Identification of tumor-reactive T cells targeting melanoma Dark Antigens™ validates this novel class of targets for development of immunotherapies

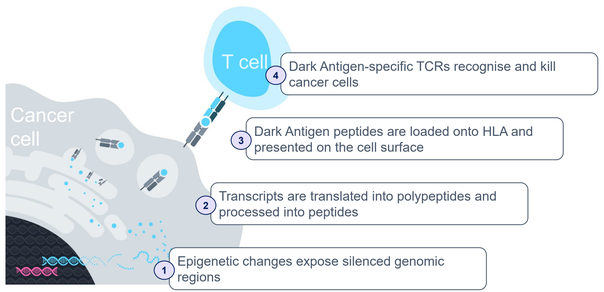
Rachel Abbott[1*], Tom Hofland[2^], Thomas M. Hulen[3^], Elizabeth M. E. Verdegaal[4^], Michael D. Crowther[3], Shawez Khan[3], Pita de Kok[4], Emily Lam[1], Giulia Masi[1], Nathifa Moyo[1], Özcan Met[3], Aimilia Schina[3], Hayden Selvadurai[1], Emily Tye[1], Marten Visser[4], Katarzyna Ward[1], Sjoerd H. van der Burg[4*], George Kassiotis[2*], Inge Marie Svane[3*], Joseph Dukes[1*]

[1] Enara Bio Ltd., Oxford, U.K.
[2] The Francis Crick Institute, London, U.K.
[3] National Center for Cancer Immune Therapy (CCIT-DK), Department of Oncology, Copenhagen University Hospital, Herlev, Denmark.
[4] Department of Medical Oncology, Oncode Institute, Leiden University Medical Centre, Leiden, The Netherlands.
^ Joint primary author *Corresponding author

Dark Antigens™ : Novel, shared, tumor-specific targets for immunotherapy

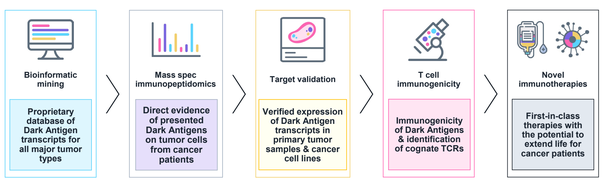
- Dark Antigens are a differentiated source of shared, tumor-specific antigens derived from genomic dark matter
- Putative Dark Antigen-encoding transcripts and open-reading frames (ORFs) can be found in all major solid tumor types
- **Epigenetically regulated** – *present across solid tumors independent of tumor mutation burden*
- **Shared across patients and tumor types** – *broader potential patient population than conventional tumor-associated antigens*
- **High degree of intratumoral homogeneity** – *attractive feature for targeted immunotherapies*

Dark Antigens enable multiple HLA-targeting opportunities that are lacking with neoantigen-directed immunotherapies

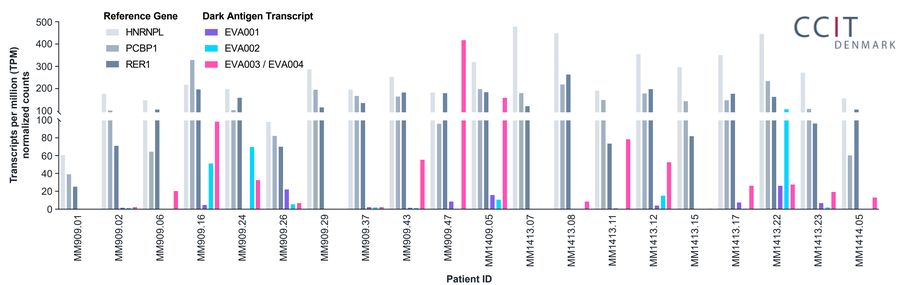


EDAPT™ pipeline for Dark Antigen discovery and validation

- Enara Bio's EDAPT (Enara Dark Antigen Platform Technology) platform probes the genomic dark matter to discover shared novel, cancer-specific antigens with validated presentation on Class I HLA of primary tumors
- Using our platform, we previously identified a number of melanoma-specific antigens, demonstrated their presentation on Class I HLA in primary tumors with mass spectrometry-based immunopeptidomics, and validated their cancer-specificity and homogenous tumor expression [1-2]

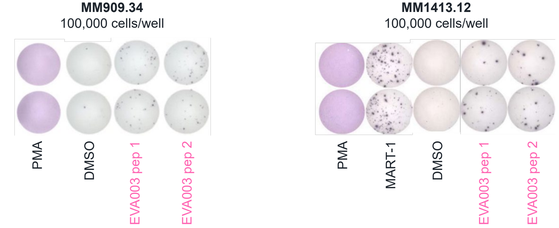


Expression of at least one Dark Antigen transcript is observed in 70% of biopsy samples from patients with metastatic melanoma



- Expression of transcripts encoding antigens EVA001, EVA002 and EVA003 / EVA004 was assessed in biopsy samples from 20 patients with metastatic melanoma via RNAseq analysis
- Samples from 14 of the 20 patients (70%) show expression of at least one of the three antigen-encoding transcripts (TPM > 5)
- The most prevalent transcript encodes two distinct melanoma-specific antigens, EVA003 and EVA004

Tumor infiltrating lymphocytes (TILs) from patients with metastatic melanoma contain T cells reactive to mass spec-validated Dark Antigen peptides



- Tumor-infiltrating lymphocytes (TILs) expanded from four melanoma patients were identified to be HLA-A*03:01+ and screened by IFNγ ELISpot for reactivity against HLA-A*03:01-restricted peptides from EVA003
- TILs from two of these four donors (MM909.34 and MM1413.12) are activated by two mass spec-validated, HLA-A*03:01-restricted peptides, demonstrating presence of Dark Antigen-reactive T cells in TIL
- RNAseq analysis of available primary tumor material (MM1413.12) confirms that this tumor is positive for expression of the EVA003/EVA004 transcript (data shown in panel to left)

Dark Antigen reactive T cells are found within peripheral blood mononuclear cells (PBMCs) from healthy donors

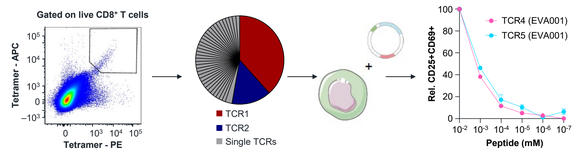
Immunogenicity of HLA-A2 peptide from antigen EVA001

Tetramer-positive cells sorted following 10 days stimulation with peptide-pulsed autologous DCs

Single-cell TCRseq data acquired from sorted cells using 10X platform

Two TCRs cloned and transduced into Jurkat cells

EVA001 specificity of TCRs confirmed with *in vitro* functional *assay*



- Naïve CD8+ T cells from healthy donor PBMCs were stimulated with autologous CD14+ monocyte-derived dendritic cells pulsed with melanoma-specific Dark Antigen peptides
- Ten days later, antigen-specific T cells were sorted using peptide-HLA (pHLA) tetramers
- T cell receptors (TCR) sequences, obtained from antigen-reactive isolated T cells using 10X Genomics, were transduced into Jurkat cells for functional assessment
- Data shared here shows identification of two TCR sequences with clear reactivity to an HLA-A*02:01 restricted peptide from EVA001, as measured by CD25 and CD69 upregulation in response to a peptide titration on T2 antigen presenting cells

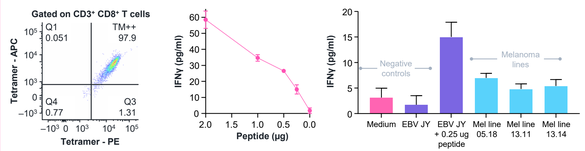
THE FRANCIS CRICK INSTITUTE

Immunogenicity of HLA-B7 peptide from antigen EVA003

T cell clone EN16.5 Tetramer Stain

T cell clone EN16.5 Peptide Titration

T cell clone EN16.5 Response to Target Cells



- Healthy donor PBMCs were assessed directly *ex vivo* for the presence of antigen-specific T cells using pHLA tetramers
- T cells that were dual stained with PE and APC pHLA tetramers were single cell and/or bulk sorted and rapidly expanded in the presence of peptide to generate (poly)clonal cultures
- Following expansion, pHLA tetramer staining was repeated and the function of the T cell cultures was assessed in IFNγ ELISAs measuring response to peptide titration and patient-derived melanoma lines
- Data shared here shows characterisation of T cell clone EN16.5 with clear specificity for an HLA-B*07:02 restricted epitope from EVA003

LUMC Leiden University Medical Center

Conclusions

- T cells that are reactive against epitopes derived from novel, melanoma-specific Dark Antigens have been identified 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
- Our EDAPT platform is now being employed to identify Dark Antigen targets in a range of other tumor types

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). The use of patient material at CCIT-DK as described was approved by the Ethics Committee of the Capital Region of Denmark and national regulations for biomedical research.

References

1. Attig, J., et al. LTR retroelement expansion of the human cancer transcriptome and immunopeptidome revealed by de novo transcript assembly. Genome Research. 2019; 29:1578-1590.
2. Jupp, R., et al. Discovery of immunogenic ERV-derived antigens as targets for melanoma immunotherapy. Society for Immunotherapy of Cancer (SITC) 34th Annual Meeting. 2019; P680.

Presented at The Society for Immunotherapy of Cancer's 37th Annual Meeting (SITC 2022)