 1 or 2.

10. The PBC according to claim 5, wherein the CIS/*cish* inhibitor comprises an miRNA and the miRNA is a short hairpin miRNA (shMIR).

11. The PBC according to claim 10, wherein the shMIR comprises SEQ ID NO: 3 or 4 or a nucleotide sequence that is at least 95% identical to SEQ ID NO: 3 or 4.

12. A host cell comprising a CIS/*cish* inhibitor and a HTRA1/*htra1* activator.

13. The host cell according to claim 12, wherein the HTRA1/*htra1* activator comprises a recombinant nucleic acid that encodes HTRA1.

14. The host cell according to claim 13, wherein the recombinant nucleic acid that encodes HTRA1 comprises a nucleotide sequence comprising SEQ ID NO: 5 or 11 or a nucleotide sequence that is at least 95% identical to SEQ ID NO: 5 or 11.

15. The host cell according to any one of claims 12-14, wherein the CIS/*cish* inhibitor comprises a small interfering RNA (siRNA), a microRNA, or an antisense nucleic acid.

16. The host cell according to claim 15, wherein the CIS/*cish* inhibitor comprises siRNA complementary to a nucleotide sequence encoding *cish*.

17. The host cell according to claim 16, wherein the siRNA is complementary to a nucleotide sequence comprising SEQ ID NO: 1 or 2.

18. The host cell according to claim 15, wherein the CIS/*cish* inhibitor comprises an antisense nucleic acid complementary to a nucleotide sequence encoding *cish*.

19. The host cell according to claim 18, wherein the antisense nucleic acid is complementary to a nucleotide sequence comprising SEQ ID NO: 1 or 2.

20. The host cell according to claim 15, wherein the CIS/*cish* inhibitor comprises an miRNA and the miRNA is a short hairpin miRNA (shMIR).

21. The host cell according to claim 20, wherein the shMIR comprises SEQ ID NO: 3 or 4 or a nucleotide sequence that is at least 95% identical to SEQ ID NO: 3 or 4.

22. A host cell comprising an anti-*cish* shMIR comprising SEQ ID NO: 3 or 4 or a nucleotide sequence that is at least 95% identical to SEQ ID NO: 3 or 4.

23. The host cell of claim 22, wherein the host cell is a PBC.

24. The host cell of claim 23, wherein the PBC is a T cell.

25. A host cell comprising a HTRA1/*htra1* inhibitor and a CIS/*cish* activator.

26. The host cell according to claim 25, wherein the CIS/*cish* activator comprises a recombinant nucleic acid that encodes CIS.

27. The host cell according to claim 25 or 26, wherein the HTRA1/*htra1* inhibitor comprises a small interfering RNA (siRNA), a microRNA (miRNA), or an antisense nucleic acid.

28. The host cell according to claim 27, wherein the HTRA1/*htra1* inhibitor comprises siRNA complementary to a nucleotide sequence encoding HTRA1.

29. The host cell according to claim 28, wherein the siRNA is complementary to a nucleotide sequence comprising SEQ ID NO: 5 or 11.

30. The host cell according to claim 27, wherein the HTRA1/*htra1* inhibitor comprises an antisense nucleic acid complementary to a nucleotide sequence encoding HTRA1.

31. The host cell according to claim 30, wherein the antisense nucleic acid is complementary to a nucleotide sequence comprising SEQ ID NO: 5.

32. The host cell according to claim 27, wherein the HTRA1/*htra1* inhibitor comprises an miRNA and the miRNA is a short hairpin miRNA (shMIR).

33. The host cell according to claim 32, wherein the shMIR comprises SEQ ID NO: 6 or a nucleotide sequence that is at least 95% identical to SEQ ID NO: 6.

34. The host cell according to claim 25 or 26, wherein the HTRA1/*htra1* inhibitor comprises NVP-LBP976.

35. The host cell according to claim 26, wherein the recombinant nucleic acid that encodes CIS comprises a nucleotide sequence comprising SEQ ID NO: 1 or 2 or a nucleotide sequence that is at least 95% identical to SEQ ID NO: 1 or 2.

36. A peripheral blood cell (PBC) comprising a HTRA1/*htra1* inhibitor.

37. The PBC according to claim 36 further comprising a CIS/*cish* activator.

38. The PBC according to claim 37, wherein the CIS/*cish* activator comprises a recombinant nucleic acid that encodes CIS.

39. The PBC according to any one of claims 36-38, wherein the HTRA1/*htra1* inhibitor comprises a small interfering RNA (siRNA), a microRNA, or an antisense nucleic acid.

40. The PBC according to any one of claims 36-39, wherein the HTRA1/*htra1* inhibitor comprises siRNA complementary to a nucleotide sequence encoding HTRA1.

41. The PBC according to claim 40, wherein the siRNA is complementary to a nucleotide sequence comprising SEQ ID NO: 5.

42. The PBC according to claim 39, wherein the HTRA1/*htra1* inhibitor comprises an antisense nucleic acid complementary to a nucleotide sequence encoding HTRA1.

43. The PBC according to claim 42, wherein the antisense nucleic acid is complementary to a nucleotide sequence comprising SEQ ID NO: 5.

44. The PBC according to claim 39, wherein the HTRA1/*htra1* inhibitor comprises an miRNA and the miRNA is a short hairpin miRNA (shMIR).

45. The PBC according to claim 44, wherein the shMIR comprises SEQ ID NO: 6 or a nucleotide sequence that is at least 95% identical to SEQ ID NO: 6.

46. The PBC according to any one of claims 36-38, wherein the HTRA1/*htra1* inhibitor comprises NVP-LBP976.

47. The PBC according to claim 38, wherein the recombinant nucleic acid that encodes CIS comprises a nucleotide sequence comprising SEQ ID NO: 1 or 2 or a nucleotide sequence that is at least 95% identical to SEQ ID NO: 1 or 2.

48. A population of cells comprising at least one PBC of any one of claims 1-11 or at least one host cell of any one of claims 12-24.

49. A population of cells comprising at least one host cell of any one of claims 25-35 or at least one PBC of any one of claims 36-47.

50. A composition comprising nanoparticles and PBCs, wherein the nanoparticles comprise an HTRA1/*htra1* activator and/or a CIS/*cish* inhibitor.

51. The composition according to claim 50, wherein the HTRA1/*htra1* activator comprises a recombinant nucleic acid that encodes HTRA1.

52. The composition according to claim 51, wherein the HTRA1/*htra1* activator that comprises a recombinant nucleic acid that encodes HTRA1 comprises a nucleotide sequence comprising SEQ ID NO: 5 or a nucleotide sequence that is at least 95% identical to SEQ ID NO: 5.

53. The composition according to claim 50, wherein the HTRA1/*htra1* activator comprises a HTRA1 protein.

54. The composition according to claim 53, wherein the HTRA1 protein comprises SEQ ID NO: 7 or an amino acid sequence that is at least 95% identical to SEQ ID NO: 7.

55. The composition according to any one of claims 50-54, wherein the CIS/*cish* inhibitor comprises a small interfering RNA (siRNA), a microRNA, or an antisense nucleic acid.

56. The composition according to claim 55, wherein the CIS/*cish* inhibitor comprises siRNA complementary to a nucleotide sequence encoding CIS.

57. The composition according to claim 56, wherein the siRNA is complementary to a nucleotide sequence comprising SEQ ID NO: 1 or 2.

58. The composition according to claim 55, wherein the CIS/*cish* inhibitor comprises an antisense nucleic acid complementary to a nucleotide sequence encoding CIS.

59. The composition according to claim 58, wherein the antisense nucleic acid is complementary to a nucleotide sequence comprising SEQ ID NO: 1 or 2.

60. The composition according to claim 55, wherein the CIS/*cish* inhibitor comprises an miRNA and the miRNA is a short hairpin miRNA (shMIR).

61. The composition according to claim 60, wherein the shMIR comprises SEQ ID NO: 3 or 4 or a nucleotide sequence that is at least 95% identical to SEQ ID NO: 3 or 4.

62. The composition according to any one of claims 50-61, wherein the PBCs are T cells.

63. A composition comprising nanoparticles and PBCs, wherein the nanoparticles comprise an HTRA1/*htra1* inhibitor and/or a CIS/*cish* activator.

64. The composition according to claim 63, wherein the CIS/*cish* activator comprises a recombinant nucleic acid that encodes CIS.

65. The composition according to claim 63, wherein the CIS/*cish* activator comprises a CIS protein.

66. The composition according to claim 65, wherein the CIS protein comprises SEQ ID NO: 8 or an amino acid sequence that is at least 95% identical to SEQ ID NO: 8.

67. The composition according to any one of claims 63-66, wherein the HTRA1/*htra1* inhibitor comprises a small interfering RNA (siRNA), a microRNA (miRNA), or an antisense nucleic acid.

68. The composition according to claim 67, wherein the HTRA1/*htra1* inhibitor comprises siRNA complementary to a nucleotide sequence encoding HTRA1.

69. The composition according to claim 67, wherein the siRNA is complementary to a nucleotide sequence comprising SEQ ID NO: 5.

70. The composition according to claim 67, wherein the HTRA1/*htra1* inhibitor comprises an antisense nucleic acid complementary to a nucleotide sequence encoding HTRA1.

71. The composition according to claim 67, wherein the antisense nucleic acid is complementary to a nucleotide sequence comprising SEQ ID NO: 5.

72. The composition according to claim 67, wherein the HTRA1/*htra1* inhibitor comprises an miRNA and the miRNA is a short hairpin miRNA (shMIR).

73. The composition according to claim 72, wherein the shMIR comprises SEQ ID NO: 6 or a nucleotide sequence that is at least 95% identical to SEQ ID NO: 6.

74. The composition according to any one of claims 63-66, wherein the HTRA1/*htra1* inhibitor comprises NVP-LBP976.

75. The composition according to claim 64, wherein the recombinant nucleic acid that encodes CIS comprises a nucleotide sequence comprising SEQ ID NO: 1 or 2 or a nucleotide sequence that is at least 95% identical to SEQ ID NO: 1 or 2.

76. A pharmaceutical composition comprising the PBC of any one of claims 1-11, the host cell of any one of claims 12-24, the population of cells of claim 48, or the composition of any one of claims 50-62, and a pharmaceutically acceptable carrier.

77. A pharmaceutical composition comprising the host cell of any one of claims 25-35, the PBC of any one of claims 36-47, the population of cells of claim 49, or the composition of any one of claims 63-75, and a pharmaceutically acceptable carrier.

78. A method of treating or preventing cancer or a chronic infectious disease in a mammal, comprising administering to the mammal the PBC of any one of claims 1-11, the host cell of any one of claims 12-24, the population of cells of claim 48, the composition of any one of claims 50-62, or the pharmaceutical composition of claim 76 in an amount effective to treat or prevent cancer or a chronic infectious disease in the mammal.

79. A method of treating or preventing an auto- and/or allo-immune disease in a mammal, comprising administering to the mammal the host cell of any one of claims 25-35, the PBC of any one of claims 36-47, or the population of cells of claim 49, the composition of any one of claims 63-75, or the pharmaceutical composition of claim 77 in an amount effective to treat or prevent an auto- and/or allo-immune disease in the mammal.

80. A method of suppressing T cell activity in a mammal, comprising administering to the mammal the host cell of any one of claims 25-35, the PBC of any one of claims 36-47, or the population of cells of claim 49, the composition of any one of claims 63-75, or the pharmaceutical composition of claim 77 in an amount effective to suppress T cell activity in the mammal.

81. A method of increasing T cell activity in a mammal, comprising administering to the mammal the PBC of any one of claims 1-11, the host cell of any one of claims 12-24, the population of cells of claim 48, the composition of any one of claims 50-62, or the pharmaceutical composition of claim 76 in an amount effective to increase T cell activity in the mammal.

82. A method of treating or preventing a disease selected from the group consisting of age-related macular degeneration, Alzheimer's disease, and familial ischemic cerebral small-vessel disease in a mammal, comprising administering to the mammal the PBC of any one of claims 1-11 and 36-47, the host cell of any one of claims 12-35, the population of cells of claim 48 or 49, the composition of any one of claims 50-75, or the pharmaceutical composition of claim 76 or 77, in an amount effective to treat or prevent the disease in the mammal.

Figure 1

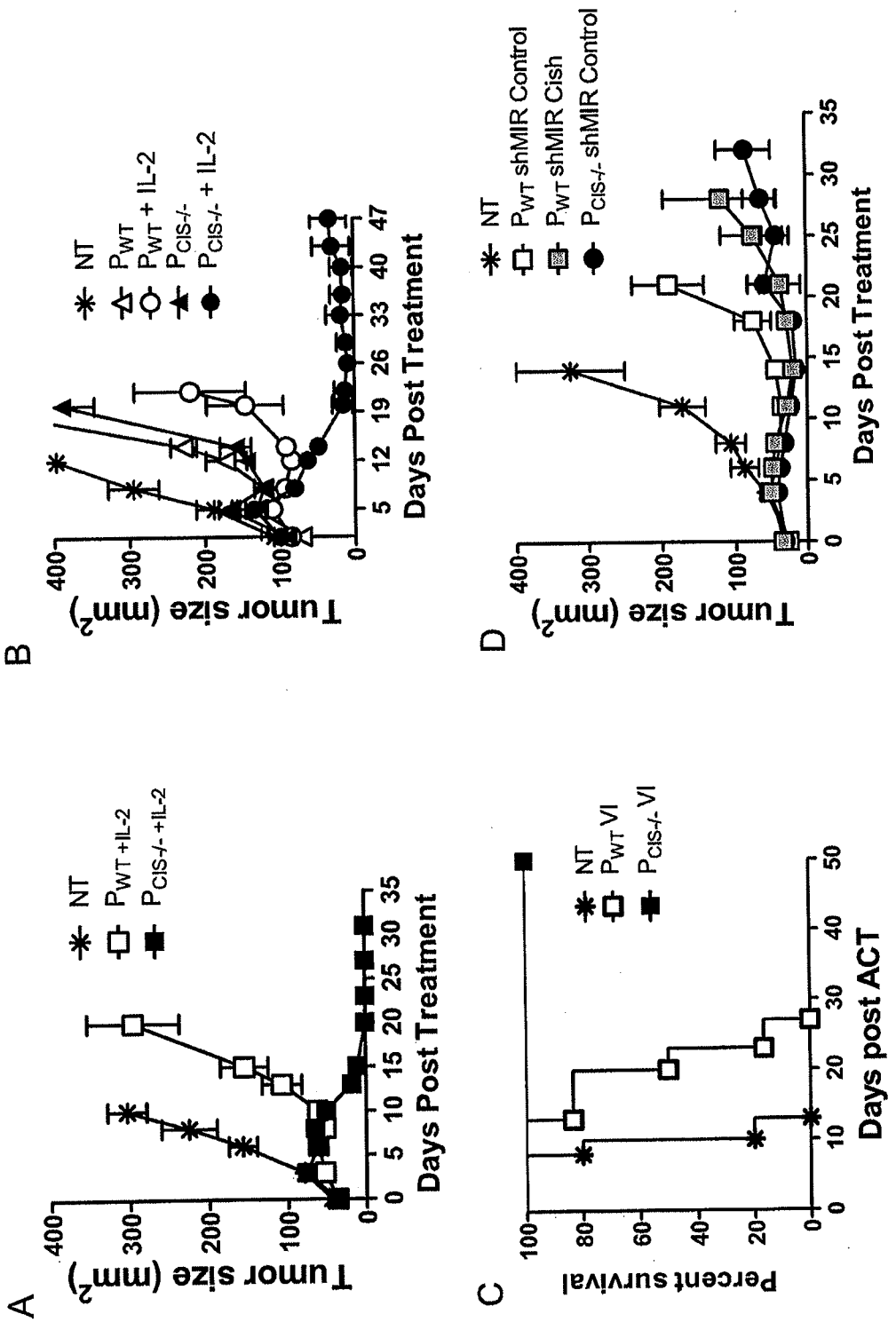

A
Tumor size (mm²)
Days Post Treatment
NT
PWT +IL-2
PCIS-/- +IL-2
B
Tumor size (mm²)
Days Post Treatment
NT
PWT
PWT + IL-2
PCIS-/-
PCIS-/- + IL-2
C
Percent survival
Days post ACT
NT
PWT VI
PCIS-/- VI
D
Tumor size (mm²)
Days Post Treatment
NT
PWT shMIR Control
PWT shMIR Cish
PCIS-/- shMIR Control

Figure 2

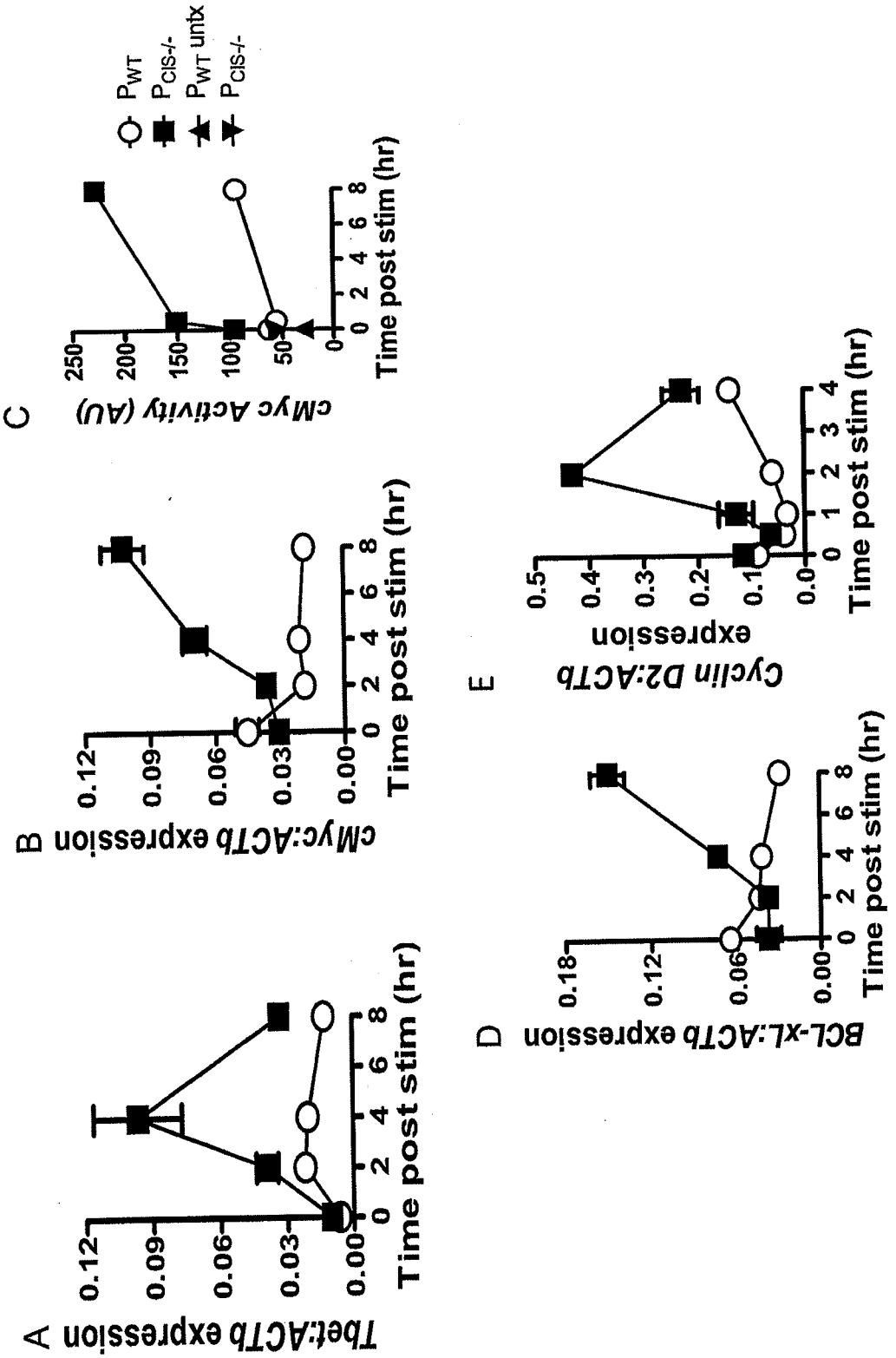

A
Tbet:ACTb expression
0.12
0.09
0.06
0.03
0.00
0 2 4 6 8
Time post stim (hr)
B
cMyc:ACTb expression
0.12
0.09
0.06
0.03
0.00
0 2 4 6 8
Time post stim (hr)
C
cMyc Activity (AU)
250
200
150
100
50
0
0 2 4 6 8
Time post stim (hr)
PWT
PCIS-/-
PWT untx
PCIS-/-
D
BCL-xL:ACTb expression
0.18
0.12
0.06
0.00
0 2 4 6 8
Time post stim (hr)
E
Cyclin D2:ACTb expression
0.5
0.4
0.3
0.2
0.1
0.0
0 1 2 3 4
Time post stim (hr)

Figure 3

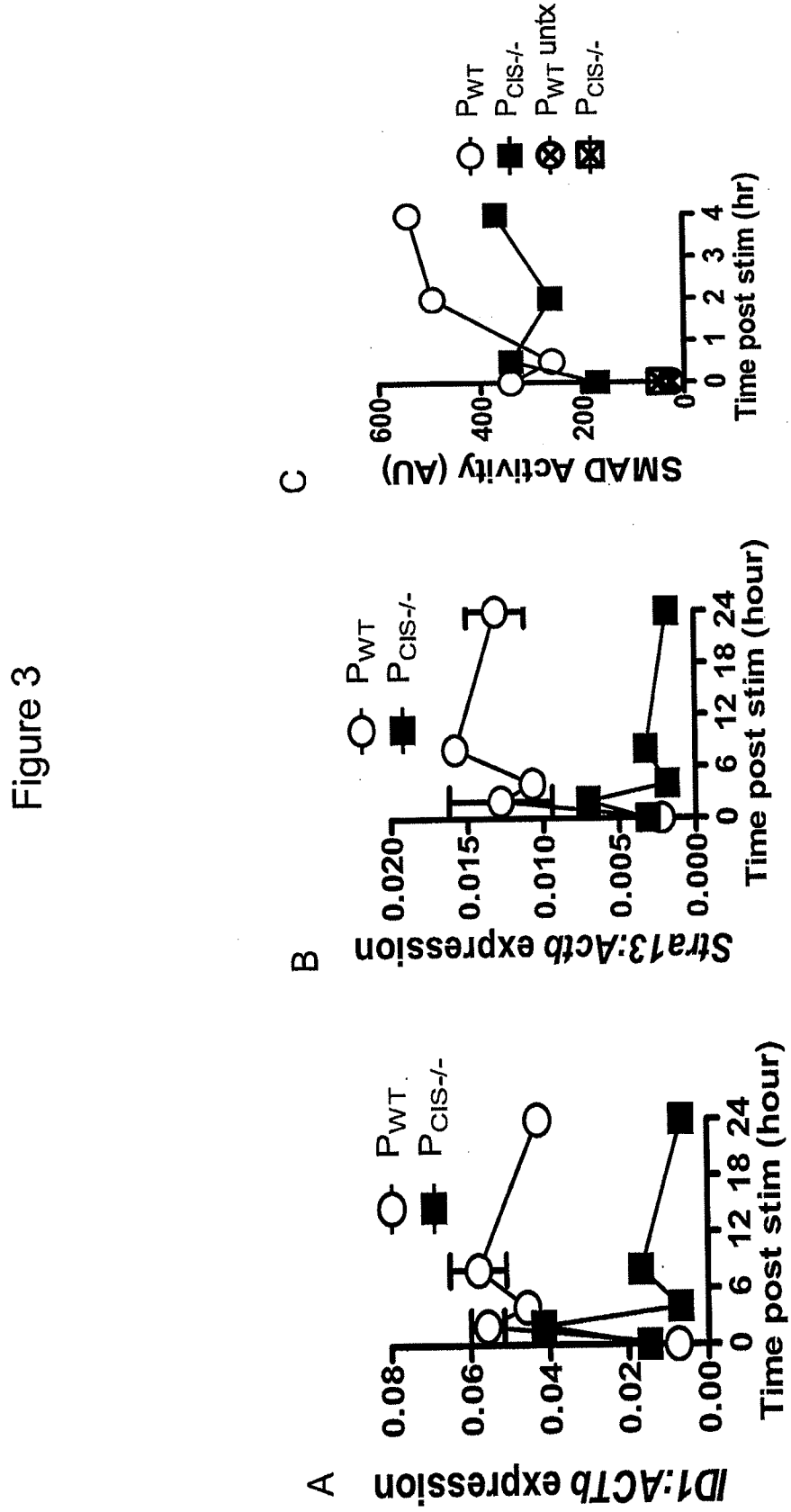

A
ID1:ACTb expression
0.08
0.06
0.04
0.02
0.00
0 6 12 18 24
Time post stim (hour)
PWT
PCIS-/-
B
Stra13:Actb expression
0.020
0.015
0.010
0.005
0.000
0 6 12 18 24
Time post stim (hour)
PWT
PCIS-/-
C
SMAD Activity (AU)
600
400
200
0
0 1 2 3 4
Time post stim (hr)
PWT
PCIS-/-
PWT untx
PCIS-/-

Figure 4

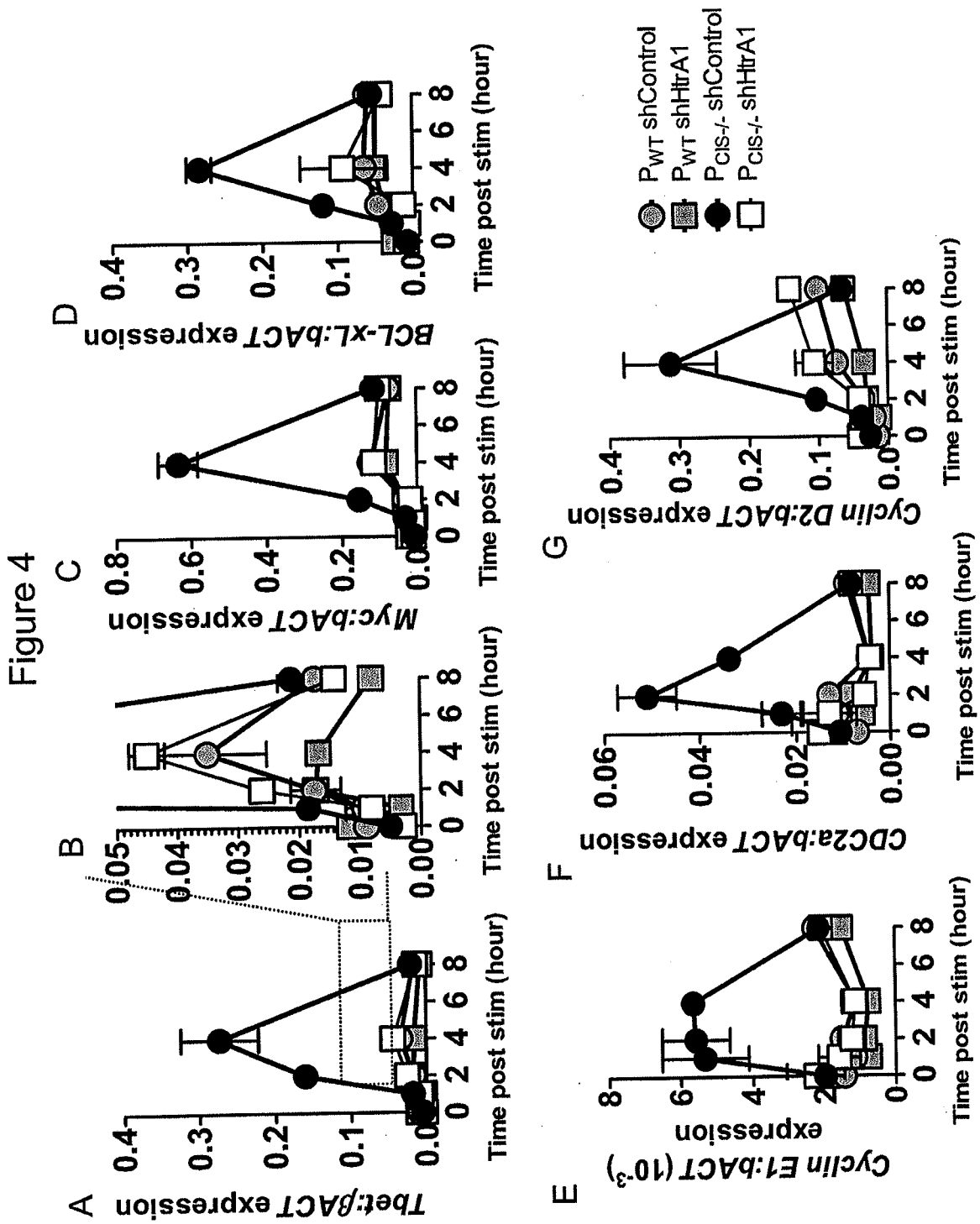

A
Tbet:βACT expression
B
C
Myc:bACT expression
D
BCL-xL:bACT expression
E
Cyclin E1:bACT (10-3) expression
F
CDC2a:bACT expression
G
Cyclin D2:bACT expression
Time post stim (hour)
PWT shControl
PWT shHtrA1
PCIS-/- shControl
PCIS-/- shHtrA1

Figure 5

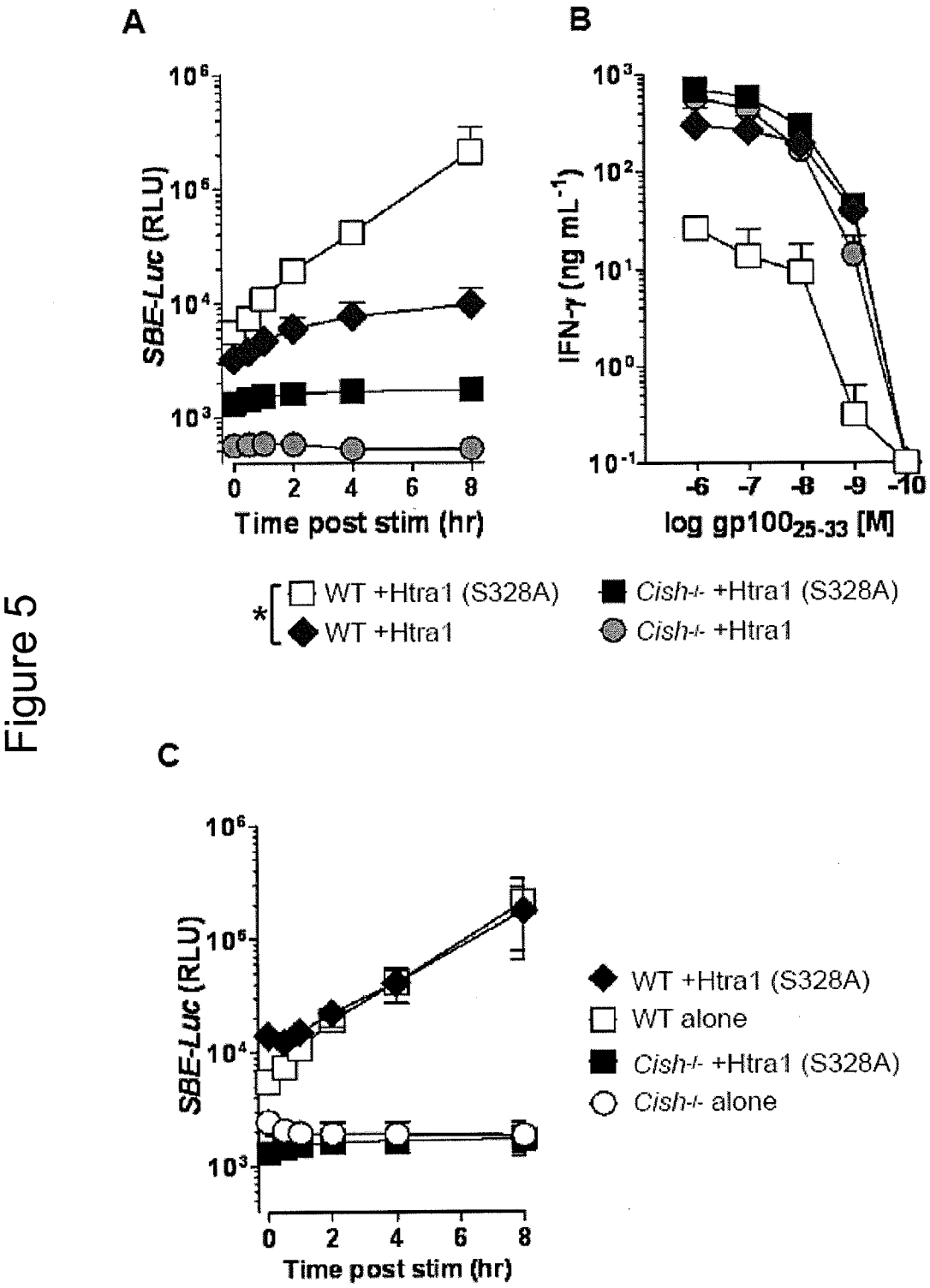

A
B
SBE-Luc (RLU)
Time post stim (hr)
IFN-γ (ng mL-1)
log gp100 25-33 [M]
WT +Htra1 (S328A)
WT +Htra1
Cish-/- +Htra1 (S328A)
Cish-/- +Htra1
C
SBE-Luc (RLU)
Time post stim (hr)
WT +Htra1 (S328A)
WT alone
Cish-/- +Htra1 (S328A)
Cish-/- alone

Figure 6

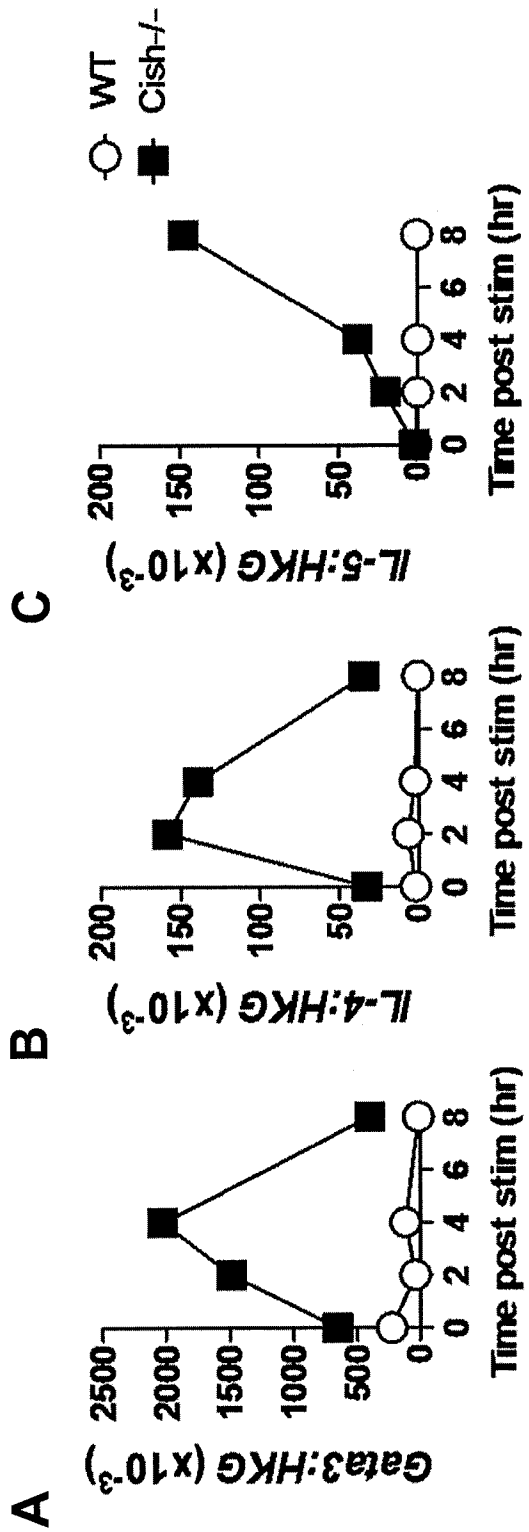

A
Gata3:HKG (x10-3)
2500
2000
1500
1000
500
0
0 2 4 6 8
Time post stim (hr)
B
IL-4:HKG (x10-3)
200
150
100
50
0
0 2 4 6 8
Time post stim (hr)
C
IL-5:HKG (x10-3)
200
150
100
50
0
0 2 4 6 8
Time post stim (hr)
WT
Cish-/-

Figure 7

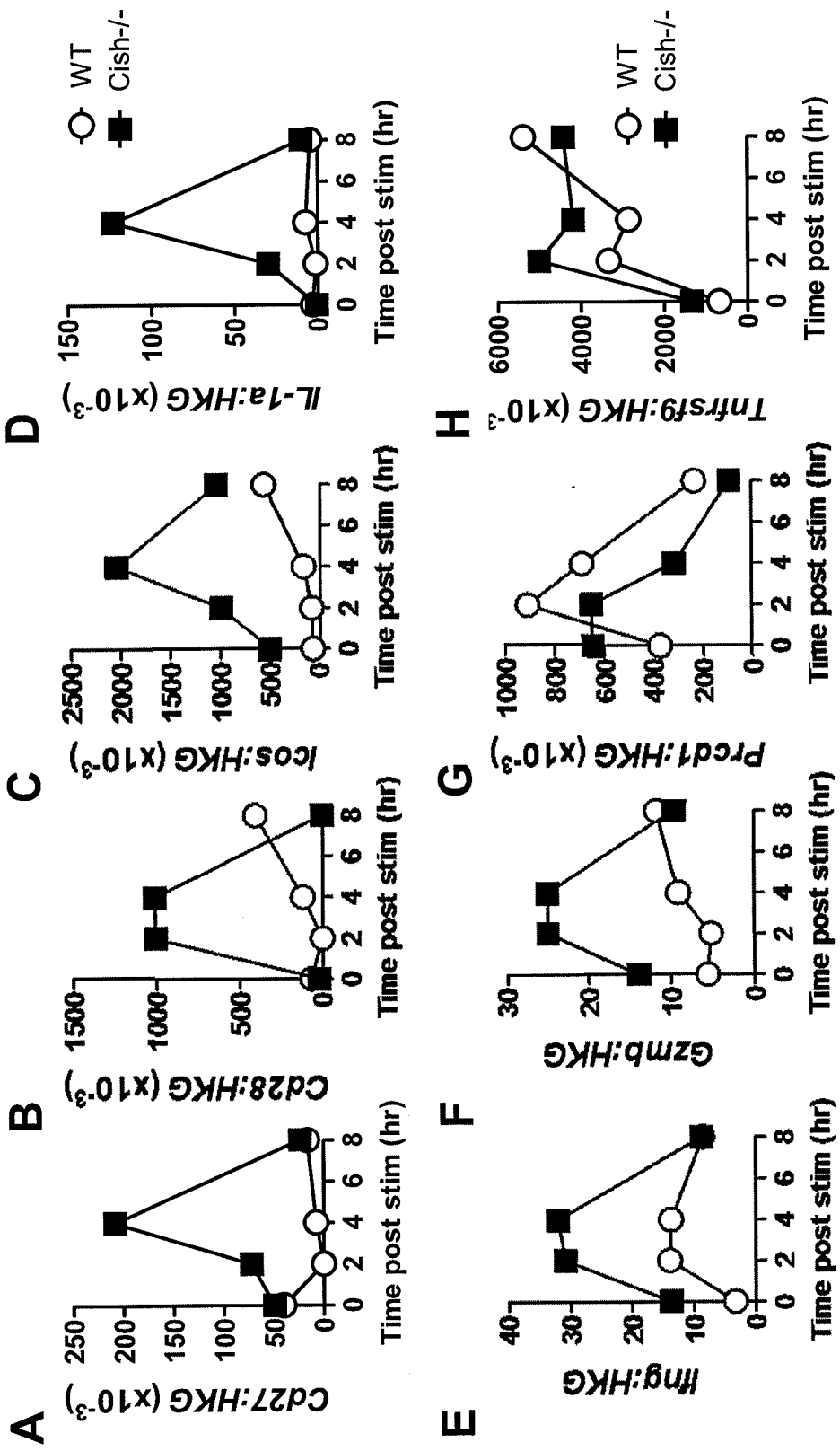

A
Cd27:HKG (x10-3)
B
Cd28:HKG (x10-3)
C
Icos:HKG (x10-3)
D
IL-1a:HKG (x10-3)
E
Ifng:HKG
F
Gzmb:HKG
G
Prcd1:HKG (x10-3)
H
Tnfrsf9:HKG (x10-3)
Time post stim (hr)
WT
Cish-/-

Figure 8

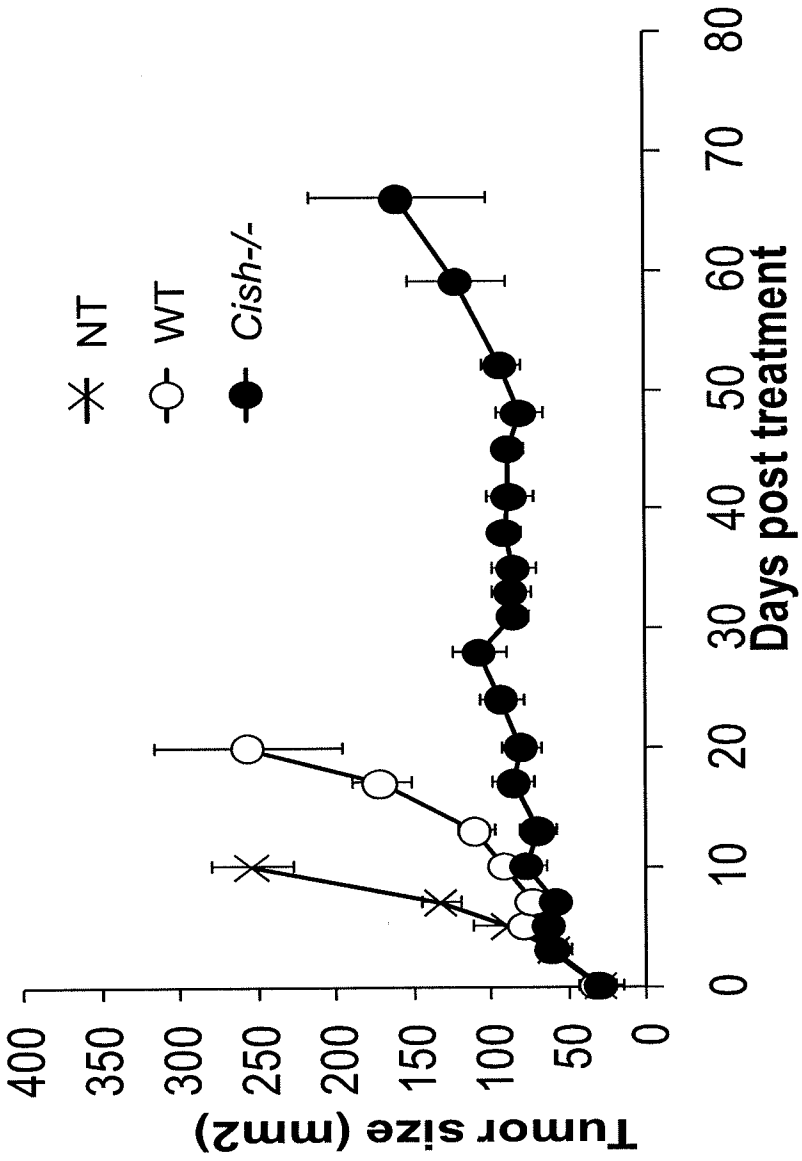
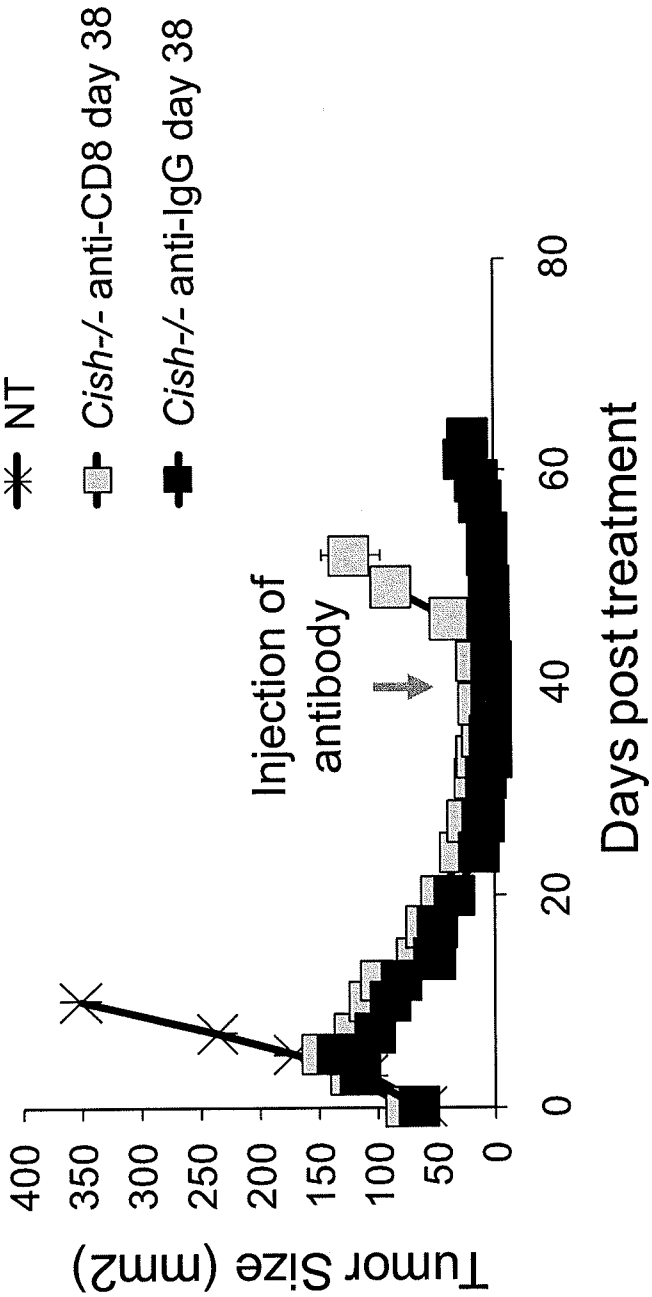

NT
WT
Cish-/-
Tumor size (mm2)
400
350
300
250
200
150
100
50
0
0
10
20
30
40
50
60
70
80
Days post treatment

Figure 9


NT
Cish-/- anti-CD8 day 38
Cish-/- anti-IgG day 38
Injection of antibody
Tumor Size (mm2)
Days post treatment
0
50
100
150
200
250
300
350
400
0
20
40
60
80

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/063375

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ZURAWA-JANICKA DOROTA ET AL: "HtrA proteins as targets in therapy of cancer and other diseases", EXPERT OPINION ON THERAPEUTIC TARGETS, ASHLEY PUBLICATIONS, LONDON, GB, vol. 14, no. 7, 1 January 2010 (2010-01-01), pages 665-679, XP008124102, ISSN: 1472-8222, DOI: 10.1517/14728222.2010.487867 page 675 - page 676 ----- -/--	1-3, 48-82

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 April 2012	04/05/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Novak-Giese, Sabine

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BALDI ALFONSO ET AL: "THE HTRA1 SERINE PROTEASE IS DOWN-REGULATED DURING HUMAN MELANOMA PROGRESSION AND REPRESSES GROWTH OF METASTATIC MELANOMA CELLS", ONCOGENE, NATURE PUBLISHING GROUP, GB, vol. 21, no. 43, 1 January 2002 (2002-01-01), pages 6684-6688, XP002976435, ISSN: 0950-9232, DOI: 10.1038/SJ.ONC.1205911 figures 1,2; tables 1,2	1-3, 48-82
X	WO 2010/068413 A1 (EMERGENT PRODUCT DEV GAITHERSB [US]; LACY MICHAEL JOSEPH [US]) 17 June 2010 (2010-06-17) paragraph [0309]; example 4	1-3, 48-82
X	KHOR CHIEA C ET AL: "CISH and Susceptibility to Infectious Diseases", NEW ENGLAND JOURNAL OF MEDICINE, vol. 362, no. 22, June 2010 (2010-06), pages 2092-2101, XP9158597, the whole document	48-82
X	CHEN SHANGWU ET AL: "Functional association of cytokine-induced SH2 protein and protein kinase C in activated T cells.", INTERNATIONAL IMMUNOLOGY, vol. 15, no. 3, March 2003 (2003-03), pages 403-409, XP9158620, ISSN: 0953-8178 page 407, column 2, paragraph 2	48-82
X	WO 2006/026807 A1 (TELETHON INST FOR CHILD HEALTH [AU]; HOLT PATRICK [AU]; SLY PETER [AU]) 16 March 2006 (2006-03-16) paragraphs [0050] - [0053]; claims 11,39	48-82
X	WO 2009/006460 A1 (ALCON RES LTD [US]; CHATTERTON JON E [US]; WAX MARTIN B [US]; ROMANO C) 8 January 2009 (2009-01-08) the whole document	25-47

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2011/063375

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010068413 A1	17-06-2010	NONE	
WO 2006026807 A1	16-03-2006	CA 2579349 A1	16-03-2006
		EP 1799849 A1	27-06-2007
		EP 2093297 A2	26-08-2009
		JP 2008512099 A	24-04-2008
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		WO 2006026807 A1	16-03-2006
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		TW 200911290 A	16-03-2009
		US 2009012030 A1	08-01-2009
		WO 2009006460 A1	08-01-2009