A synthetic cytokine receptor platform for producing cytotoxic innate lymphocytes as "off-the-shelf" cancer therapeutics

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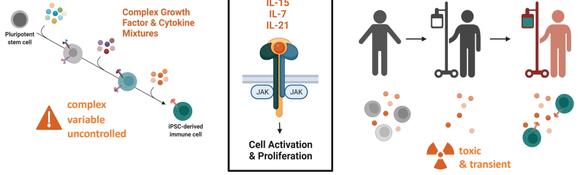
Executive Summary

Umoja BioPharma is a multi-platform immune-oncology company focused on "off the shelf" curative cell therapies for hematologic and solid tumors.

Umoja's iPSC cell therapy 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 tumors.

Three integrated technology platforms to make next-generation allogeneic CAR cell product candidates:
- Engineered iPSCs (ARM): Precision editing of induced pluripotent stem cells (iPSCs), intended to provide a renewable starting material for the scalable manufacturing of synthetic allogeneic CAR cell products.
- RACR (EXPAND): A synthetic cytokine receptor that has the potential to enable cytokine-free manufacturing and remove the need for lymphodepletion through simultaneous protection and expansion of cell product *in vivo*.
- TumorTag (TARGET): Combinatorial targeting designed to address tumor heterogeneity, antigen escape and immunosuppressive tumor microenvironment (TME).

Common gamma chain cytokines are essential for lymphoid cell growth

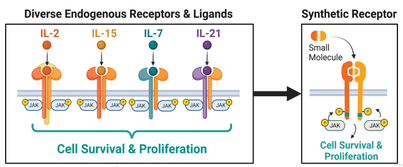


The family of common gamma chain cytokines (IL-2, IL-15, IL-7, IL-21) are required to support cell growth during both *ex vivo* manufacturing as well as for *in vivo* expansion. Diverse inputs all to induce cell activation and proliferation.

Cell Therapy Manufacturing: To derive an immune cell from iPSCs requires addition of many different growth factors to push the cells down the desired differentiation pathway. Common gamma chain cytokines are essential during the transition from hematopoietic progenitors to a fully differentiated lymphoid cells, such as an innate lymphoid cell (ILC) or natural killer (NK) cell. The requirement for four different GMP cytokine inputs at different times during differentiation makes this process complex, expensive, and hard to control.

In Vivo Expansion: These cytokines are also essential for cell therapy products to expand and grow *in vivo* but must compete with the cells of the patient's endogenous immune system that also require these cytokines for growth. In order to remove this competition, a toxic prior treatment regiment called lymphodepletion (LD) is used to deplete the endogenous immune system, opening up a "cytokine niche" for the cell therapy product to expand. However, LD is a transient solution and the patient's immune system quickly reconstitutes, thus reducing the free cytokines in the system as well as introducing anti-graft responses against the cell therapy product. Multiple rounds of LD are required for re-dosing patients, and due to the toxic nature of LD patients can become ineligible for additional rounds of therapy.

A synthetic cytokine receptor can replicate the signal of endogenous cytokine receptors



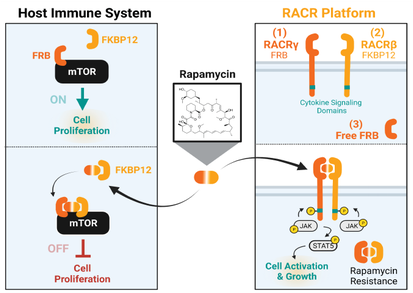
We have engineered a synthetic cytokine receptor that can mimic the JAK/STAT signal downstream of common gamma chain cytokines, thus inducing cell survival and growth. This receptor is called the RACR, Rapamycin Activated Cytokine Receptor.

RACR: Rapamycin Activated Cytokine Receptor

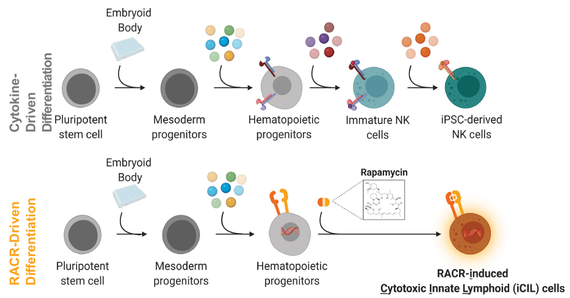
Rapamycin is an FDA approved drug that blocks cell proliferation through inhibition of mTOR (molecular target of rapamycin), an essential pathway for cell growth. Rapamycin is a small molecule that acts as a molecular glue, first binding to FKBP12 in cells and then inducing dimerization of the rapamycin-FKBP12 complex with the FRB domain of mTOR and subsequent block of kinase activity.

We have co-opted the natural dimerization ability of rapamycin to create a receptor, with FRB and FKBP12 domains expressed extracellularly and cytokine signaling domains expressed intracellularly. Thus, rapamycin dimerizes this receptor and induces a JAK/STAT signal for cell proliferation and growth.

The third component of the RACR platform is a "free FRB" that is expressed in the cytoplasm and neutralizes any intracellular rapamycin-FKBP12 complexes and prevents mTOR suppression of RACR-containing cells.



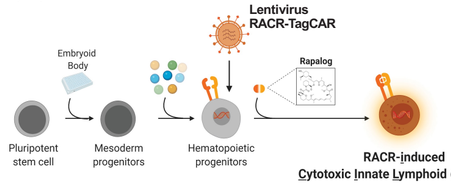
RACR platform provides simplified and controlled manufacturing of synthetic cells



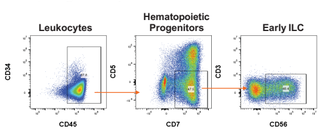
The process of deriving differentiated cells from induced pluripotent stem cells (iPSCs) requires addition of many different exogenous growth factors at different times during differentiation. Common gamma chain cytokines are some of the most important cytokines used in deriving innate lymphocytes (ILCs, NKs) or T cells and require multi-step addition of these cytokines during cell production. Using constitutive RACR signaling to drive cell differentiation and growth, we have derived synthetic cells, termed RACR-iCILs, that grow independent of endogenous cytokine receptor expression.

RACR-differentiation and expansion from iPSC-derived progenitor cells

A) Experimental Outline

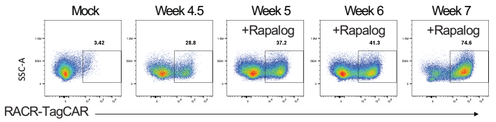


B) Progenitors present at 4 weeks of culture

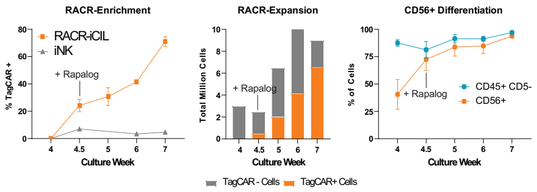


A) iPSCs were differentiated into hematopoietic progenitors and transduced with lentivirus containing RACR-TagCAR. Cells were then placed in rapalog in the absence of cytokines and tested for RACR-mediated differentiation and expansion. **B)** Flow plots demonstrating progenitors present at 4 weeks of culture prior to transduction. Most cells are blood-derived as indicated by CD45 expression, of these cells there are common lymphoid progenitors (CD7+ CD5+), innate lymphoid cell (ILC) progenitors (CD7+ CD5-), and other (CD5-CD7-). Of the ILC progenitors, we see a subset expressing CD56, suggesting early differentiation into ILCs or NK cells. **C)** Flow plots indicating RACR-TagCAR expression over time showing rapalog-mediated enrichment of RACR-TagCAR+ cells. **D)** Quantification of RACR-TagCAR enrichment (increased %) and expansion (total cell counts). Lastly, we measured differentiation as indicated by CD56 expression over time, suggesting RACR can drive cell differentiation in place of common gamma chain cytokines.

C) Flow plots demonstrating enrichment of RACR-TagCAR positive cells



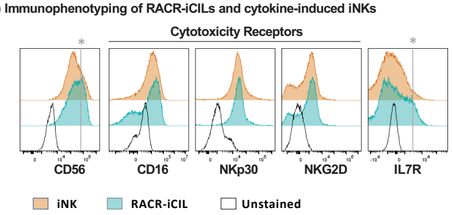
D) Quantification of RACR-TagCAR enrichment and expansion of CD56+ cells



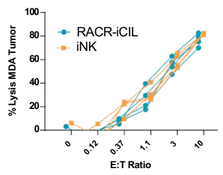
This data suggests that RACR can be used to replace common gamma chain cytokines during differentiation to generate highly controlled synthetic cells, termed RACR-induced cytotoxic innate lymphocytes (RACR-iCILs).

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

A) Immunophenotyping of RACR-iCILs and cytokine-induced iNKs



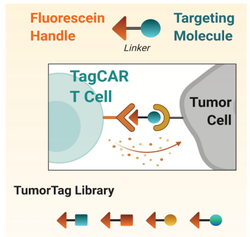
B) RACR-iCILs are highly cytotoxic and comparable to cytokine-derived iNKs



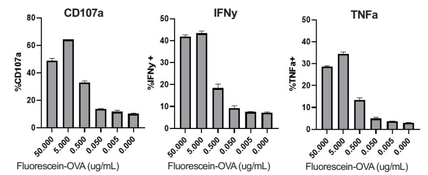
A) RACR-iCILs have increased CD56 and IL7R expression compared to cytokine-dervied iNKs, suggesting a potentially more proliferative state. Both cells show high expression of cytotoxicity receptors, NKp30 and NKG2D. **B)** Comparable innate killing of MDA breast cancer line by NK and RACR-iCILs.

RACR-iCILs exhibit potent anti-tumor activity driven by TagCAR & TumorTag adapters

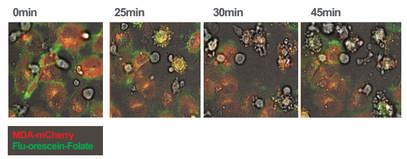
A) Universal TagCAR and TumorTag Platform



B) TagCAR-mediated granule and cytokine release of RACR-iCILs



C) TagCAR-mediated killing of MDA tumor line by RACR-iCILs

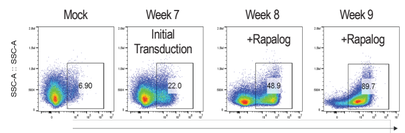


A) Diagram of the TumorTag Platform, consisting of the universal TagCAR and our suite of small molecule TumorTag adapters. The TagCAR recognizes a fluorescein handle that is linked to a targeting molecule. We have a suite of these fluorescein-conjugated small molecules that enable diverse antigen targeting with a single TagCAR construct. **B)** CD107a, IFNγ, and TNFa release from TagCAR RACR-iCILs in response to plate bound fluorescein-ligand indicating robust TagCAR function. **C)** Images of MDA breast cancer cells (red) coated in TumorTag adapter Fluorescein-Folate UBTT170 (green) being targeted by TagCAR RACR-iCILs.

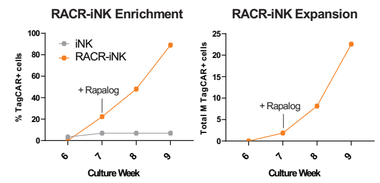
These data demonstrate the ability of RACR-iCILs to kill tumor cells via both innate- and TagCAR-mediated targeting.

RACR expansion of cytokine-derived NK cells

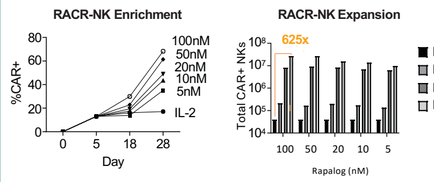
A) Flow plots demonstrating enrichment of RACR-TagCAR+ iNK cells



B) Quantification of RACR-iNK enrichment and expansion

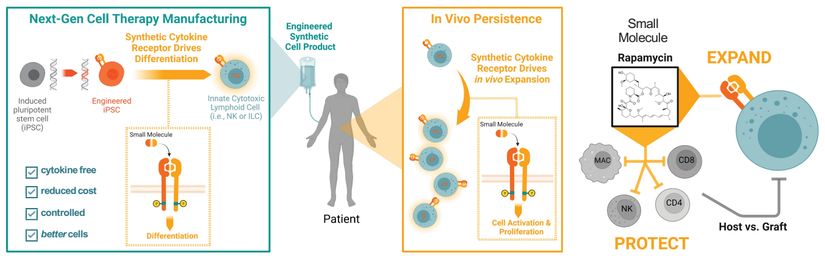


C) Dose-responsive RACR-expansion of NK cells



A) iPSC-derived NK cells were generated using cytokines (~6 weeks) and then transduced with lentivirus containing RACR-TagCAR. Initial transduction was checked at Week 7 and cells were placed into rapalog for expansion. Flow plots of RACR-TagCAR iNK growth over time demonstrating rapalog-mediated enrichment of RACR-TagCAR positive iNK cells. **B)** Quantification of RACR-TagCAR enrichment (increased % RACR-TagCAR+) and expansion (total cell counts) over time. **C)** Lastly, we tested multiple doses of rapalog and showed dose-responsive RACR enrichment and expansion (>625x) of RACR containing NK cells. **These data demonstrate the ability of RACR to replace cytokines and its potential to drive in vivo expansion.**

Engaging a synthetic cytokine receptor (RACR) for next-gen cell products



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.

If successfully developed, our synthetic cell product should enable *in vivo* expansion of the engrafting cells without the need for endogenous or exogenous cytokines. We expect that rapamycin will simultaneously expand and protect our cells through both simulating RACR signaling as well as suppressing host anti-graft responses, all without the need for toxic lymphodepletion regimens.