Abstracts

1. Thomas M Hulen*, Michael D Crowther, Shavez Khan, Aimilia Schina, Duncan Howie, Ozcan Met, Inge Svaen. Center for Cancer Immune Therapy, Herlev, Denmark; National Center for Cancer Immune Therapy (CCIT-DK), Department of Oncology, Copenhagen University Hospital, Herlev, Denmark; Enara Bio Ltd, Oxford, UK

Background: Adoptive cell therapy (ACT) with autologous tumor infiltrating lymphocytes (TILs) is an effective therapy for advanced melanoma, with response rates between 30–50%. However, the majority of patients do not respond, and immunological targets of TIL TCRs remain elusive.

Dark Antigens are cancer-specific targets discovered in regions of the genome historically considered noncoding. Due to their intratumoral homogeneity and shared expression between individuals, they make attractive immunotherapeutic targets. Using the EDAPT® (Enara Dark Antigen Platform Technology) platform, Enara Bio has identified numerous melanoma-specific translated peptides, derived from Dark Antigens, and presented on Class I MHC by primary tumors.

We sought to demonstrate that patient-derived melanoma cell lines express Dark Antigen transcripts, identify Dark Antigen-reactivity in TILs from these patients, and identify and validate the reactivity of anti-Dark Antigen TCRs.

Methods: mRNA sequencing data from patient-derived melanoma cell lines was evaluated for Dark Antigen transcript expression. TILs derived from these patients were stimulated with Dark Antigen peptide and screened in ELISPOT for IFNγ production. Dark Antigen reactive TILs from one patient were isolated and TCR sequences of reactive cells were obtained by integration of gene expression and VDJ single cell sequencing data (10X Genomics). Through lentiviral transduction selected TCRs were expressed in healthy donor T cells (TCR-Ts) and functionally evaluated using ELISPOT, intracellular cytokine staining (ICS), and chromium-51 (Cr51) killing assay.

Results: Dark Antigen transcripts EVA001, EVA002, or EVA003 are expressed in 70% of patient-derived melanoma lines investigated from our biobank. Peptide #1, derived from EVA003, stimulated significant IFNγ release from TIL isolated from a single patient. Three Peptide #1-reactive candidate TCRs were identified by single cell sequencing. One TCR demonstrated EVA003-Peptide #1 specific functional activity as a derivative TCR-T through production of cytokines IFNγ and TNFα, upregulation of activation markers CD137 and CD107a and cytolysis of HLA-A3-positive target cells pulsed with Dark Antigen Peptide #1.

Conclusions: Using clinical material from our melanoma biobank, we have confirmed the broad expression of Dark Antigen transcripts across patients, identified Dark Antigen reactive TILs, and validated the discovery of a specific Dark Antigen reactive TCR. Ongoing investigations will utilize clinical tumor samples as target cells to evaluate TCR-T specific reactivity against in situ Dark Antigen expression. This study highlights the potential of Dark Antigens to address the need for novel cancer-specific targets for solid tumors.

REFERENCES:

Ethics Approval: All work involving the use of human tissue was approved by the NHS Health Research Authority North-west Haydock Research Ethics Committee (reference number 19/NW/0216). This study was conducted using TILs from patients enrolled in a clinical studies conducted at CCIT-DK. All patients signed a written consent form according to the Declaration of Helsinki. The studies were approved by the local ethics committee for the capital region of Denmark (Region H).

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0363