RTX-224, AN ENGINEERED ALLOGENEIC RED CELL THERAPEUTIC EXPRESSING 4–1BBL AND IL-12, ACTIVATES IMMUNE CELLS IN BLOOD AND SPLEEN TO PROMOTE TUMOR GROWTH INHIBITION IN MICE


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-224, an allogeneic red cell therapeutic genetically engineered to express hundreds of thousands of copies of 4-1BBL and IL-12 in their natural conformation on the cell surface. RTX-224 is designed to activate four key target cell types: CD4+ and CD8+ T cells, antigen presenting cells and NK cells for a broad and effective anti-tumor response while providing improved safety due to the restricted biodistribution of red blood cells to the vasculature and spleen. Here we investigated the potential efficacy and mechanism of action of RTX-224 using the mouse surrogate mRBC-224.

Methods mRBC-224 was administered intravenously (i.v.) to normal or tumor-bearing mice (B16F10 tumor models). Blood, spleen and tumors were harvested and the pharmacodynamic effects of mRBC-224 on immune cells were evaluated.

Results mRBC-224 administered to mice inoculated i.v. with B16F10 melanoma reduced the number of metastases (p<0.0001 and 76.8% tumor growth inhibition on Day 14). This was accompanied by increased proliferation (Ki67+) and cytotoxicity (GzmB+) of tumor-infiltrating CD8+ T cells and NK cells, and an increased CD8+ effector memory (TEM) phenotype. Similarly, mRBC-224 reduced tumor growth in the B16F10 s.c. model (p<0.0001 and 56.2% tumor growth inhibition on Day 9), and this was associated with increased frequency of activated (MHC-II+) tumor-infiltrating macrophages. Consistent with the known biodistribution of red cells, mRBC-224 did not distribute to the tumor but was predominantly localized in the blood and spleen raising the question about mRBC-224 mechanism of action in mediating antitumor responses. In normal and B16F10 s.c. tumor-bearing mice, mRBC-224 induced the activation of CD8+ T cells, NK cells and monocytes/macrophages in blood and spleen in a dose-dependent manner. PD studies in the tumor suggest that these activated immune cells are capable of trafficking from blood/spleen to the tumor. These results align with published data suggesting that activated T cells in the spleen or blood can replenish exhausted tumor-infiltrating cells.

Conclusions Taken together, these data unveil the mechanism of action of mRBC-224 and suggest that mRBC-224 activate immune cells in the spleen and blood, leading to their trafficking into the tumor microenvironment to promote efficacy.

http://dx.doi.org/10.1136/jitc-2021-SITC2021.208