Synthetic Receptor Enabled Differentiation (ShRED), a novel platform for manufacturing of iPSC-derived cytotoxic innate lymphocytes for "off-the-shelf" cancer immunotherapies

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Executive Summary

Disruptive early clinical stage multi-platform immuno-oncology company focused on "off the shelf" curative cell therapies for hematologic and solid tumors

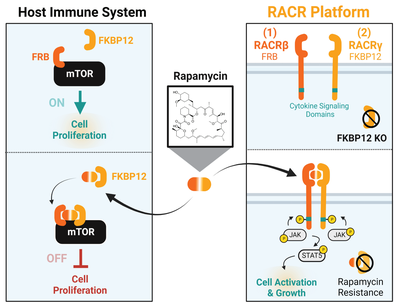
Best in class iPSC-based allogeneic cell therapy platform
- Synthetic Receptor Enabled Differentiation (ShRED) manufacturing process employs a synthetic receptor system to direct differentiation to cytotoxic innate lymphocytes at unprecedented yields, overcoming major CMC challenges
- Synthetic receptor can be activated in vivo to enhance engraftment, expansion, and persistence of therapeutic cells in patients without requirements for toxic lymphodepleting chemotherapy

Ongoing active collaborations speak to our leadership in the field:
- Next-gen bioreactor- and encapsulation-technology for scalable iPSC GMP manufacturing: collaboration with TreeFrog Therapeutics

induced cytotoxic innate lymphocyte (iCIL) therapeutic platform applicable to hematologic malignancies
- Scalable process allows for repeated dosing of high cell numbers that may correlate with deeper, more durable responses

The Rapamycin Activation Cytokine Receptor (RACR): A synthetic STAT5 receptor

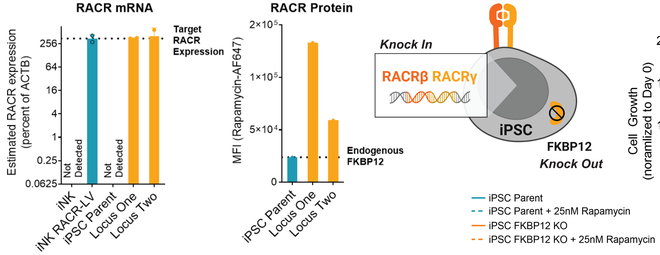
We have engineered a synthetic cytokine receptor that can mimic the JAK/STAT signal downstream of important cytokine and growth factor receptors that are required for immune cell differentiation and expansion.



Rapamycin: 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 forming a rapamycin-FKBP12 complex, which will then bind to the FRB domain of mTOR and subsequently block kinase activity. Rapamycin is commonly used to protect patients' allogeneic solid organ transplant from anti-graft responses.

The rapamycin activated cytokine receptor (RACR): We have co-opted the natural dimerization ability of rapamycin to create a synthetic 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. Lastly, to protect RACR-containing cells from rapamycin suppression we remove endogenous FKBP12 to render cells resistant to rapamycin.

CRISPR-based Editing of iPSCs with the RACR Platform



Arming iPSCs with RACR: iPSCs are genetically modified via CRISPR-based editing to express the RACR. Shown are plots of RACR expression after genetic knock-in at two different loci. **Left** bar-graph shows RACR mRNA levels as measured by qPCR in iPSCs comparison to iNK cells lentivirally transduced to express RACR. **Right** bar-graph is protein level in comparison to unedited iPSC cells as measured by flow cytometry after staining with rapamycin-AF647.

FKBP12 Knock Out: In addition to RACR knock-in, we have also rendered RACR-iPSC cells resistant to rapamycin suppression via removal of endogenous FKBP12. **Above** line-graph shows cell growth and resistance of iPSC edited with FKBP12 KO when grown in 25nM rapamycin compared to unedited control cells. We have observed no changes morphology or growth of iPSCs with loss of FKBP12.

ShRED: Using RACR to drive differentiation & expansion of RACR-induced cytotoxic innate lymphocytes (iCILs)

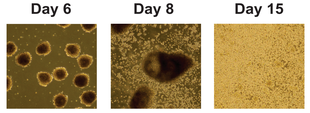
Conventional allogeneic manufacturing process relies on multiple complex raw materials and variable endogenous receptor expression.

Umoja has developed a Synthetic Receptor Enabled Differentiation (ShRED) protocol that employs RACR technology to drive differentiation and expansion in a controlled and consistent manner.

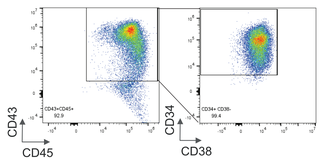
ShRED process generates unprecedented cell yields

ShRED generates Hematopoeitic Progenitor (CD34+) cells well beyond any previously described approach

Embryoid bodies (EB) transition to more than 93% hematopoietic progenitors (HP) by ~2 weeks. High purity of HPs removes any need for filtration or CD34 selection.



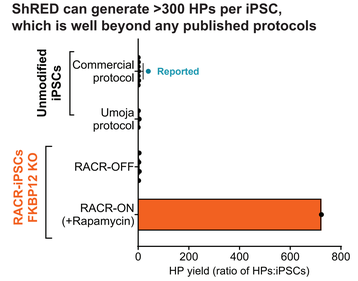
Day 15 HPs are >93% CD45+ CD43+ CD38- CD34+



Left images show iPSC-based aggregates are initially formed (still visible at D6), and by D8 of differentiation these aggregates begin to dissociate as hematopeiotic progenitors (HPs) emerge, resulting in a single-cell culture of HPs by D15.

Left representative flow plots show the high purity of HPs at D15, cells are positive for CD45/CD43/CD34 and CD38neg.

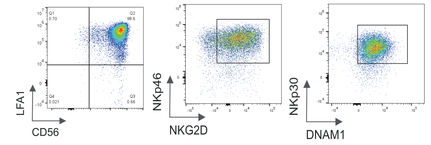
ShRED can generate >300 HPs per iPSC, which is well beyond any published protocols



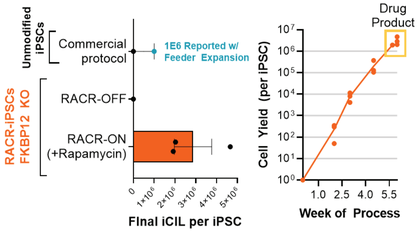
Right bar-graph shows fold expansion yields of HPs per starting iPSC with standard commercially available cell culture protocols in unmodified iPSCs compared to RACR-engineered iPSCs in the presence (ShRED; "RACR on") or absence ("RACR off") of rapamycin. Blue data point represent a reported expansion from public presentations.

Final RACR-iCIL Expansion at ~5 weeks: ShRED generates >95% pure RACR-iCILs at unmatched yields

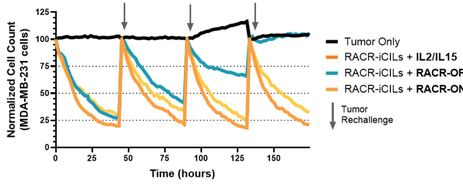
RACR-iCILs are highly pure with high expression of cytotoxicity receptors



ShRED generates >4 million-fold expansion from one initial RACR-iPSC

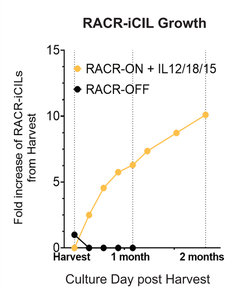


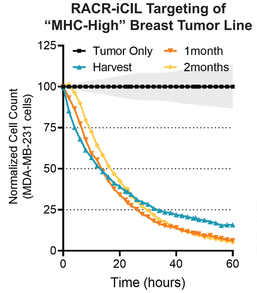
RACR-activation increases RACR-iCIL's serial killing of "MHC-high" breast tumor cell line

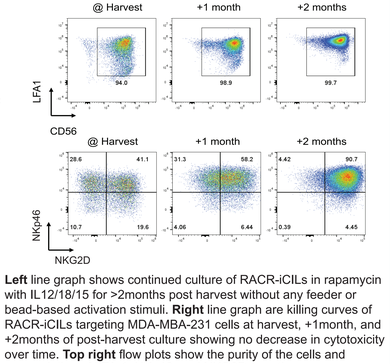


Left flow plots show phenotypic analysis of RACR-iCIL cell markers CD56, LFA1, NKG2D, and NKp46 of ShRED-derived RACR-iCIL cells demonstrates a highly pure and mature phenotype. **Left** line-graph shows serial kill assay of RACR-iCILs targeting breast tumor cell line MDA-MBA-231 and RACR-activation increasing cell killing similar to cytokine support. **Right** fold expansion yields of RACR-iCIL cells per starting iPSC with standard commercially available cell culture protocols in unmodified iPSCs compared to RACR-engineered iPSCs in the presence (ShRED) or absence of rapamycin. Blue data point represent a reported expansion from public presentations. Line graph is total ShRED cell expansion process.

RACR-iCILs cultured >2 months post-harvest in "feeder-free culture" retain function



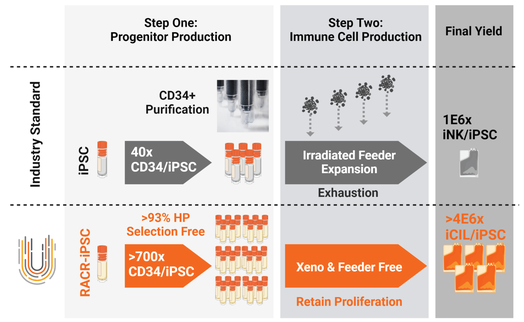




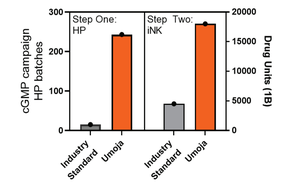
Left line graph shows continued culture of RACR-iCILs in rapamycin with IL12/18/15 for >2months post harvest without any feeder or bead-based activation stimuli. **Right** line graph are killing curves of RACR-iCILs targeting MDA-MBA-231 cells at harvest, +1month, and +2months of post-harvest culture showing no decrease in cytotoxicity over time. **Top right** flow plots show the purity of the cells and up-regulation of LFA-1, NKp46, and NKG2D over time.

ShRED generates higher cell yields and potentially more proliferative cells than iNK industry standard

High cell yields enable cost-effective repeat dosing for potentially more durable responses *in vivo*.



Theorectical Clinical Batches

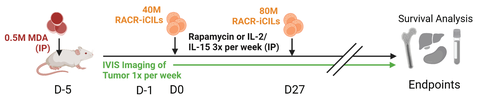


ShRED increases the number of total cells produced through massive HP expansion and the high purity of resulting HPs removes the need for complex and time-consuming filtration or CD34 purification.

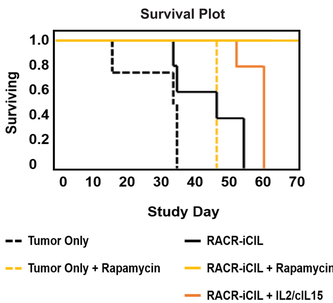
ShRED does not require feeder cells to induce effector cell expansion, thus retaining the proliferative capacity of cells over ones that have gone through mulitple rounds exhaustive feeder-expansion.

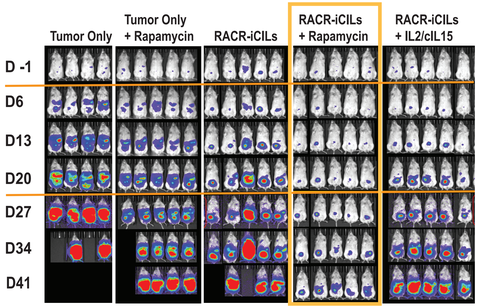
RACR-activation enhances RACR-iCIL-mediated breast tumor clearance *in vivo*

RACR-iCIL *in vivo* PoC study design

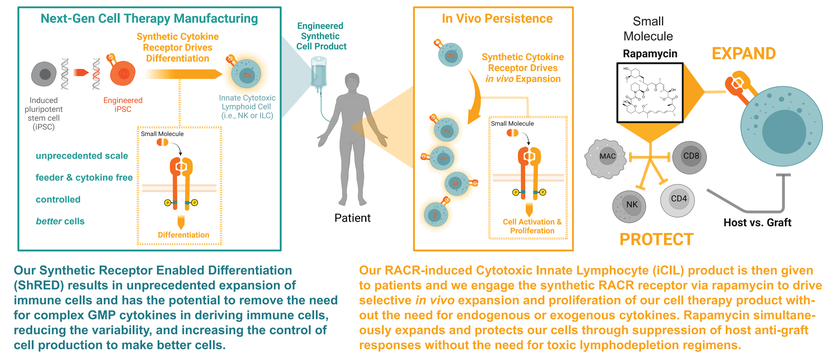


Top Left: NSG MHC I/II DKO mice were injected with FFLUC+ MDA-MBA-231 breast adenocarcinoma tumors via intraperitoneal (IP) injection at D -5. 40M RACR-iCILs were then injected IP at D0. Rapamycin or IL2/cIL15 were injected IP 3x per week and IVIS imaging was performed 1x per week. At D27 an additional 80M RACR-iCILs were injected IP. **Bottom Left:** Survival plot shows mice treated with rapamycin and RACR-iCILs have 100% survival at D70. **Bottom Right:** Tumor burden shown below from weekly IVIS imaging.





Summary: Harnessing the RACR to create next-generation cell therapy products



Our Synthetic Receptor Enabled Differentiation (ShRED) results in unprecedented expansion of immune cells and 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 RACR-induced Cytotoxic Innate Lymphocyte (iCIL) product is then given to patients and we engage the synthetic RACR receptor via rapamycin to drive selective *in vivo* expansion and proliferation of our cell therapy product without the need for endogenous or exogenous cytokines. Rapamycin simultaneously expands and protects our cells through suppression of host anti-graft responses without the need for toxic lymphodepletion regimens.