Identification of novel non-small cell lung cancer (NSCLC) Dark Antigens™, with expression in multiple tumor types, as promising targets for immunotherapies

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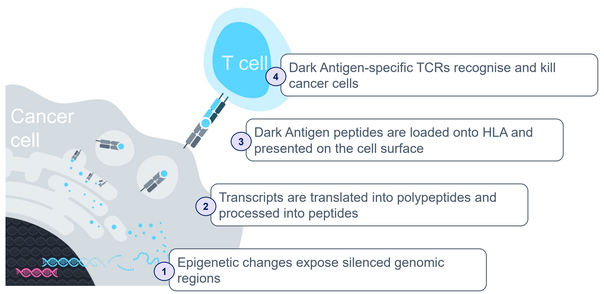
Dark Antigens™ : Novel, shared, tumor-specific targets for immunotherapy

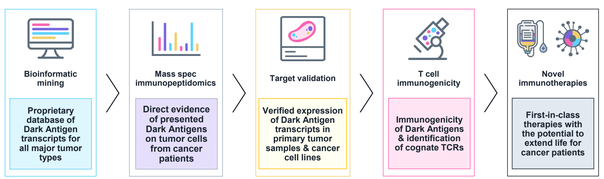
- Dark Antigens are a differentiated source of shared, tumor-specific antigens derived from genomic dark matter
- Putative Dark Antigen-encoding transcripts and open-reading frames (ORFs) can be found in all major solid tumor types
- **Epigenetically regulated** – *present across solid tumors independent 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

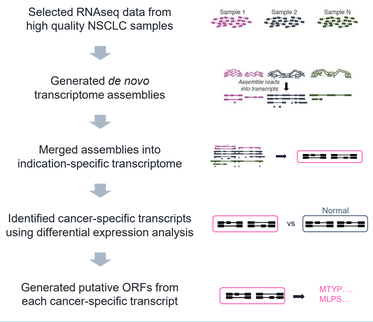


EDAPT™ pipeline for Dark Antigen discovery and validation

- 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
- Our platform is fed by *de novo* indication-specific transcriptome assemblies filtered for differential expression between cancer and normal tissue. Cancer-specific transcripts are filtered through a sequence of activities focused on validating their presentation by HLA on the cell surface, cancer-specificity, homogeneity, and immunogenicity



Bioinformatics workflow generated putative cancer-specific ORFs from NSCLC samples



Source Data:

TCGA

GTEx

Number of putative cancer-specific transcripts per assembly:
***Adenocarcinoma*: Up to ~5,900**
***Squamous*: Up to ~5,300**

Number of putative ORFs per assembly:
***Adenocarcinoma*: Up to ~42,000**
***Squamous*: Up to ~47,000**

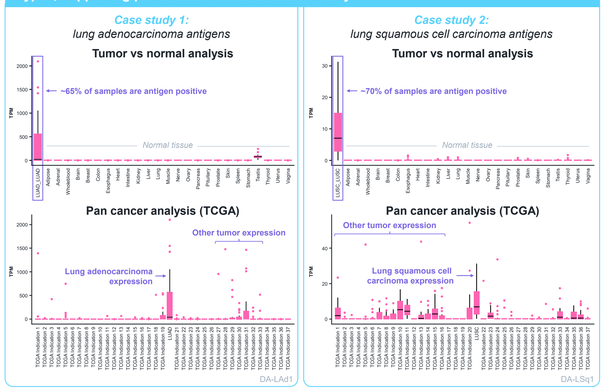
Immunopeptidomics workflow provided experimental evidence of cancer-specific ORFs presented on primary NSCLC tumor tissues

Number of mass spec identified NSCLC tumor-specific ORFs:
***Adenocarcinoma*: 440** **_Squamous_: 1,121**

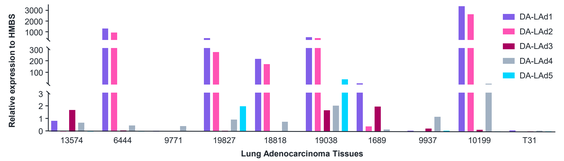
Mass spec (MS)-validated ORFs with high prevalence in NSCLC were selected for further experimental validation

Number of NSCLC tumor-specific ORFs selected for experimental validation:
***Adenocarcinoma*: 12** **_Squamous_: 9**

Selected candidate antigens displayed promising expression across additional tumor types, supporting potential for broader clinical utility

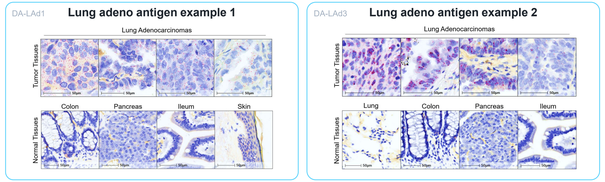


Assessing lung adenocarcinoma antigen transcript expression by qPCR confirmed prevalence is consistent with initial TCGA discovery data



Dark Antigens are homogenously expressed within tumors

- RNA-ISH confirmed tumor-specificity and intra-tumoral homogeneity at the transcript level, with little to no transcript expression identified across healthy tissues



Identification of T cells reactive against the MS-identified peptides confirms immunogenicity of the NSCLC Dark Antigens

- Immunogenicity of Dark Antigens is assessed by IFNγ ELISpot assay following priming and repeated stimulation of naïve CD8 T cells from healthy subjects with MS-identified epitope peptides
- Presence of Dark Antigen reactivity within the naïve T cell compartment of healthy subjects indicates a lack of central-tolerance deletion of T cells specific for these antigen-derived peptides, supporting the lack of normal tissue expression

Case study 1: Healthy subject T cell response to lung adenocarcinoma peptides

	250,000 T cells/well	100,000 T cells/well
Peptide pool	46* 41*	14* 15*
DA-LAd3_Pep1	4* 0	3* 1
DA-LAd1 / DA-LAd2_Pep1	0 0	1 0
DA-LAd4_Pep1	23* 28*	14* 11*
DA-LAd4_Pep2	4* 3*	0 1
DA-LAd6_Pep1	0 0	0 1

	250,000 T cells/well	100,000 T cells/well
DA-LAd5_Pep1	0 1	1 0
DA-LAd5_Pep2	6* 8*	1 0
DA-LAd7_Pep1	3* 0	1 0
No peptide (T cells alone)	1 0	0 0
PMA	1168* 1	766* 813*
CD3/CD28	383* 0	208* 226*

IFNγ ELISpot reveals a clear response to the pool of 8 lung adeno peptides that had previously been used to prime and restimulate the naïve CD8 T cells isolated from peripheral blood PBMCs (pink box), as well as at least one of the individual peptides (DA-LAd4_Pep1; purple box)

Conclusions

- EDAPT pipeline enabled discovery of multiple novel, cancer-specific Dark Antigens with expression in multiple tumor types and validated peptide-HLA presentation on the surface of primary NSCLC tumor cells
- T cells with specificity for these antigens can be detected in healthy subjects
- Dark Antigens are promising candidates for the development of targeted immunotherapies such as cancer vaccines, TCR-T cell therapies and bispecific T cell engagers
- EDAPT pipeline workflow can be applied across all tumor types to enable discovery of additional Dark Antigens

Acknowledgements

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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).

References

1. Attig et al, 2019. LTR retroelement expansion of the human cancer transcriptome and immunopeptidome revealed by de novo transcript assembly. Genome Research.

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