Editorial

Fishing therapeutic T-cell receptors in healthy donor blood, is safety predictable?

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Adoptive transfer of genetically modified cells is close to becoming mainstream therapy for hematological malignancies (1). It entails the addition of targeting receptors in an effector cell (T cell or NK cell). To date, two main types of receptors have been exploited: a synthetic construct known as chimeric antigen receptor (CAR) which is comprised of the fusion of an antibody binding domain with a signaling transmembrane receptor or a T-cell receptor (TCR) which is the natural targeting protein of a T cell. Both receptors are expected to guide effector cells to recognize the tumour and to kill it. Astonishing clinical successes were reported using CAR targeting the B-cell lineage marker CD19, whereas TCRs have been harder to exploit (1). The main advantages of CARs over TCRs reside in their high affinity and MHC-independent binding, the latter rendering their use universal. However, CAR being restricted to membrane proteins, only 1% of the proteome can be targeted. In addition, not all hematological malignancies, such as multiple myeloma, express CD19 antigen, thus novel specific targets need to be identified, but the search for other extracellular proteins has been somewhat disappointing. Jahn and colleagues in a recent issue of *Blood* (2) achieved two goals: (I) they identified a novel target for $CD19^-$ hematological malignancies, including multiple myeloma; and (II) they isolated a specific antigen receptor and performed a pre-clinical validation of this molecule. Since their target was intracellular, they took advantage of the ability of the inherent TCR unrestricted spatial recognition of their ligand. Finally, since this target protein is also expressed in healthy B cells, precluding the existence of TCR in patients due to clonal deletion of autoreactive thymocytes, they used unmatched healthy donor blood to find reactive T cells.They elegantly present the feasibility of exploiting such an alternative approach for isolating therapeutic material, but also the difficulty of finding a safe candidate.

The authors analyzed a microarray gene expression database and identified *POU2AF1* as a candidate gene whose expression was B-cell lineage restricted, but undetectable in $CD34^+$ hematopoietic progenitor cells. *POU2AF1* encodes a specific transcription factor, BOB1 (also known as OCA-B or OBF-1). They then searched for potential BOB1 peptides binding frequent MHC alleles and found four candidate peptides, one restricted to HLA-A*02:01 and three to HLA-B*07:02, both being frequent alleles in the Caucasian population. In order to find reactive TCRs, they isolated TCRs from non-matched MHC donors, a strategy of such outsourcing of the immune response first reported by Sadovnikova and colleagues (3). These so-called alloreactive TCRs have been shown to have a high affinity for their target. The isolation protocol described by Jahn and colleagues was based on peptide-MHC tetramer recognition and after a laborious screening process, eleven BOB1 peptides-specific clones were isolated. Among those, two demonstrated specific recognition of peptide-pulsed target cells. However,

only one T-cell clone, 4G11, HLA-B*07:02 restricted, could efficiently recognize endogenously processed and presented BOB1. Furthermore, this clone was reactive against different malignant cells, including primary samples of multiple myeloma. Next, the 4G11 TCR sequence was identified and optimized by genetic modifications (4) and transferred to recipient T cells. *In vitro* experiments confirmed its specificity and, finally, the authors used a multiple myeloma xenograft mouse model to validate the *in vivo* antitumor efficacy of their TCR.

The paper by Jahn and colleagues supports BOB1 as an attractive target in the treatment of B-cell malignancies such as ALL, CLL, MCL but, most importantly, multiple myeloma which does not have any available curative treatment. The authors openly discussed the different aspects of an orientated treatment and important points are developed. It is worth noting that, as for CD19-based targeting, removal of expressing cells might also lead to B-cell aplasia. Therefore, a transient or controlled system should be favored. Will target cells be able to reduce BOB1 expression and escape immune surveillance during treatment, as observed with CD19 (5)? Here the authors speculate on the critical role of BOB1 and the necessity for malignant cells to express it. Alloreactive TCR represents a smart alternative to fish high avidity TCRs since autoreactive TCRs should not be available. However, since specific TCRs reactive for non-mutated tumour antigens such as MART-1 have been identified in patients and healthy donors, it would be interesting, if such TCRs exist for BOB1, to compare them to the alloreactive ones. Since the autoreactive TCRs have already been screened in the thymus, they might be less cross-reactive. The paper of Jahn and colleagues also demonstrated the rather poor outcome of a peptide-MHC multimer-based screen. The authors mentioned that "thousands of T cells" were first enriched, but they finished their study with a single clone. One can ask if the method of selection was sound. Indeed, the use of software prediction algorithms and bacterially produced MHC to evaluate peptide recognition might be the source of the problem. First, the prediction tools might miss interesting candidates. This was the case when the clinically validated TGFbRII frameshift peptide was analyzed in by public algorithms (6) (and our unpublished data). Second, the *in vitro* peptide loading on the multimer might not be efficient enough to reproduce the intracellular loading. We have recently reported such a defect for the TGFbRII frameshift peptide (7): although well recognized by a specific TCR when loaded as a peptide or endogenously generated through proteasome degradation, this peptide could never be prepared in a peptide MHC multimer format. Consequently, if the TGFbRII frameshift protein had been analyzed using Jahn and colleagues' method, this peptide and its cognate TCR would never have been identified: their fishing rod would miss a valuable fish. A selection based on a less artificial platform might have led to a greater amount of real positive clones. Alternatively, one could select for cell-generated peptides and thereby reduce the number of false positives and increase the number of reactive T cells (8). In addition, the poor amount of specific T cells isolated from a healthy donor raises questions about the safety of using alloreactive clones and TCR in a clinical setting. It also leads to a crucial issue about the selected TCR 4G11: is it specific or are we not equipped to detect its cross-reactivity? Although TCR4G11 did not show any reactivity against a broad panel of $BOB1^-$ $HLA\text{-}B*07:02^+$ stimulator cells and any cross-reactivity against other HLA class I and II alleles tested, the cross-reactivity of a TCR is nearly impossible to determine since it is an unfeasible task to test every existing type of cells and MHC alleles. It is important to emphasize that the authors have performed a significant number of relevant tests to verify the specificity of the 4G11 clone. Nevertheless, the difficulty of identifying cross-reactive peptides has recently been reported and has been shown to carry the risk of causing dramatic outcomes (9). In conclusion, the possibility of off-target or on-target off-tumor toxicity can only be assessed when tested in a human being… and still, cross-reactivity could be patient specific due to infrequent polymorphisms or mutations. Therefore, although the method commented herein is smart, inventive and elegant, alloreactive TCRs targeted against non-mutated antigens should be combined with safety systems (10). Thus, Jahn *et al.* provide an attractive yet risky perspective to treat incurable diseases like multiple myeloma without the need for hunting for neoantigens.

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Footnote

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