New platform provides deep insights into T cell responses against novel cancer vaccine

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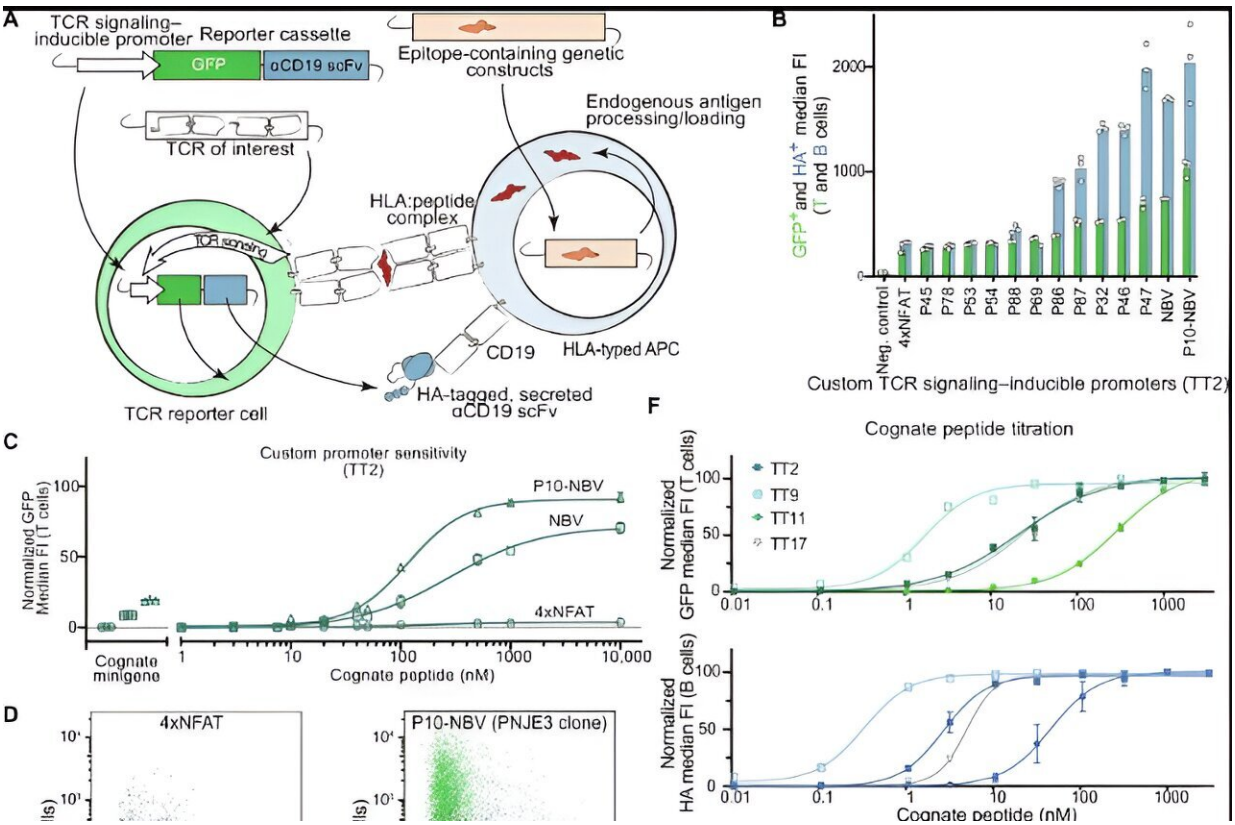

Developing and benchmarking a TCR activation reporter system. (A) Schematic depicting construction of a TCR reporter cell line (left) transgenically modified with an inducible promoter and TCR of interest. Upon cognate ligand interaction, the reporter cell produces cytoplasmic GFP and an HA-labeled secreted antibody fragment specific for CD19, a B cell-specific surface protein. B cells (right) used as APCs are transduced with epitope containing minigenes or exogenously loaded with peptide epitopes. Upon reporter T cell activation, the B cells are labeled by the secreted αCD19 scFv. (B) Median fluorescent GFP

(green) and αHA (blue) signals upon cognate ligand interaction for TT2-transgenic Jurkat cells with bespoke TCR signaling–inducible promoters. Negative control, noncognate interaction (from P10-NBV promoter as an example). (C) Minigene and peptide dilution curves of a cognate epitope for P10-NBV, NBV, and 4xNFAT transposon-integrated inducible reporter cassettes (bulk T cell population before single-clone selection). (D) Superimposed dot plots depicting T cell GFP expression (y axis, green) and B cell anti-CD19 scFv labeling (x axis, blue) in a single cognate-target coculture. Black dots, GFP fluorescence in a noncognate control coculture. Left: 4xNFAT promoter. Right: P10-NBV promoter-transgenic single clone PNJE3. (E) TCR-independent titration curves for two benchmarking TCRs (TT7 and TT11) using an αCD3 antibody (OKT-3). (F) Titration curves of PNJE3 activation of four benchmarking TCRs (TT2, TT9, TT11, and TT17) with cognate peptide epitopes from the Clostridium tetani tetanospasmin protein. Upper plot, T cell GFP expression; lower plot, B cell anti-CD19 scFv labeling. Error bars indicate SD from three replicates. (G) Activation of PNJE3 in coculture with BOLETH cells following cognate peptide and full-length tetanus toxoid protein electropulsing, or transduction with an epitope-containing 400–amino acid minigene. Representative data from two independent titration experiments. Credit: *Science Advances* (2024). DOI: 10.1126/sciadv.adk3060

BioMed X and the Universitätsmedizin Mannheim (UMM) announced the publication of two manuscripts in the field of cancer immunology in the journal *Science Advances*. The main objective of the collaboration was to understand the role of specific T cell responses in a patient with an aggressive subtype of diffuse glioma who showed sustained remission after receiving a neoepitope peptide vaccine at the University Hospital Mannheim.

The work is based on a collaboration between both institutions and researchers at the German Cancer Research Center (DKFZ), the Heidelberg University, and the Helmholtz Institute for Translational Oncology (HI-TRON).

T cells are critical for maintaining human health by eliminating tumor cells from the body—a process which is driven by the specific interaction of unique receptors on the surface of T cells (T cell receptors or TCRs) to mutant peptides (antigens) on the surface of tumor cells. The identification of such cancer-specific antigens, and the TCRs that bind them, underlies current efforts to develop targeted cancer therapies. Until now, functionally robust high-throughput approaches to this challenge were lacking.

Dr. John M. Lindner and his research team at the BioMed X Institute in Heidelberg designed the T-FINDER platform to solve this problem. The platform is capable of rapidly screening thousands of potential interactions between TCRs and antigens on the surface of potential target cells for their ability to activate T cells.

Driven by a trio of lead scientists (Miray Cetin, Dr. Veronica Pinamonti, and Dr. Theresa Schmid), the team first generated a highly sensitive reporter cell line for T cell activation. This reporter lies at the heart of T-FINDER, which can sensitively read out the specificity of any number of T cell-activating receptors (for example, CAR-T receptors in addition to classical TCRs) and ligands.

For the latter, Dr. Pinamonti provided solutions to key aspects of enabling the detection of class II HLA-presented peptides during her Ph.D. thesis work, leading to the development of novel strategies for boosting antigen processing and presentation by the target cell. This class of ligand was until now very challenging to investigate experimentally, and together with its superior sensitivity form the foundation of T-FINDER's advantages in the field.

In a companion manuscript, the team collaborated with a research team led by Dr. Ed Green from the Clinical Cooperation Unit Neuroimmunology and Brain Tumor Immunology (Head: Prof. Michael

Platten) at DKFZ and University Hospital Mannheim. The researchers applied T-FINDER to decode the immune response of two diffuse midline glioma patients vaccinated against the H3 mutation which was driving their cancer.

Patients receiving the H3-vaccine have shown promising but heterogeneous results, and with the aid of T-FINDER, the two groups could precisely map the functional immune response of a patient in remission to TCRs binding HLA class II-presented epitopes of mutant H3. This work provides key insights into the mechanism of anti-tumor T cell responses in these patients, and will support ongoing vaccination studies.

"We are excited about the publication of the results of our collaboration with Ed Green, Michael Platten, and colleagues," said John Lindner, Head of Immunology Discovery at the BioMed X Institute. "Our joint research demonstrated the unique sensitivity, flexibility, and overall performance of our new T-FINDER platform, especially for class II HLA-presented targets, which have always been a challenge in the past."

"Previously, we have been limited in the tools we could use to study class II-presented epitopes such as mutant H3. The T-FINDER platform has enabled us to identify and benchmark dozens of H3-reactive TCRs, allowing us to track patient responses to vaccination today, as well as benchmark TCRs for the autologous cell therapies of tomorrow," said Ed Green, Head of the ImmunoGenomics team in Michael Platten's lab at the DKFZ.

More information: Miray Cetin et al, T-FINDER: A highly sensitive, pan-HLA platform for functional T cell receptor and ligand discovery, *Science Advances* (2024). DOI: 10.1126/sciadv.adk3060

Tamara Boschert et al, H3K27M neoepitope vaccination in diffuse

midline glioma induces B and T cell responses across diverse HLA loci of a recovered patient, *Science Advances* (2024). DOI: 10.1126/sciadv.adi9091

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