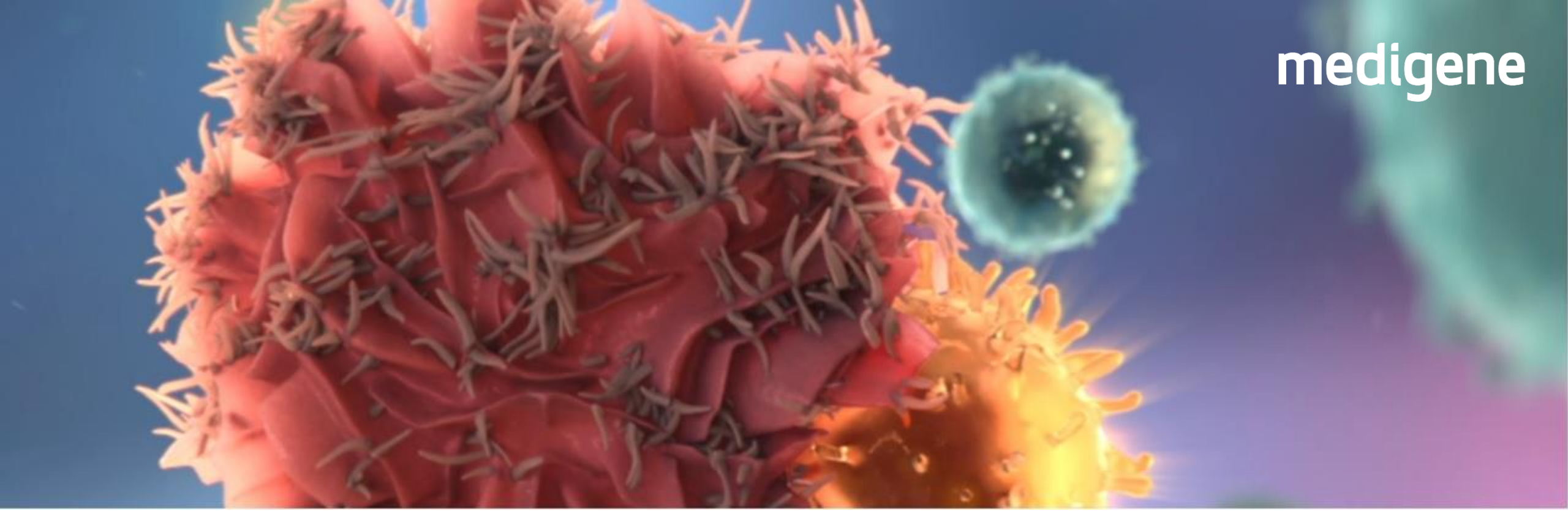

Identify Peptide Targets for TCR-Ts Derived from Non-Coding RNA Sequences

Next Generation CAR-TCR
Engineering Smarter Adoptive Cell Therapies

Justyna Ogonek, Senior Scientist, Medigene Immunotherapies GmbH

Forward looking statements disclaimer

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Different neoantigens offer a spectrum of targets for T cells

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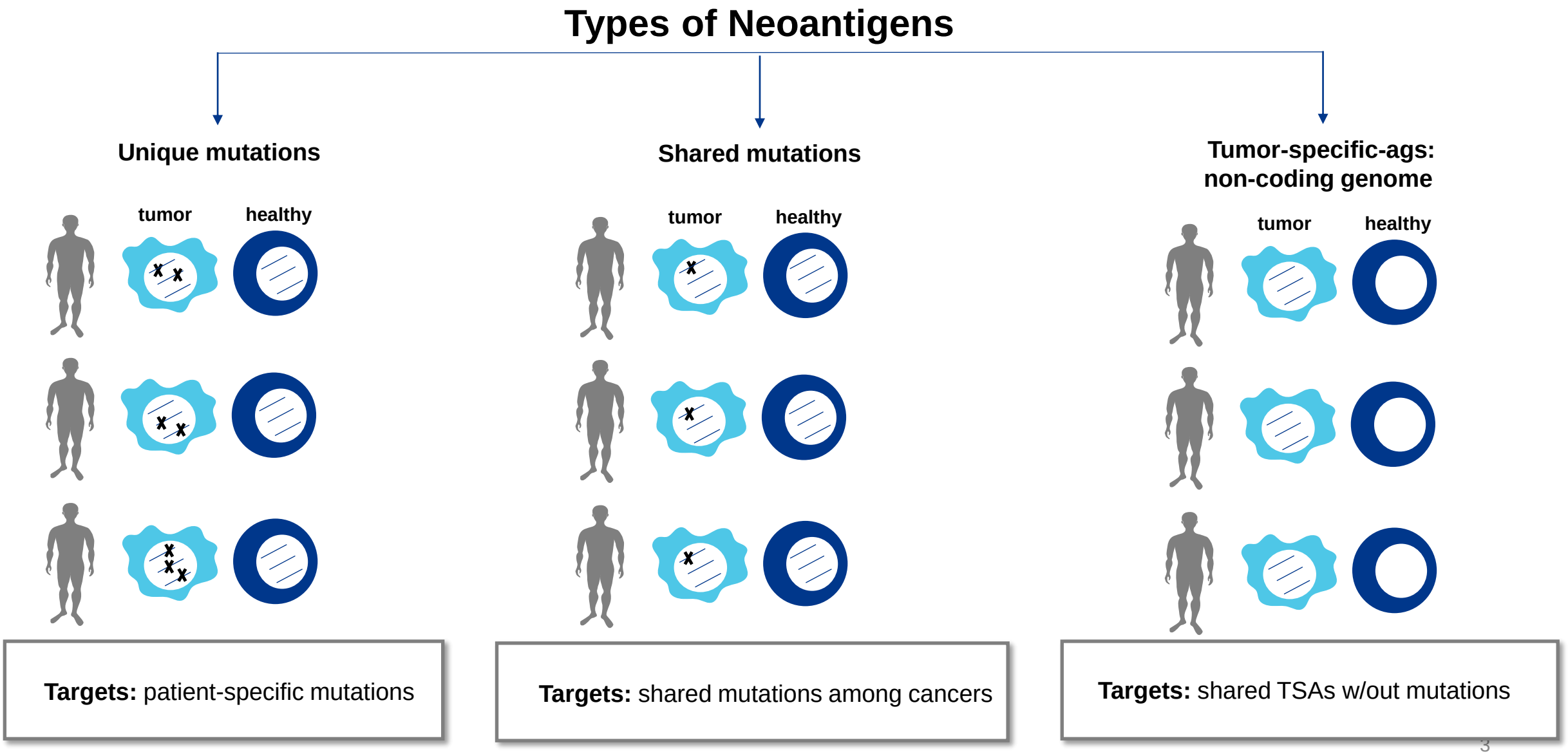
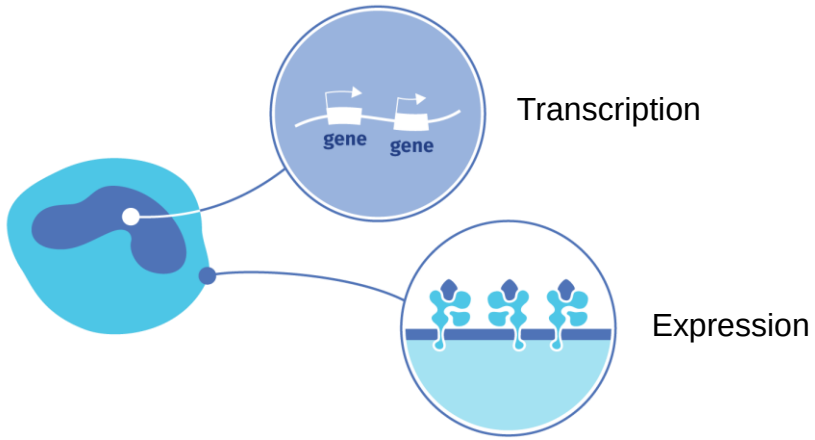
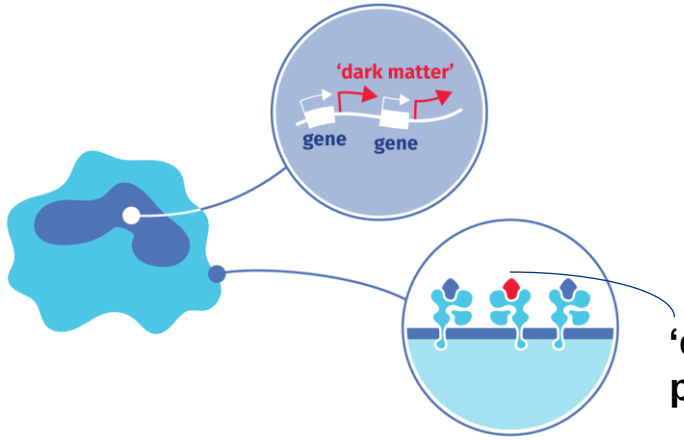


Novel tumor-specific antigens from non-coding regions of the genome – cancer's 'dark matter' - can be targeted by TCRs

Healthy cells: exome-encoded peptides



Tumor cells: peptides from exome and non-coding genome

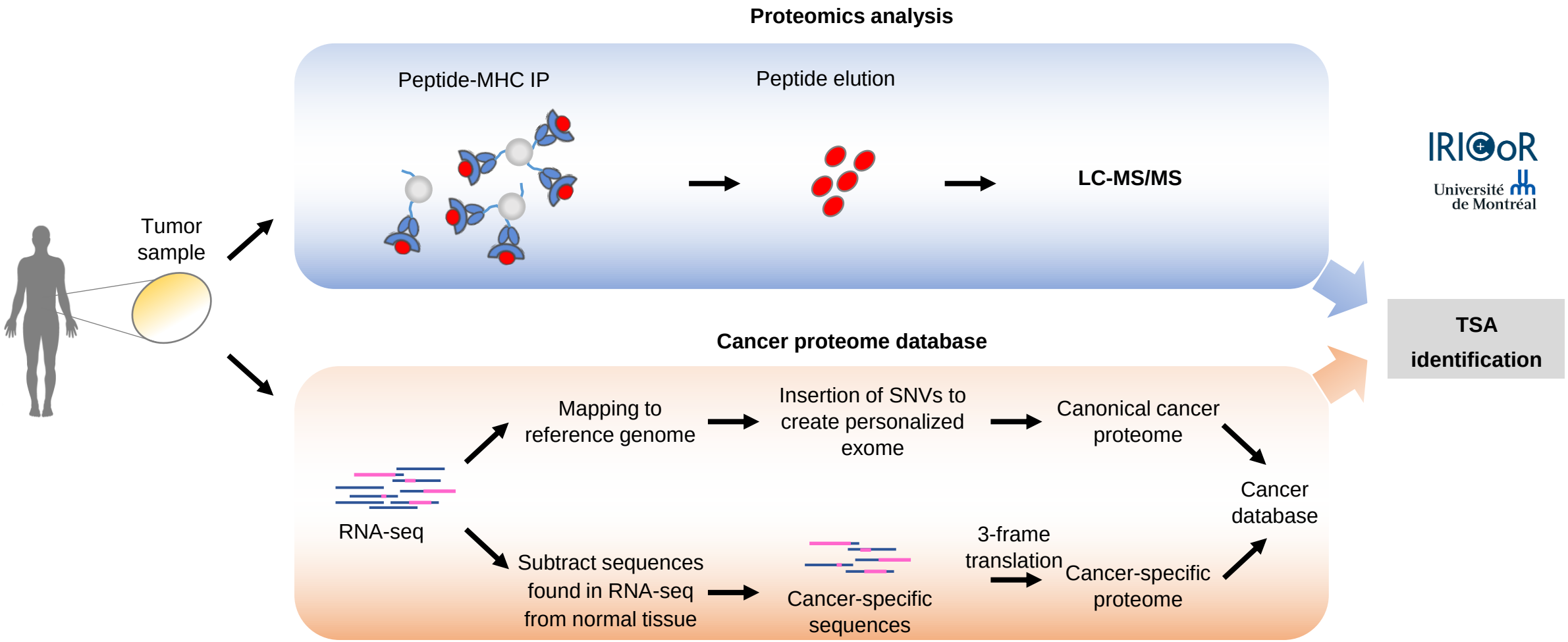


'dark matter" TSA-derived peptide presented by HLA

- Tumor-specific antigen (TSA)-derived peptides are present on cancer cells but not on healthy tissues
- Non-coding genomic regions ('dark matter') are not transcribed in healthy cells but some 'dark matter' regions are transcribed as TSAs and their peptides bound by HLA can be presented on the surface of cancer cells
- 47* TSA-derived 'dark matter' peptides eluted from surface of cancer cells are evaluated to identify those seen by T cells with TCRs that recognize tumor cells but not healthy tissues
- Medigene can acquire exclusive rights for the five best targets through collaboration with University of Montréal / IRICoR

IRICoR: Pan-Canadian drug discovery research commercialization center; * Thereof 44 non-coding and 3 exomic sequences; *Zhao et al. 2020 Cancer Immunol Res; 8:544–55*

Non-mutated tumor-specific antigens (TSAs) were identified by proteogenomics at University of Montreal



For details: *Zhao et al. 2020 Cancer Immunol Res; 8:544–55*

TSA candidates derive from non-mutated non-coding genomic regions and are shared across several cancer indications

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TSA #	HLA	Cancer types	Genomic origin
1	A*01:01	OC	Intronic
2	A*01:01	OC, BC, LC	Intronic
3	A*01:01	OC, BC, LC	Frameshift
4	A*01:01	OC, BC, LC	Intergenic
5	A*01:01	OC, BC, LC	5'UTR
6	A*01:01	OC	ncRNA
7	A*02:01	OC	Intronic
8	A*02:01	OC, BC, LC	Intergenic
9	A*02:01	OC, LC	Intergenic
10	A*02:01	OC	Intergenic
11	A*02:01	OC, BC, LC	Intronic
13	A*02:01	OC, BC, LC	Intronic
14	A*02:01	OC, LC	Antisense
15	A*02:01	OC	Intronic
16	A*03:01	OC, LC	Intergenic
17	A*03:01	OC	Intergenic
18	A*03:01	OC, BC, LC	5'UTR
19	A*03:01	OC	5'UTR
20	A*03:01	OC	ncRNA
21	A*03:01	OC	ncRNA
22	A*03:01	OC, BC	5'UTR
23	A*03:01	OC, BC, LC	Frameshift
24	A*03:01	OC	Intronic

TSA #	HLA	Cancer types	Genomic origin
25	A*03:01	OC, BC, LC	ncRNA
26	A*03:01	OC, LC	5'UTR
27	A*11:01	OC	Intergenic
28	A*11:01	OC, LC	ncRNA
29	A*11:01	OC, BC, LC	Intergenic
30	A*11:01	OC	ncRNA
31	A*11:01	OC	Intronic
32	A*11:01	OC	Intronic
34	A*11:01	OC, BC, LC	Frameshift
35	A*11:01	OC, BC	Intergenic
36	B*08:01	OC	Intergenic
37	B*08:01	OC	Intronic
38	B*08:01	OC, BC, LC	Intergenic
39	B*08:01	OC	Intronic
40	B*08:01	OC, LC	Intergenic
41	B*08:01	OC, BC, LC	Intronic
42	B*08:01	OC	Intergenic
43	B*08:01	OC, BC	Intronic
45	B*08:01	OC, BC, LC	5'UTR
46	B*08:01	OC, LC	Intronic
47	B*08:01	OC	Frameshift

OC = ovarian cancer
BC = breast cancer
LC = lung cancer

Systematic study of peptides as targets for cancer immunotherapy

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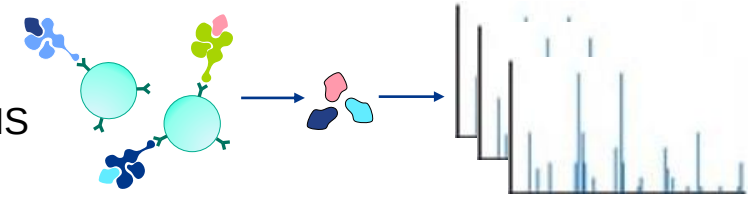
Methods

Peptide detection

MHC-associated peptide LC-MS/MS

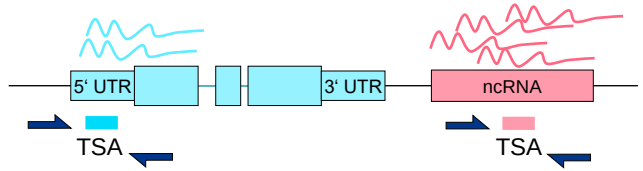


Identification of genomic origin

Transcript expression

RNA-seq

RT-qPCR



Target expression in cancer

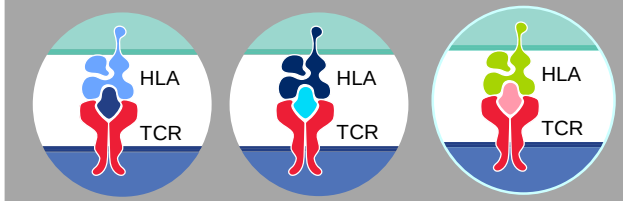
- Expression in cancer samples (UdeM)
- Expression in cancer databases
- Peptide vs RNA expression
- *in vitro* models for functional testing

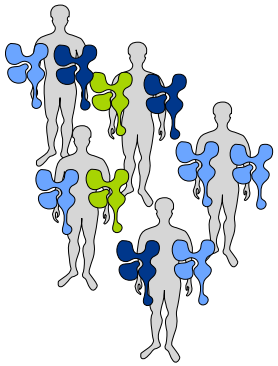
Target safety

- Expression in databases of healthy tissues
- *in vitro* models for functional testing

Target immunogenicity

TCR Discovery Platform





Prevalence of HLA allotypes

Target selection

Healthy donor T cells identify leads for TCRs bypassing the need for patient samples

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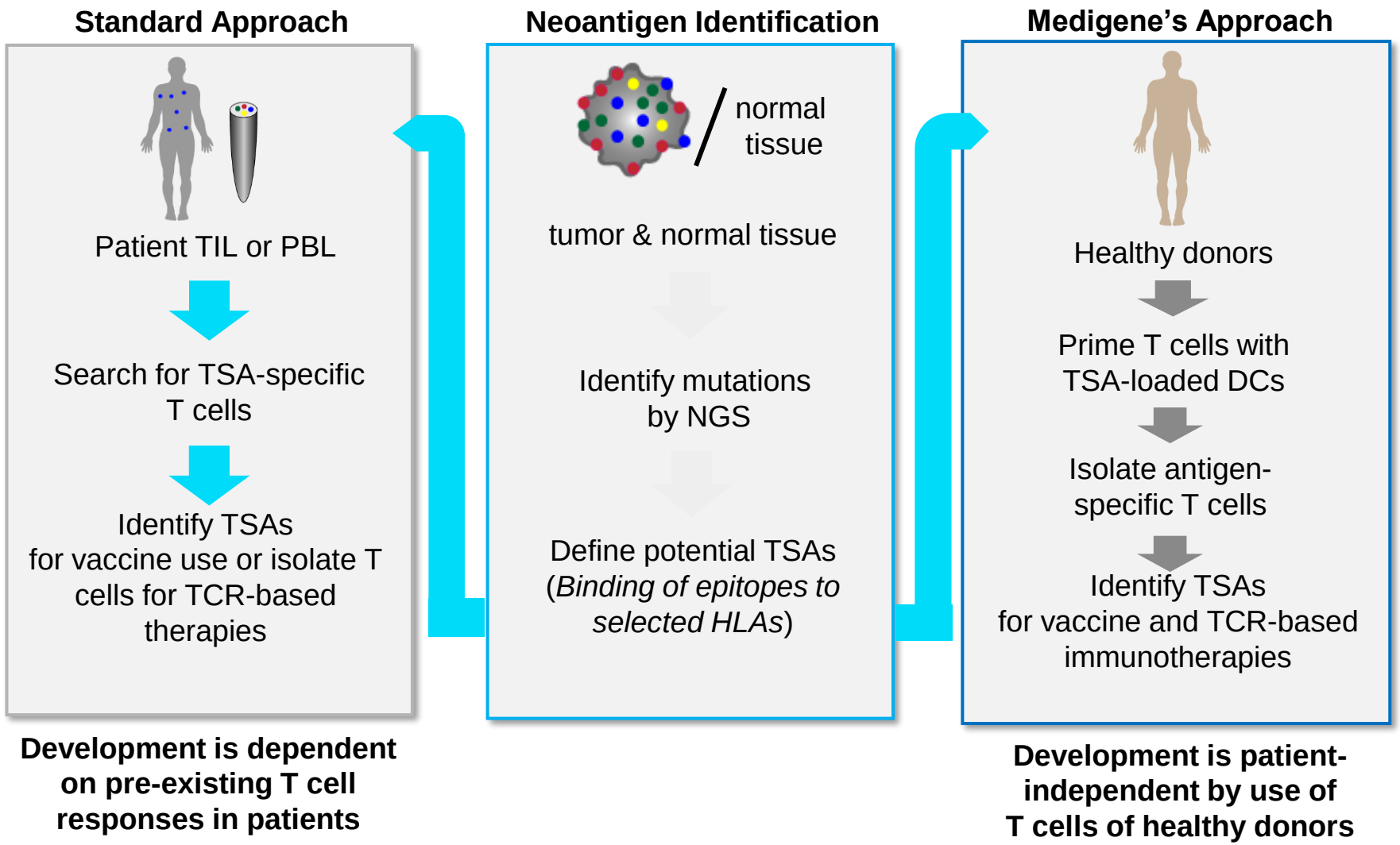
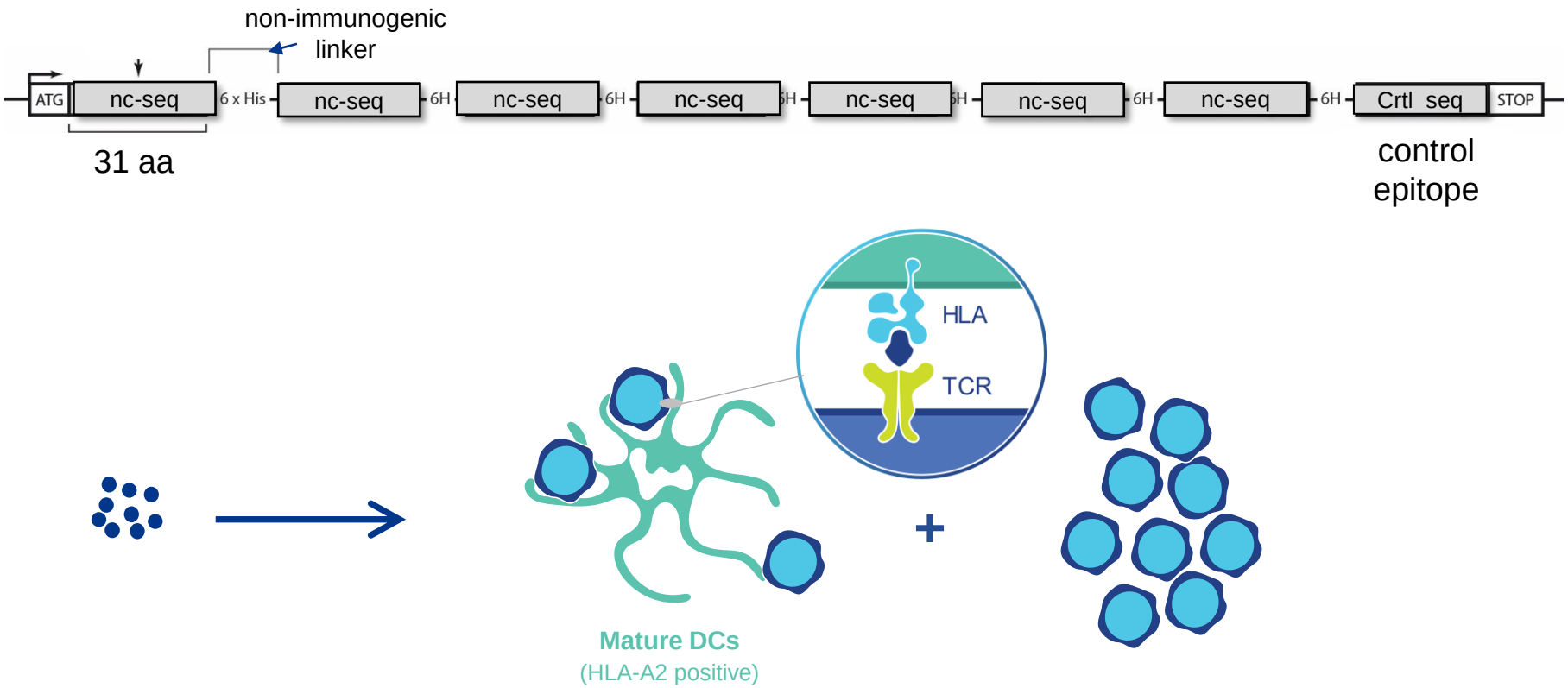


T cells are primed with autologous mDCs expressing *ivt*-RNA

ivt-RNA - epitopes cloned into a minigene-string construct

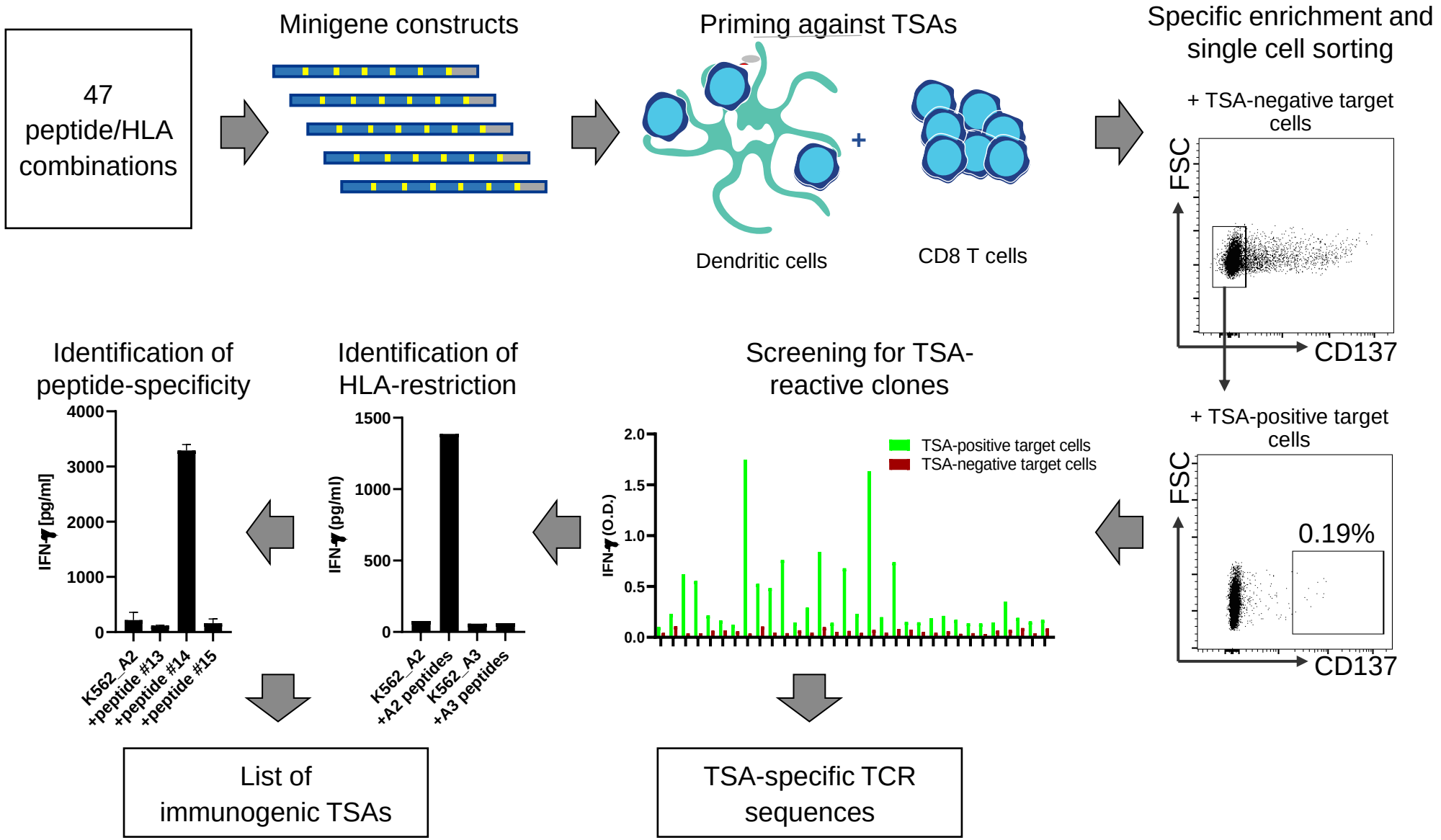


ivt-RNA

mDCs from healthy HLA-relevant donors

autologous (naïve) $CD8^+$ T cell repertoire

Procedure for immunogenicity screen of dark matter peptides



Several “dark matter” peptides are immunogenic for T cells and presented by different common HLA allotypes

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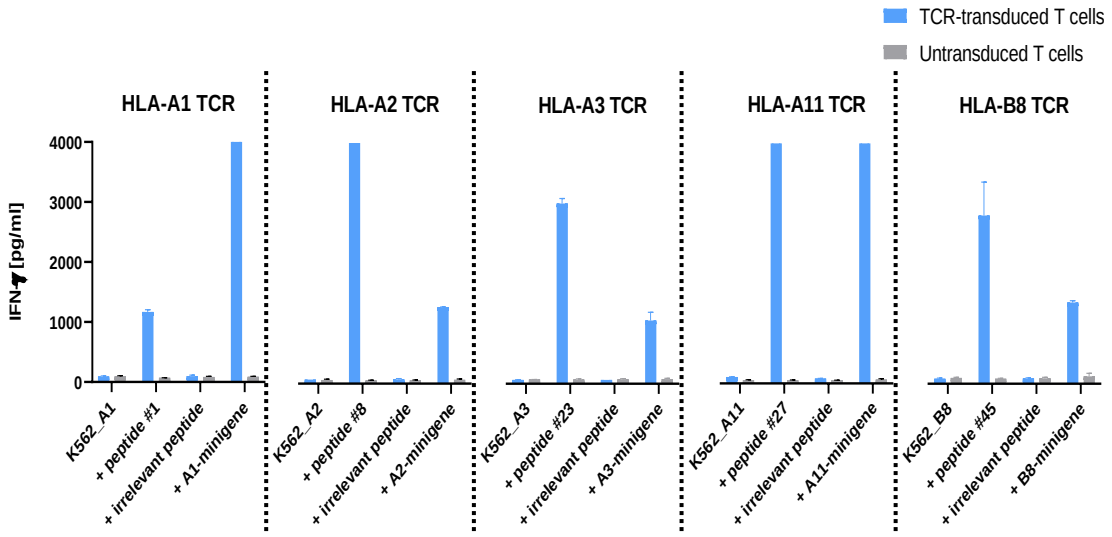
TSA sequence	HLA	Cancer types	Genomic origin	HLA affinity (nM)	No. of specific clones and donors	Recognition of processed peptide
Peptide 1	**A*01:01**	OC	Intronic	4.0	1 (1/4 donor)	confirmed
Peptide 2	**A*01:01**	OC, BC, LC	5’UTR	2.6	3 (2/4 donors)	confirmed
Peptide 3	**A*02:01**	OC, BC, LC	Intergenic	2.7	139 (8/11 donors)	confirmed
Peptide 4	**A*02:01**	OC, LC	Antisense	15.0	2 (2/11 donor)	
Peptide 5	**A*02:01**	OC	Intronic	28.4	1 (1/11 donor)	confirmed
Peptide 6	**A*03:01**	OC	ncRNA	10.6	4 (1/4 donor)	confirmed
Peptide 7	**A*03:01**	OC, BC, LC	Frameshift	12.5	1 (1/4 donor)	
Peptide 8	**A*11:01**	OC	Intergenic	33.3	14 (4/4 donor)	confirmed
Peptide 9	**A*11:01**	OC, LC	ncRNA	22.4	1 (1/4 donor)	confirmed
Peptide 10	**B*08:01**	OC, BC, LC	5'UTR	142.6	21 (3/4 donors)	

- Peptides were identified by MS on cancer samples of ovarian cancer (OC), breast cancer (BC) and lung cancer (LC)

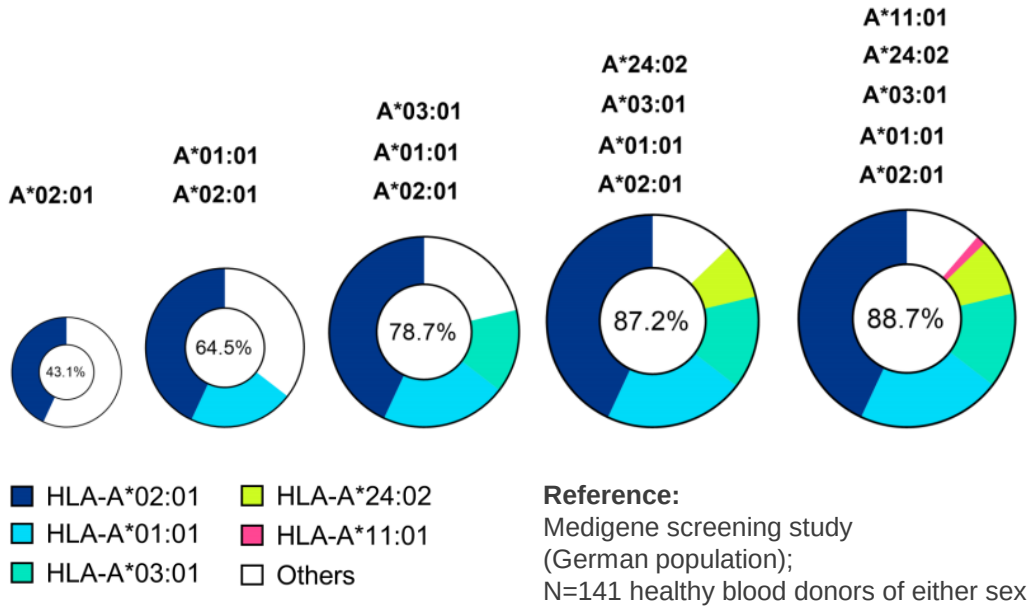
Addressing diversity: Multiple HLAs to cover world populations

- **TCR-T products addressing five common HLA-A allotypes give broad population coverage**
- **Commercial opportunity maximized with different peptide-HLA combinations per indication**

Unique TCRs recognize “dark matter” TSA-derived peptides presented by different HLAs



Coverage of patients worldwide can be extended from ~40% to almost 90% with different HLAs*



Reference:
Medigene screening study (German population);
N=141 healthy blood donors of either sex

***example given here for HLA frequencies in German population**

Ovarian cancer cell line SK-OV3 with endogenous transcript for dark matter TSA was recognized by four HLA-A*11:01-restricted TCR-Ts

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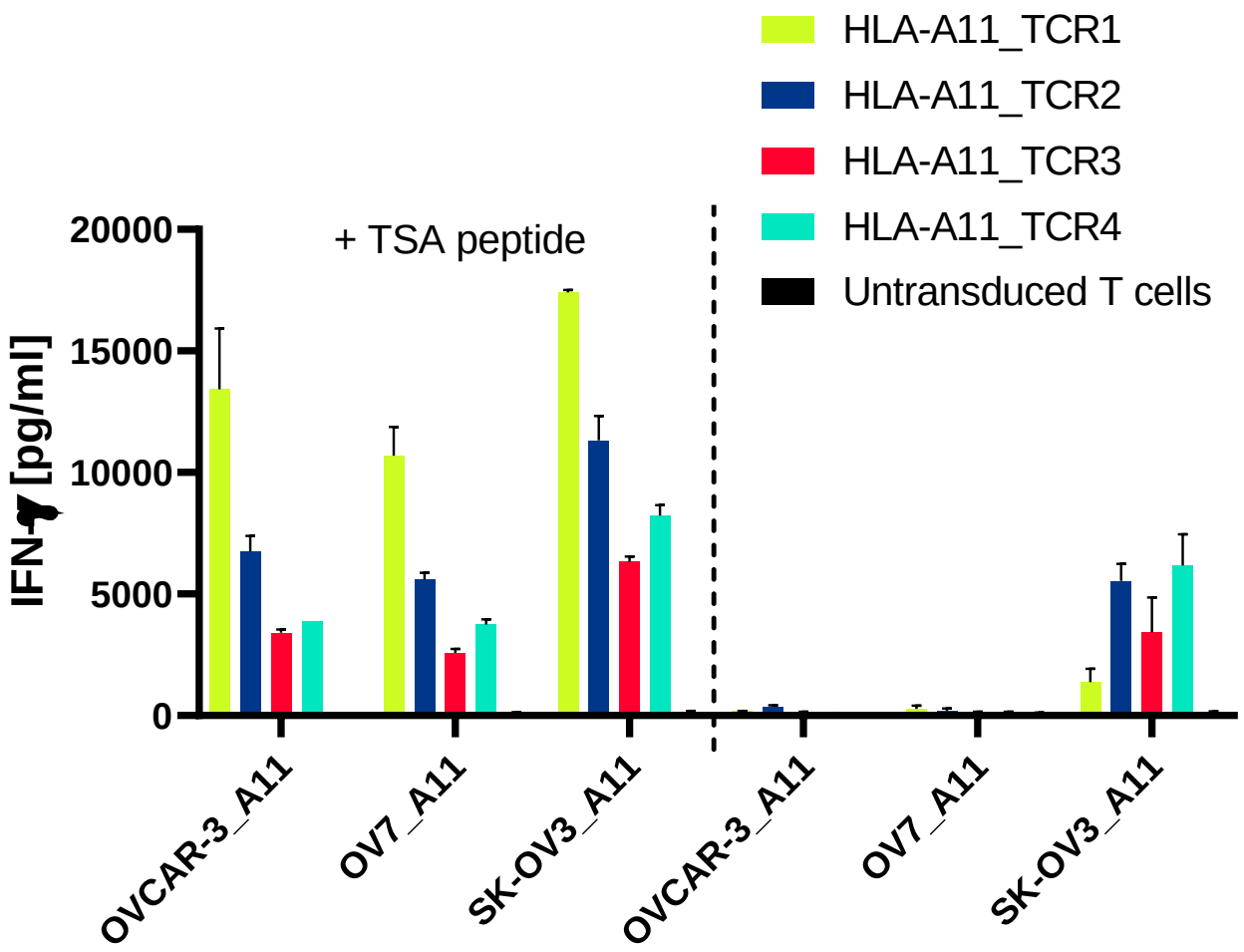
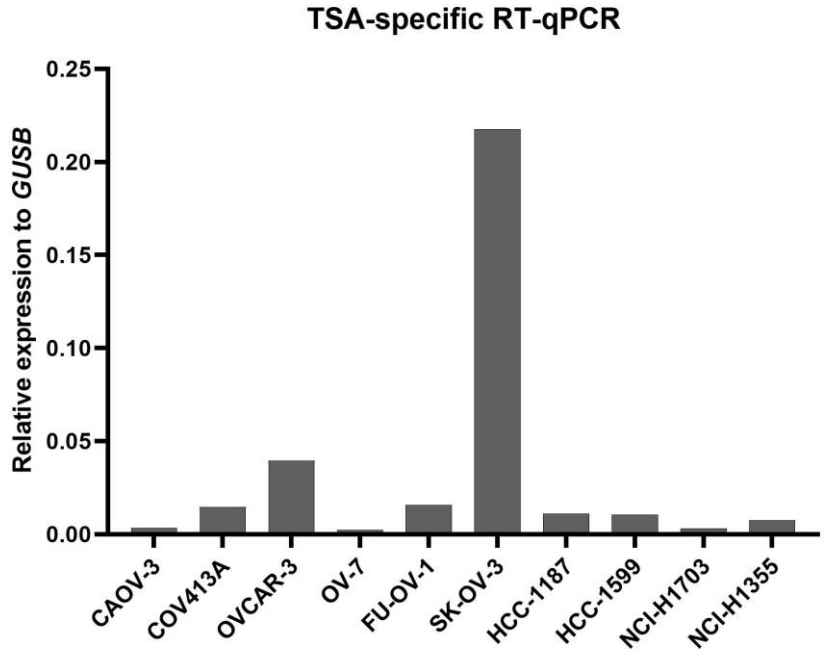
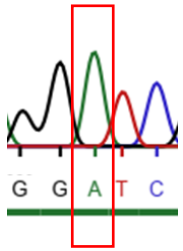


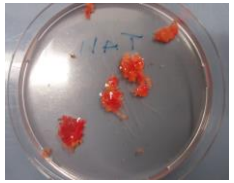


- High expression in SK-OV3 cell line observed by antigen-specific RT-qPCR and confirmed by subsequent sequencing.
- Weaker expression was seen for the other two OC cell lines.

Cancer organoids are recognized by two TSA-specific TCRs recognizing the same „dark matter“ peptide

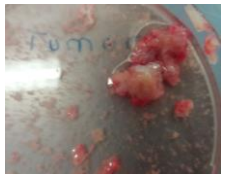
Matched sequence* in fresh specimen and cell line





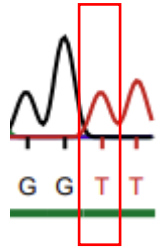
Healthy

2D cell line

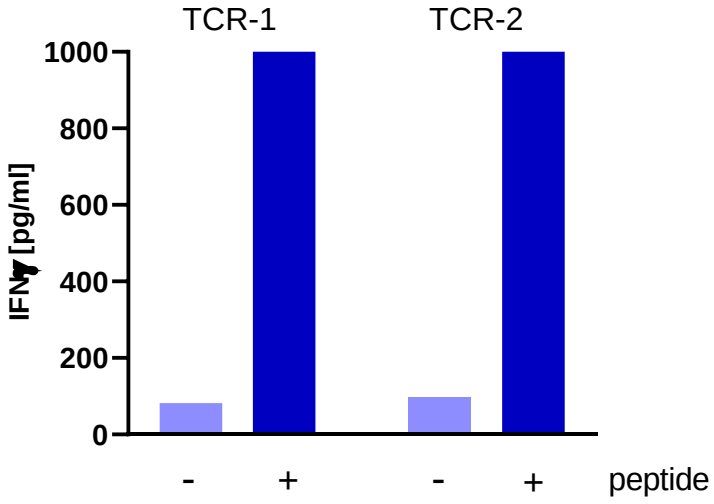


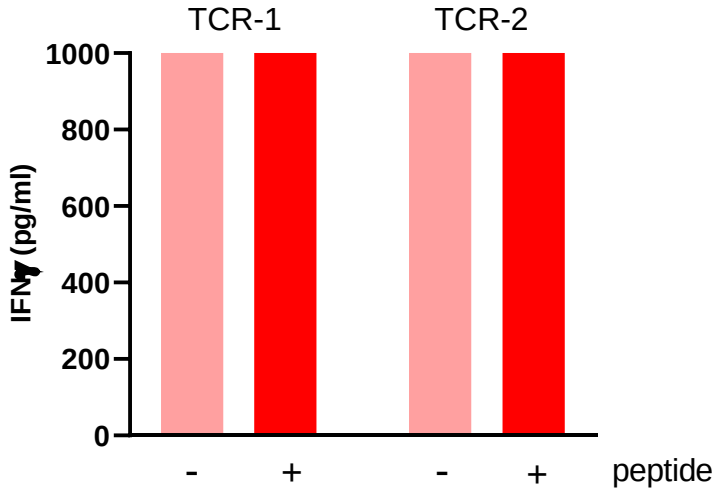
Tumor

3D organoid



Matched sequence* in fresh specimen and organoid





WES data: tumor specific SNV A:T DNA **allelic fraction 87.3%*

Summary

- Novel tumor-specific antigens (TSAs) from non-coding regions of the genome – cancer's **'dark matter'** –were identified by proteogenomics
- Biomarker studies of multiple cancer specimens using MS of peptides eluted from class I molecules and transcriptomics were used to identify a collection of potential target epitopes and predict their HLA presenting allotypes
- Additional biomarker studies are used to establish deep information about the safety profile of targets in healthy tissue
- Functional studies address T cell immunogenicity and establish that some peptides can serve as targets for cancer immunotherapy

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Thank you