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

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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 in order to generate soluble and highly potent bispecific T cell receptor therapeutics.

Key aspects of Immatics' TCR maturation platform

- Native TCRs against peptide-HLA targets can be converted into stable single-chain TCRs (scTvs).
- Affinity maturation of the stable scTv by yeast surface display selection of degenerate CDR libraries strongly increases binding towards peptide-HLA target.
- Specificity of the affinity maturated scTv variants is retained by negative selection against similar peptides.
- Combination of the best CDR variants with the stable scaffold results in a highly affine scTv serving as targeting moiety for bispecific TCR generation.

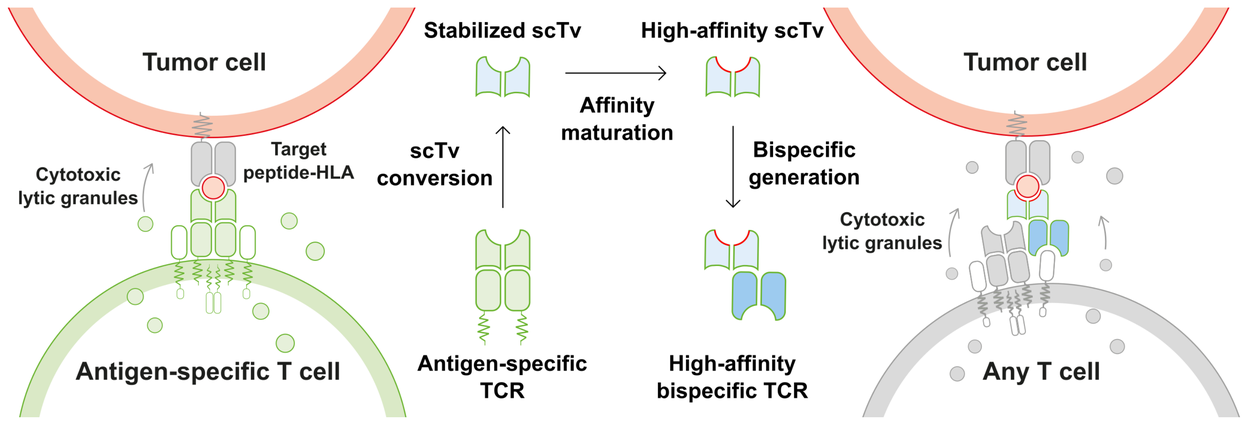

Figure 1: From native TCR to soluble bispecific TCR. Native TCRs with validated and specific recognition of XPRESIDENT® peptide-HLA targets are converted into scTv and further stabilized by introducing framework mutations via yeast surface display. Stabilized scTvs with increased binding affinity are selected from scTv yeast display libraries with combinatorial mutations in CDR regions. To maintain binding specificity during the affinity maturation the scTvs are counter-selected against HLA complexes presenting peptides from normal tissues, which have high sequence similarity to the tumor target peptide.

If you want to find out more about Immatics' bispecific molecules, please visit our poster:

Development of an optimized scaffold for bispecific T cell receptor therapeutics

Ag008-01 – a strictly tumor-associated peptide target

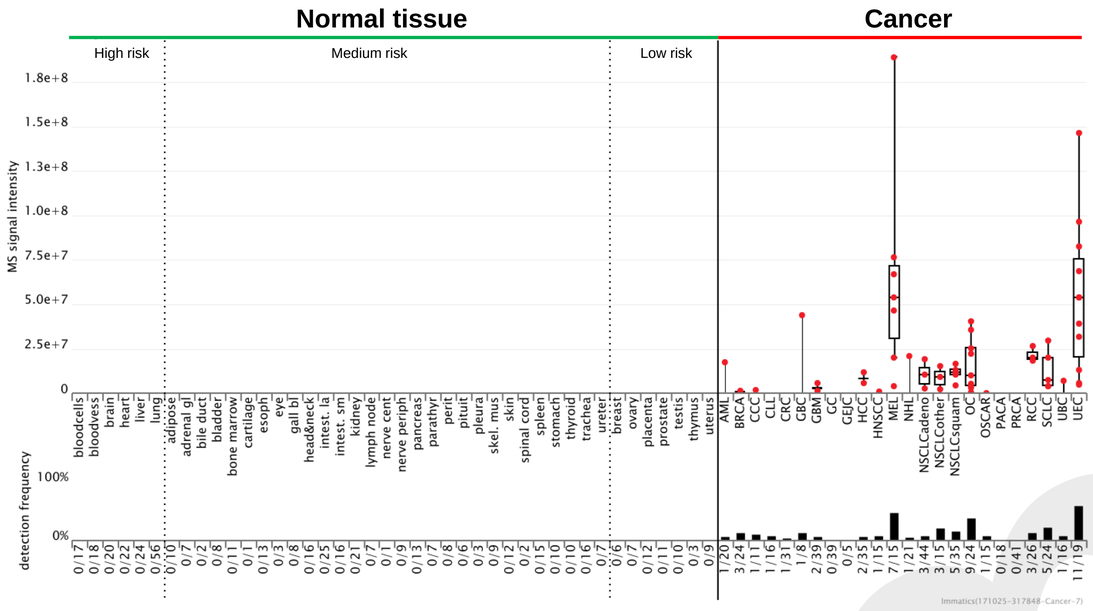

Figure 2: HLA-A*02-dependent presentation of Ag008-01 in cancer and in normal tissues. Upper panel: median normalized mass spectrometry signal intensities from technical replicate measurements are plotted as red dots for single HLA-A*02 positive tumor samples on which the target peptide Ag008-01 was detected. No detection on HLA-A*02 positive normal tissues (n≈500) was observed. Lower panel: The relative peptide detection frequency in every organ is shown as spine plot.

Native TCR P2 is highly specific for Ag008-01

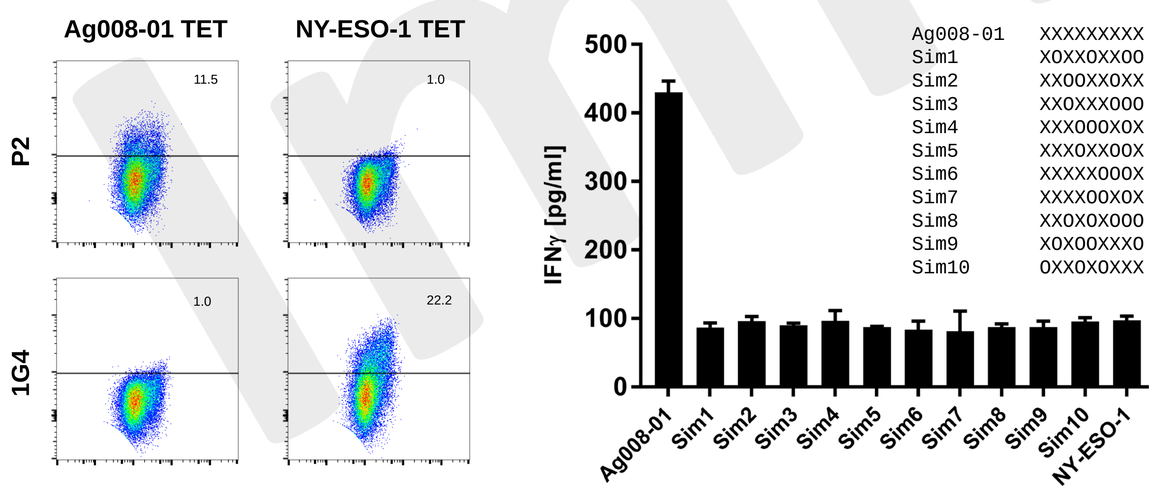

Figure 3: TCR P2 specifically recognizes the tumor-associated peptide Ag008-01. TCR P2, discovered from the T cell repertoire of a human donor, was reexpressed on human CD8+ T cells through electroporation of *in vitro* transcribed alpha/beta chain mRNA. Left panel: CD8+ T cells expressing TCR P2 or NY-ESO-1-specific control TCR 1G4 were stained with HLA-A*02 tetramers (TET) comprising Ag008-01 or NY-ESO-1 peptide. Right panel: IFNγ release of TCR P2 expressing CD8+ T cells upon coculture with T2 cells loaded with cognate Ag008-01 peptide, similar peptides (Sim1-10) or unrelated control peptide (NY-ESO-1). The similar peptides were selected based on presentation on normal tissues and degree of sequence similarity to Ag008-01 (identical amino acid residues are shown as X).

Conversion of native TCR P2 into a stabilized scTv

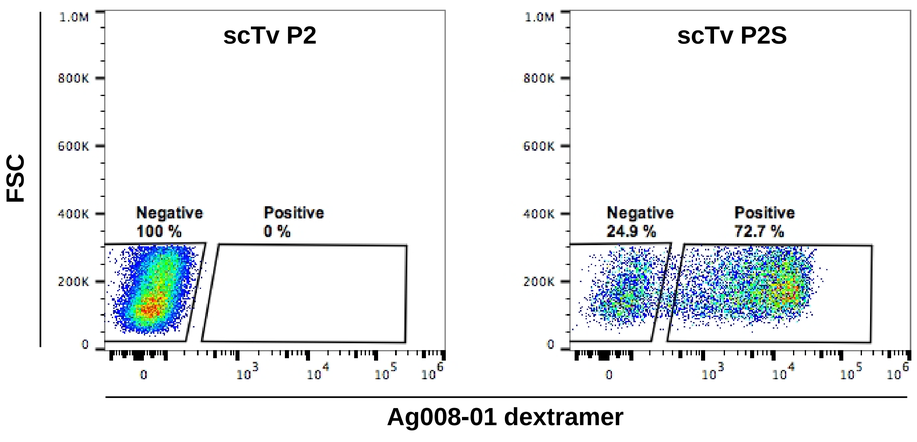

Figure 4: Conversion and selection of stable scTv variants of TCR P2 by yeast surface display. Alpha and beta chain variable domains of the TCR P2 were connected by a glycine serine linker and expressed on yeast cells. To increase scTv stability different mutations were introduced into the scTv scaffold and the resulting scTv library was sorted for 3 rounds to identify clones with increased expression and Ag008-01 binding. Left panel: Parental scTv P2 without stabilizing mutations stained with Ag008-01 dextramers. Right panel: Stabilized scTv P2S with 4 framework mutations stained with Ag008-01 dextramers.

Peptide-HLA toolbox for scTv conversion and affinity maturation

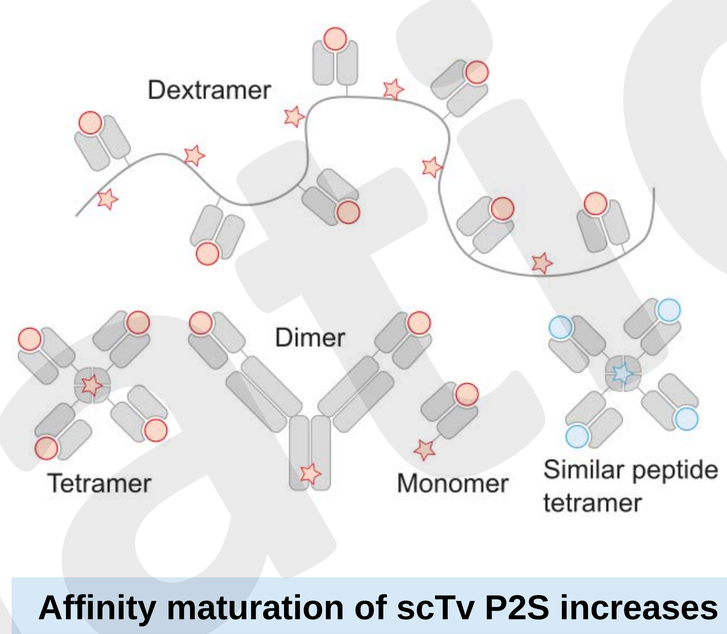

Figure 5: Toolbox for the selection of best performing scTvs in yeast surface display. Various peptide-HLA formats (with tumor-associated peptide in red) are used for the selection of the best binder. Starting with a low affinity TCR, a high-avidity approach is necessary to achieve staining of the yeast cells. Thus, a multivalent dextramer (Immudex) with up to 28 peptide-HLA binding sites can be used. The higher the scTv affinity due to the mutated CDR residues gets, the lower the valency of the tools can become, decreasing from tetramer with up to four binding sites, to dimers (BD™ DimerX) with two and finally monomers with only one binding site. To assure a high level of specificity, similar peptides (in blue) can be used for simultaneous negative selection with higher valency than used for the target peptide.

Affinity maturation of scTv P2S increases Ag008-01 binding

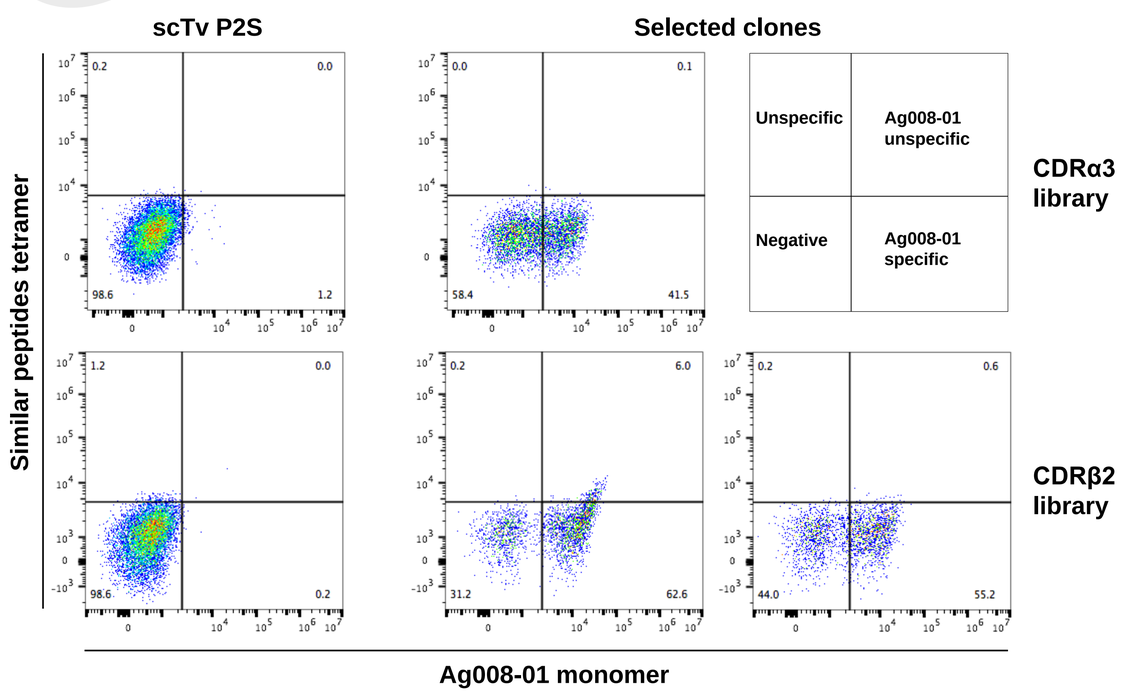

Figure 6: Affinity maturation and selection of specific scTv P2S variants. Based on stabilized scTv P2S, 6 different yeast libraries were generated, each with one degenerate CDR1, CDR2 or CDR3 sequence. The libraries were stained with Ag008-01 peptide-HLA-A*02 tetramers, dimers or monomers to select for best scTv binders. Simultaneous negative selection with a mix of HLA-A*02 tetramers comprising 10 different similar peptides (see Fig. 3) was performed. Left panel: Stabilized scTv P2S with unmodified CDRα3 and CDRβ2 sequence. Right panel: Affinity maturated scTv P2S variants with mutant CDRα3 or CDRβ2 sequence.

Mutant CDR combination leads to high-affinity scTv for bispecific TCR

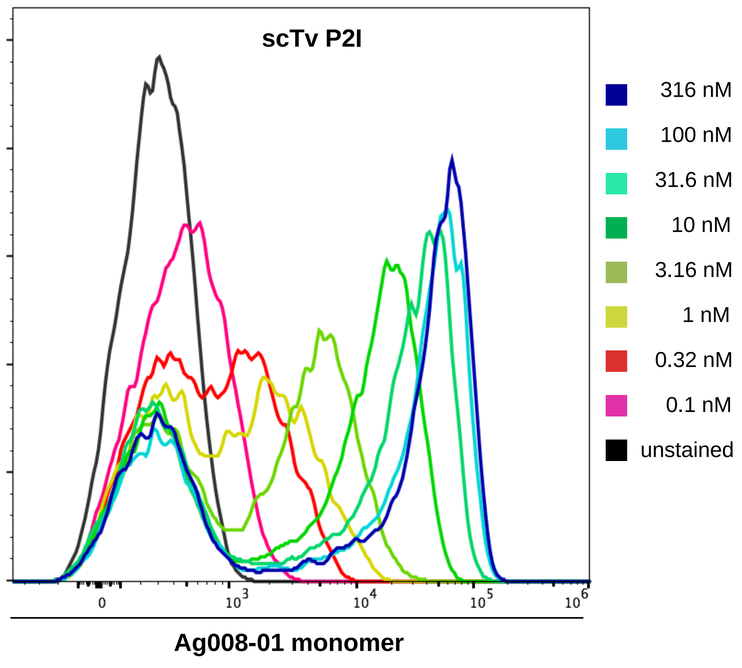

Figure 7: Combination of selected CDR variants for titration series of Ag008-01 monomer. The new scTv P2I was generated by incorporation of selected CDRα3 and CDRβ2 variants with favorable antigen binding properties and high degree of binding specificity, into the optimized scTv scaffold. The resulting scTv was expressed on the yeast surface and revealed drastically improved binding properties against the target Ag008-01 as monomeric peptide-HLA-A*02 with an EC_{50} of yeast surface binding of 23 nM.

The unmodified scTv P2 did hardly show any expression on yeast and even the stable scaffold scTv P2S could not bind to Ag008-01 monomer at all. With the now selected scTv P2I we will be able to create novel bispecific molecules with potent activity against Ag008-01 positive cancer cells.