specific response with reduced tonic signaling, potentially indicating that our novel CAR-T cells may show improved clinical efficacy on solid tumor.

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Background Bioengineered T cell treatments for acute myeloid leukemia (AML) are challenged by near universal expression of leukemia antigens on normal hematopoietic stem/progenitor cells. \(^1\) \(^2\) on target/off tumor activity may cause myelosuppression while sustained antigen exposure can lead to T cell exhaustion. \(^3\) In addition, splicing variants may allow antigen escape. We hypothesize that by using a novel CD33-C2-specific single domain VHH antibody as the antigen targeting domain in dimersing agent-regulated immunoreceptor complex T cells (DARIC T cells), we will enable pharmacologically-controllable targeting of CD33, allowing eradication of leukemia expressing either of the major splice variants of CD33: i.e., full-length CD33 or CD33ΔE2.

Methods We engineered DARIC-expressing lentiviral vectors containing encoded separating CD33-C2-specific antigen binding and 41BB-CD3ζeta signaling chains that heterodimerize following addition of rapamycin via embedded FKB12 and FRB\(^*\) domains. \(^4\) Peripheral blood mononuclear cells were stimulated with IL-2, anti-CD3, and anti-CD28 antibodies 24h prior to transduction with DARIC33 lentivector. Surface expression of antigen binding or signaling chains was assessed using biotinylated CD33, or antibodies to VHH-domains or FRB\(^*\) respectively. Rapamycin-dependent in vitro activity was measured by IFNγ release. To evaluate in vivo activity, NSG mice injected with \(1 \times 10^5\) MOLM-14/luc cells were treated 5-7 days later with \(1 \times 10^7\) DARIC33 T cells in the presence or absence of rapamycin and tumor progression followed by luciferase activity.

Results DARIC33+ T cells bound biotinylated-CD33, anti-VHH and anti-FRB\(^*\) antibodies. Rapamycin addition increased expression of both signaling and antigen-recognition chains, suggesting augmented receptor stability in the presence of dimersing drug. In the presence of rapamycin, coculture of DARIC33 T cells with cell lines expressing either full length or CD33ΔE2 \(^5\) showed equalrapamycin-dependent activation, demonstrating DARIC33 responds to both splice variants. Titration experiments showed rapamycin-dependent activation with EC\(_{50}\) = 25pM. Negligible IFNγ release was observed in the absence of drug. DARIC33 T cells significantly extended survival of AML-bearing mice, but only when treated with rapamycin. The DARIC33 T cells were activated in vivo by sub-immunosuppressive rapamycin dosing, as weekly or 0.1 mg/kg QOD dosing led to similar levels of tumor suppression.

Conclusions DARIC33 T cells appear to be potent antileukemic agents: they are activated by AML cell lines in vitro as demonstrated by cytokine release and cytotoxicity, and significantly extend survival in an aggressive xenograft model. Temporal control provided by the DARIC architecture promises to enhance safety and potentially efficacy of CAR T therapy for AML, for example by enabling hematopoietic recovery or providing T cell rest.

REFERENCES


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Background Engineered T cell therapies have revolutionized treatment of relapsed refractory haematological malignancies, however the cost of treatment for autologous products remains a significant challenge to their widespread use. The high cost is driven largely by the need for personalized manufacturing of autologous cell products. A non-conventional class of T cells, the gamma/delta T cell, can be safely transplanted into an unrelated recipient without inducing graft-versus host disease, \(^1\) making them an ideal candidate for mass-manufactured off-the-shelf T cell therapies. We have previously described a novel method of directing conventional alpha/beta T cells towards tumour targets by co-opting the T cell receptor using the T cell Antigen Coupler (TAC) receptor. \(^2\) Here, we describe the use of TAC receptors to engineer antigen-specific reactivity into gamma/delta T cells, resulting in highly potent anti-tumor cytotoxicity.

Methods Engineered gamma/delta T cells were manufactured by activating PBMCs with Zoledronate and IL-2. The TAC transgene was introduced into T cells using either VSV-G pseudotype lentivirus or GALV-pseudotyped gamma-retrovirus vectors. Through optimization studies, we determined transduction was highest 24 hours post-activation for lentivirus and 72 hours post-activation for gamma-retrovirus. Cultures were fed with IL-2 supplemented media every 2 – 3 days and enriched on Day 14 to >99% gamma/delta T cell purity using CD4/CD8 magnetic-activated cell sorting depletion (Miltenyi Biotech).

Results Both methods of gene transfer tested for our pilot study yielded excellent gene transduction (40% - 70%). Using lentivirus-engineered gamma/delta T cells, we demonstrated that the TAC receptor re-directs gamma/delta T cells to attack tumors in an antigen-specific manner. The presence of the TAC receptor did not interfere with lysis of tumor cells via the natural tumor-reactive gamma/delta T cell receptors.