

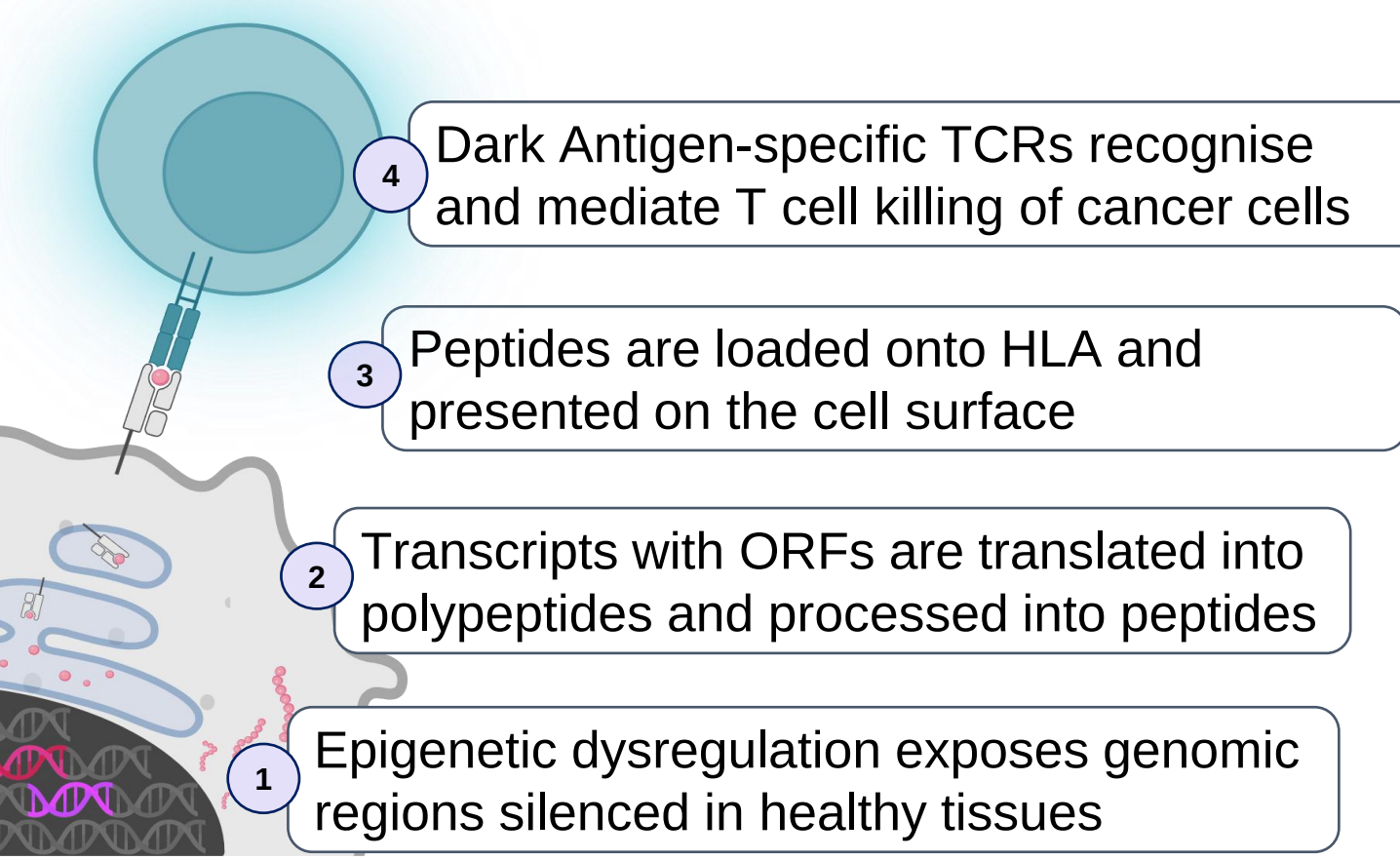
Identification of Dark Antigen® Reactive T cell Receptors from Tumor-Infiltrating Lymphocytes

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



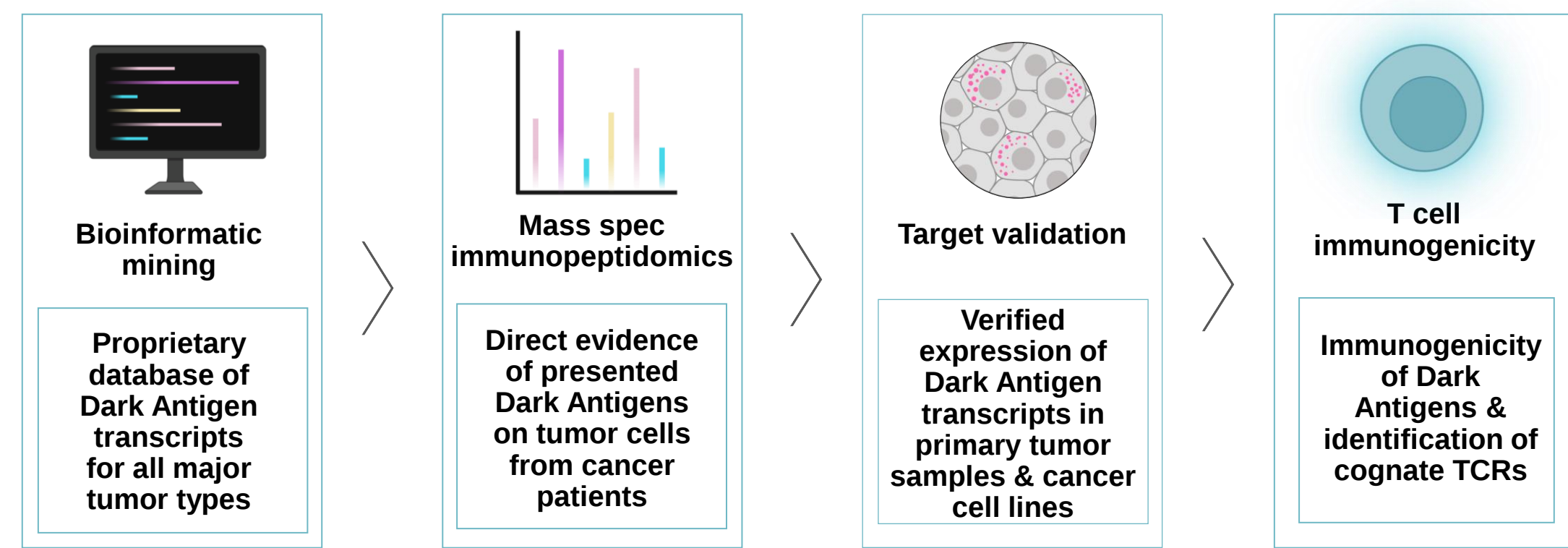
Dark Antigens® are a novel class of shared, tumor-specific antigens

- Derived from regions of the genome historically considered noncoding called genomic 'dark matter'
- Dark Antigen-encoding transcripts are found in all major solid tumors irrespective of their immune phenotype

Dark Antigens have several attractive characteristics as targets for novel immunotherapies:

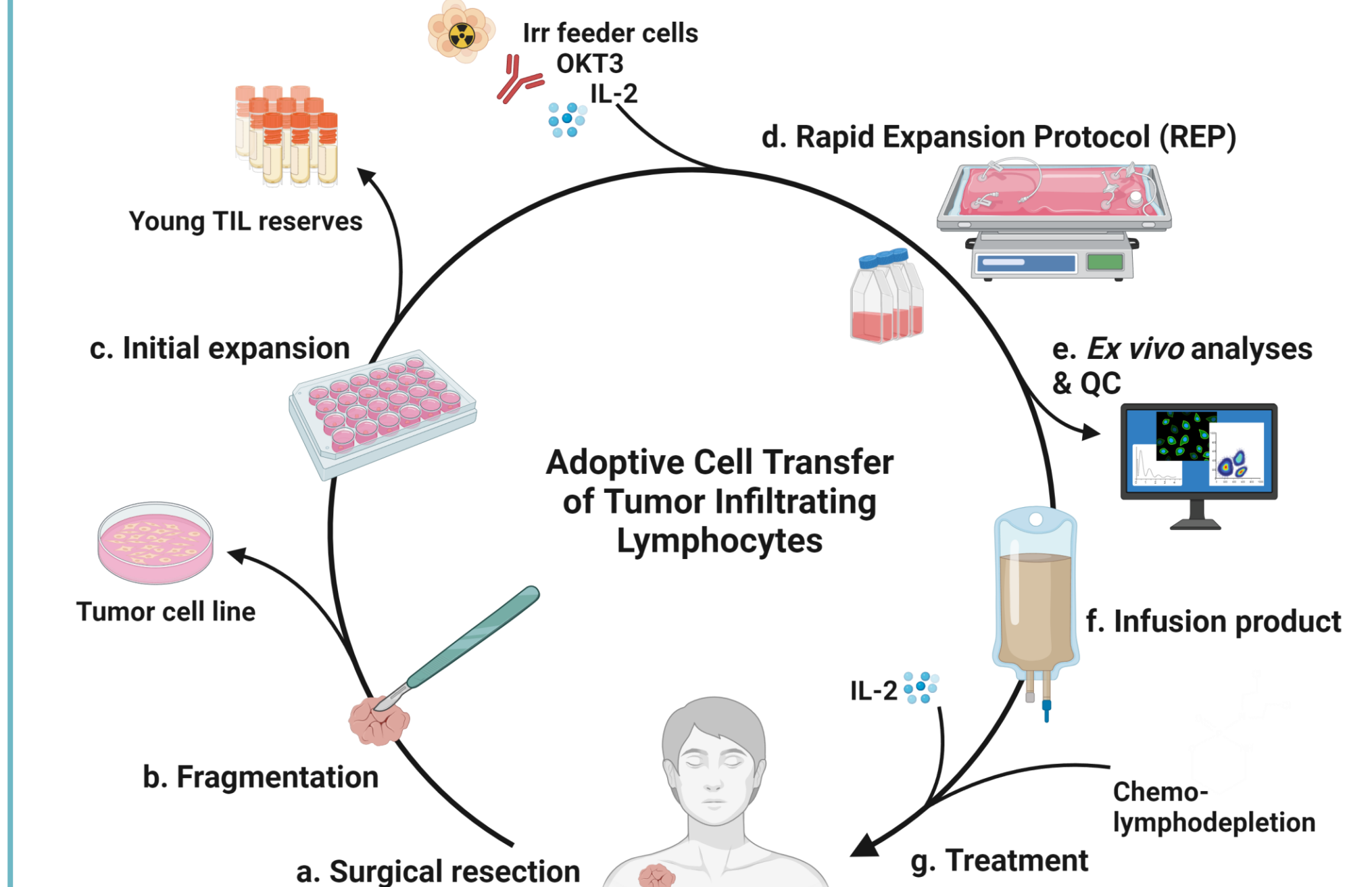
- Tumor-specific expression – *altered cellular processes in cancer cells lead to the transcription of Dark Antigen-encoding sequences that are usually silenced in healthy cells, minimizing the risk of on-target toxicity*
- High degree of intratumoral homogeneity – *increases the likelihood of clinical efficacy*
- Shared across patients and tumor types – *a broader population of patients will be eligible for treatment than with conventional tumor-associated antigens and mutation-derived neoantigens*

EDAPT® pipeline for Dark Antigen discovery and validation



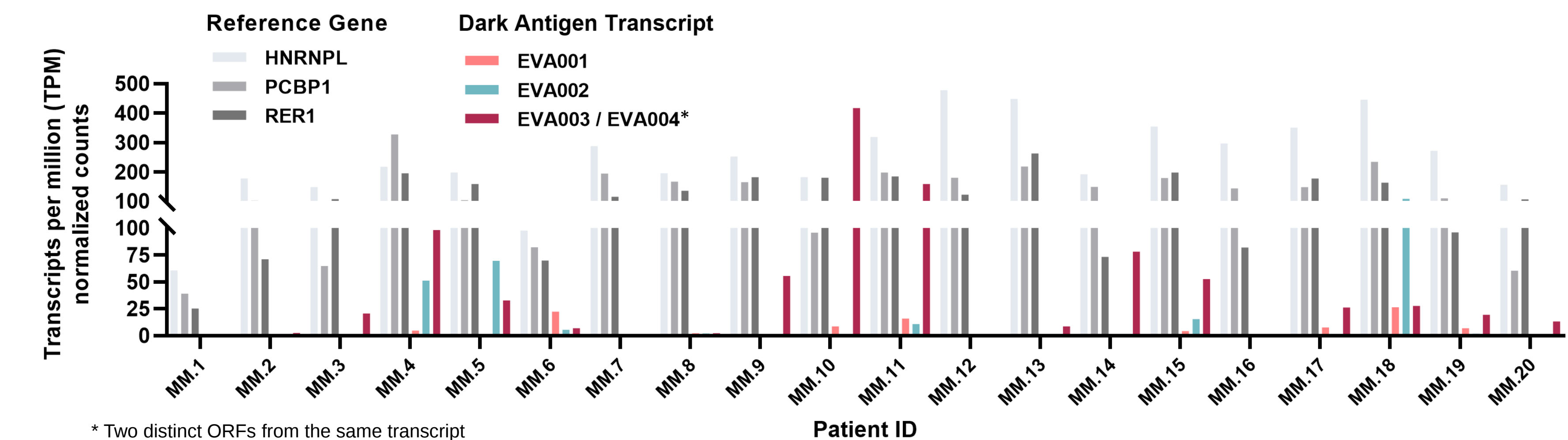
- Enara Bio's EDAPT® platform probes the genomic dark matter to discover novel, cancer-specific antigens
- A series of melanoma-specific antigens have been identified using EDAPT
- Class I HLA restriction of Dark Antigen-derived peptides on primary tumors has been confirmed by mass spectrometry-based immunopeptidomics [1-2]
- Cancer-specificity and homogenous tumor expression were validated using RNA *in situ* hybridisation methods [1-2]

Tumor-Infiltrating Lymphocytes for Adoptive Cell Therapy



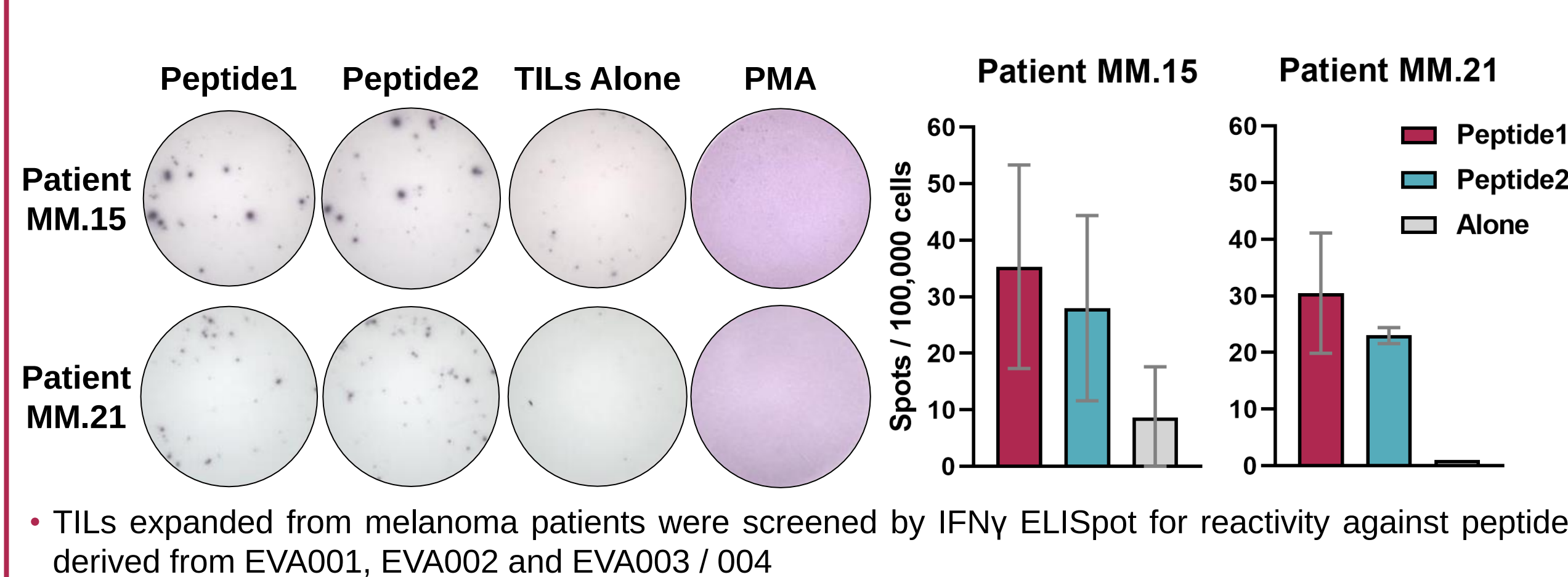
- Adoptive cell therapy with tumor-infiltrating lymphocytes (TILs) is an effective therapy for metastatic melanoma, with response rates between 30-50% [3, 4, 5].
- However, the immunological targets that mediate successful outcome remain largely elusive
- Tumor cell lines are produced in conjunction with TIL production for downstream research applications

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



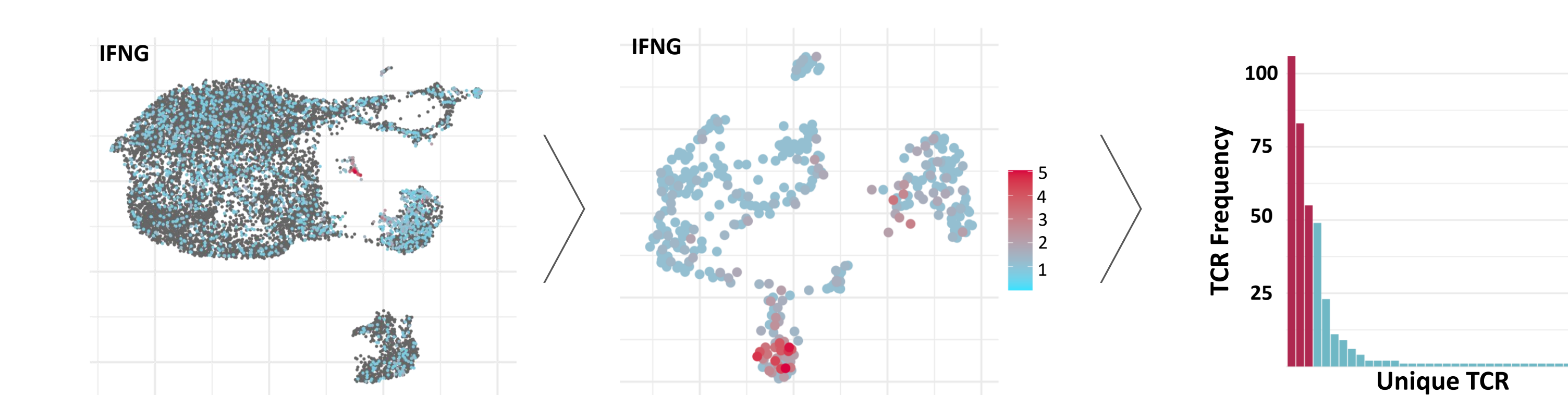
- Expression of EVA001, EVA002 and EVA003 / EVA004 transcripts was assessed in biopsy samples from 20 metastatic melanoma patients via RNAseq analysis
- Samples from 14 of the 20 patients (70%) showed expression of at least one Dark Antigen transcript (TPM > 5)
- The most prevalent transcript encodes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

TILs from patients with metastatic melanoma react to Dark Antigen peptides



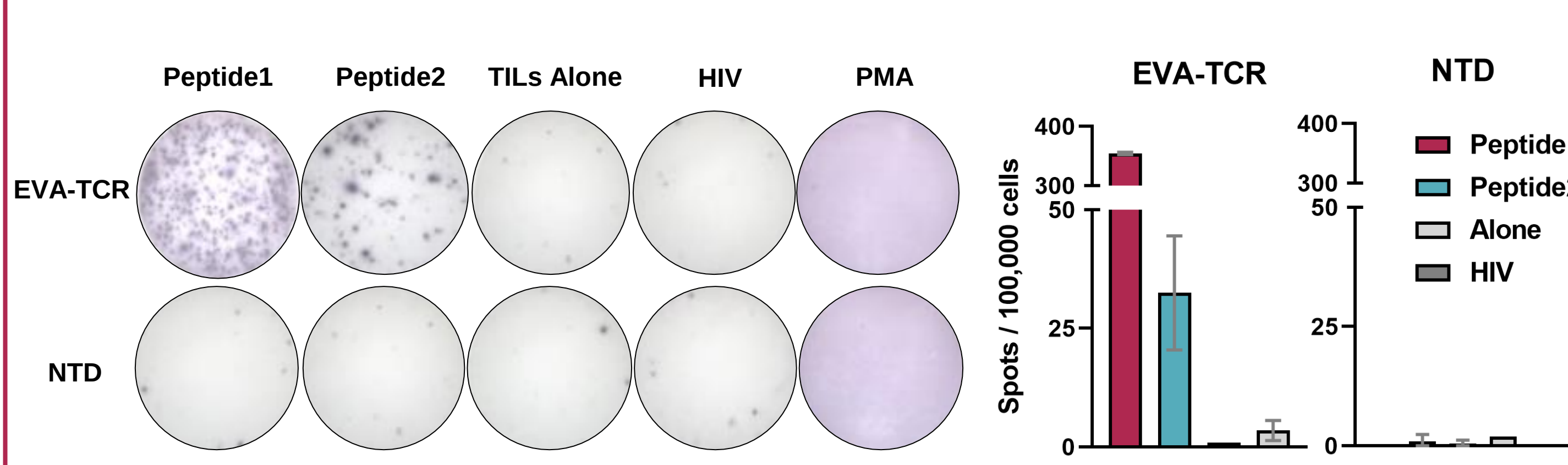
- TILs expanded from melanoma patients were screened by IFNγ ELISpot for reactivity against peptides derived from EVA001, EVA002 and EVA003 / 004
- TILs from two HLA-matched patients (MM.15 and MM.21) respond to the same pair of overlapping HLA-A*03:01-restricted, mass spec-validated peptides (Peptide1 and Peptide2) from EVA003, demonstrating the presence of Dark Antigen-reactive T cells in TILs
- RNAseq analysis of the biopsy from patient MM.15 showed clear expression of the EVA003/EVA004 transcript (analysis was not performed on patient MM.21 due to lack of biopsy sample)

Dark Antigen peptide stimulation and IFNγ-focused single cell sequencing reveals reactive TCR candidates



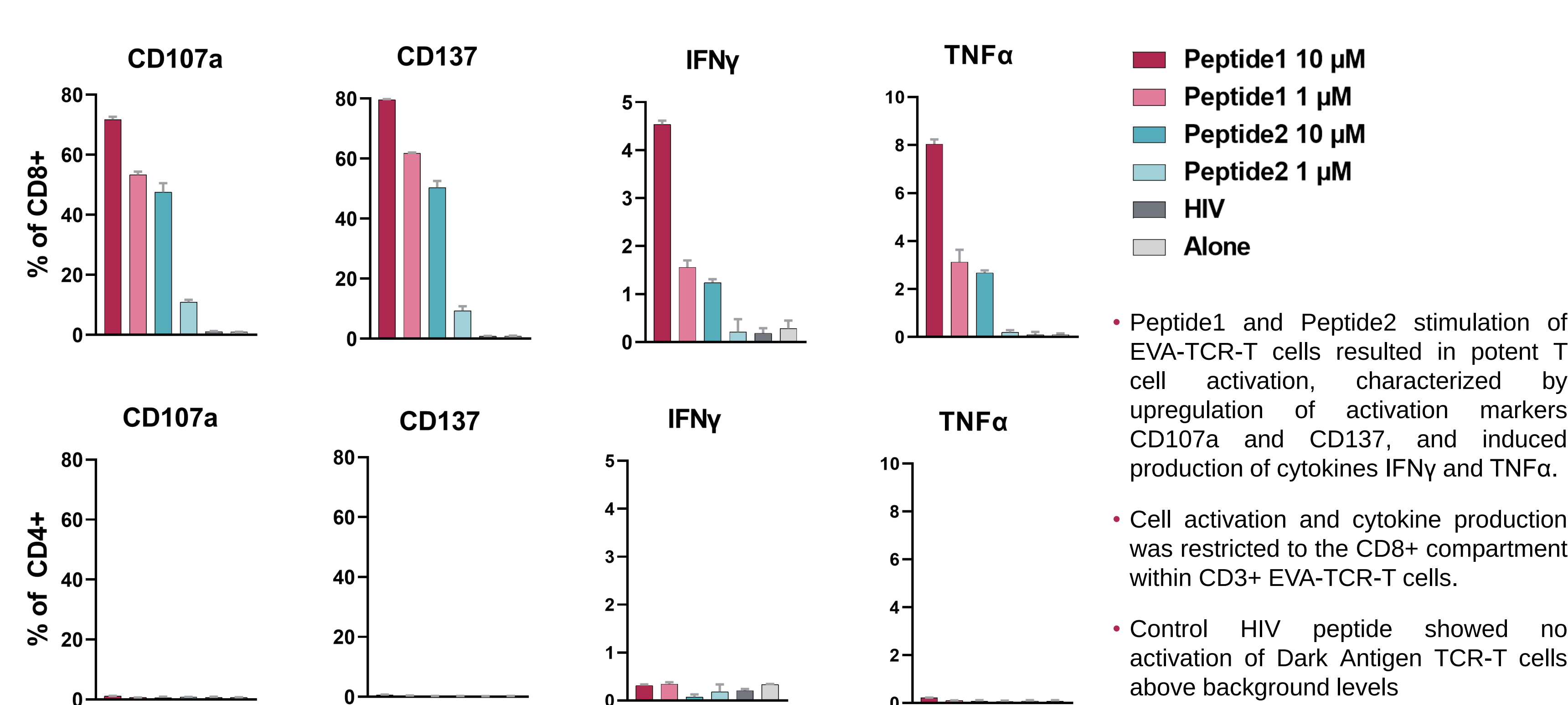
- TILs from patient MM.15 were stimulated with EVA003-derived peptides, enriched on IFNγ secretion (Miltenyi IFNγ enrichment kit) and following a rest, were restimulated with a mix of the peptides and autologous tumor. Expanded T cells were processed through a single cell 10X Genomics workflow to identify TCR sequences and associated gene expression profiles
- Gene expression data was overlaid with V(D)J sequencing data to find TCR clonotypes within reactive TIL populations. IFNγ was the primary parameter for measurement of activation
- TCR sequences derived from three separate clonotypes that exhibited the highest IFNγ expression were synthesized and cloned into a lentiviral expression construct and virally packaged for transduction

Healthy donor T cells transduced with these TCRs show Dark Antigen-dependent activation

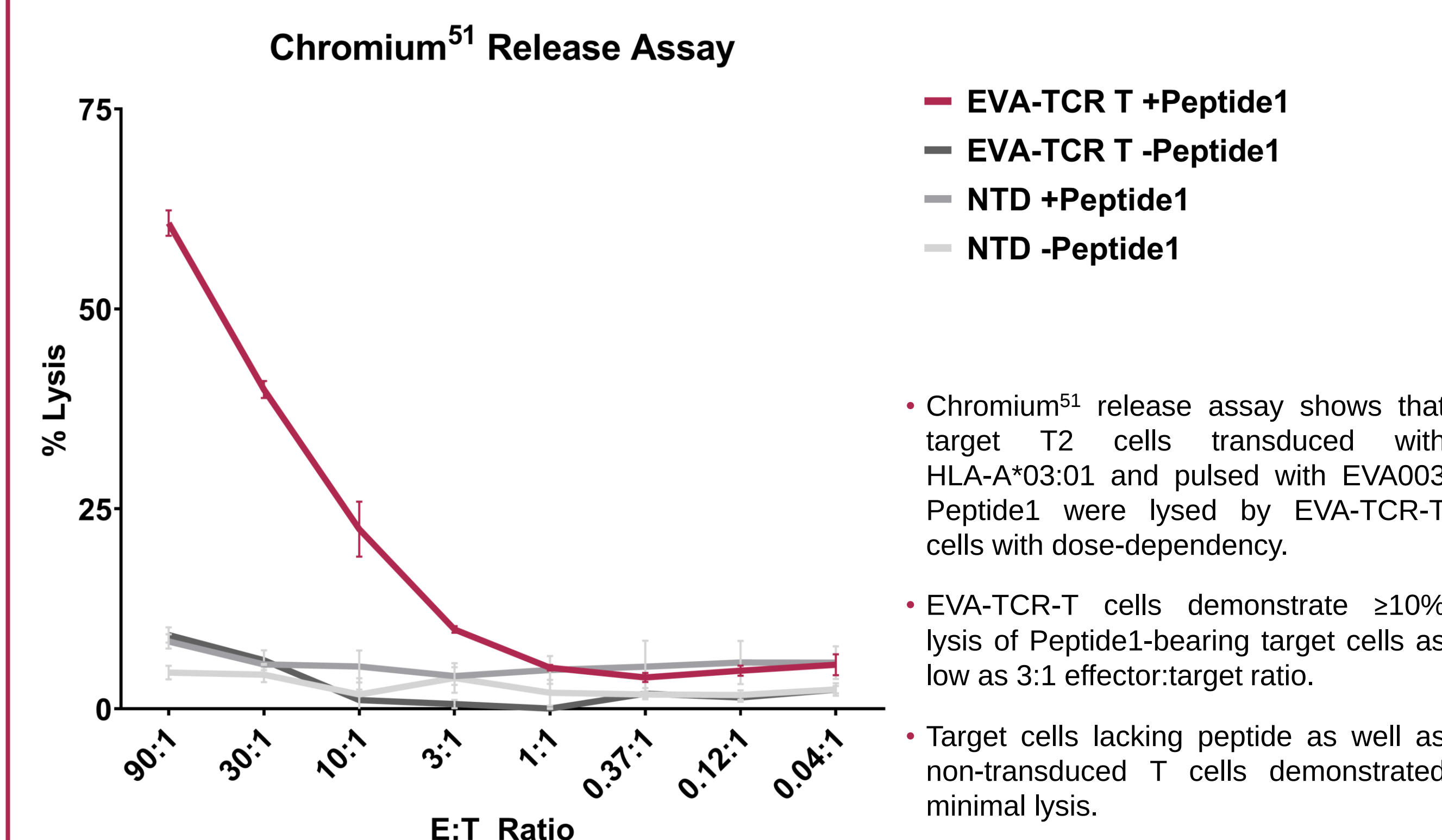


- A transduction efficiency of 98% was reached with the main TCR of interest to produce Dark Antigen-reactive TCR-transduced T cells (EVA-TCR-T cells)
- EVA-TCR-T cells demonstrated IFNγ release when stimulated with Peptide1 and Peptide2 in ELISPOT assays
- Non-transduced control PBMCs (NTD) show minimal background in all conditions
- PMA was used as positive control

Dark Antigen TCR-T cells demonstrate antigen-specific functional activity



Dark Antigen TCR-T cells are cytotoxic against peptide-pulsed target cells



Summary & Conclusions

- Using clinical material from our melanoma biobank, we have confirmed shared expression of Dark Antigen transcripts in metastatic melanoma patients, and identified TILs that are reactive against epitopes derived from these transcripts, supporting their relevance as novel cancer antigens.
- Single cell sequencing of Dark Antigen-reactive TILs identified candidate TCRs that were suspected to be responsible for Dark Antigen-dependent T cell activation.
- When virally transduced into healthy donor T cells, one of these TCRs demonstrated strong antigen-specific functional activity, as shown by production of cytokines IFNγ and TNFα, upregulation of activation markers CD137 and CD107a, and cytolysis of peptide-pulsed target cells.

Implications & Future Directions

- This work highlights the promise of Dark Antigens as a novel class of targets for the development of immunotherapies such as cancer vaccines, TCR-T cell therapies and bispecific T cell engagers.
- Ongoing studies are evaluating the cytotoxicity of Dark Antigen TCR-T cells against patient-derived metastatic melanoma cell lines from CCIT's biobank.
- Other candidate TCRs are currently being tested to validate Dark Antigen-reactivity and killing of target cells, while the Enara Bio EDAPT platform is being employed to identify novel Dark Antigens that are expressed across multiple major solid tumor types.

Presenter and Affiliation Information



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). 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).

References

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