312 IDENTIFICATION OF NOVEL NSC LUNG CANCER DARK ANTIGENS™ WITH EXPRESSION IN MULTIPLE TUMOR TYPES, AS PROMISING TARGETS FOR IMMUNOTHERAPIES

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Background Recent advances in immunotherapy have led to the development of multiple therapeutic modalities that harness T cells to recognize and eliminate cancer cells, bringing benefit to cancer patients with unmet need. However, targeted immunotherapies have utilized traditional tumor-associated antigens, which lack cancer specificity and broad expression across patient populations.

We utilized Enara Bio's EDAPT™ (Enara Dark Antigen Platform Technology) platform to probe the genomic dark matter (genomic regions previously thought to be non-coding) and discover novel, cancer-specific antigens with validated presentation on Class I HLA of primary tumors.

Methods We created a *de novo* pan-cancer transcriptome assembly with RNA-seq reads from The Cancer Genome Atlas (TCGA) non-small cell (NSC) lung cancer projects. Transcript sequences were subject to differential expression analysis selecting those with enriched expression in tumors compared to a comprehensive panel of healthy tissues.

To discover *bona fide* Dark Antigens™ encoded by tumor-specific transcripts, we translated all possible open reading frames (ORFs) and interrogated these against mass spectrometry-based immunopeptidomics data, from primary NSC lung cancer samples, to identify peptides solely mapping to putative ORF sequences. Subsequent filtering was performed to extract those antigens with robust immunopeptidomic support, transcript expression prevalence, and cancer specificity.

Confirmation of tumor-specific expression of antigen-encoding transcripts was carried out using RNA *in situ* hybridization (ISH) and RT-qPCR. The immunogenicity of detected epitopes was assessed by characterization of antigen-specific T cell responses from healthy donors.

Results Our *de novo* assemblies identified >1,000 transcripts encoding >15,000 ORFs. Interrogation of these ORFs against MS immunopeptidomic datasets mapped HLA-bound peptides to hundreds of ORFs, demonstrating presentation of these novel antigens in multiple patient tumor samples. Many candidate antigens displayed promising expression across additional tumor types, supporting potential for broader clinical utility.

RNA ISH revealed tumor-specificity and intra-tumoral homogeneity at the transcript level, with little to no transcript expression identified across healthy tissues. Assessment of immunogenicity in healthy subject peripheral blood cells revealed reactive T cells, indicating a lack of central-tolerance deletion of T cells specific for these antigen-derived peptides.

Conclusions We identified a suite of candidate Dark Antigens™ that are expressed in a cancer-specific manner with evidence of processing and HLA presentation on the surface of tumor cells. T cells with specificity for these antigens can be detected in healthy subjects, and thus these antigens show promise as candidates for the development of targeted immunotherapies such as cancer vaccines, TCR-T cell and bi-specific T cell engager therapies.

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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).

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