IDENTIFICATION OF TUMOR-REACTIVE T CELLS
TARGETING MELANOMA DARK ANTIGENS™ VALIDATES
THIS NOVEL CLASS OF TARGETS FOR DEVELOPMENT OF
IMMUNOTHERAPIES

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Background Better solid tumor targets are needed to help realise the potential for TCR-directed immunotherapy. Dark Antigens™, encoded by regions of the genome previously thought to be non-coding, are promising targets for immunotherapy due to their cancer-specific expression and broad patient coverage.

Using our EDAPT™ (Enara Dark Antigen Platform Technology) platform, we have previously identified a number of melanoma-specific antigens, demonstrated their presentation on Class I HLA molecules of primary tumors using mass spectrometry-based immunopeptidomics data, validated their cancer-specificity and demonstrated homogenous tumor expression using RNA-Scope.1-2

We sought to demonstrate that antigen-reactive T cells can be found in both the periphery of healthy donors and melanoma patient tumor material, and to further characterize these T cell responses.

Methods Tumor-infiltrating lymphocytes (TILs) expanded from twenty-one melanoma patients were screened by IFNγ ELISpot for reactivity against peptides from four different melanoma-specific antigens.

Healthy donor PBMCs were assessed for the presence of antigen-specific T cells using peptide-HLA tetramers, either directly ex vivo or following a period of peptide-specific stimulation and expansion.

T cell receptors (TCR) sequences were obtained from antigen-reactive isolated T cells using either iRepertoire or 10x Genomics, and are being screened for function against patient-derived tumor lines with endogenous expression of the cognate antigen.

Results We have shown that TILs expanded from two melanoma patients are reactive to a common epitope within EVA003, a melanoma-specific antigen. The autologous tumor lines derived from both patients were shown to be EVA003-positive.

Furthermore, we have identified T cells specific for two melanoma-specific antigens, EVA001 and EVA003, from PBMCs of several healthy donors.

TCRs have been sequenced, for further functional characterization, from T cells reactive to HLA-A3 and HLA-B7-restricted peptides from EVA003 and to an HLA-A2-restricted peptide from EVA001.

Conclusions We have identified T cells that are reactive against epitopes derived from melanoma-specific antigens in both patient TILs and peripheral blood of healthy subjects, supporting their relevance as cancer-specific antigens. TCRs isolated from these T cells are currently being assessed for reactivity against antigen-positive patient-derived melanoma tumor lines. This work highlights the promise of Dark Antigens™ as a novel class of targets for the development of targeted immunotherapies such as cancer vaccines, TCR-T cell and bi-specific T cell engager therapies, and our EDAPT™ platform is now being employed to identify Dark Antigen™ targets in a range of other tumor types.

REFERENCES


Ethics Approval All work involving the use of human tissue was approved by the NHS Health Research Authority Northwest Haydock Research Ethics Committee (reference number 19/NW/0216), London Bridge Research Ethics Committee (reference number 20/PR/0400), and the Medical Ethics Committee of the Leiden University Medical Center (reference number P04.085).