Umoja's RACR technology drives Synthetic Receptor Enabled Differentiation (ShRED) of iPSCs, a proprietary and uniquely scalable process for manufacturing potent and persistent engineered immune effector cells

Umoja is developing an allogeneic cell therapy platform that employs precision-edited induced pluripotent stem cells (iPSCs) to enable scalable and simplified manufacturing of immune effector cells.

Clinical Experience with Pluripotent Stem Cells Demonstrates Vast Potential Applications

Human pluripotent stem cells first received FDA clearance for use in a clinical trial in 2009. In a systematic multi-database analysis published in 2020, 131 clinical trials worldwide were identified that involved human pluripotent stem cells[1]. While several early clinical trials employed human embryonic stem cells, trials employing iPSCs are progressing, with applications for regenerative medicine as well as oncology. Several active, interventional clinical trials evaluating the therapeutic benefit of iPSC-derived cells, as of 2020[1], are summarized here based on target disease:

- Retinal diseases, including age-related macular degeneration (using autologous and allogeneic iPSCs in small trials; 5-6 patients enrolled)
- Heart failure and ischemic cardiomyopathy using iPSC-derived cardiomyocytes (allogeneic iPSCs in small trials; 5 patients enrolled)
- Parkinson's disease using iPSC-derived dopaminergic progenitors (small trials; 7 patients enrolled)
- Beta-thalassemia using iPSC-derived hematopoietic stem cells (autologous iPSCs in small trials; 2 patients enrolled)
- Graft vs host disease using iPSC-derived mesenchymal stem cells (allogeneic iPSCs, with 16 patients enrolled)
- Solid tumors using iPSC-derived NK cells (allogeneic iPSCs in large trials; 76 patients enrolled)
- Other target diseases have preclinical data using iPSC-derived cells, including diabetes using iPSC-derived pancreatic islet/beta cells

Most of these studies are in early phases, however, several late-stage clinical trials across multiple iPSC-derived cell therapies in the oncology space are expected to initiate in the coming 6-12 months.

Umoja's iPSC platform

Umoja's iPSC platform involves engineering iPSCs to express Umoja's proprietary Rapamycin Activated Cytokine Receptor (RACR™) technology, which endows the cells to undergo Synthetic Receptor Enabled Differentiation (ShRED). ShREDding is a controlled differentiation process that drives iPSCs to become cytotoxic innate lymphocytes at unprecedented yields (**Figure 1**).

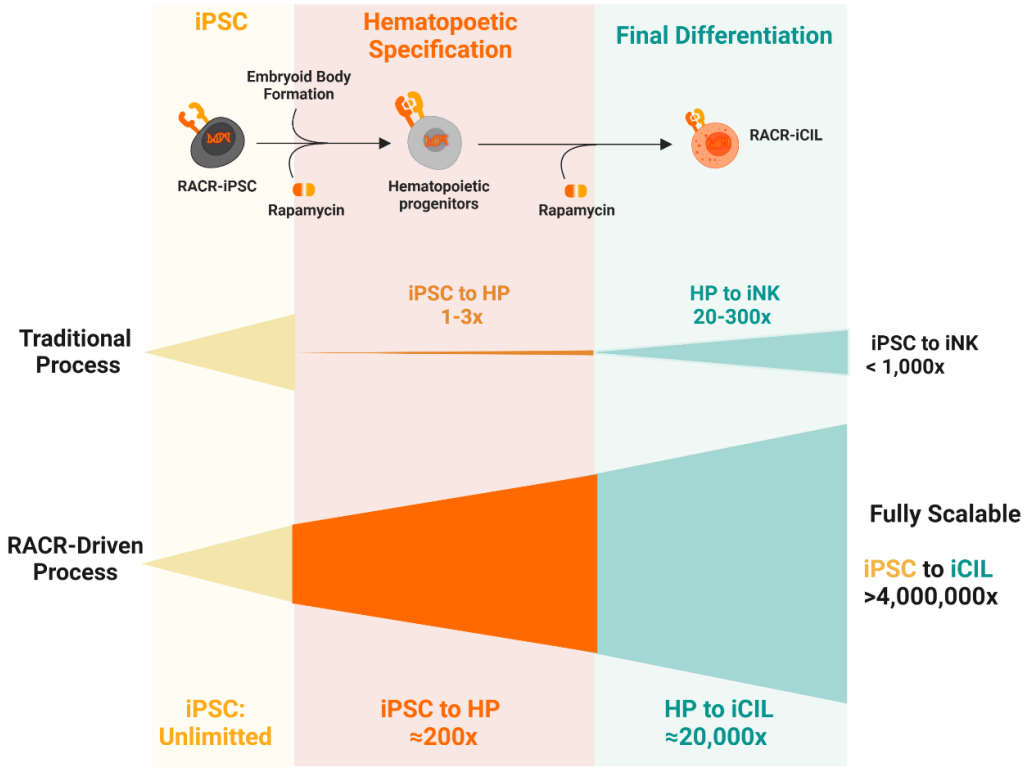


Figure 1. Using the ShRED process, Umoja can generate unprecedented yields of cancer-killing cells, called RACR-induced Cytotoxic Innate Lymphoid (RACR-iCIL) cells. The ShRED process overcomes key bottlenecks in the field faced using traditional processes to produce iPSC-derived Natural Killer (iNK) cells, such as low yields of Hematopoietic Progenitors (HPs) per initial iPSC.

We anticipate that ShRED-based manufacturing will overcome many existing Chemistry, Manufacturing, and Controls (CMC) challenges in generating tumor-fighting lymphocytes from iPSCs, reducing costs and improving patient access to cutting-edge, off-the-shelf cell therapies. Furthermore, the RACR system can be activated *in vivo*, where it may enhance engraftment, expansion, and persistence of therapeutic cells in patients without requiring toxic lymphodepleting chemotherapy.

Features of Umoja's engineered iPSC platform:

1. **iPSC starting material:** iPSCs are pluripotent stem cells that can be generated by reprogramming adult cells into a cellular state akin to embryonic stem cells. These cells are thought to be capable of differentiating into all the cell types found in the human body. iPSCs possess an unlimited expansion capacity, meaning they can proliferate indefinitely, generating a nearly endless supply of starting material supportive of process scalability. iPSCs are also amenable to precision multiplex genome editing, allowing safe, controlled introduction of multiple genetic modifications. Additionally, iPSCs provide a consistent starting material, originating from a single

cell (clone), enabling consistency in genome integrity, differentiation, and the final cell product. Lastly, iPSCs have been tested in human clinical trials, possessing an established history and clinical validation. Umoja has in-licensed a qualified iPSC line as the starting cell source for its ShRED-based process.

2. **Engineered anti-tumor specificity and effector functions:** iPSCs can be modified via CRISPR-based precision genome editing to express a Chimeric Antigen Receptor (CAR), including conventional CARs or Umoja's universal TagCAR. Umoja's TagCAR is designed to be used in conjunction with Umoja's TumorTag™ platform to overcome challenges associated with targeting heterogeneous solid tumor microenvironments.
3. **Synthetic receptor platform engineering:** Umoja's engineered iPSCs are modified via CRISPR-based precision genome editing to express Umoja's RACR system (**Figure 2**) and be resistant to rapamycin-mediated immunosuppression. Activation of RACR by rapamycin dosing mimics IL-2/IL-15 STAT5 signaling. Rapamycin is a potent proliferation inhibitor of the host immune system, acting through mTOR inhibition, and an FDA-approved small molecule immunosuppressant with a decades' long history of safe use in solid organ transplantation. FKBP12 knockout engineering is intended to protect RACR-expressing cells from rapamycin-mediated mTOR inhibition. Thus, when cells expressing RACR are exposed to rapamycin, these engineered cells are selectively expanded. In patients, rapamycin is expected to simultaneously suppress the anti-allograft response against these engineered cells.

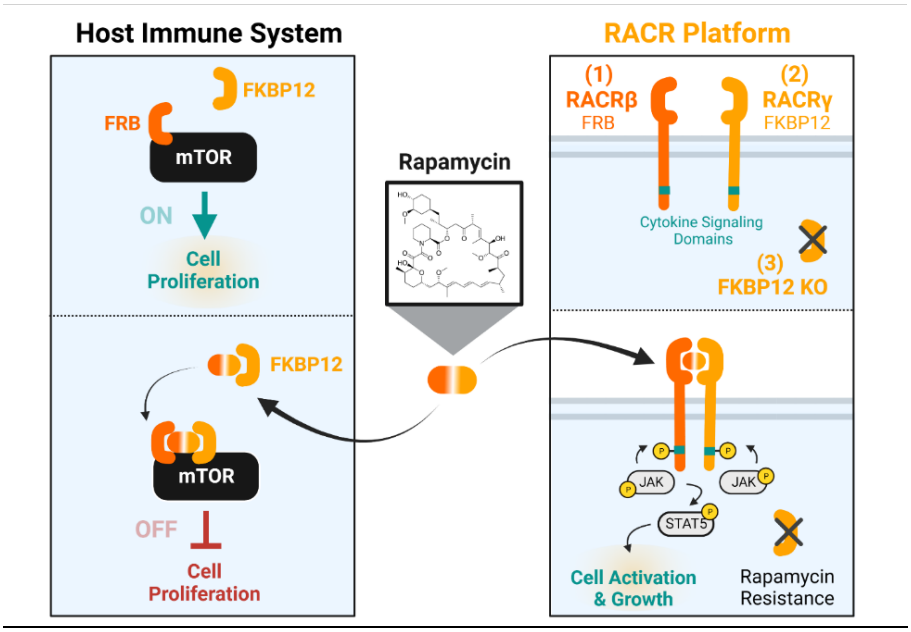


Figure 2. The proprietary RACR system includes two components: RACRβ (FRB) and RACRγ (FKBP12). While rapamycin dosing suppresses the host immune system through mTOR inhibition, when cells expressing RACR are dosed with rapamycin, these rapamycin-resistant cells (made resistant through FKBP12 deficiency) activate JAK/STAT5 signaling. This provides a path for selective expansion and activation of the RACR-containing cells.

4. **Improved manufacturing:** During ShRED-based manufacturing, RACR activates multiple JAK/STAT signals that drive iPSC differentiation into hematopoietic progenitors (HPs) and then into immune effector cells termed RACR-induced Cytotoxic Innate Lymphoid (**RACR-iCIL**) cells. The ShRED approach eliminates the need to add several growth factors and cytokines, reducing manufacturing complexity and cost. Additionally, the process is feeder cell-free, xenogeneic material-free, and scalable in a dynamic suspension culture system.
5. **Engraftment in the absence of lymphodepleting chemotherapy:** It is expected that once RACR-iCIL cells are administered to the patient, RACR activation may support engraftment, persistence, and effector function of therapeutic cells without toxic lymphodepletion.

Current Cell Therapy Paradigm: Manufacturing

Current approaches to cell therapy in oncology include either autologous or allogeneic cellular starting material from which a tumor-targeting therapeutic cell product is engineered. Allogeneic cells can be further broken down into donor- or iPSC-derived starting materials. Donor-derived starting materials are generally sourced from the circulation or cord blood of a healthy donor, and the therapeutic cell type (e.g., natural killer or NK cells) is subsequently harvested and expanded in a complex cell culture process that generally includes multiple cytokines, growth factors, gene engineering, and feeder cells to generate many cellular doses. Alternatively, iPSC-based approaches generally require multiple complex cell culture conditions implemented in a stepwise fashion to drive cells through the necessary progenitor stages to ultimately obtain the intended immune effector cell type, typically NK cells. This approach is inefficient in generating necessary intermediate progenitor cells, resulting in a low initial yield of NK cells that then requires feeder cell-driven expansion. This feeder cell-driven expansion can dramatically reduce the proliferative capacity of the final cell therapy product, thus requiring high (≈1 billion cells) and repeat dosing, coupled with repeated cycles of lymphodepleting chemotherapy, to achieve the necessary engraftment and exposure leading to an anti-tumor effect.

Current Cell Therapy Paradigm: Clinical Efficacy of Autologous and Allogeneic Approaches

The gold standard for clinical efficacy for cell-based cancer therapies is autologous CAR T cells in hematologic malignancy, where unprecedented durable remission rates have been observed in lymphoma and multiple myeloma. Importantly, CAR T cell efficacy requires lymphodepleting chemotherapy to eliminate sinks for survival factors such as IL-15. The allogeneic cell therapy field has shown promising early clinical results; however, the durable response profile has been generally poor in comparison to autologous CAR T cell therapies, despite employing multiple high intensity lymphodepletion cycles. This may be due to limitations of the drug product cell type, manufacturing processes, as well as anti-allograft responses against the therapeutic cells. For example, NK cells are a common therapeutic immune effector cell type employed in allogeneic cell therapy. NK cells are short-lived and current manufacturing processes require extensive feeder cell-driven expansion of NK cells, which can reduce their proliferative capacity once in the patient.

ShREDing iPSCs into iCILs: Solutions to Challenges with Current Cell Therapy Paradigms

The ability of the ShRED process to generate extremely high yields of RACR-iCILs, overcoming differentiation bottlenecks in the field (**Figure 1**), coupled with anticipated enhanced engraftment properties of the RACR-iCILs, is expected to provide solutions to the key challenges in CMC and clinical durability faced by the current allogeneic cell therapy space. **Table 1** provides a comparison of the different approaches to cell therapy in oncology.

Table 1. Comparison of autologous and allogeneic cell therapy approaches

Attribute	Cell Therapy Product Starting Material			
	Autologous cells	Allogeneic Cell Therapy Approaches		
		Donor-derived	Conventional iPSC	Umoja's RACR-iCIL
Turnaround Time	Weeks to months	• Off-the-shelf • Days required for lymphodepleting chemotherapy	• Off-the-shelf • Days required for lymphodepleting chemotherapy	• Off-the-shelf • No lymphodepletion required
Scalability	• Least scalable • Requires scale-out process	• Scale-up ability limited by terminally differentiated starting material	• Unlimited starting material • Progenitor differentiation inefficient • Final differentiated cell type typically requires significant feeder cell-based expansion	• Unlimited starting material • Progenitor differentiation highly efficient • Minimal expansion needed for final cell type
Gene Editing Compatibility	• Inefficient editing • Product-to-product variability	• Inefficient editing • Product-to-product variability	• Efficient editing • Consistent, homogeneous product	• Efficient editing • Consistent, homogeneous product
Cell Culture Conditions	Necessary to achieve sufficient yields: • Feeder cells • Cytokines/growth factors	Necessary to achieve sufficient yields: • Feeder cells • Cytokines/growth factors	Necessary to achieve sufficient yields: • Feeder cells • Cytokines/growth factors	• Xenogeneic-free • Feeder cell-free • Minimal complex raw materials required
Clinical Safety	• Requires lymphodepletion • CRS and ICANS are common • Requires inpatient administration	• Requires lymphodepletion • CRS and ICANS are less common and less severe	• Requires lymphodepletion • CRS and ICANS are less common and less severe	TBD but anticipated: • No lymphodepletion • Likely very low CRS and ICANS profile, similar to other allogeneic NK products
Product Persistence and Engraftment	• Requires lymphodepletion • Potential for long-term persistence	• Requires lymphodepletion • Hypoimmune engineering • Minimal persistence • Repeat dosing required	• Requires lymphodepletion • Hypoimmune engineering • Minimal persistence • Repeat dosing required	TBD but anticipated: • No lymphodepletion • RACR activation designed to promote engraftment, expansion, and minimize or eliminate hypoimmune engineering requirements

ShREDing iPSCs into iCILs: Improved Manufacturing

The engineered iPSCs platform utilizes a unique ShRED-based manufacturing process wherein RACR activation (through rapamycin dosing) drives RACR-iCIL cell production (**Figure 3**).

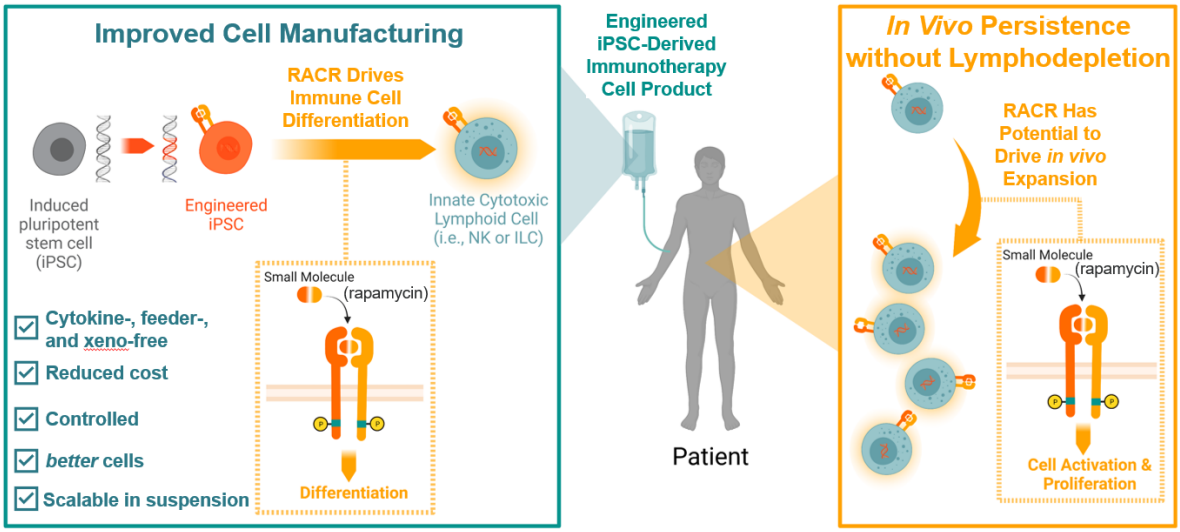


Figure 3. Engaging RACR improves cell manufacturing (left) and has the potential to enhance cell therapy engraftment and pharmacokinetics following administration to the patient (right) without lymphodepleting chemotherapy by concomitantly driving proliferation and survival of the RACR-iCIL cells and suppressing the anti-allograft response in the presence of rapamycin.

The key features of this process include:

1. **Controllable cell production** through rapamycin dosing and activation of RACR in a dose-dependent manner. This controlled process results in a more reproducible differentiation process and homogeneous cell product.
2. **Potentially reduced cost** as RACR activation is projected to eliminate the need to add expensive growth factors, cytokines, and other raw materials, reducing manufacturing cost.
3. **Unprecedented yields of highly pure HPs and iCILs** generated through the ShRED process has the potential to further reduce costs and improve feasibility of repeated patient dosing of RACR-iCIL cells.
    a. The ShRED platform consistently generates highly pure HPs as an intermediate progenitor population, as quantified by high expression of CD43, CD45, and CD34 (**Figure 4**).

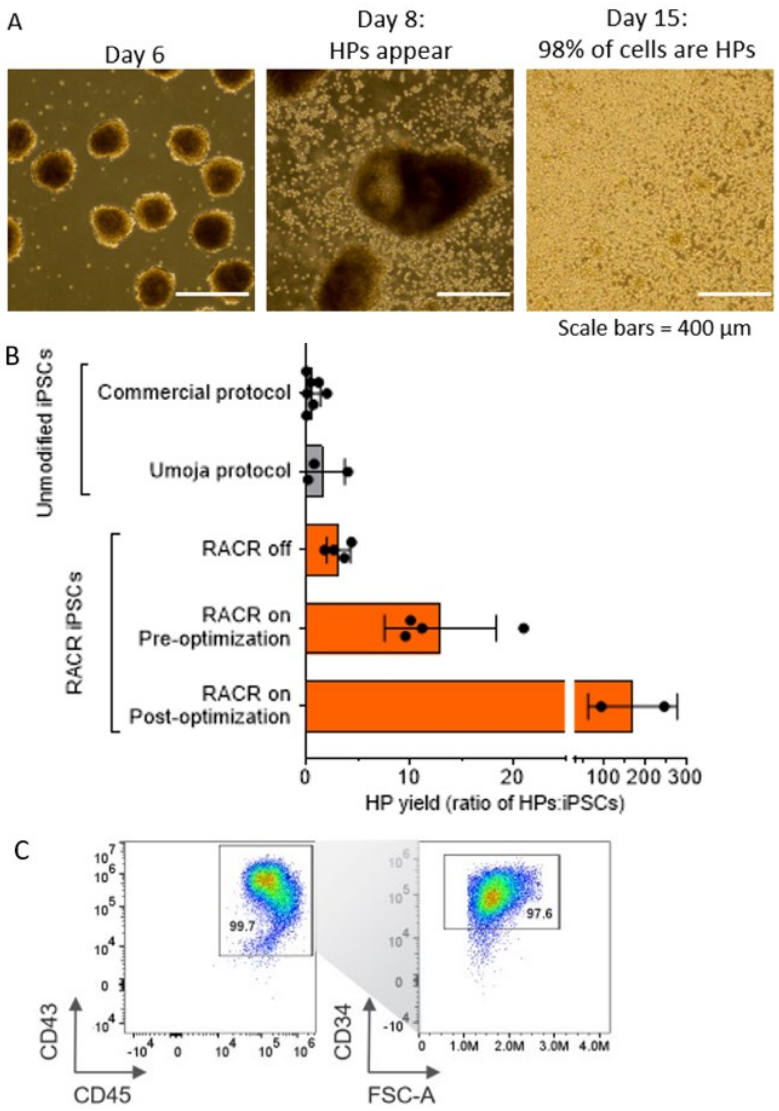


Figure 4. ShRED enables highly efficient generation of pure HPs from iPSCs. (A) iPSC-based aggregates are initially formed (still visible at day 6 of differentiation), and by day 8 of differentiation these aggregates begin to dissociate as hematopoietic progenitors (HPs) emerge, resulting in a single-cell culture of HPs by day 15. (B) 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. (C) Phenotypic analysis of HP cell markers CD43, CD45, and CD34 of ShRED-derived HPs demonstrates a highly pure HP phenotype.

b. The resultant, harvested RACR-iCIL cells are highly pure and phenotypically mature, as quantified by high expression of CD56, LFA1, NKG2D and NKp46 (**Figure 5**).

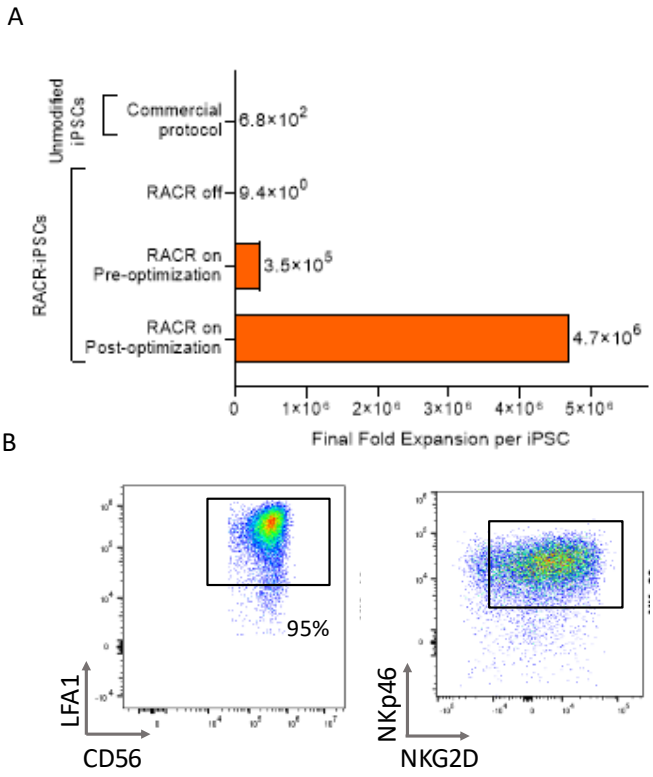


Figure 5. ShRED enables efficient generation of highly pure RACR-iCIL cells from iPSCs. (A) Fold expansion yields of RACR-iCIL cells per starting iPSC with standard commercially available cell culture protocols in unmodified iPSCs compared to RACR-engineered iPSCs in the presence (ShRED) or absence of rapamycin. (B) 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.

4. **Consistent and controlled raw materials and manufacturing** process; we aim to make it completely feeder cell-free and xenogeneic-free.
5. **Scalable in suspension,** utilizing a novel suspension (i.e., dynamic)-based ShRED process to produce cells in a manner compatible with scalability.

ShREDing iPSCs into iCILs: Enhanced in vivo Anti-Tumor Activity

Through RACR activation via rapamycin dosing of the patient, the administered RACR-iCIL cells have the potential to result in better clinical outcomes. This includes the following key features:

1. **Improved engraftment, persistence, and effector functions** of therapeutic RACR-iCIL cells.
    a. *In vitro* cytotoxicity assays consistently demonstrate potent tumor cell killing of a difficult-to-kill MDA-MB-231 breast adenocarcinoma cell line. This killing is enhanced by

the addition of activating cytokines or through RACR activation via addition of a rapamycin analog (rapalog) **(Figure 6).**

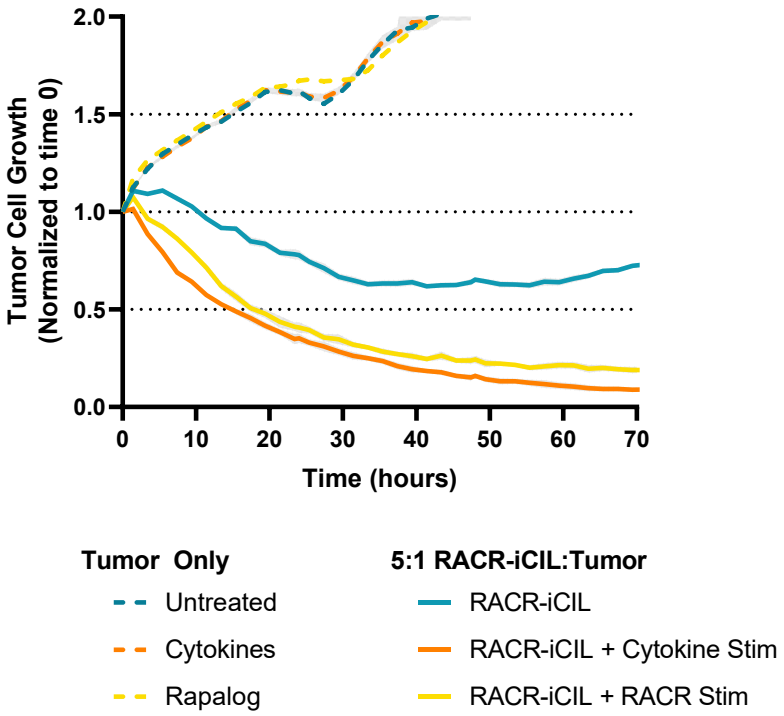


Figure 6. RACR activation in RACR-iCIL cells enhances tumor cell killing *in vitro* in co-culture with MDA-MB-231 breast adenocarcinoma cell line. Tumor cells were left untreated or cultured with RACR-iCIL cells alone, or with RACR-iCIL cells in the presence of cytokine stimulation or RACR stimulation (via addition of a rapamycin analog, rapalog).

b. *In vivo* anti-tumor activity against the MDA-MB-231 breast adenocarcinoma cell line is being evaluated in an ongoing study in an immune compromised mouse tumor xenograft model. Results thus far demonstrate RACR-iCIL cell-mediated anti-tumor activity that is enhanced by co-administration of activating cytokines or rapamycin (**Figure 7**).

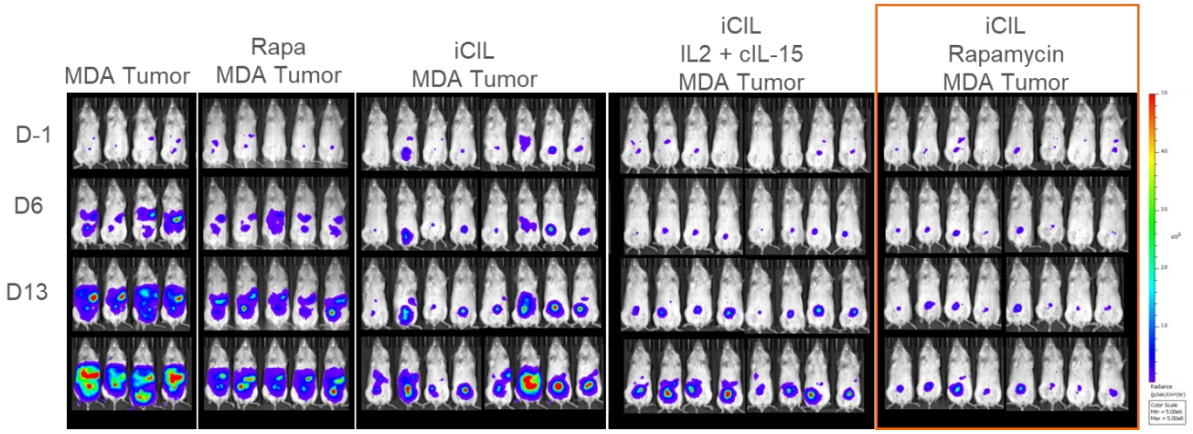
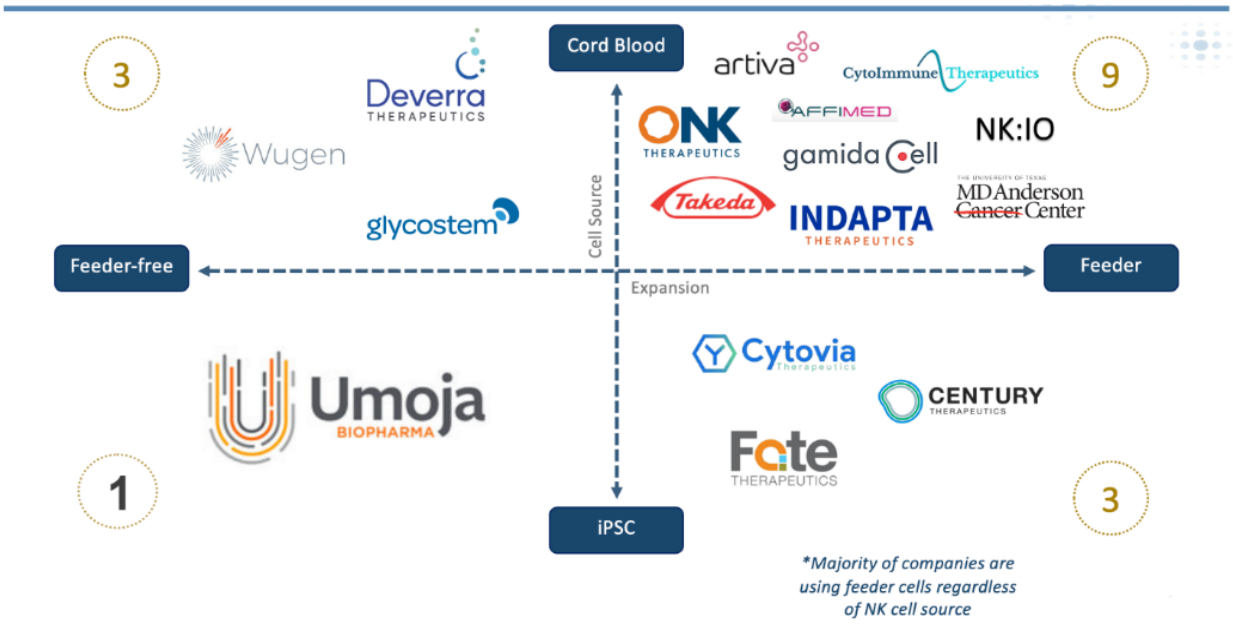


Figure 7. RACR activation enhances RACR iCIL cell-mediated MDA-MB-231 tumor cell killing *in vivo*. Immune compromised NSG MHCI/II knockout mice were injected with MDA-BM-231 firefly luciferase-expressing tumor cells intraperitoneally (IP) on Day -5. Tumor imaging was performed on Day -1 (top row), and 40x10^6 ShRED-generated RACR-iCIL cells were administered via IP injection on Day 0. Administration of rapamycin (to activate RACR) or activating cytokines (IL-2 plus IL-15/IL-15Ra complex) was initiated on Day 0 in two separate cohorts of tumor-bearing mice that also received RACR-iCIL cells. The first post-RACR iCIL cell administration tumor imaging was performed on study Days 6, 13, and 20 (bottom row).

2. **Inhibition of the host immune response** via rapamycin dosing, enabling engraftment of the RACR-iCIL cells by suppressing the host-mediated anti-allograft response, minimizing requirements for hypo-immune modifications.
3. **Potentially avoiding toxic lymphodepletion** by utilizing the RACR system to selectively support RACR-iCIL cell expansion and survival.



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1. Deinsberger, J., Reisinger, D., and Weber, B. Global trends in clinical trials involving pluripotent stem cells: a systematic multi-database analysis. (2020). *NPJ Regenerative Medicine*. 5(15). https://doi.org/10. 1038/s41536-020-00100-4