T Cell Receptor Gene Therapy Targeting the Intracellular Transcription Factor Bob1 for the Treatment of Multiple Myeloma and Other B Cell Malignancies

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Therapeutic reactivity of CD20-specific monoclonal antibodies (mAb) or CD19-specific chimeric antigen receptor (CAR)-transduced T cells is exerted by targeting extracellular antigens. In contrast to mAbs and CARs, T cell receptors (TCRs) recognize antigen-derived peptides that are bound to human leukocyte antigen (HLA) molecules on the cell surface. Since HLA molecules constantly sample the entire endogenous proteome of a cell, extracellular and intracellular antigens are presented and can thus be recognized by a TCR.

Here, we identified the intracellular transcription factor Bob1 encoded by gene POU2AF1 as a suitable target for immunotherapy. Bob1 is highly expressed in CD19⁺ B cells, acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL) and multiple myeloma (MM) and is absent in the non-B lineages including CD34⁺ hematopoietic progenitor cells (HPCs), T cells, fibroblasts, keratinocytes and gastrointestinal tract. Bob1 is localized intracellularly but HLA-presented Bob1-derived peptides are accessible on the cell surface to TCRs and can thus be recognized by T cells. From the HLA-presented ligandome (Mol Cell Proteomics, 2013;12:1829) we identified naturally processed Bob1-derived peptides displayed in HLA-A*0201 (HLA-A2) and in HLA-B*0702 (HLA-B7). Since auto-reactivity towards self-antigens such as Bob1 is prevented by depleting high-
avidity T cells recognizing self-antigens in self-HLA, we exploited the immunogenicity of these peptides presented in allogeneic HLA. From a HLA-A2/B7-negative healthy individual we isolated T cell clone 4G11 demonstrating high sensitivity and specificity for Bob1-derived peptide Bob144 presented in HLA-B7. Bob1-dependent recognition was demonstrated by transduction of Bob1 into cell lines that otherwise lack Bob1 expression. No harmful toxicities of clone 4G11 were observed against a wide panel of Bob1-negative stimulator cells including HLA-B7-positive CD34+ HPCs, T cells, monocytes, immature and mature dendritic cells, and fibroblasts even under simulated inflamed conditions. Furthermore, stringent HLA-B7-restricted recognition was observed for clone 4G11 when tested against a stimulator panel expressing a wide range of common and rare HLA class I and II molecules. Clone 4G11 demonstrated clinical applicability by efficiently recognizing HLA-B7+ primary ALL, CLL and MCL. Furthermore, reproducible strong recognition of purified primary HLA-B7+ MM could be demonstrated.

Therefore, the TCR of clone 4G11 may be used for immunotherapy by administering TCR-transduced T cells to patients suffering from B cell malignancies including multiple myeloma. Retroviral gene transfer of TCR 4G11 led to efficient cell surface expression demonstrated by binding of TCR-transduced CD8+ T cells to pMHC-tetramer composed of peptide Bob144 bound to HLA-B7. TCR-modified CD8+ T cells strongly recognized Bob1-expressing HLA-B7+ multiple myeloma cell lines U266 and UM9, and ALL cell lines. TCR-modified T cells efficiently lysed HLA-B7+ primary ALL, CLL and MCL at very low effector-to-target ratios. In addition, highly purified primary multiple myeloma samples were also readily lysed. Furthermore, TCR-transduced T cells strongly proliferated in an antigen-specific manner when stimulated with primary malignant cell samples including ALL, CLL, and MCL or MM cell lines. As expected,
TCR-transduced T cells also lysed autologous primary and CD40L-stimulated B cells since these targets cells also express Bob1. In contrast, no lysis of Bob1-negative autologous primary and activated T cells, or monocytes was observed when co-cultured with TCR-transduced T cells.

In summary, we identified the intracellular transcription factor Bob1 encoded by gene POU2AF1 as a suitable target for TCR-based immunotherapies of B cell malignancies. Bob1-specific T cell clone 4G11 efficiently recognized primary B cell leukemia and multiple myeloma. Gene transfer of TCR of clone 4G11 installed Bob1-reactivity and specificity onto recipient T cells shown here by cytolytic capacity and proliferation upon antigen encounter. TCR gene transfer approaches using this Bob1-specific TCR can bring novel treatment modalities and possibly curative therapy to patients with B cell malignancies including multiple myeloma.

Characters (without spaces): 3,711/3,800 max.

**Category:**

700s – Transplantation: 703 Adoptive Immunotherapy

Includes pre-clinical in vitro and animal models of adoptive immunotherapy including T cells, regulatory cells, dendritic cells and other cell populations designed to treat malignancies or infections, and vaccine therapies.

Or

600s – Hemological Malignancy: 652. Myeloma: Pathophysiology and Pre-Clinical Studies, excluding Therapy
Includes signal transduction studies, animal and pre-clinical models as well as preclinical studies of novel emerging therapies.