Development of an optimized scaffold for bispecific T-cell receptor therapeutics

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Introduction

Bispecific T-cell receptor (TCR)-antibody fusion proteins against tumor-specific targets represent a promising class of cancer therapeutics. The utilization of a TCR moiety is a major advantage of these molecules as it allows targeting of human leucocyte antigen (HLA)-bound peptides derived from virtually all proteins of the tumor cell regardless of their extracellular or intracellular location. Immatics is developing TCR bispecifics against tumor associated peptide-HLA targets, which have been identified and validated by its proprietary target discovery engine XPRESIDENT®. Immatics has further established a portfolio of technologies to discover and engineer TCRs originating from the natural repertoire of human donors. After affinity maturation of single chain TCRs (scTv), the mutant scTv candidates displaying enhanced stability and affinity serve as building blocks for the generation of soluble and highly potent bispecific T cell receptor therapeutics.

Here we present the development efforts to generate an optimized scaffold for the construction of such bispecific TCR molecules. Six different TCR-antibody fusion scaffolds with various variants thereof were designed utilizing an affinity maturated scTv specific for the HIV-derived SL9 peptide (SLYNTVATL) presented on HLA-A*02. For engagement of T-cells, two different antibody-derived domains were tested. All candidate molecules were purified from CHO supernatants and subjected to thoroughly *in vitro* testing including stress studies. The scaffolds were ranked according to developability characteristics including productivity and stability as well as efficacy and safety data to finally determine the molecular architecture of Immatics' bispecific T-cell receptor therapeutics platform.

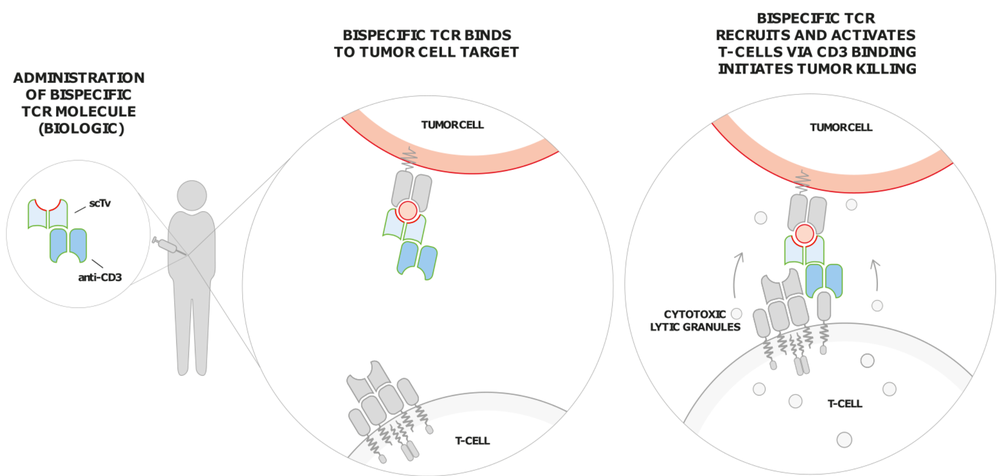


Figure 1: Anticipated mode-of-action of bispecific T-cell receptor therapeutics. Bispecific TCR molecules consist of an affinity maturated single-chain T-cell receptor (scTv) combined with variable domains derived from an effector cell-recruiting antibody. The cartoon schematically shows the binding of the bispecific TCR therapeutic to a tumor cell and the subsequent recruitment and activation of a T-cell. Finally this results in tumor cell lysis induced by the T-cell.

Bipecific TCR scaffold design

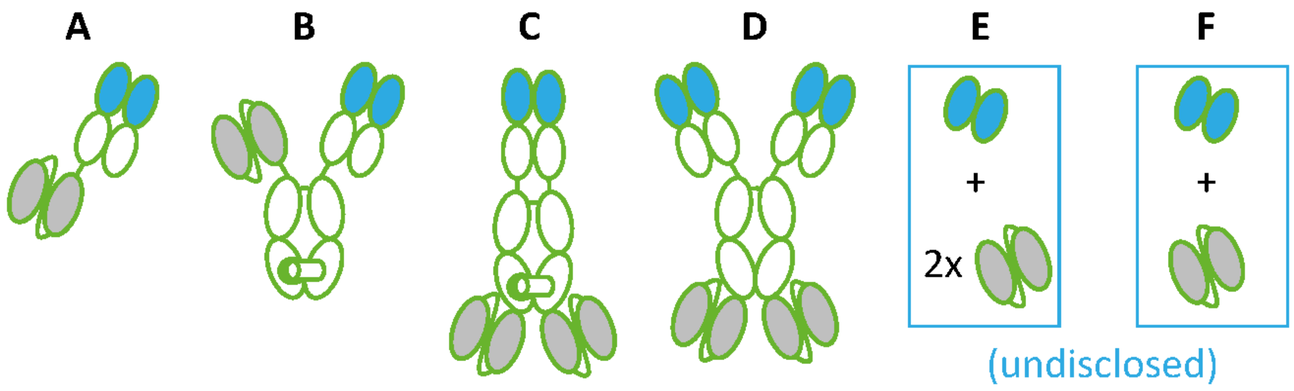


Figure 2: Schematic design of bispecific TCR scaffolds used in the study. Six different molecular designs of bispecific TCR molecules (A-F) were generated based on a public domain single-chain TCR (filled in grey, 868Z11 scTv against HIV-specific SLYNTVATL:HLA-A*02) and humanized variants of different T-cell recruiting antibodies comprising variable domain (filled in blue) and IgG1-derived constant domains (non-filled). Distinct mutations were introduced into IgG1 heavy chain constant domain 2 to ablate binding to Fc gamma receptors. Scaffolds (B) and (C) utilize knob-into-hole technology for improved heterodimerization. Scaffolds (E) and (F) are not disclosed.

Key facts

- Affinity maturated single-chain T-cell receptors (scTvs) can serve as building blocks for bispecific TCR-antibody fusion proteins.

  If you want to learn more about Immatics' TCR maturation platform please visit our poster:

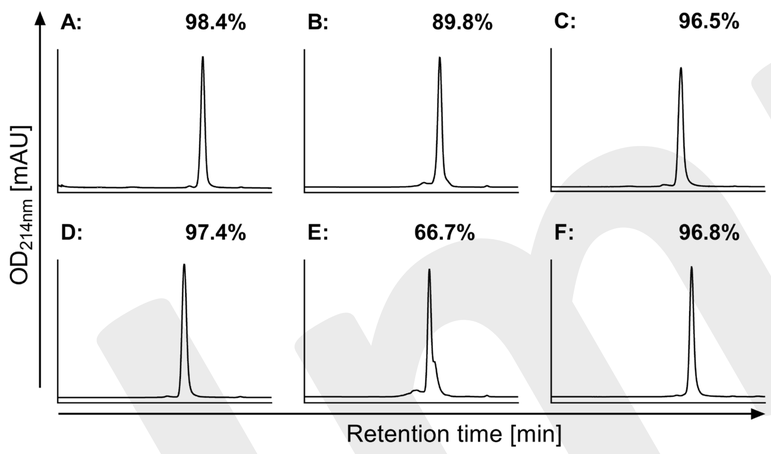
  Setup and validation of a T cell receptor maturation platform resulting in high-affinity binders for engineering of bispecific molecules

- IgG-based bispecific TCR molecules can be produced and purified utilizing standard techniques established for antibody production.

- The presented molecular scaffolds provide the basis for the generation of stable and highly potent bispecific TCR therapeutics.

- Immatics' proprietary platform for bispecific TCR molecules enables the optimization of mode-of-action by exchanging different effector cell-recruiting domains.

Production and purification of bispecific TCR molecules



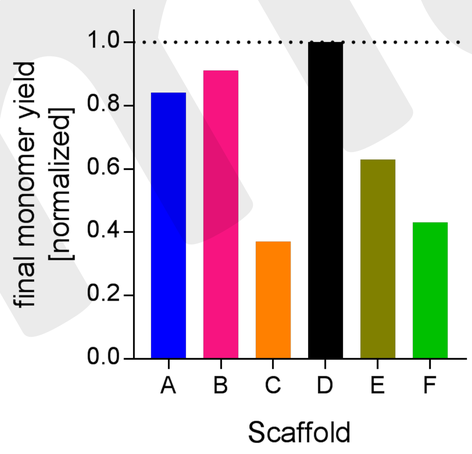


Figure 3: Production, purification and monomer yield of bispecific TCR molecules. Bispecific TCR molecules were produced by transient expression in CHO cells (ExpiCHO, Thermo) and purified with a capture column (MAbSelect SuRE or HiTrap Protein L) followed by preparative size exclusion chromatography (SEC, Superdex 200) on an Äkta Pure 25 L system (all GE Lifesciences). Left panel: SEC retention profiles and calculated monomer content of purified bispecific TCR molecules as determined by analytical SEC (MAbPac SEC-1 column on a Vanquish UHPLC-System, Thermo). Right panel: Final monomer yields of different bispecific TCR molecules shown as normalized graph.

Potency of target cell lysis is dependent on molecular scaffold

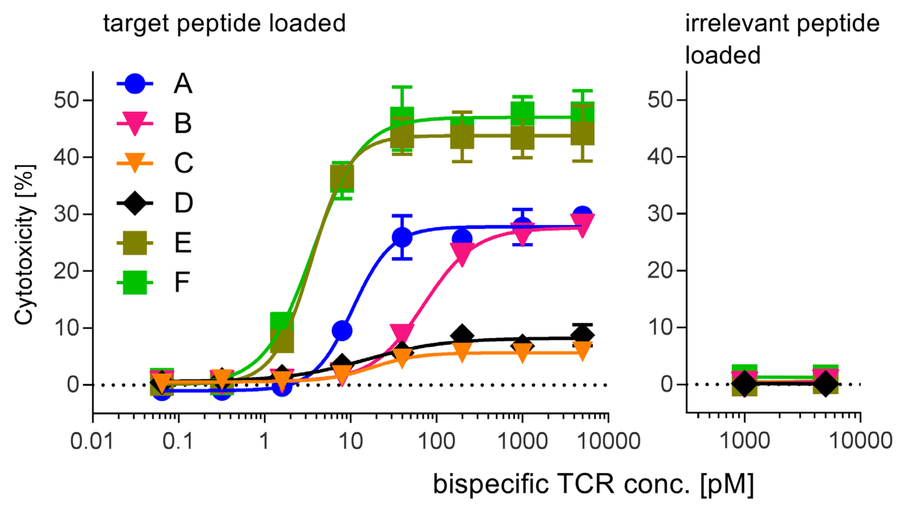


Figure 5: Cytotoxicity induced by bispecific TCR-molecules. Target cell lysis induced by bispecific TCR molecules was quantified utilizing unstimulated PBMC of healthy donors. These effector cells were cocultured with T2 cells (E:T = 10:1) loaded with low densities of either target peptide (10 nM SLYNTVATL, left panel) or irrelevant peptide (right panel), respectively. Cytolysis of T2 cells was quantified by measuring LDH release after 24 hours.

Activity and stress stability of bispecific TCR molecules tested by potency assay

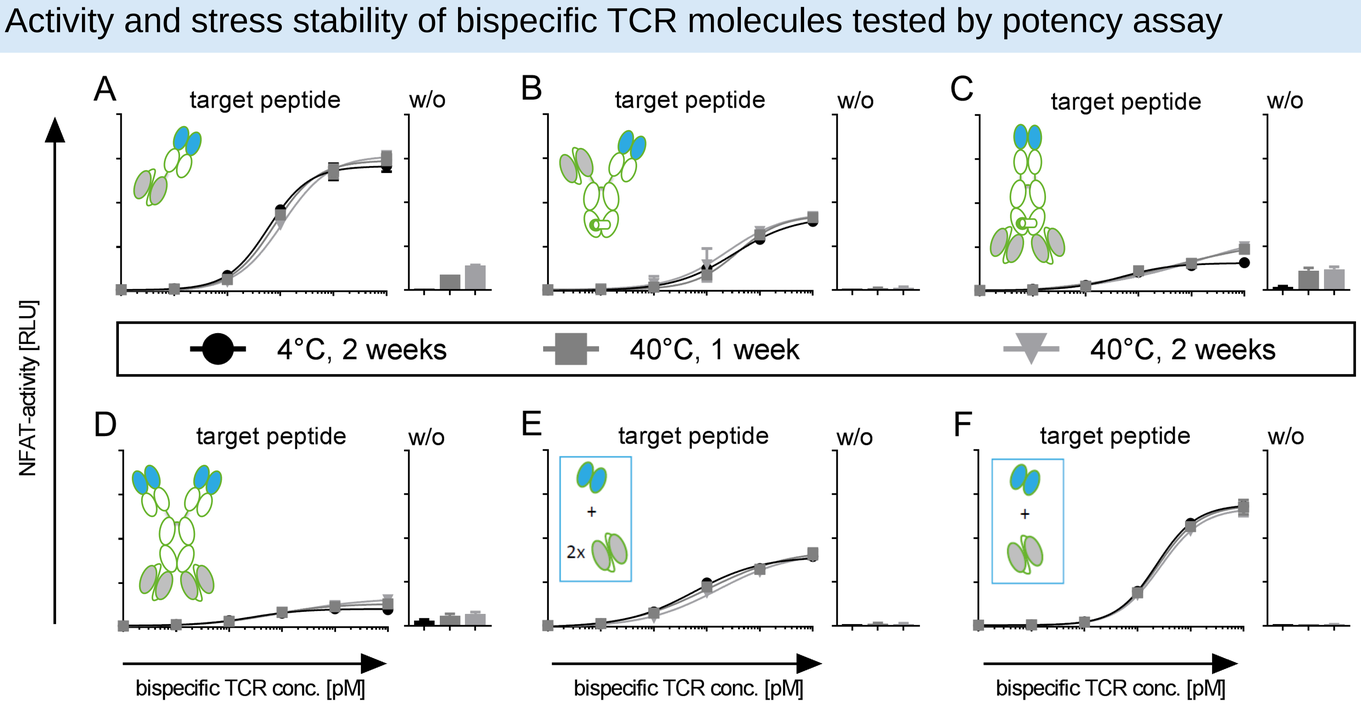


Figure 4: Activity of bispecific TCR scaffolds in absence and presence of thermal stress. The different bispecific TCR scaffolds A-F were assessed for their potency to activate the NFAT Reporter Jurkat Cell Line (Promega) containing a firefly luciferase gene under the control of an NFAT response element. For the assay, the Jurkat cells were cocultured with HLA-A*02 positive T2 cells (E:T = 1:1) loaded with 10 nM SLYNTVATL (target peptide, low density) or left unloaded (w/o), each in the presence of bispecific TCR molecules. After 24 hours NFAT activation was determined by quantification of luminescence signals. To assess the thermal stress resistance of the molecules, the different bispecific TCR scaffolds A-F, formulated at 1 mg/ml in PBS, were incubated for one or two weeks at 40°C prior to the potency assessment. Despite all scaffolds showed an increased content of aggregates after incubation at 40°C (HPLC-SEC, data not shown) the bispecific TCR scaffolds (B), (E) and (F) did not show aggregate-induced activation of reporter cells in absence of loaded target peptide.

Bispecific TCR scaffold F is suitable as platform

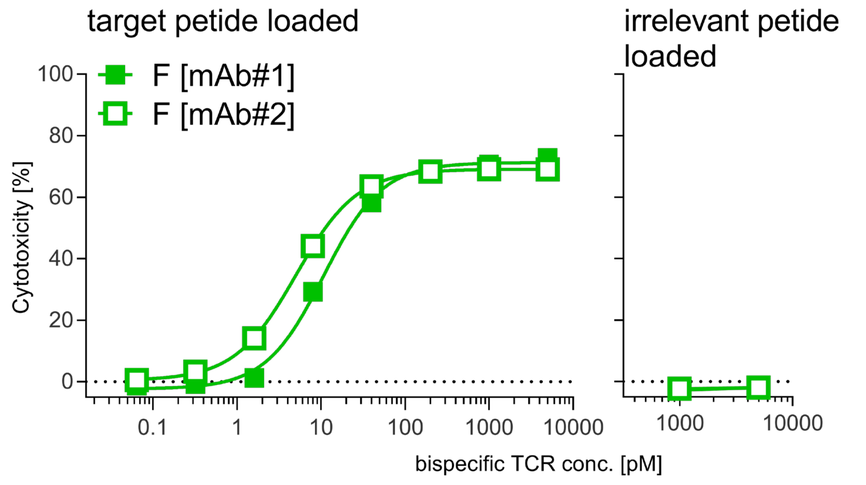


Figure 6: T-cell recruiting domains are interchangeable within molecular scaffold F. The variable domains of the T-cell-recruiting antibody in scaffold F (mAb#1) were exchanged against a second T-cell-recruiting antibody (mAb#2) and a bispecific TCR molecule was generated and purified. As with mAb#1 the bispecific TCR with mAb#2 induced target cell lysis in a highly potent and strictly target-dependent manner.