A Synthetic Cytokine Receptor Platform for Producing Cytotoxic Innate Lymphocytes as Off-the-Shelf Cancer Therapeutics

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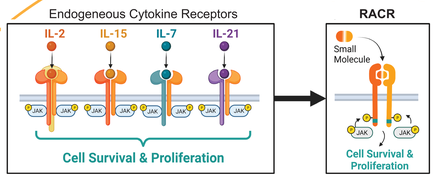
Teisha Rowland, Samantha D. O'Hara, David Vereide, Ryan Koning, Michele Hoffmann, Ashley Yingst, Chris Nicolai, Mark Pankau, Kristen Mittelsteadt, Kathryn Michels, Seungjin Shin, Laurie Beitz, Byoung Ryu, Ryan Crisman, Andrew Scharenberg, Chris Garbe, and Ryan P. Larson. **Umoja BioPharma, Boulder, CO & Umoja BioPharma, Seattle, WA**

For questions about this poster, contact:
Teisha Rowland, Principal Scientist
Teisha.Rowland@umoja-biopharma.com

Introduction

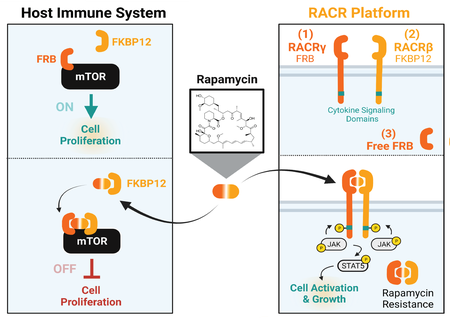
• While **chimeric antigen receptor (CAR) T cell therapies** have revolutionized the treatment of blood-forming tissue cancers (i.e., hematologic malignancies), **significant challenges** with using patient-derived materials remain, including: limited expansion capacity and scalability, manufacturing complexity, high cost, variability from patient to patient, and patient access. As part of our mission to deliver "off-the-shelf" cancer therapies to overcome these challenges, Umoja Biopharma is developing an **Engineered iPSC Platform**. This platform aims to utilize iPSCs to enable scalable, virtually unlimited, and simplified manufacturing of precisely engineered, cancer-fighting cytotoxic innate lymphocytes.
• Other key challenges in the allogeneic therapy space include **engraftment and persistence**, both necessary for tumor remission but challenging to achieve due to the host's anti-allograft response. Consequently, such treatments usually require toxic chemotherapy administration. To address this, we are developing a synthetic cytokine receptor system, the rapamycin-activated cytokine receptor (RACR), or **RACR platform**.

RACR Platform: A synthetic receptor platform that replicates proliferative cytokine signaling



• We have engineered a synthetic chimeric receptor system, the **RACR platform**. Upon **small molecule rapamycin** binding, RACR induces a signal analogous to IL-2 and IL-15 cytokine signaling, mimicking proliferative JAK/STAT signaling. Thus, rapamycin addition causes selective cell survival and proliferation.

• Umoja Biopharma's engineered iPSCs are modified to express RACR. Rapamycin addition drives differentiation and expansion of these lymphocytes we term **RACR-induced Cytotoxic Innate Lymphoid (iCIL) cells**, giving iCIL cells a growth advantage while eliminating the need to add expensive cytokines and other raw materials.
• ***Why rapamycin?*** Rapamycin is an FDA-approved drug that suppresses proliferation of immune cells that are not expressing RACR through inhibition of mTOR (molecular target of rapamycin) by binding FKBP12 and mTOR's FRB domain. In a patient, rapamycin inhibits the host immune response. Simultaneously, rapamycin is expected to bolster iCIL cell expansion *in vivo*.
• **To create RACR**, we combined the FRB and FKBP12 rapamycin-binding domains extracellularly with cytokine signaling domains intracellularly. Thus, rapamycin administration induces cytokine-like cell proliferation. A "free FRB" is also incorporated to neutralize rapamycin -FKBP12 complexes, prevening mTOR suppression.

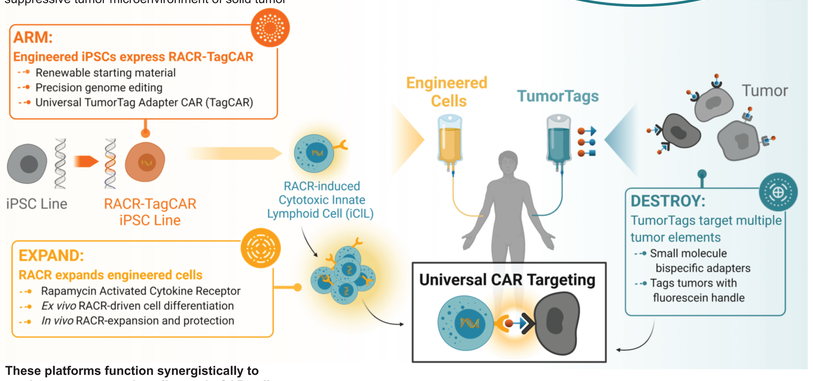


TumorTag Platform: Targeting cancer cells

• RACR-iCIL cells are designed to target and destroy tumor cells through a TagCAR that engages with cells labeled with Umoja Biopharma's **TumorTags**.
• While a significant challenge in the immunotherapy field is unpredictable efficacy of solid tumor treatments due to hostile tumor microenvironments (TME) and heterogeneity, our **TumorTag platform** aims to improve efficacy by labeling TME targets with a cocktail of TumorTags.

Summary

Umoja Biopharma's Engineered iPSC Platform has the potential to address key limitations of current allogeneic CAR cell therapies:
• Reducing manufacturing complexity, cost, and variability to make better cells
• Eliminating lymphodepletion and enhancing *in vivo* persistence of cells
• Universal CAR technology for combinatorial targeting of tumor antigens and the suppressive tumor microenvironment of solid tumor



These platforms function synergistically to produce next-generation allogeneic CAR cell product candidates:

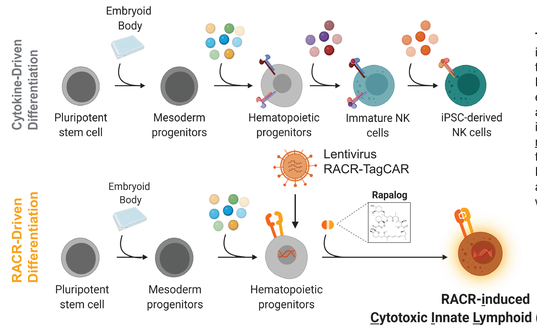
• **Engineered iPSCs (ARM)**: Our modified iPSCs have the potential to provide a renewable starting material for scalable manufacturing of synthetic allogeneic CAR cell products, here termed RA-CR-iCIL cells.

• **RACR (EXPAND)**: RACR has the potential to enable cytokine-free manufacturing and remove the need for toxic lymphodepletion through protection and expansion of RA-CR-iCIL cells in patients.

• **TumorTag (DESTROY)**: Our TumorTag Platform has the potential to enable combinatorial targeting of patient tumors, addressing challenges with tumor heterogeneity, antigen escape, and the immunosuppressive tumor microenvironment.

Methods

Generating RACR-induced Cytotoxic Innate Lymphoid (RACR-iCIL) cells



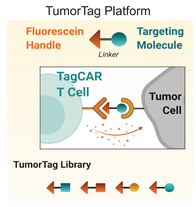
To generate RACR-iCIL cells from human iPSCs, iPSCs were differentiated into hematopoietic progenitors (HPs) and transduced with lentivirus containing RACR-TagCAR construct. Rapalog dosing of transduced cells induced RACR signaling, driving RACR-mediated differentiation and growth to generate RACR-iCILs. Normally, deriving innate lymphoid cells (ILCs), natural killer (NK) cells, or T cells requires multi-step, time-sensitive addition of growth factors and cytokines; RACR-iCILs generated here grew independent in the absence of cytokines. Differentiation and expansion was quantified using flow cytometry analysis.

Quantifying TagCAR and TumorTag-mediated anti-cancer activity of RACR-iCIL cells

To quantify innate anti-cancer activity of RACR-iCIL cells, expression of cytotoxicity receptors was assessed via flow cytometry and cancer cell killing via co-culture with an MDA breast cancer line.

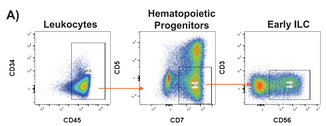
To quantify TagCAR and TumorTag-mediated anti-cancer activity of RACR-iCIL cells, RACR-iCIL cells expressing a universal TagCAR were co-cultured with MDA breast cancer cells and TumorTags added. These TumorTags (UB-TT170) are small molecule adapters containing a fluorescein tag recognized by the TagCAR and a targeting molecule that is bound by a tumor cell receptor (folate was used to target folate receptors). Upon binding the tumor cell, RACR-iCILs initiate killing. Cells were observed fluorescently to quantify cancer killing.

Umoja Biopharma's TumorTag Platform includes a suite of TumorTags with various targeting molecules. These fluorescein-conjugated targeting molecules are designed to enable diverse, multi-antigen targeting with a TagCAR construct.

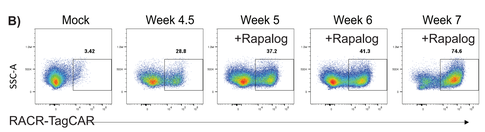


Results

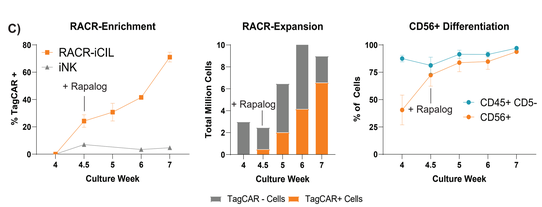
RACR activation mediates selective expansion and differentiation of RACR-iCILs



A) Prior to transduction with lentivirus containing RACR-TagCAR, the iPSC-derived differentiating cells (following ~4 weeks of diferentiation) contained a mix of mostly blood-derived progenitors, based on CD45+ expression via flow cytometry analysis. Of the CD45+ cells, there were common lymphoid progenitors (CD7+ CD5+), ILC progenitors (CD7+ CD5-), and others (CD5- CD7-). Of the ILC progenitors, a subset expressed CD56, suggesting early differentiation into ILCs or NK cells.

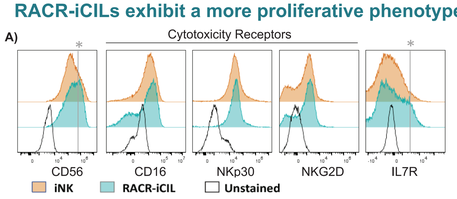


B) Following transduction with lentivirus containing RACR-TagCAR (at week 4), RACR-TagCAR+ cells were enriched for over time using rapalog dosing to activate RACR, shown here via flow cytometry analysis.

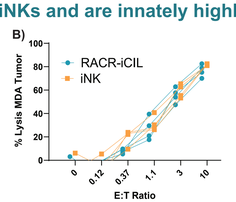


C) Following transduction with lentivirus containing RACR-TagCAR (at week 4), RACR activation enriched, expanded, and differentiated RACR-expressing, CD56+ RACR-iCILs. (**left panel**) Rapalog dosing of transduced cells enriched for RACR-TagCAR+ cells over time, as measured by percent TagCAR+, compared to non-transduced iPSC-derived NK (iNK) cells. (**middle panel**) Rapalog dosing of transduced cells selective expanded RACR-TagCAR+ cells over time, as measured by total cell counts of TagCAR+ and TagCAR- cells. (**right panel**) Rapalog dosing of transduced cells supported differentiation of CD56+ cells, suggesting RACR can drive cell differentiation in place of common gamma chain cytokines.

RACR-iCILs exhibit a more proliferative phenotype than iNKs and are innately highly cytotoxic



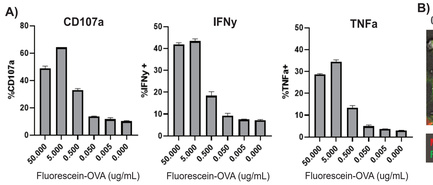
A) RACR-iCILs had increased CD56+ and IL7R+ expression compared to cytokine-dervied iNKs, suggesting a potentially more proliferative state. Both cells showed high expression of cytotoxicity receptors NKp30 and NKG2D. Immunophenotyping performed via flow cytometry analysis.



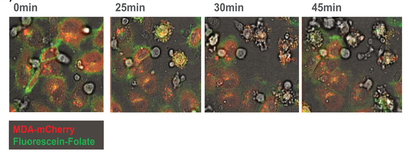
B) RACR-iCILs were innately highly cytotoxic, with innate killing of an MDA breast cancer line comparable to that of cytokine-derived iNKs.

Results cont.

RACR-iCILs exhibit potent anti-tumor activity mediated by TagCAR and TumorTag

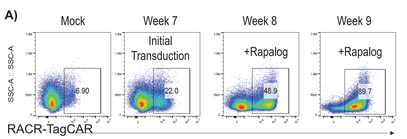


A) RACR-iCILs expressing TagCAR released graules and cytokines (CD107a, IFNγ, and TNFa) in response to being exposed to plate-bound fluorescein. Because the TagCAR recognizes fluorescein, these data indicate that RACR-iCILs possess robust TagCAR function.

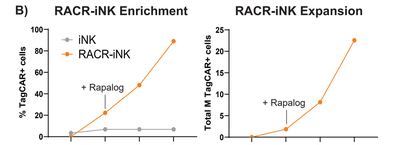


B) RACR-iCILs expressing TagCAR co-cultured with MDA breast cancer cells (red) with the addition of TumorTag (fluorescein-folate UBTT170; green) were targeted and killed by the RACR-iCILs, demonstrating TagCAR-mediated cancer cell killing of RACR-iCILs.

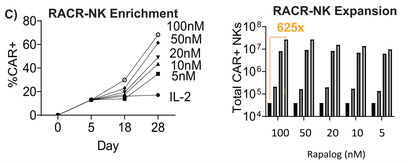
RACR activation mediates selective expansion and enrichment of transduced iNKs



A) iPSCs were differentiated into NK cells (iNKs) using cytokines (~6 weeks) and then transduced with lentivirus containing RACR-TagCAR. At week 7 (1 week following transduction), RACR-TagCAR+ iNKs were enriched for over time using rapalog dosing to activate RACR, shown here via flow cytometry analysis.



B) Following transduction with lentivirus containing RACR-TagCAR (at week 6), RACR activation via rapalog dosing enriched and expanded RACR-expressing iNKs, with enrichment measured by percent TagCAR+, compared to non-transduced iNKs, and expansion measured by total cell counts of TagCAR+ cells.



C) iNKs transduced with RACR-TagCAR showed enrichment and expansion of TagCAR+ iNKs in a rapalog dose-dependent manner, with >625 fold expansion of TagCAR+ iNKs from day 7 to 28 of rapalog dosing.

Conclusions

RACR

• RACR-iCILs can be selectively expanded, enriched, and CD56+ differentiation supported via RACR activation through rapalog dosing
• RACR-iCILs possess a proliferative phenotype
• iNKs transduced with RACR-TagCAR can be similarly selectively expanded and enriched via RACR activation through rapalog dosing
• Taken together, these data demonstrate the ability of RACR to replace cytokines and its potential to drive *in vivo* expansion

TumorTag

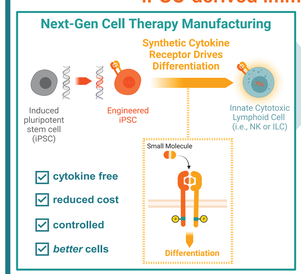
• RACR-iCILs are innately highly cytotoxic, and exhibit potent targeted anti-tumor activity when used in combination with Umoja Biopharma's TagCAR and TumorTag Platforms

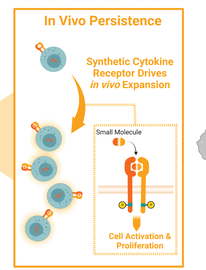
Engineered iPSCs

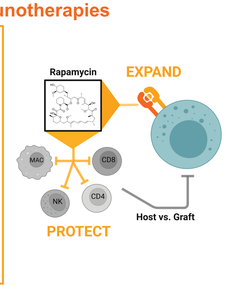
In summary, these data suggest that RACR activation can be used to replace cytokine addition during differentiation to generate highly-controlled, cytoxic cells, termed here RACR-induced cytotoxic innate lymphoid (RACR-iCILs) cells.

Future Steps:

Enaging RACR for simplified and controlled manufacturing of precisely engineered, iPSC-derived immune cell products for cancer immunotherapies







The RACR has the potential to remove the need for complex GMP cytokines in deriving immune cells, reducing the variability, and increasing the control of cell production to make better cells.

Our synthetic cell product is then given to patients and we engage the RACR for in vivo expansion driving selective proliferation of our cell therapy product without the need for endogenous or exogenous cytokines. Rapamycin simultaneously expands and protects our cells through RACR-signaling and suppression of host anti-graft responses without the need for toxic lymphodepletion regimens.

References

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• Juillerat A, Marechal A, Fihol JM, et al. Design of chimeric antigen receptors with integrated controllable transient functions. Sci Rep. 2016;6:18950.