Abstract: P1008

Title: TARGETED T CELLS AGAINST HEMATOPOIETIC CELLS EXPRESSING ONCOGENIC CALRETICULIN MUTANTS

Abstract Type: Poster Presentation

Topic: Myeloproliferative neoplasms - Biology & translational research

Background:

Disease modifying treatment options are an unmet medical need for myeloproliferative neoplasms (MPNs). The discovery of calreticulin mutations in essential thrombocythemia and primary myelofibrosis, however, provided a new molecular target that is specific for MPN cells. Due to the novel C-terminal amino acid composition and the surface presentation of mutant CALR (mutCALR) by the thrombopoietin receptor (MPL), mutCALR constitutes a unique antigen for immunotherapy. Therefore, we generated a chimeric antigen receptor (CAR) targeting mutCALR and examined its efficacy in murine models.

Aims:

We explored the use of CAR-T cells in murine MPN models.

Methods:

We generated a fully murine anti-mutCALR CAR by incorporating single chain variable fragment derived from a mouse monoclonal antibody targeting mutCALR into an established CAR-backbone by Kochenderfer et al. CAR-T cells were produced by retroviral transduction of CD3/CD28-activated splenic T cells and used on day 3-7 post isolation. We tested the efficacy of these CAR-T cells against a modified Ba/F3 cell line overexpressing human CALRdel52 and the human thrombopoietin receptor (MPL) *in vitro* and *in vivo* in a bioluminescence-based NSG-xenograft model. Overall survival was assessed by using the Kaplan-Meier method and curves were compared with a Log-rank test. We further used a transgenic mouse model established in our group to test CAR-T cells against in a physiologically relevant immunocompetent model *ex vivo* and *in vivo* by using a competitive bone marrow transplantation model and assessing mutant:wild type chimerism.

Results:

Murine anti-mutCALR CAR-T cells selectively killed Ba/F3 cells overexpressing mutCALR in cell culture experiments, while cells with wild type (wt) CALR were unaffected. To assess the efficacy of CAR-T cells in vivo, we engrafted NSG mice with mutCALR-expressing Ba/F3 cells by i.v. injection and treated mice with either Mock-T cells or CAR-T cells. While all mice showed Ba/F3 cell engraftment at the start of treatment, CAR-T cell treated mice achieved bioluminescence negativity indicative of tumor-cell clearance for 25-63 days. Further, with a cohort of five mice each, median overall survival was significantly improved by CAR-T cell treatment (21 days in the CAR-T cell cohort vs. 48 days in the Mock-T cell cohort, $p = 0.0062$). CAR-T cells further led to specific clearance of CALR-mutated stem cells harvested in *ex vivo* co-culture experiments using a mixture of lineage negative cells isolated from wild type and transgenic animals. On the contrary, the *in vivo* treatment of immunocompetent chimeric animals after competitive bone marrow transplantation did not result in decrease of mutant cells and CAT-T cell activation. Analysis of recovered CAR-T cells showed decreased staining index using fluorescently labelled peptide carrying the target mutCALR epitope suggesting the secreted mutCALR may prevent CAR-T cell activation in vivo.

Summary/Conclusion:

Here we generated the first CAR targeting mutCALR. CAR-T cells could effectively eliminate Ba/F3 cells overexpressing mutCALR *in vitro* and *in vivo*. Further, CAR-T cells recognized primary CALR-mutated stem cells and led to their eradication in *ex-vivo* co-culture experiments. While this proves that CAR-T cells can effectively target primary CALR-mutated cells, *in vivo* treatment of chimeric animals did not result in disease clearance.

Therefore, we are further investigating mechanisms hindering CAR-T cells in this fully immunocompetent model.

Keywords: Mouse model, Myeloproliferative disorder, CAR-T