Understanding and Navigating Immune Responses to Cas Proteins Used in Gene Editing

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14th Open scientific EIP symposium on Immunogenicity of Biopharmaceuticals, Lisbon

April 27th 2023

With gratitude to

Vijaya Simhadri

Wojciech Jankowski

Daniel Lagassé

Yun-Jong Park

Justine Noel

Joseph McGill

Pratima Bajgain

Atul Rawal

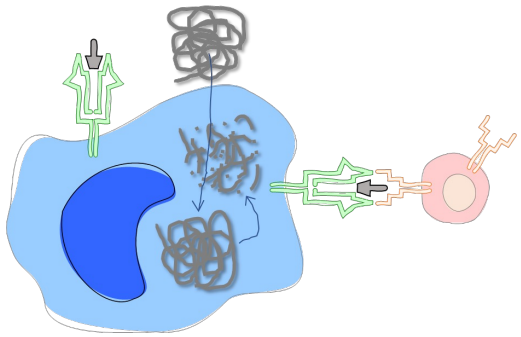
Jiayi Ou

Chris Kidchob

Biologics can be perceived as foreign and elicit immune responses

- When anti-drug antibodies (ADAs) develop, these may or may not affect the activity of the drug
- Antibodies that affect drug activity by binding to active protein domains are called neutralizing ADAs (NABs)
- Non-neutralizing antibodies are not necessarily benign as they can affect the PK/PD (activity) profile and causing loss of tissue targeting
- ADAs can also cross-react with endogenous proteins or elicit anaphylactic reactions

Immunogenicity: The players and their complexity



The antigen

Depending on the size of the antigen 100s or 1000s of peptides can potentially be generated and presented to the immune system

The protein processing machinery

The antigen (protein) has to be processed into peptide fragments
Not every possible peptide can be generated

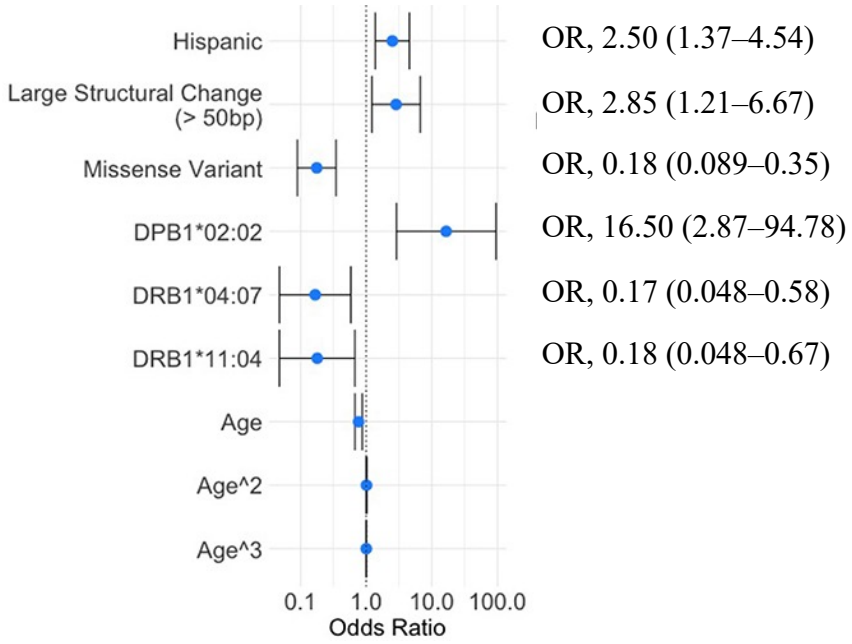
The major histocompatibility complex (MHC) -called HLA in humans

The MHC is polygenic: every individual contains several MHC genes
The MHC is polymorphic: The population has variants of each gene
The MHC genes are the most polymorphic genes in the human genome
Each MHC molecule binds different peptides with different affinities

Identifying variables associated with immunogenicity

The My Life, Our Future (MLOF) project [a collaboration between the American Thrombosis and Hemostasis Network (ATHN), National Hemophilia Foundation (NHF), Bloodworks Northwest and Bioverativ] provided free hemophilia genotype analysis for participants in the United States. As the MLOF collaboration did not HLA type the participants, we HLA typed 1,000 participants for whom *F8* genotype and clinical and demographic information was available

Ethnicity, some HLA variants and large structural changes in the *F8* gene are associated with a higher odds-ratio for ADAs



McGill, Simhadri & Sauna ***Frontiers in Medicine*** 8: 663396, 2021

Neo-sequences: Small changes can have significant consequences

Company Announcement

28 September 2012

novo nordisk®

Novo Nordisk discontinues development of vatreptacog alfa following analysis of phase 3 results

Novo Nordisk today announced the decision to discontinue the development of vatreptacog alfa, a fast-acting recombinant factor VIIa analogue for haemophilia patients with inhibitors. The decision follows analysis of the data from the phase 3a trial adept™2. On 9 August, Novo Nordisk announced that a few patients in the trial had developed anti-drug antibodies to vatreptacog alfa, one patient with a potentially neutralising effect.

Factor VIIa

NO reports of anti-FVIIa antibodies in hemophilia patients

FVIIa variant, Vatreptacog alfa
{V158D, E296V, M298Q}

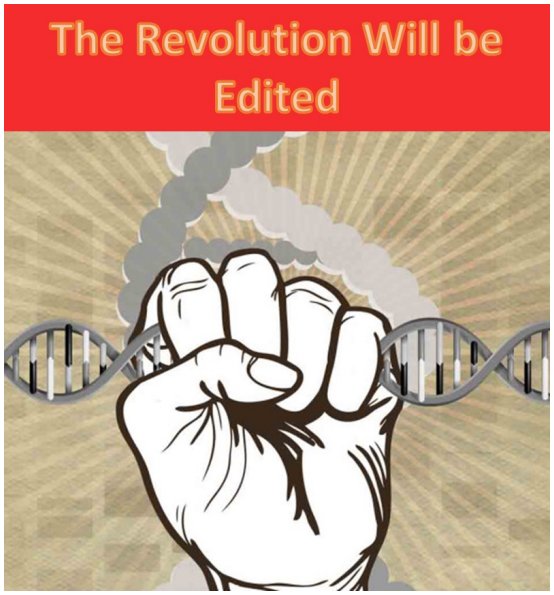
Incidence of anti-FVIIa antibodies = **11.1**%

Post-hoc assessment of Vatreptacog alfa immunogenicity

ASSAY/METHOD	RESULTS
Do mutant peptides bind HLA-II molecules with high affinity (in silico)?	Mutant peptides bind with high affinity to some but not all HLA-II variants
Do mutant peptides bind HLA-II molecules with high affinity (in vitro)?	Confirmed in silico findings
Are mutant peptides presented on HLA-II molecules (MAPPs)?	YES
Do mutant peptides that bind with high affinity elicit a T-cell response?	YES
Are there any associations with clinical outcomes?	ADA-positive patients carry HLA-II that bind to mutant peptide with high affinity

Lamberth, Reedtz-Runge, Simon, Klementyeva, Pandey, Padkjær, Pascal, León, Gudme, Buus & Sauna.
Science Transl. Med. 9: eaag1286, 2017

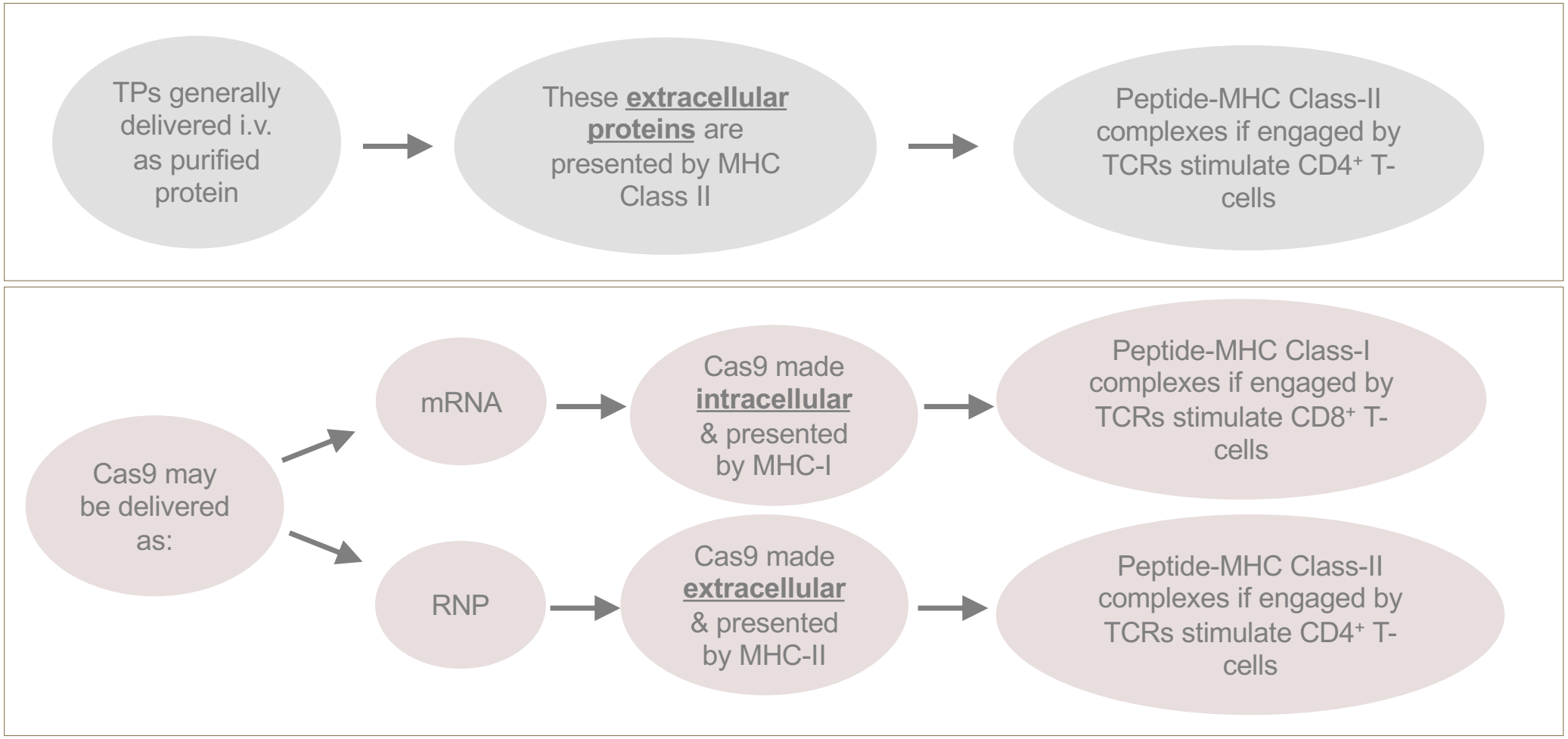
Novel modalities: The CRISPR/Cas-system & immunogenicity



- An important research focus of CRISPR Cas technology, and a regulatory (safety) concern is the off-target effects because these are unpredictable and could lead to lethal consequences
- Technological approaches to resolve off target effects have moved CRISPR-Cas mediated gene editing to the clinic
- As in vivo clinical applications expand immunogenicity is likely to be a key regulatory concern:
  - Cas-proteins are of bacterial origin; thus, in the high immunogenicity risk category per FDA Guidance
  - Cas9 is derived from Staphylococcus aureus or Streptococcus pyogenes which are common pathogens and pre-existing antibodies to Cas9 have been identified in human subjects

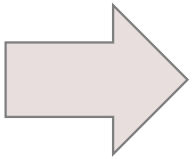
Simhadri, McGill, McMahon, Wang, Jian & Sauna. **Mol Ther Methods Clin Dev**. 10: 105-112, 2018
Simhadri, Hopkins, McGill, Duke, Mukherjee, Zhang, & Sauna. **Nat. Commun.** 12: 5090, 2021

Cas-immunogenicity: Not the same as therapeutic proteins



Experimental identification of biologically relevant T-cell epitopes

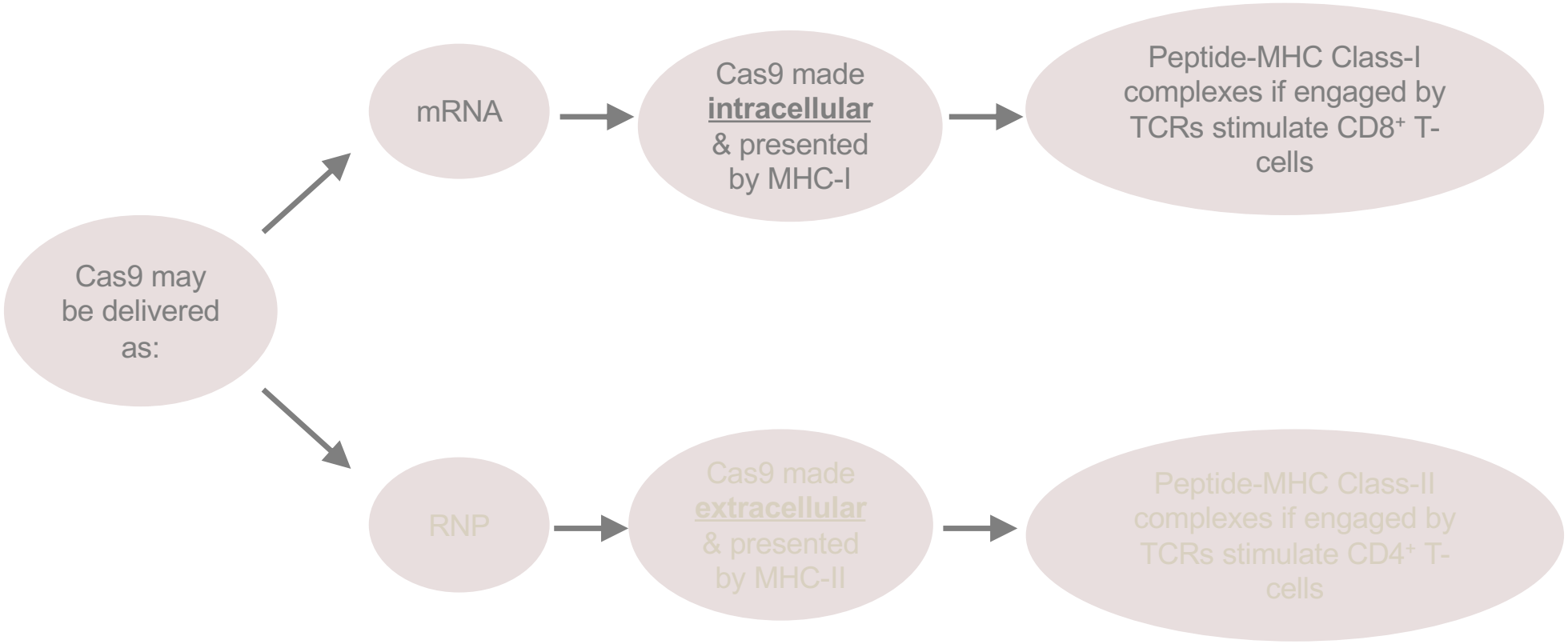
- Peptide presentation (on HLA Class II) measured in an MHC-Associated Peptide Proteomics (MAPPs) assay
- Activation of CD4+ T cells measured by flow cytometry using the markers: IFN-γ, TNF-α, IL-2



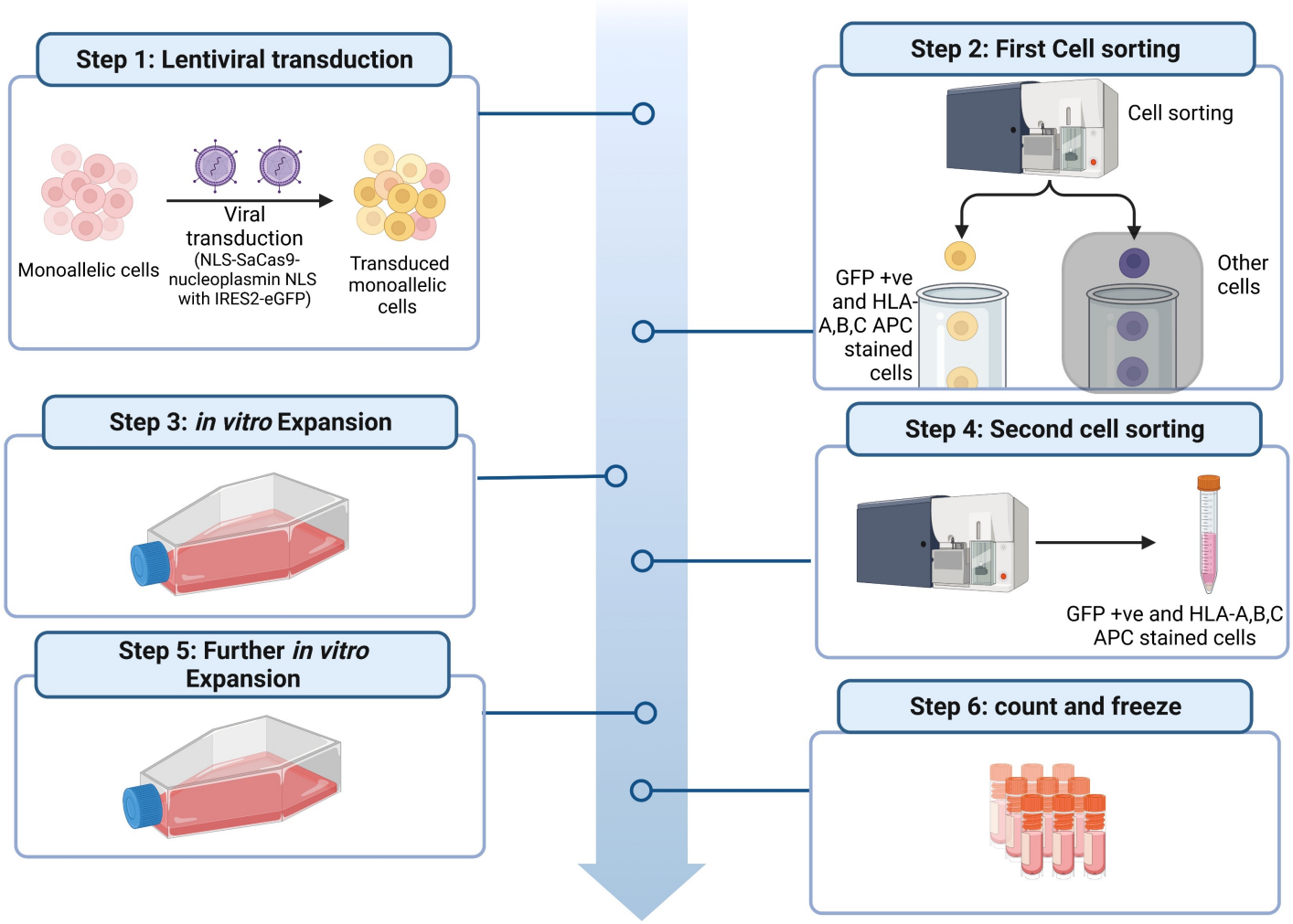
Sr #	Peptide	Position (SaCas9)
1	LFDYNLLTDHSELSGINPYEARV	71 - 93
2	SVKYAYNADLYNALNDL	246 - 262
3	NADLYNALNDLNNLVITRDENEKLE	252 - 276
4	KEILVNEEDIKGYR	301 - 314
5	LDQIAKILTIYQSSE	348 - 362
6	NLNSELTQEEIEQISNLKGYTGTHN	370 - 394
7	AINLILDELWHTNDNQIA	399 - 416
8	ILDELWHTNDNQIAIFNR	403 - 420
9	TNDNQIAIFNRLKLVPK	410 - 426
10	LVDDFILSPVVKRSFIQS	440 - 457
11	IQSIKVINAIIKKYGLPND	455 - 473
12	LPNDIIIELAREKNSKDA	470 - 487
13	EGKCLYSLEAIPLEDL	531 - 546
14	NYEVDHIIPRSVSFDNSFNN	552 - 571
15	TPFQYLSSSDSKISYE	587 - 602
16	KDDKGNTLIVNNLNGLYDKDNDKL	793 - 816
17	LLMYHHDPQTYQK	827 - 839
18	DEKNPLYKYYEETGNYLTKYS	849 - 869
19	GNYLTKYSKKDNGPV	862 - 876
20	LDNGVYKFVTVKNLDVIK	918 - 935
21	KENYYEVNSKCYEEAK	936 - 951
22	ISNQAEFIASFYNNDLIK	956 - 973

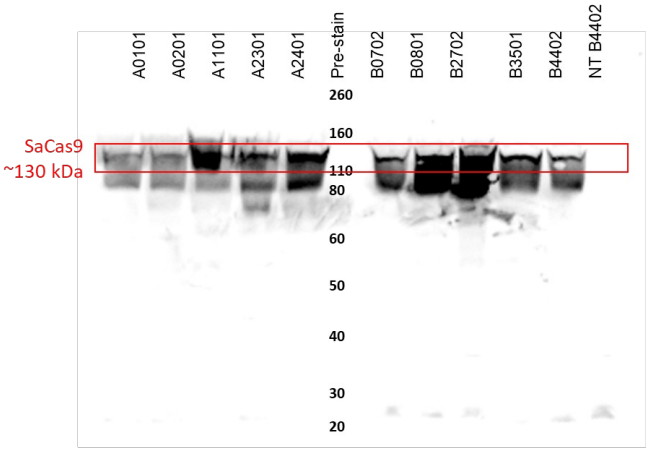
Simhadri, Hopkins, McGill, Mukherjee, Zhang & Sauna. **Nature Communications** 12: 5090 (2021)

Cas-immunogenicity: Peptides that engage with MHC-I and elicit CD8+ T cell responses



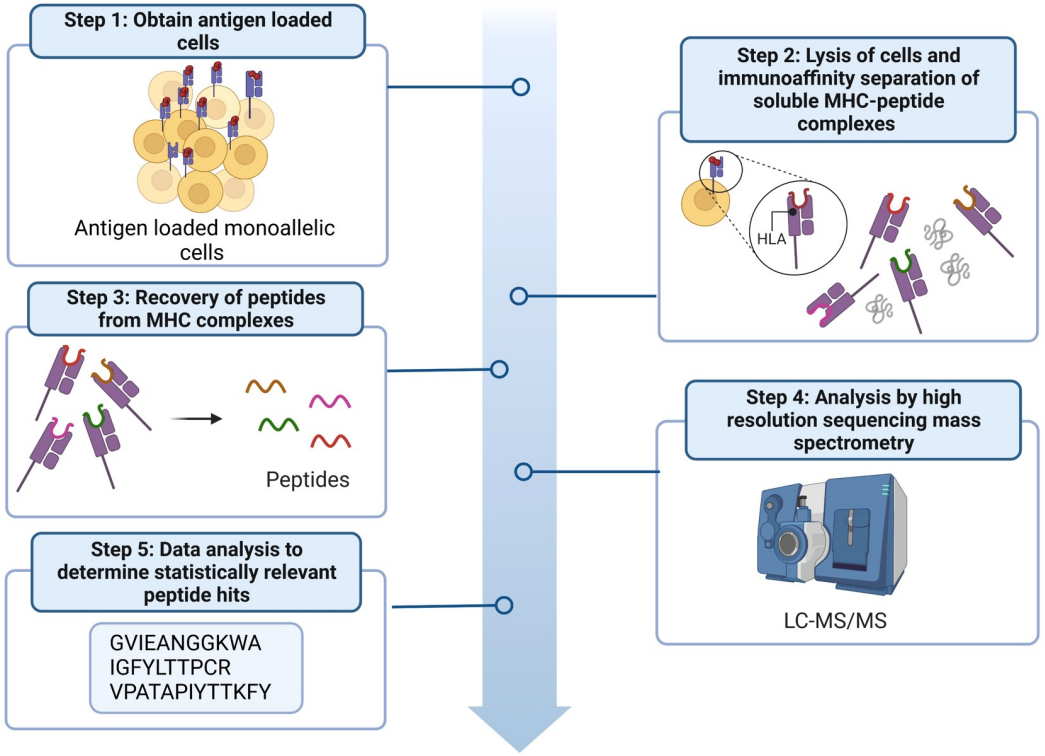
Intracellular expression of Cas9





Monoallelic cells: Devin B. Keskin, Broad Institute of MIT and Harvard, Cambridge, MA (Sarkizova et al. 2020 Nature Biotechnology)

Identification of Cas9 peptides presented by MHC-I



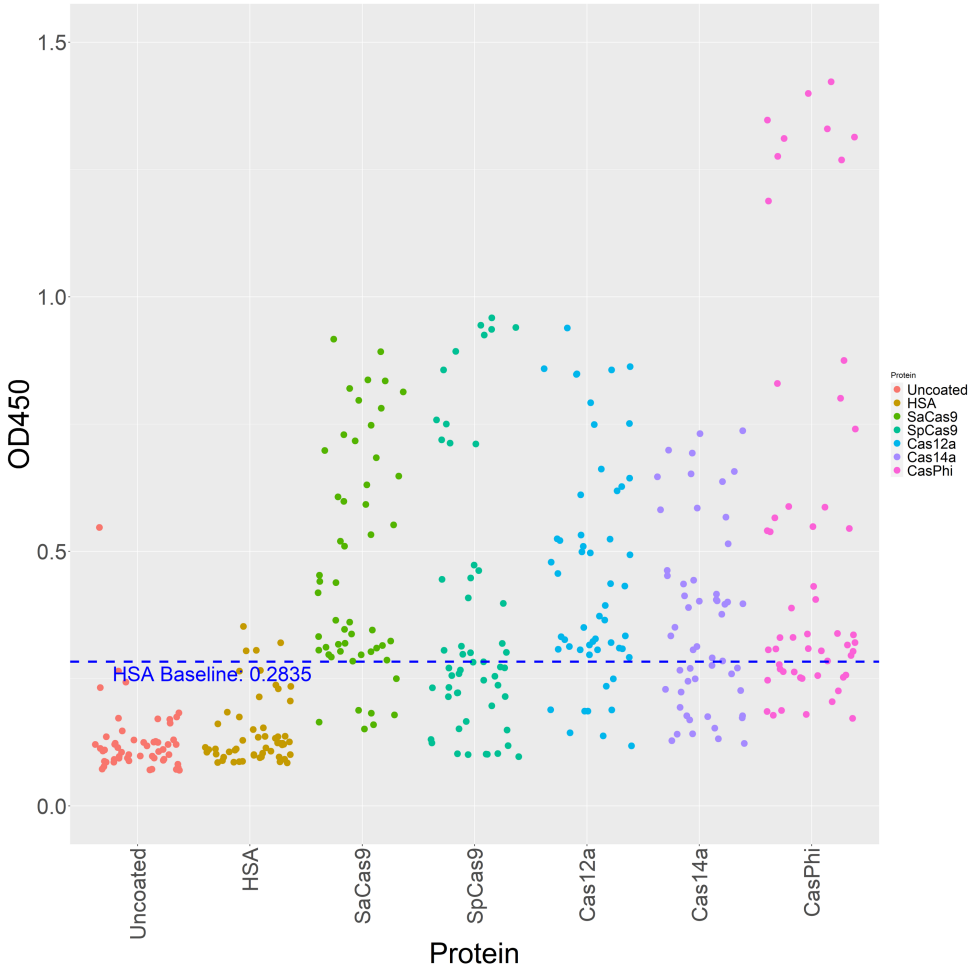
Cas9 peptides identified on MHC-I variants

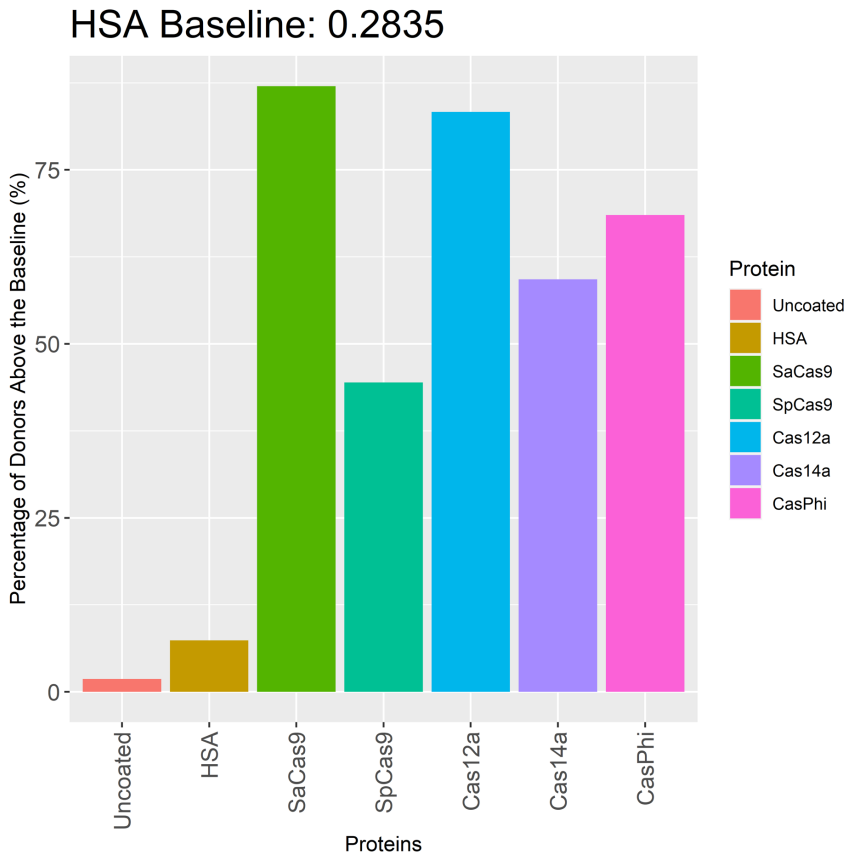
Unique Peptides	Amino acid (start-end)	Donor-1	Donor-2	Donor-3	Donor-4	Donor-5	Donor-6	Donor-7	Donor-8	Donor-9	Donor-10	
		*01:01	*02:01	*11:01	*23:01	*24:02						HLA_A1
		*01:01	*02:01	*11:01	*23:01	*24:02						HLA_A2
							*35:01	*08:01	*07:02	*27:02	*44:02	HLA_B1
							*35:01	*08:01	*07:02	*27:02	*44:02	HLA_B2
EEIEQISNLKGY	378 - 389											
YLIEKIKL	519 - 526											
HIIPRSVSF	557 - 565											
SINGGFTSFLR	675 - 685											
MPEIETEQEY	741 - 750											
VYLDNGVYKF	916 - 925											
GVYKFVTVK	921 - 929											
NRIEVNMIDITY	990 - 1001											

Alternatives to Cas9

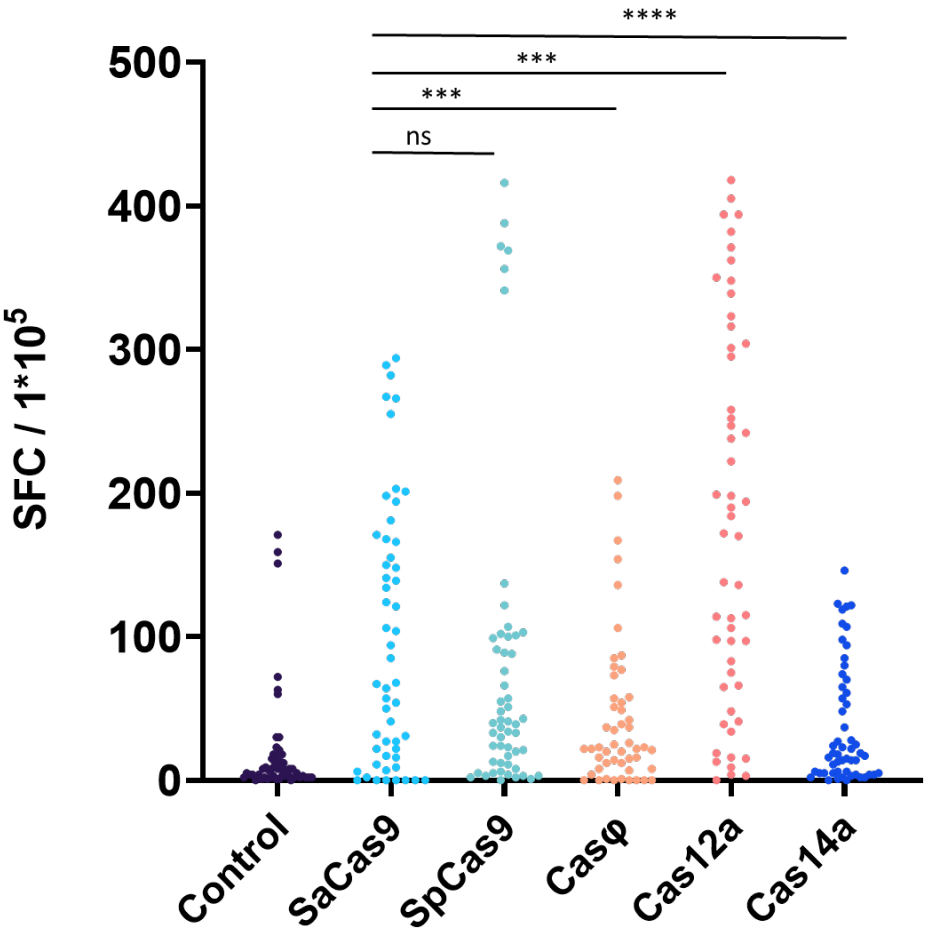
SpCas9	**SaCas9**	**Cas12a**	**Cas14a**	**Casφ**
Streptococcus pyogens	*Staphylococcus aureus*	*Acidaminococcus sp*	*DPANN Archaea*	Biggiephage
1368 amino acid residues	1053 amino acid residues	~1300 amino acid residues	~400-700 amino acid residues	~750 amino acid residues

Pre-existing antibodies to alternatives to Cas9





T-and B-cell responses to alternatives to Cas9



Donor #	SaCas9		SpCas9		CasPhi		Cas12a		Cas14a	
	ELISA	ELISPOT	ELISA	ELISPOT	ELISA	ELISPOT	ELISA	ELISPOT	ELISA	ELISPOT
D192										
D033										
D025										
D163										
D064										
D200										
D940										
D620										
D580										
D695										
D556										
D2717										
D2812										
D586										
D995										
D770										
D303										

Immunogenicity risk assessment

"Take calculated risks.
That is quite different from being rash."
-GEORGE S PATTON

Estimating the probabilities of an immune response to a protein used therapeutically is only half the story.

We also need to know:

What are the clinical consequences of the immune response(s)?

RISK:

Probability of occurrence

x

Severity of the consequence

Glimpses into likely clinical consequences of Cas9 immunity

In vivo consequences of pre-existing immunity to SaCas9 were evaluated in the context of liver genome editing with AAV packaging CRISPR-Cas9 in a mouse model.

Efficient genome editing occurred in mouse liver **with pre-existing SaCas9 immunity**.

HOWEVER:

Genome editing was accompanied by an increase in $CD8^+$ T cells in the liver and a cytotoxic T cell response.

Results: Hepatocyte apoptosis, loss of recombinant AAV genomes, and complete elimination of genome-edited cells.

Thank you!