CD229 CAR T cells eliminate multiple myeloma and tumor propagating cells but show limited targeting of normal T cells

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BACKGROUND

The SLAM receptor CD229/LY9 is strongly expressed on multiple myeloma (MM) cells and is required for their growth, rendering it a potential target for the treatment of MM.

METHOD

We used antibody phage display to develop the first fully human antibody targeting CD229, clone 2D3. Using various screening assays, as well as biochemical characterization and functional assays, we evaluated this antibody and T cells expressing a chimeric antigen receptor (CAR) based on this antibody. Low-affinity antibodies were generated using a single site saturation variant library and characterized using high-throughput surface plasmon resonance.

RESULTS

CD229 is strongly expressed in the malignant plasma cells of patients with newly diagnosed and relapsed/refractory MM (Fig. 1A). In contrast to BCMA, CD229 expression can also be detected on memory and transitional B cells (Fig. 1B), a potential reservoir for clonotypic MM cells. We generated a CD229-specific antibody 2D3 specifically binding CD229 (Fig. 1C and 1D). CD229 CAR T cells show potent killing of MM cell lines (Fig. 2A). In vivo treatment with a single dose of CD229 CAR T cells resulted in tumor eradication or delayed tumor growth and significantly prolonged survival (Fig. 2B). We further observed significantly reduced colony formation by CD34neg bone marrow cells from patients with MM after treatment with CD229 CAR T cells compared to BCMA CAR T cells (Fig. 2C), indicating more efficient targeting of tumor propagating cells. While we observed that CD229 CAR T cells target resting CD229high T cells (Fig. 3A), they spared CD229neg/low T cells. CD229neg/low T cells show the same phenotype as CD229high T cells and conventional effector functions in response to common pathogens (data not shown). If my be possible to increase the selectivity of CD229 CAR T cells for MM cells by reducing the binding domains affinity due to the different CD229 expression levels on T cells and MM cells (Fig. 3B). We generated low-affinity CD229 antibodies (Fig. 3C) and show that CAR T cells based on the these antibodies have increased selectivity for MM cells over normal T cells (Fig. 3D).

CONCLUSIONS

CD229 CAR T cells can be manufactured efficiently and are highly active against MM, including tumor propagating cells. CD229 CAR T cells showed limited fratricide during CAR T cell production, spare a functional CD229neg/low T cell population, and reducing antibody affinity further enhances their selectivity for MM. CAR T cells targeting CD229 are a promising new approach for the treatment of MM.