Targeting T-cell receptor β-constant domain for immunotherapy of T-cell malignancies

AUTHORS

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T-cell lymphomas and leukemias are aggressive, treatment-resistant cancers with poor prognosis. Immunotherapeutic approaches have been limited by a lack of target antigens discriminating malignant from healthy cells. While treatment of B-cell cancers has been enhanced by targeting pan B-cell antigens, an equivalent approach is not possible for T-cell malignancies since profound T-cell depletion, unlike B-cell depletion, would be prohibitively toxic. We propose an immunotherapeutic strategy for targeting a pan T-cell antigen without causing severe depletion of normal T-cells.

The α/β T-cell receptor (TCR) is a pan T-cell antigen, expressed on >90% of T-cell lymphomas and all normal T-cells. An overlooked feature of the TCR is that the β-constant region comprises 2 functionally identical genes: TRBC1 and TRBC2. Each T-cell expresses only one of these. Hence, normal T-cells will be a mixture of individual cells expressing either TRBC1 or 2, while a clonal T-cell cancer will express TRBC1 or 2 in its entirety.

Despite almost identical amino acid sequences, we identified an antibody with unique TRBC1 specificity. Flow cytometry (FACS) of T-cells in normal donors (n = 27) and patients with T-cell cancers (n = 18) revealed all subjects had TRBC1 and 2 cells in both CD4 and CD8 compartments, with median TRBC1 expression of 35% (range 25-47%). In addition, we examined viral-specific T-cells in healthy volunteers, by generation of Epstein Barr virus-specific primary cytotoxic T-cell lines (3 donors) or by identification of cytomegalovirus- (3 donors) or adenovirus- (5 donors) specific T-cells by peptide stimulation. We demonstrated similar TRBC1: 2 ratios in viral-specific cells, suggesting that depletion of either subset would not remove viral immunity. Next, using FACS and immunohistochemistry, we showed that TCR+ cell lines (n = 8) and primary T-cell lymphomas and leukemias (n = 55) across a wide range of histological subtypes were entirely restricted to one compartment (34% TRBC1).

As proof of concept for TRBC-selective therapy, we developed anti-TRBC1 chimeric antigen receptor (CAR) T-cells. After retroviral transduction of healthy donor T-cells, comprising mixed TRBC1/2 populations, 90% of T-cells expressed CAR on the surface. No detectable TRBC1 T-cells remained in the culture, suggesting selective depletion of this population. Anti-TRBC1 CAR T-cells secreted interferon-γ in response to TRBC1-expressing target cell lines ($P<0.001$) and autologous normal TRBC1+ cells, and not TRBC2 cell lines or autologous normal TRBC2 cells. Anti-TRBC1 CAR killed multiple TRBC1 cell lines and autologous normal TRBC1cells, and not TRBC2 cell lines or autologous normal TRBC2-cells. These cell line based findings were confirmed using primary cells from two patients with TRBC1+ adult T-cell

leukaemia/lymphoma. We demonstrated specific tumour kill by allogeneic or autologous T-cells *in vitro*, despite partial downregulation of surface TCR by tumour cells. We developed a xenograft murine model of disseminated T-cell leukemia by engrafting engineered firefly luciferase+ TRBC1+ Jurkat cells in NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG) mice. Bioluminescent imaging and FACS of marrow at 5 days following IV T-cell injection showed that mice treated with anti-TRBC1 CAR T-cells and not non-transduced (NT) T-cells had disease clearance ($p<0.0001$). In a further model, mice were engrafted with equal proportions of TRBC1-Jurkat and TRBC2-Jurkat cells. FACS analysis of bone marrow at 5 days post T-cells demonstrated specific eradication of TRBC1 and not TRBC2 tumour by anti-TRBC1 CAR ($p<0.001$).

In summary, we have demonstrated a novel approach to investigation and targeting of T-cell malignancies by distinguishing between two possible TCR β-chain constant regions. Using CART-cells targeting TRBC1 we have demonstrated proof of concept. Unlike non-selective approaches targeting the entire T-cell population, TRBC targeting could eradicate a T-cell tumour while preserving sufficient normal T-cells to maintain cellular immunity.