Improved Anti-tumor Activity of Next-generation TCR-engineered T cells through CD8αβ Co-expression

Gagan Bajwa, Justin Gunesch*, Inbar Azoulay-Alfaguter, Melinda Mata, Ali Mohamed, Mamta Kalra#, Steffen Walter#*

Next-generation TCR-T through CD8 Co-Expression

- Successful targeting of solid tumors with TCR-engineered T cells (TCR-T) requires eliciting of an antigen-specific, multi-dimensional, sustained anti-tumor immune response by infused T cells while overcoming the suppressive tumor microenvironment.
- First-generation TCR-T approaches have demonstrated clinical efficacy in some solid cancers. However, effective treatment across several solid tumor indications may require next-generation engineered T cells with enhanced anti-tumor activity.
- One of the next-generation approaches is to redirect CD4 T cells to target HLA class I-specific tumor antigens by introduction of a CD8 co-receptor.
- Effective engagement of CD4 T cells may enhance anti-tumor immune response and potentially improve the clinical outcome of TCR-T therapies in solid tumor patients.

Harnessing the Anti-tumor Potency of CD4 T cells

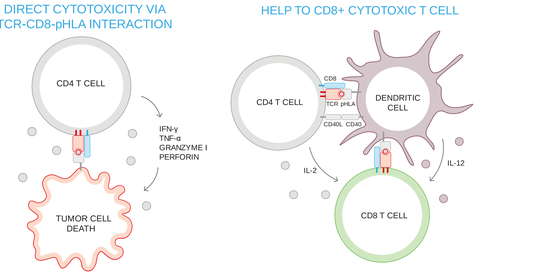


Figure 1: Polyfunctional role of CD4 T cells in anti-tumor response: Upon antigen-specific activation, CD4 T cells elicit effector cytokines and mediate direct cytotoxicity. In addition, CD4 T cells provide help to cytotoxic CD8 T cells through direct and indirect mechanisms.

CD8 Co-receptor Exists as a Homodimer and a Heterodimer

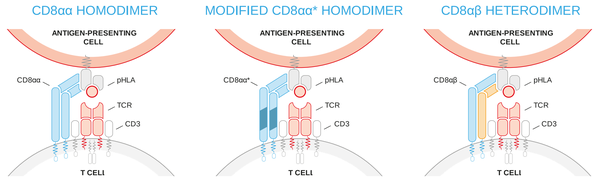


Figure 2: Schematic of different isoforms of CD8 co-receptor and interaction with peptide HLA (pHLA) complex for antigen recognition via TCR. Naturally, the CD8 receptor exists as a homodimer and a heterodimer differentially expressed in various immune cell subsets. A proprietary modified version of CD8α homodimer (CD8αα*) was engineered for a comprehensive functional evaluation in comparison with the wild type homodimer and heterodimer isoforms.

Study Objectives

We evaluated the impact of co-expression of different types of CD8 co-receptors (CD8 homodimer, modified homodimer and heterodimer) on the functionality of CD4+ and CD8+ T cells engineered with a TCR directed to an HLA-A*02-presented PRAME peptide (IMA203 TCR) and determined the depth and durability of anti-tumor response *in vitro*.

CD8 Co-receptor Enables pHLA Tetramer Binding of HLA Class I-specific TCR in CD4+ T cells

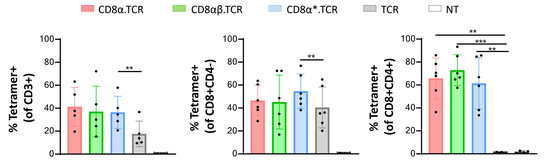


Figure 3: Healthy donor PBMC were transduced with a vector expressing TCR and CD8α homodimer (CD8α.TCR) or CD8α* homodimer (CD8α*.TCR) or CD8αβ heterodimer (CD8αβ.TCR) or TCR alone (TCR) and antigen-specific tetramer binding was measured by flow cytometry. Cumulative data on tetramer+ cells among total CD3+ cells (left), total CD8+ (middle) and total CD4+CD8+ cells (right) are shown. NT=Non-transduced control. N=5-6, mean±SD, p values based on one-way ANOVA/Tukey's multiple comparisons (**p<0.01, ***p<0.001).

CD8αβ.TCR+ and CD8α*.TCR+ CD4+ T cells Elicit Broader Anti-tumor Response upon Antigen Stimulation as Compared to CD8α.TCR+ cells

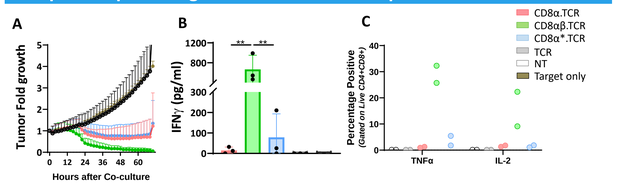


Figure 4: CD8α.TCR+, CD8αβ.TCR+, or CD8α*.TCR+ CD4+ T cells were co-cultured with target-positive UACC257-RFP tumor cell line at an E:T ratio of 4:1 and **(A)** Tumor fold growth was measured using IncuCyte® live-cell analysis system, n=4 mean±SD. **(B)** IFNγ quantification in the supernatants after 24 h of coculture using ELISA, n=3, mean±SD; **p<0.01 based on one-way ANOVA/Tukey's multiple comparisons. **(C)** Intracellular TNFα and IL-2 expression on CD4+CD8+ subset measured by flow cytometry, n=2.

Based on the functionality of CD4 T cells, further evaluation focused on CD8αβ.TCR and CD8α*.TCR only

CD8αβ.TCR+ T cells Are Polyfunctional & Secrete Higher Levels of Th1 Cytokines in Response to Antigen Stimulation

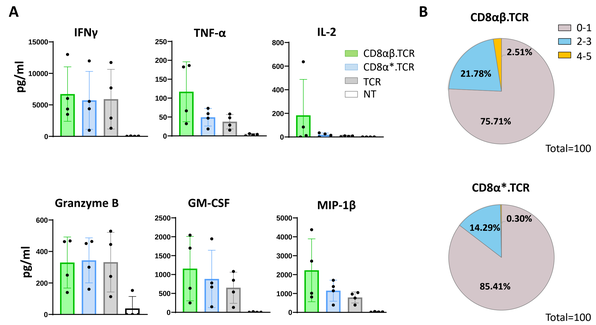
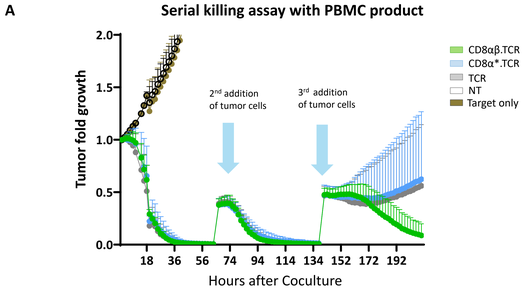
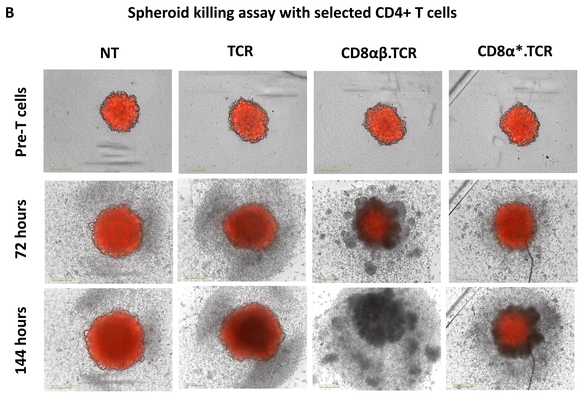


Figure 5: (A) CD8αβ.TCR+, CD8α*.TCR+, TCR+ or NT T cells were co-cultured with UACC257 tumor cells at an E:T ratio of 4:1 (effectors normalized to %CD3+Tet+) for 24 h and cytokines were quantified in the supernatants by multiplex cytokine assay; n=4, mean±SD. **(B)** Polyfunctional responses (assessed by intracellular cytokine staining) of CD4+CD8+ T cells from PBMC-derived products transduced with either CD8αβ.TCR (top) or CD8α*.TCR (bottom) co-cultured with target-positive UACC257 tumor cells. Depicted are the percentages of cells expressing 0-1, 2-3, or 4-5 effector molecules; n=3.

CD8αβ.TCR+ T cells Show Sustained Suppression of Tumor Growth in Long-term Killing Assays *in vitro*





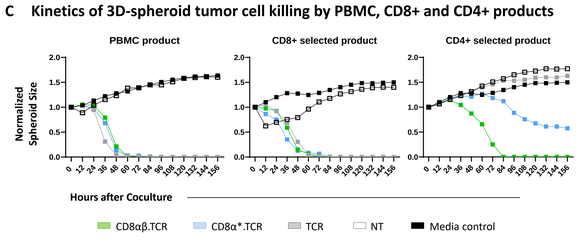


Figure 6: (A) CD8αβ.TCR+, CD8α*.TCR or TCR+ T cells were co-cultured with UACC257-RFP tumor cell line at an E:T ratio of 4:1 (effectors normalized to %CD3+Tet+) for 10 days, every 3 days T cells were rechallenged with fresh tumor cells and tumor fold growth (normalized to day 0) was analyzed using IncuCyte® live-cell analysis system, n=4, mean±SD. **(B)** Representative bright field and fluorescent images of UACC257-RFP spheroids after co-culture with selected CD4+ T cells from transduced PBMC products. **(C)** Spheroid size analysis over 156 hours post addition of PBMC-derived products or CD8+ or CD4+ selected T cells. Data shown normalized to last acquired time point prior to T cell addition. Data shown for one of three donor products.

CD8αβ.TCR+ CD4+ T cells Demonstrate More Efficient Engagement with Dendritic Cells and Modulation of Pro-inflammatory Cytokines

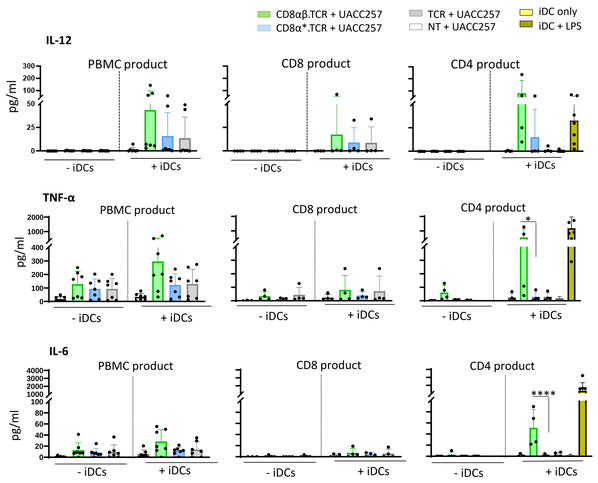


Figure 7: Tri-cocultures of PBMC- or CD8+ selected- or CD4+ selected-product with UACC257 tumor cell line in the presence or absence of autologous monocyte-derived immature dendritic cells (iDCs) followed by cytokine quantification using multiplex assay; iDCs alone or with LPS as controls, n=4-7, mean±SD, p values based on 2-way ANOVA (*p<0.5, ****p<0.0001)

Engaging CD4+ T cells via CD8αβ Co-expression Potentiates Anti-tumor Activity of HLA class I-specific TCR-T cells

- Differential functional profile of TCR-T cells co-expressing either CD8α, CD8α* or CD8αβ suggests that optimizing the type of co-receptor is relevant to maximize anti-tumor response and reveals CD8αβ to be the optimal co-receptor for the IMA203 PRAME TCR.
- Engaging CD4+ PRAME-directed T cells via CD8αβ co-expression
  - imparts cytotoxic potential to CD4+ T cells to suppress tumor growth in serial killing assays and 3D spheroid models *in vitro*
  - confers CD4+ T cells polyfunctionality to secrete Th1 cytokines and cytotoxic effector molecules upon antigen stimulation
  - induces efficient engagement with antigen-presenting cells and modulation of pro-inflammatory cytokine response
- Pleiotropic effects mediated by activated CD4+ T cells including acquired cytotoxicity and helper activity may potentially improve outcomes in solid tumor patients in clinical settings.
- We plan to implement CD8αβ co-expression into IMA203 clinical development via a next-generation IMA203CD8 cohort in 2022.

Acknowledgements

The authors acknowledge contributions of Siyu Ren, Kelsey Howard, Xavier Legras, Victor Cardenas, and Erik Farrar in completion of this work and Sabrina Schecher for significant support in preparation of this poster.

*Joint-First Authorship, # Joint-Last Authorship

Immatics Biotechnologies GmbH
Immatics US, Inc.

www.immatics.com
info@immatics.com

Improved Anti-tumor Activity of Next-generation TCR-engineered T cells through CD8αβ Co-expression

Gagan Bajwa, Justin Gunesch*, Inbar Azoulay-Alfaguter, Melinda Mata, Ali Mohamed, Mamta Kalra#, Steffen Walter#*

Next-generation TCR-T through CD8 Co-Expression

- Successful targeting of solid tumors with TCR-engineered T cells (TCR-T) requires eliciting of an antigen-specific, multi-dimensional, sustained anti-tumor immune response by infused T cells while overcoming the suppressive tumor microenvironment.

- First-generation TCR-T approaches have demonstrated clinical efficacy in some solid cancers. However, effective treatment across several solid tumor indications may require next-generation engineered T cells with enhanced anti-tumor activity.

- One of the next-generation approaches is to redirect CD4 T cells to target HLA class I-specific tumor antigens by introduction of a CD8 co-receptor.

- Effective engagement of CD4 T cells may enhance anti-tumor immune response and potentially improve the clinical outcome of TCR-T therapies in solid tumor patients.

*Joint-First Authorship, # Joint-Last Authorship

Immatics Biotechnologies GmbH
Immatics US, Inc.

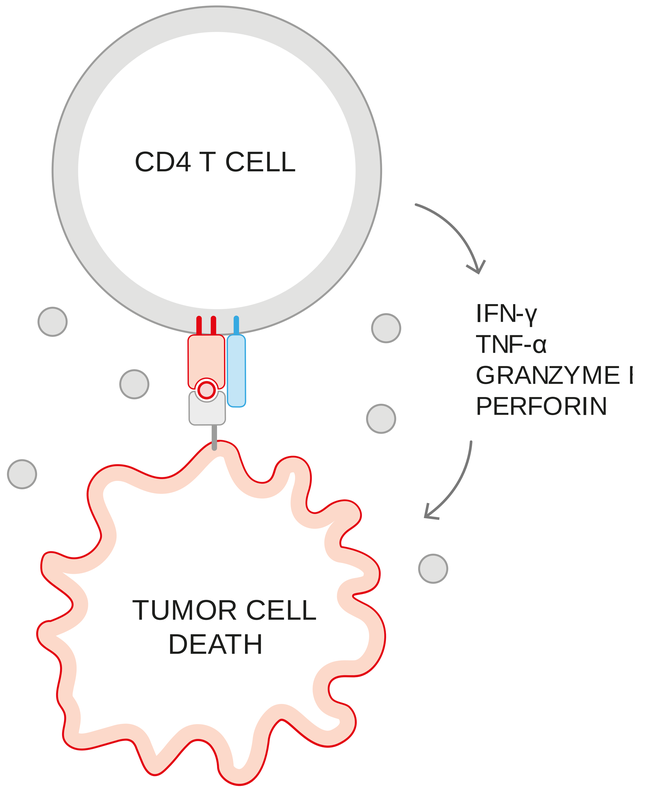
www.immatics.com
info@immatics.com

Improved Anti-tumor Activity of Next-generation TCR-engineered T cells through CD8αβ Co-expression

Gagan Bajwa, Justin Gunesch*, Inbar Azoulay-Alfaguter, Melinda Mata, Ali Mohamed, Mamta Kalra#, Steffen Walter#*

Harnessing the Anti-tumor Potency of CD4 T cells

DIRECT CYTOTOXICITY VIA TCR-CD8-pHLA INTERACTION



HELP TO CD8+ CYTOTOXIC T CELL

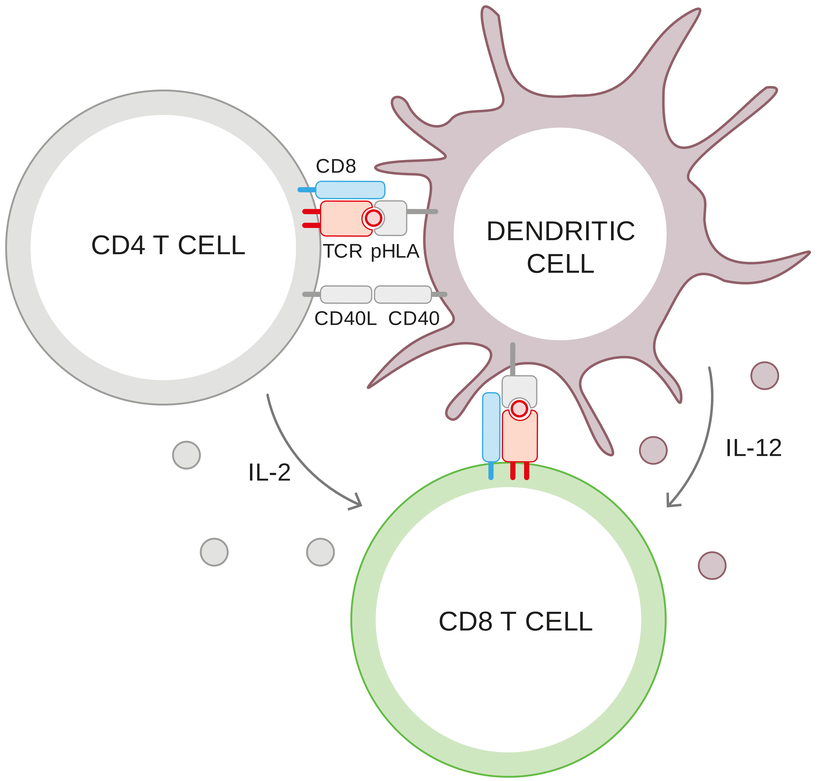


Figure 1: Polyfunctional role of CD4 T cells in anti-tumor response: Upon antigen-specific activation, CD4 T cells elicit effector cytokines and mediate direct cytotoxicity. In addition, CD4 T cells provide help to cytotoxic CD8 T cells through direct and indirect mechanisms.

*Joint-First Authorship, # Joint-Last Authorship

Immatics Biotechnologies GmbH
Immatics US, Inc.

www.immatics.com
info@immatics.com

Improved Anti-tumor Activity of Next-generation TCR-engineered T cells through CD8αβ Co-expression

Gagan Bajwa, Justin Gunesch*, Inbar Azoulay-Alfaguter, Melinda Mata, Ali Mohamed, Mamta Kalra#, Steffen Walter#*

CD8 Co-receptor Exists as a Homodimer and a Heterodimer

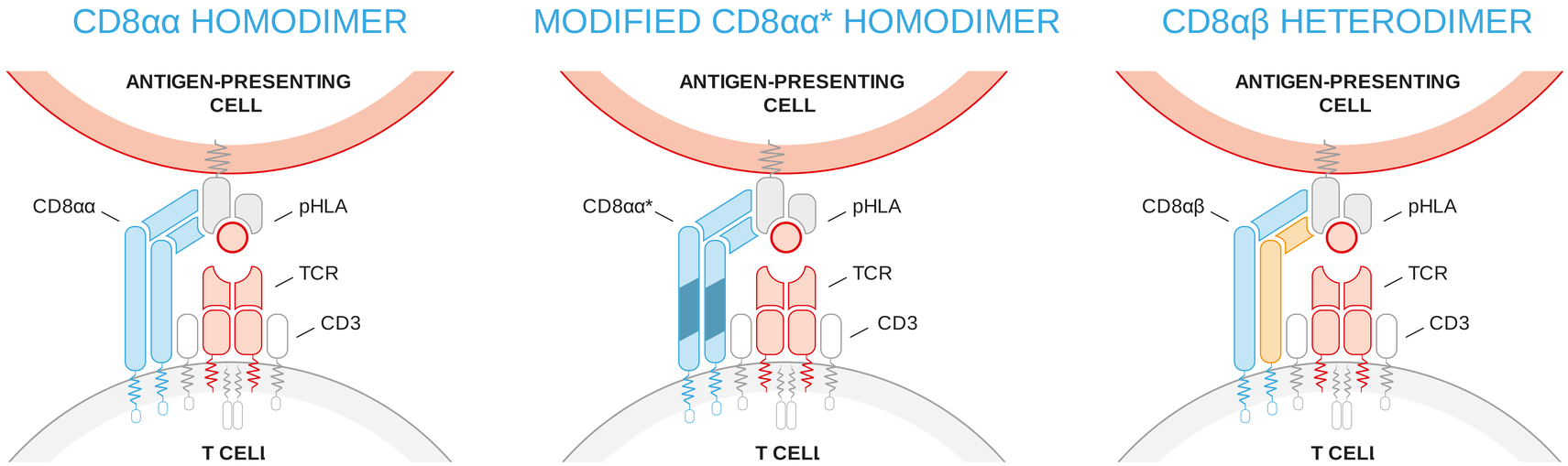


Figure 2: Schematic of different isoforms of CD8 co-receptor and interaction with peptide HLA (pHLA) complex for antigen recognition via TCR. Naturally, the CD8 receptor exists as a homodimer and a heterodimer differentially expressed in various immune cell subsets. A proprietary modified version of CD8α homodimer (CD8αα*) was engineered for a comprehensive functional evaluation in comparison with the wild type homodimer and heterodimer isoforms.

Study Objectives

We evaluated the impact of co-expression of different types of CD8 co-receptors (CD8 homodimer, modified homodimer and heterodimer) on the functionality of CD4+ and CD8+ T cells engineered with a TCR directed to an HLA-A*02-presented PRAME peptide (IMA203 TCR) and determined the depth and durability of anti-tumor response *in vitro*.

*Joint-First Authorship, # Joint-Last Authorship

Immatics Biotechnologies GmbH
Immatics US, Inc.

www.immatics.com
info@immatics.com

Improved Anti-tumor Activity of Next-generation TCR-engineered T cells through CD8αβ Co-expression

Gagan Bajwa, Justin Gunesch*, Inbar Azoulay-Alfaguter, Melinda Mata, Ali Mohamed, Mamta Kalra#, Steffen Walter#*

CD8 Co-receptor Enables pHLA Tetramer Binding of HLA Class I-specific TCR in CD4+ T cells

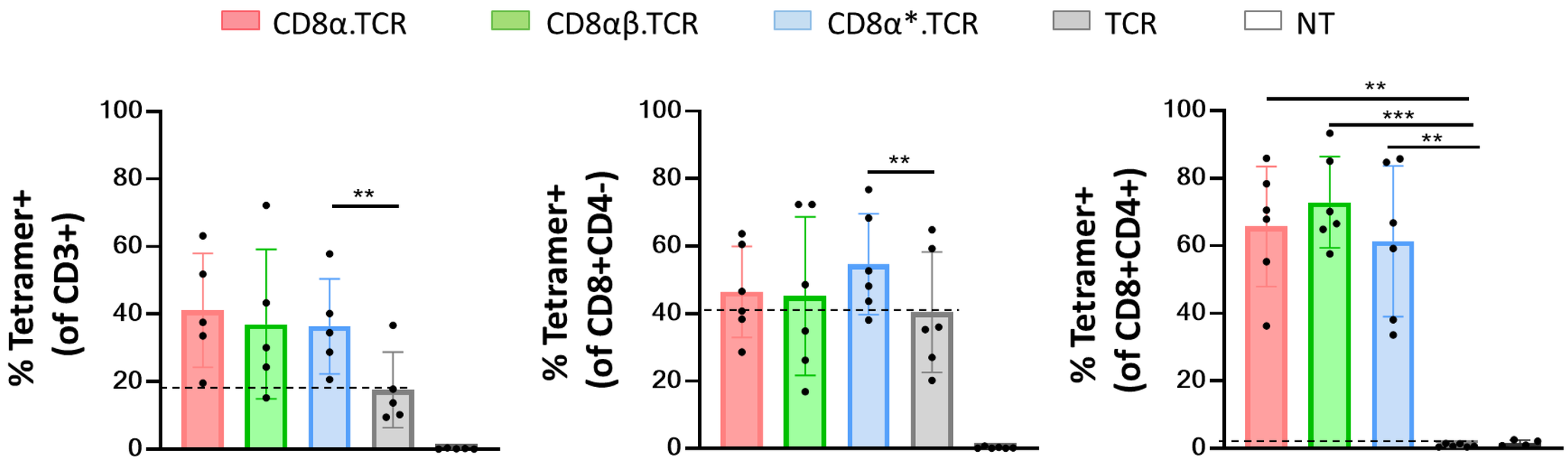


Figure 3: Healthy donor PBMC were transduced with a vector expressing TCR and CD8α homodimer (CD8α.TCR) or CD8α* homodimer (CD8α*.TCR) or CD8αβ heterodimer (CD8αβ.TCR) or TCR alone (TCR) and antigen-specific tetramer binding was measured by flow cytometry. Cumulative data on tetramer+ cells among total CD3+ cells (left), total CD8+ (middle) and total CD4+CD8+ cells (right) are shown. NT=Non-transduced control. N=5-6, mean±SD, p values based on one-way ANOVA/Tukey's multiple comparisons (**p<0.01, ***p<0.001).

*Joint-First Authorship, # Joint-Last Authorship

Immatics Biotechnologies GmbH
Immatics US, Inc.

www.immatics.com
info@immatics.com

Improved Anti-tumor Activity of Next-generation TCR-engineered T cells through CD8αβ Co-expression

Gagan Bajwa, Justin Gunesch*, Inbar Azoulay-Alfaguter, Melinda Mata, Ali Mohamed, Mamta Kalra#, Steffen Walter#*

CD8αβ.TCR+ and CD8α*.TCR+ CD4+ T cells Elicit Broader Anti-tumor Response upon Antigen Stimulation as Compared to CD8α.TCR+ cells

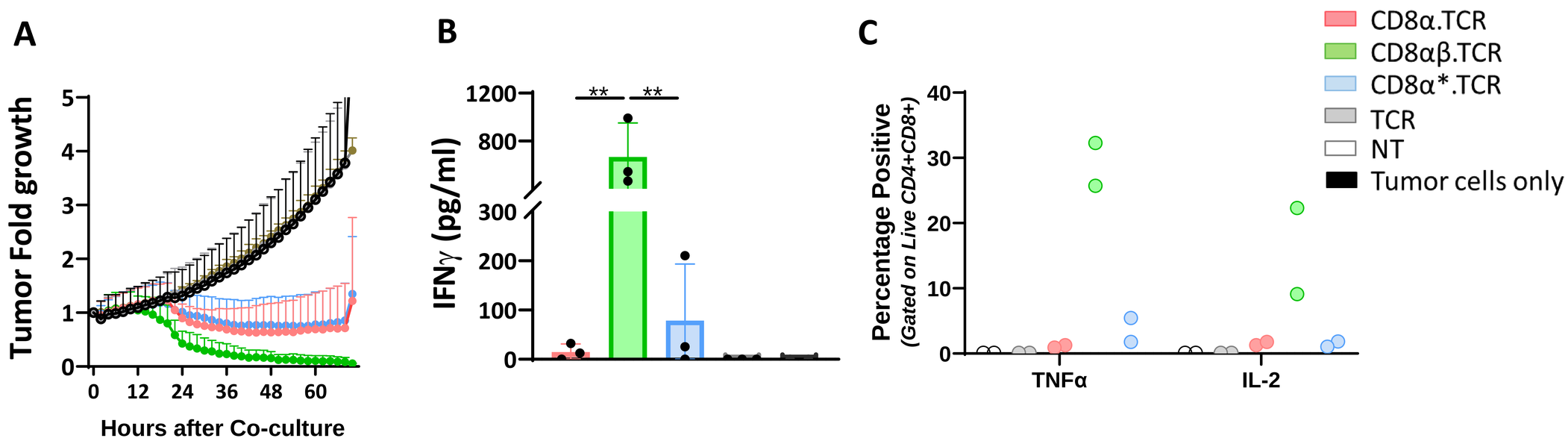


Figure 4: CD8α.TCR+, CD8αβ.TCR+, or CD8α*.TCR+ CD4+ T cells were co-cultured with target-positive UACC257-RFP tumor cell line at an E:T ratio of 4:1 and **(A)** Tumor fold growth was measured using IncuCyte® live-cell analysis system, n=4 mean±SD. **(B)** IFNγ quantification in the supernatants after 24 h of coculture using ELISA, n=3, mean±SD; p values based on one-way ANOVA/Tukey's multiple comparisons. **(C)** Intracellular TNFα and IL-2 expression on CD4+CD8+ subset measured by flow cytometry, n=2.

Based on the functionality of CD4 T cells, further evaluation focused on CD8αβ.TCR and CD8α*.TCR only

*Joint-First Authorship, # Joint-Last Authorship

Immatics Biotechnologies GmbH
Immatics US, Inc.

www.immatics.com
info@immatics.com

Improved Anti-tumor Activity of Next-generation TCR-engineered T cells through CD8αβ Co-expression

Gagan Bajwa, Justin Gunesch*, Inbar Azoulay-Alfaguter, Melinda Mata, Ali Mohamed, Mamta Kalra#, Steffen Walter#*

CD8αβ.TCR+ T cells Are Polyfunctional & Secrete Higher Levels of Th1 Cytokines in Response to Antigen Stimulation

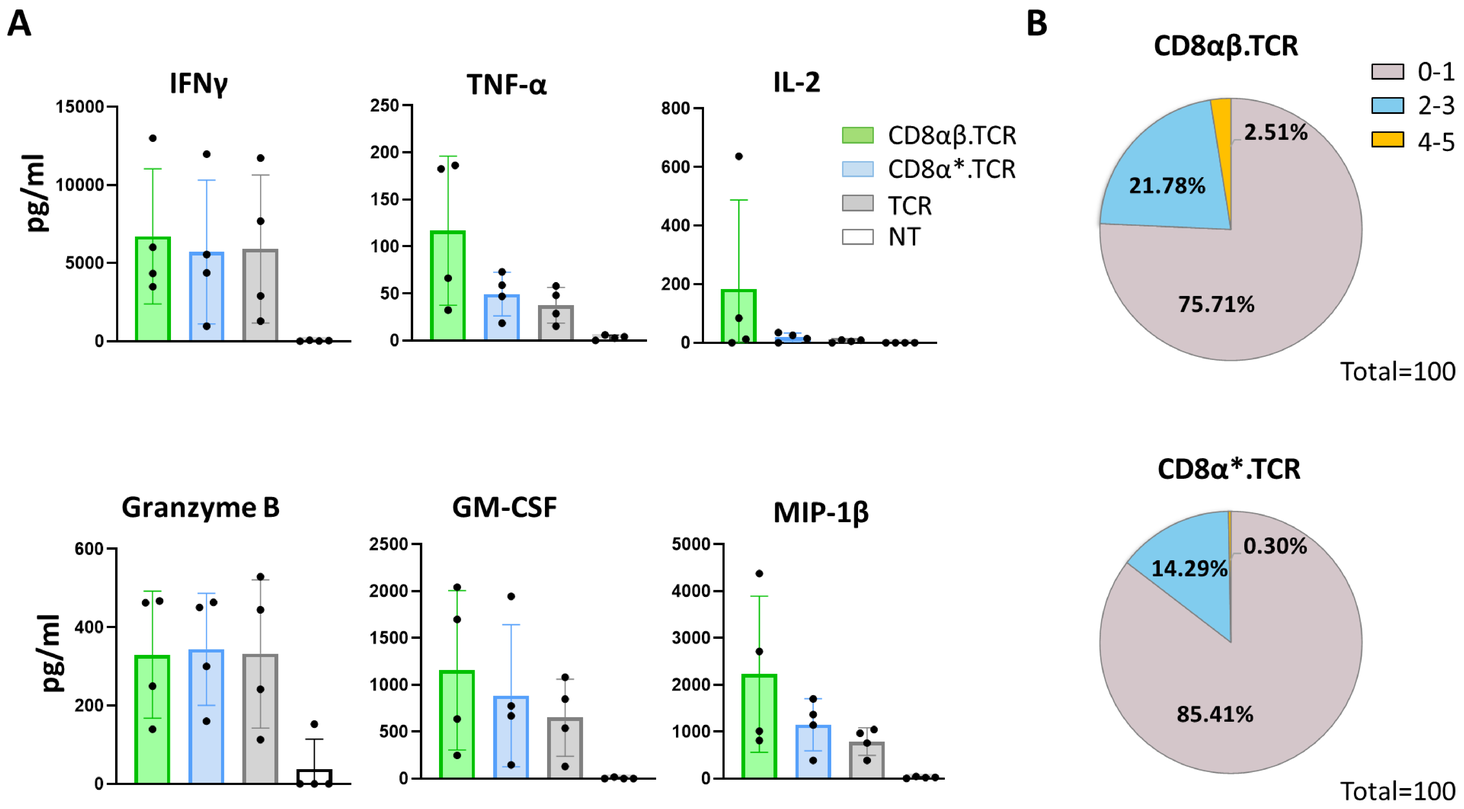


Figure 5: (A) CD8αβ.TCR+, CD8α*.TCR+, TCR+ or NT T cells were co-cultured with UACC257 tumor cells at an E:T ratio of 4:1 (effectors normalized to %CD3+Tet+) for 24 h and cytokines were quantified in the supernatants by multiplex cytokine assay; n=4, mean±SD. **(B)** Polyfunctional responses (assessed by intracellular cytokine staining) of CD4+CD8+ T cells from PBMC-derived products transduced with either CD8αβ.TCR (top) or CD8α*.TCR (bottom) co-cultured with target-positive UACC257 tumor cells. Depicted are the percentages of cells expressing 0-1, 2-3, or 4-5 effector molecules; n=3.

*Joint-First Authorship, # Joint-Last Authorship

Immatics Biotechnologies GmbH
Immatics US, Inc.

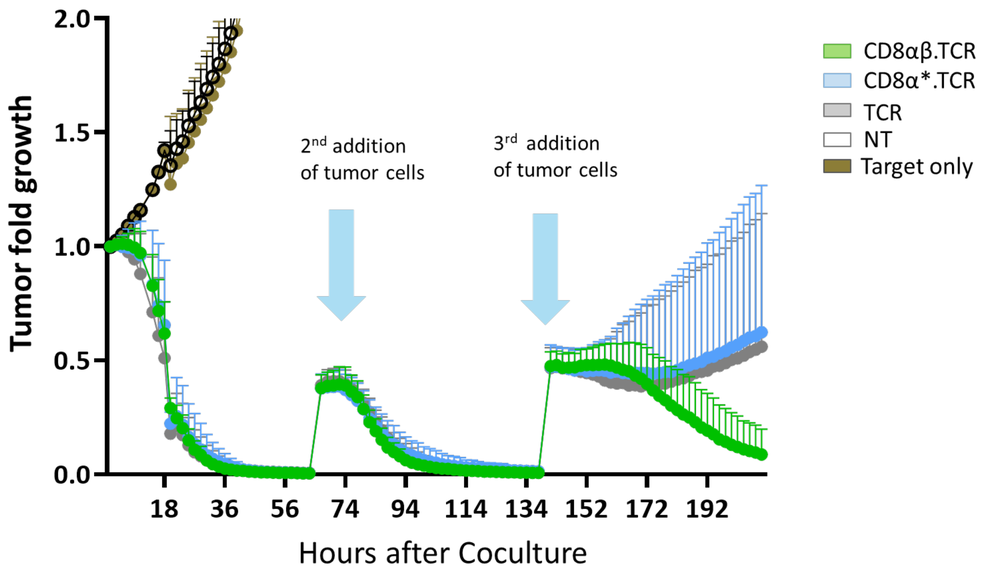
www.immatics.com
info@immatics.com

Improved Anti-tumor Activity of Next-generation TCR-engineered T cells through CD8αβ Co-expression

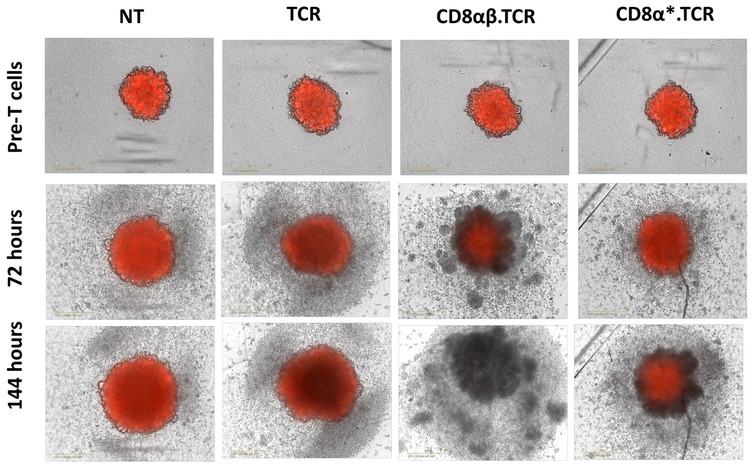
Gagan Bajwa, Justin Gunesch*, Inbar Azoulay-Alfaguter, Melinda Mata, Ali Mohamed, Mamta Kalra#, Steffen Walter#*

CD8αβ.TCR+ T cells Show Sustained Suppression of Tumor Growth in Long-term Killing Assays *in vitro*

A **Serial killing assay with PBMC product**



B **Spheroid killing assay with selected CD4+ T cells**



C **Kinetics of 3D-spheroid tumor cell killing by PBMC, CD8+ and CD4+ products**

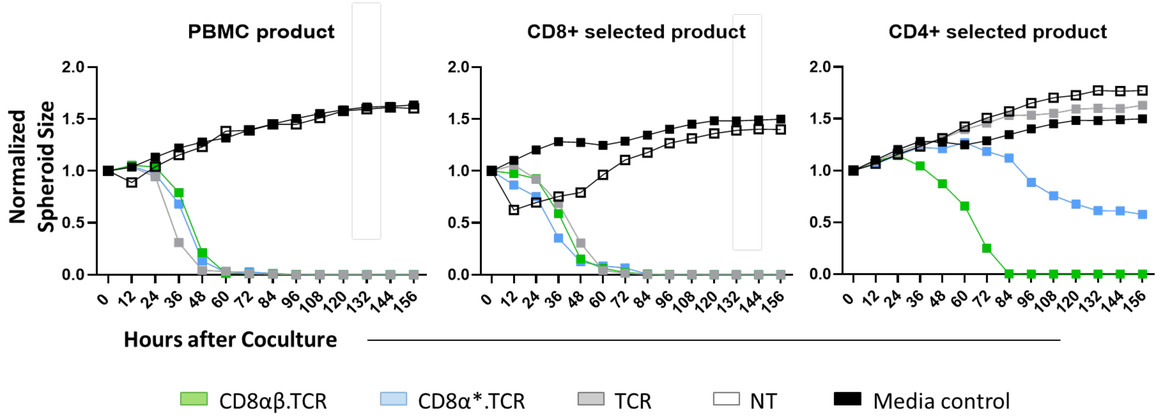


Figure 6: (A) CD8αβ.TCR+, CD8α*.TCR or TCR+ T cells were co-cultured with UACC257-RFP tumor cell line at an E:T ratio of 4:1 (effectors normalized to %CD3+Tet+) for 10 days, every 3 days T cells were rechallenged with fresh tumor cells and tumor fold growth (normalized to day 0) was analyzed using IncuCyte® live-cell analysis system, n=4, mean±SD. **(B)** Representative bright field and fluorescent images of UACC257-RFP spheroids after co-culture with selected CD4+ T cells from transduced PBMC products. **(C)** Spheroid size analysis over 156 hours post addition of PBMC-derived products or CD8+ or CD4+ selected T cells. Data shown normalized to last acquired time point prior to T cell addition. Data shown for one of three donor products.

*Joint-First Authorship, # Joint-Last Authorship

Immatics Biotechnologies GmbH
Immatics US, Inc.

www.immatics.com
info@immatics.com

Improved Anti-tumor Activity of Next-generation TCR-engineered T cells through CD8αβ Co-expression

Gagan Bajwa, Justin Gunesch*, Inbar Azoulay-Alfaguter, Melinda Mata, Ali Mohamed, Mamta Kalra#, Steffen Walter#*

CD8αβ.TCR+ CD4+ T cells Demonstrate More Efficient Engagement with Dendritic Cells and Modulation of Pro-inflammatory Cytokines

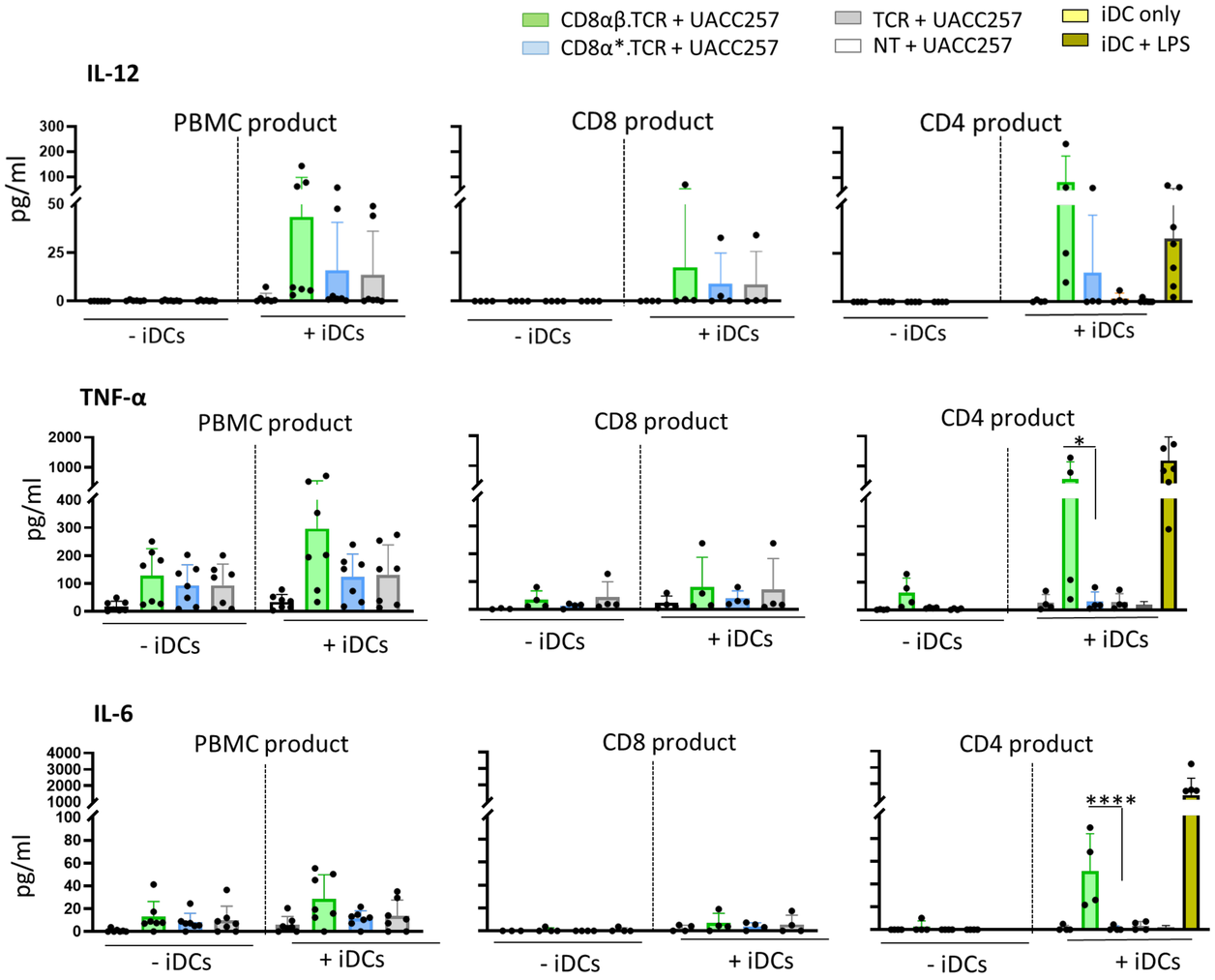


Figure 7: Tri-cocultures of PBMC-, CD8+ selected- or CD4+ selected-product with UACC257 tumor cell line in the presence or absence of autologous monocyte-derived immature dendritic cells (iDCs) followed by cytokine quantification using multiplex assay; iDCs alone or with LPS as controls, n=4-7, mean±SD, p values based on 2-way ANOVA (*p<0.5, ****p<0.0001)

*Joint-First Authorship, # Joint-Last Authorship

Immatics Biotechnologies GmbH
Immatics US, Inc.

www.immatics.com
info@immatics.com

Improved Anti-tumor Activity of Next-generation TCR-engineered T cells through CD8αβ Co-expression

Gagan Bajwa, Justin Gunesch*, Inbar Azoulay-Alfaguter, Melinda Mata, Ali Mohamed, Mamta Kalra#, Steffen Walter#*

Engaging CD4+ T cells via CD8αβ Co-expression Potentiates Anti-tumor Activity of HLA class I-specific TCR-T cells

- Differential functional profile of TCR-T cells co-expressing either CD8α, CD8α* or CD8αβ suggests that optimizing the type of co-receptor is relevant to maximize anti-tumor response and reveals CD8αβ to be the optimal co-receptor for the IMA203 PRAME TCR.
- Engaging CD4+ PRAME-directed T cells via CD8αβ co-expression
  - imparts cytotoxic potential to CD4+ T cells to suppress tumor growth in serial killing assays and 3D spheroid models *in vitro*
  - confers CD4+ T cells polyfunctionality to secrete Th1 cytokines and cytotoxic effector molecules upon antigen stimulation
  - induces efficient engagement with antigen-presenting cells and modulation of pro-inflammatory cytokine response
- Pleiotropic effects mediated by activated CD4+ T cells including acquired cytotoxicity and helper activity may potentially improve outcomes in solid tumor patients in clinical settings.
- We plan to implement CD8αβ co-expression into IMA203 clinical development via a next-generation IMA203CD8 cohort in 2022.

Acknowledgements

The authors acknowledge contributions of Siyu Ren, Kelsey Howard, Xavier Legras, Victor Cardenas, and Erik Farrar in completion of this work and Sabrina Schecher for significant support in preparation of this poster.

*Joint-First Authorship, # Joint-Last Authorship

Immatics Biotechnologies GmbH
Immatics US, Inc.

www.immatics.com
info@immatics.com