

are shown on the upper panel. Each of this population is gated and forward scatter vs JOVI-1 staining are shown on the Y and X-axes respectively. These data show that both CD4+ and CD8+ compartments contain cells which are TRBC1 +ve and -ve. This is representative data from one donor.

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FIGURE 7: TRBC1+ T-cells in several normal donors. Ten normal donors were bled and peripheral blood mononuclear cells were stained as described in figure 4 above. The aggregate data of the proportion of TRBC1 T-cells in both CD4 and CD8 compartments is shown in a bar graph along with median and range. All donors had

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FIGURE 8: T-cell malignancy derived cell lines stained with JOVI-1. Several cell lines have been derived from T-cell malignancies. Many of these cell lines still express the TCR. We selected Jurkats (A T-cell leukaemia cell line), HPB-ALL (another T-cell leukaemia cell line) and HD-Mar-2 (a T-cell lymphoma cell line) for study. By staining these cell lines with a pan-TCR/CD3 antibody, we were able to demonstrate that all three express TCR (left panels, staining overlaid over isotype control staining). Next, by staining with JOVI-1 we were able to determine that these T-cell lines are either TRBC1 negative or positive. Only Jurkats cells (TRBC1+) and not HPB-ALL or HD-Mar-2 (TRBC2+) cells stain with JOVI-1, supporting exclusive expression of either TRBC1 or

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FIGURE 9: Selective Killing of TRBC1 T-cells with JOVI-1 mAb. Wild-type Jurkat T-cells (CD34-, TRBC1+) were mixed with TCR $\alpha\beta$ knock-out Jurkat T-cells transduced with TRBC2 co-expressed with the CD34 marker gene (CD34+TRBC2+). These cells were incubated with JOVI-1 alone or incubated with JOVI-1 and complement for 1 hour. Cells were washed and stained for CD34, Annexin V and 7-AAD. Cells were analysed by flow-cytometry. Shown below is CD34 expression in the live population as defined by Annexin-V negative and 71AAD dim population. (a) JOVI-1 alone; (b) JOVI-1 with complement. Selective killing of TRBC1 T-cells (CD34-) is observed.

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FIGURE 10: Polyclonal Epstein Barr Virus (EBV) specific T-cells can be split into two approximately equal TRBC1/2 populations. Using well established methods, the inventors selectively expanded EBV specific T-cells from the peripheral blood of a normal donor. (a) The subsequent line had a high degree of selectivity against autologous EBV infected B-cells (auto LCLs), and no activity against allogeneic EBV

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infected T-cells (allo LCLs), and no non-specific NK activity (as measured by testing against K562 cells). Such a line is representative of the donor's EBV immunity. (b) When stained with JOVI-1, these T-cells typed approximately equally for TRBC1 and TRBC2.

5 **FIGURE 11: Annotated sequence of JOVI-1 VH and VL.** The hypervariable regions are underlined and in bold.

FIGURE 12: Demonstration that a peripheral T-cell lymphoma is TRBC restricted, but normal circulating T-cells are not. Peripheral blood T-cells from a patient with
 10 circulating T-cell lymphoma cells were drawn. Peripheral mononuclear cells were isolated and stained with a panel of antibodies including CD5, TCR and JOVI-1. Normal and malignant T-cells could be differentiated on flow by CD5 expression intensity. CD5 bright (normal T-cells) had approximately equal TRBC1 and 2 populations. The CD5 intermediate and dim populations (the tumour) were all TRBC2 positive. If this patient
 15 had a TRBC2 directed therapy, the lymphoma would be eradicated and approximately half of their T-cells would be spared.

FIGURE 13: Demonstration that the VH and VL derived from JOVI-1 were correct and that they can fold as a single-chain variable fragment. Original hybridoma
 20 supernatant, recombinant JOVI-1 antibody and scFv-Fc generated from transfected 293T cells were used to stain a number of cell lines: Jurkat TCR knock-outs, wild-type Jurkats, Jurkat TCR knock-out transduced with a TRBC1 TCR in a vector co-expressing eBFP2; Jurkat TCR knock-out transduced with a TRBD2 TCR in a vector co-expressing eBFP2. Staining was analysed by flow cytometry. Both the recombinant antibody and
 25 the scFv bound cells expressing TRBC2.

FIGURE 14: JOVI-1 based CARs in different formats. CARs typically comprise of a binding domain, a spacer, a transmembrane domain and an intracellular signalling domain. In this study CARs were generated which comprise of the JOVI-1 scFv; a
 30 spacer derived from either (a) the CD8 stalk, (b) the hinge-CH2-CH3 domain of human IgG1 with mutations which remove FcR binding; or (c) a spacer derived from human IgG1.

FIGURE 15: Function of JOVI-1 based CAR. Normal donor peripheral blood T-cells
 35 were transduced with the different CARs described above. T-cells were also transduced with a CD19-specific CAR as a control. These T-cells were then challenged with target cells: Jurkats – TCR knock-out and Jurkats wild-type and Raji

262	Solid-phase, indirect immobilisation	TRBC1	Round-2	186	93
263	Solid-phase, indirect immobilisation	TRBC2	Round-2	186	68
264	Solution-phase	TRBC1	Round-2	186	83
265	Solution-phase	TRBC2	Round-2	186	29
266	Solution-phase	TRBC1	Round-3	94	47
267	Solution-phase	TRBC2	Round-3	94	33
268	Solid-phase, direct immobilisation (BSA/OA)	TRBC1	Round-2	94	32
269	Solid-phase, direct immobilisation (BSA/OA)	TRBC2	Round-2	94	9

Example 13 – TRBC polyclonal antibody production via peptide immunisation of rabbits

In order to generate antibodies which distinguish between TRBC2 and TRBC1, 2 peptides that cover the principle area of differentiation between the two TRBC isoforms were synthesized and used for the immunization of rabbits. The following peptide sequences were used:

TRBC1: VLEDLNKVPPEVAVC (SEQ ID No. 38)

TRBC2: VLEDLKNVPPEVAVC (SEQ ID No. 39).

15 mg of TRBC1 and TRBC2 peptides were synthesized. Keyhole Lympet Hemocyanin was conjugated to TRBC1 and TRBC2 peptides via C terminal cysteines present on the peptides. For each peptide 2 New England rabbits were immunized a total of three times with KLH conjugated TRBC1 or TRBC2 peptide. After the third immunization rabbits were sacrificed and bled, and the serum collected for purification. The crude serum obtained from the rabbits were passed through a crosslinked beaded agarose resin column coupled with the peptide used for immunization to collect antibodies specific for the common segments and the TRBC isoform specific epitope of the peptide. The initially purified supernatant was then