Abstract 142 Figure 1 Rb-340-1 performance in vitro and in vivo

Conclusions Intrinsic conditional regulation of checkpoint expression in CAR T cells provides a simplified approach to combination therapies that limits systemic toxicities and reduces costs. Since the expression of multiple genes can be simultaneously controlled by CRISPRi, broader applications can be envisioned in the future.

Ethics Approval Not Applicable

Consent Not applicable

REFERENCES


144 RTX-240, AN ALLOGENEIC ENGINEERED RED BLOOD CELL EXPRESSING 4-1BBL AND IL-15TP, PROMOTES NK CELL FUNCTIONALITY IN VITRO AND IN VIVO

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Background Agonist antibodies and recombinant cytokines have had limited success in the clinic due to three factors: severe toxicity leading to a narrow therapeutic index, the diminished activity of an agonistic antibody compared with natural ligand, and the lack of multiple signals needed to effectively activate most cell types. To address these limitations, Rubius Therapeutics has developed RTX-240, an allogeneic cellular therapy using red blood cells genetically engineered to express 4-1BBL and IL-15/IL-15Ra fusion (IL-15TP) in their natural conformation on the cell surface. RTX-240 is designed to recapitulate human biology by broadly stimulating adaptive and innate immunity to generate an anti-tumor response and provide improved safety due to the restricted biodistribution of red blood cells to the vasculature. Here we demonstrate that RTX-240 is highly active in preclinical models.

Methods PBMCs or NK cells were treated with RTX-240 in vitro. mRBC-240 was used for in vivo studies.

Results Treatment of either PBMCs or isolated NK cells with RTX-240 induced a dose-dependent increase in NK cell activation, proliferation and functionality. These effects were further enhanced with increased 4-1BBL and IL-15TP expression on the surface of RTX-240. NK cell counts, Nkp44 and Trail expression were increased 150, 4.6 and 6-fold over media control, respectively. Activation of NK cells with RTX-240, followed by incubation with K562 targets enhanced NK cell cytotoxicity (1.3-2.8 over control), that was accompanied by increased NK cell activation (CD69) and degranulation (CD107a) (3.1-fold and 1.9-fold, respectively). RTX-240-activated NK cells showed higher frequency of CD56dim/CD16+ NK cells, which have been reported to induce natural and ADCC-dependent cytotoxicity. Correspondingly, RTX-240-promoted enhanced ADCC-induced killing of Raji cells when combined with anti-CD20 mAb (1.4-fold over control). Intravenous administration of mRBC-240 to a B16F10 intravenous lung metastases model led to NK cell expansion on Day 4 (3.8-fold over control). These NK cells were cytotoxic (Granzyme B+) and highly proliferative (Ki67+) (1.4-fold and 18.8-fold over control, respectively). Treatment with mRBC-240 increased the frequency of terminally differentiated NK cells (NK1.1+/CD11b−/CD27−/KLRG1+) in the tumor (2.1-fold increase over control). Terminally differentiated NK cells are highly cytotoxic and their frequency in the tumor was strongly correlated with efficacy in this model (p=0.0001).

Conclusions Taken together, these data indicate that RTX-240 promotes NK cell activity and functionality in preclinical models. RTX-240 has now entered a first-in-human Phase 1 trial for the treatment of patients with relapsed/refractory or locally advanced solid tumors, with a planned arm evaluating RTX-240 in relapsed/refractory acute myeloid leukemia.

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145 PRECLINICAL DEVELOPMENT OF EDIT-201, A MULTIPLEXED CRISPR-CAS12A GENE EDITED HEALTHY DONOR DERIVED NK CELLS DEMONSTRATING IMPROVED PERSISTENCE AND RESISTANCE TO THE TUMOR MICROENVIRONMENT

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Background Natural killer (NK) cells distinguish tumor from healthy tissue via multiple mechanisms, including recognition of stress ligands and loss of MHC class I expression. However, effector function of allogeneic NK cells can be diminished by the lack of functional persistence, as well as tumor-intrinsic immunosuppressive mechanisms, such as production of TGF-β. We developed a next-generation allogeneic NK cell therapy using CRISPR-Cas12a gene editing to enhance NK cell function through knockout of the CISH and TGFBR2 genes. We hypothesized that knockout of CISH, a negative regulator of IL-2/IL-15 signaling, would improve NK cell effector function, while knockout of the TGF-β receptor gene,
TGFB2, would render NK cells resistant to TGF-β mediated suppression.

Methods NK cells were expanded from CD3-PBMC starting material in the presence of 20 ng/mL IL-15 for 14 days. A variety of methods were performed to assess the effects of CRISPR-Cas12a gene editing on primary human NK cells including NGS to assess editing efficiency, flow cytometry, in vitro spheroid killing assays and an in vivo NSG tumor model. These methods were performed consistent with protocols widely accepted in the field.

Results Following editing optimization, we achieved greater than 80% in/dels at both targets in NK cells in both single and double gene knockout (KO, DKO) contexts. Using flow cytometry-based assays we demonstrated that TGFB2 KO NK cells phosphorylated less SMAD2/3 relative to unedited control NK cells in response to TGFB-β, while CISH KO NK cells showed enhanced pSTAT3 and pSTAT5 upon IL-15 stimulation. We next explored the ability of these single knockouts demonstrated improved cytotoxicity against tumor targets in the presence of exogenous TGF-β (p<0.0001 for both single KOs). Importantly, in both the SK-OV-3 and PC-3 tumor spheroid killing assays, DKO NK cells demonstrated superior rapid and sustained tumor killing compared to either single knockouts or unedited control NK cells (n=7 independent experiments, 4 unique NK cell donors, p<0.001), demonstrating additive effects of simultaneously targeting both pathways. Relative to control NK cells, DKO NK cells had increased expression of CD107a, CD25, CD69, and NKP44 after exposure to tumor cells and produced higher concentrations of TNF-a and IFN-g (p<0.01). In an in vivo NSG mouse xenograft model, where SK-OV-3 cells are injected i.p. one week prior to i.p. NK cell injection, Sorted T-allo10-derived Tr1 cells and non-Tr1 cells, as well as control effector T cells (Teff) and parental CD4+ T cells, were analyzed by TCR- and RNA- seq. Protein expression for key differentially expressed genes were validated, and the functional roles for IL-10, CTLA-4 and PD-1 in T-allo10-mediated suppression were confirmed in a suppression assay. These methods were performed consistent with protocols widely accepted in the field.

Conclusions Our data shows that the T-allo10 cell product is: i) enriched for Tr1 cells, ii) anergic in response to alloantigen re-challenge, but not to non-specific stimuli or 3rd party antigens, and iii) suppresses host-reactive T cells, but not T cell responses to other antigens. Furthermore, T-allo10-derived, isolated Tr1 cells had a restricted TCR repertoire, suggesting they clonally expand in response to alloantigens. T-allo10-derived Tr1 cells have a distinct signature compared to non-Tr1 cells, and, in addition to IL-10, express high levels of CTLA-4 and PD-1 (but not FOXP3). Notably, blockade of CTLA-4 and the PD-1 pathway completely abolishes T-allo10-mediated suppression of T cell responses.

Ethics Approval The patient study was approved by Administrative Panels on Human Subjects in Medical Research, Stanford University, Tallo10 eProtocol # 38734. Healthy donor samples were purchased as deidentified human blood products from the Stanford Blood Center, and are thus exempt.

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CD155 BLOCKADE BOOSTS ALLOREACTIVE NATURAL KILLER CELL ANTITUMOR EFFECTS AGAINST OSTEOSARCOMA


Background Pediatric patients with relapsed and refractory osteosarcoma have poor prognoses with few treatment options. Alloimmune bone marrow transplant (BMT) has not yet shown a graft-versus-tumor (GVT) effect for osteosarcoma. Natural killer (NK) cells demonstrate antitumor activity against osteosarcoma, but adoptively transferred NK cells have limited