GRAdHIVNE1: A Gorilla Adenoviral HIV Vaccine Encoding Highly Networked CD8⁺ T cell Epitopes

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Matthew A. Getz[1], Angelo Raggioli[2], Michela Gentile[2], Shaown Bhattacharyya[1], Benjamin H. Goldberg[1], Hsinyen Huang[1], Ryan Yim[1], Aljawharah Alrubayyi[1], Alessandra Soares-Schanoski[1], Anusha Nathan[1], Alton Gayton[1], Charles Crain[1], Sandra Vo[1], Maria Ssozi[1], Ali Albowaidey[1], Usha Nair[1], Facundo D. Batista[1], Marco Soriani[2], Stefano Colloca[2], Stefania Capone[2], Gaurav D. Gaiha[1,3]

[1] Ragon Institute of Mass General Brigham, MIT, and Harvard, Cambridge, United States, [2] ReiThera srl - Castel Romano, Rome, Italy, [3] Massachusetts General Hospital, Division of Gastroenterology, Boston, United States

ABSTRACT

Background:
Adenoviral vectors have long been utilized in HIV vaccine development to elicit CD8⁺ T cell responses. However, recent trials have been limited by pre-existing vector immunity, skewed immunodominance hierarchies, and the emergence of viral escape variants. To address these barriers, we developed GRAdHIVNE1, a replication-incompetent gorilla adenovirus serotype 32 (GRAd32) vector encoding a T cell vaccine insert composed of structurally constrained, highly networked HIV epitopes - regions critical to viral fitness and less prone to escape.

Methods:
The GRAdHIVNE1 cassette was developed after iterative testing of 12 immunogens and is comprised of an N-terminal endoplasmic reticulum (ER) insertion signal sequence, 46 networked regions from HIV clade B and C separated by furin cleavage sites, and a universal CD4⁺ T helper epitope. Immunogenicity was evaluated in BALB/c mice, HLA-A*02:01 and B*07:02 knock-in mice, and New Zealand white rabbits. Animals received a single dose (1×10^8 viral particles), followed by evaluation of antigen-specific CD8⁺ T cell responses using ELISpot and intracellular cytokine staining for assessments of polyfunctionality.

Results:
GRAdHIVNE1 elicited robust, polyfunctional CD8⁺ T cell responses against two H-2K(d)/D(d)-restricted epitopes in BALB/c mice (Gag AI9 and protease VI9). In HLA-A*02:01 and B*07:02 knock-in mice, vaccination generated strong CD8⁺ T cell responses to distinct HLA-A*02:01 and B*07:02 restricted networked epitopes derived from key regions of the protease core, reverse transcriptase active site, envelope V1V2 stem, Gag p24 interface and Nef core. Importantly, responses were appropriately MHC/HLA-restricted and polyfunctional, reflecting the capacity of the GRAdHIVNE1 immunogen to process and present epitopes within distinct MHC backgrounds. Preliminary studies in rabbits have further confirmed immunogenicity, supporting cross-species potency.

Conclusion:
GRAdHIVNE1 is a newly developed T cell vaccine candidate capable of inducing strong MHC/HLA-restricted CD8⁺ T cell responses directed against highly networked HIV epitopes in diverse pre-clinical models. These results establish proof-of-concept for the GRAd platform and provide the rationale for human clinical trials currently underway in sub-Saharan Africa (NCT06617091), where novel vaccine strategies remain urgently needed.

GRAdHIVNE1 is a novel HIV T cell vaccine that drives potent, HLA-restricted CD8⁺ T cell responses against mutationally constrained epitopes and is currently being evaluated in human clinical trials in southern Africa.

BACKGROUND AND METHODS

Generation of highly networked HIV T cell epitope vaccines

Clade B – (23 Networked Epitope Regions - 12 Shared Clade B + C Regions, 11 Unique Clade B Regions)



Clade B + Clade C – (46 Networked Epitope Regions - 24 Shared Clade B + C Regions, 22 Unique Clade B and C Regions)



Clade C – (23 Networked Epitope Regions - 12 Shared Clade B + C Regions, 11 Unique Clade C Regions)

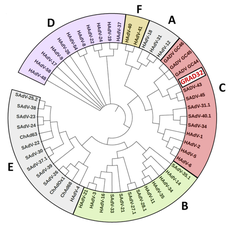


= Shared Clade B + Clade C = Unique Clade B = Unique Clade C = Furin Cleavage Sequence

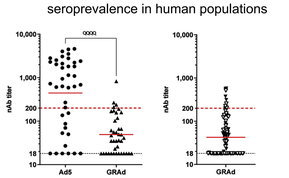
The Clade B and Clade C networked HIV T cell vaccine cassettes are comprised of 23 networked epitope regions (12 Shared Clade B + C Regions, 11 Unique Clade B or C Regions) encompassing 46 networked HIV epitopes that are separated by furin cleavage sequences. Additionally, one design combined the full Clade B and Clade C regions (24 Shared Clade B + C Regions, 22 Unique Clade B and C Regions). The networked epitope regions are flanked by an endoplasmic reticulum insertion signal sequence (ERISS) and a universal CD4+ T cell helper epitope (from tetanus and diphtheria toxoids).

GRAd32 is a novel group C simian adenovirus

GRAd32 shares group C lineage with human Ad5



GRAd32 has minimal baseline seroprevalence in human populations



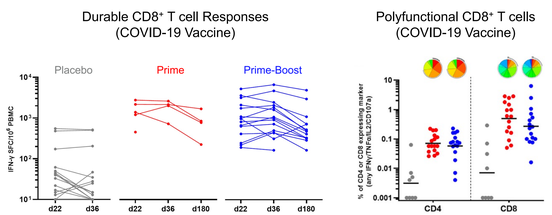
titer	Ad5	GRAd
<18	6 (15%)	13 (32.5%)
18-200	7 (17.5%)	23 (57.5%)
>200	27 (67.5%)	4 (10%)

	younger	older	total
<18	16 (40%)	22 (48.9%)	40 (44.4%)
18-200	21 (46.7%)	19 (42.2%)	40 (44.4%)
>200	6 (13.3%)	4 (8.9%)	10 (11.1%)

Capone et al., *Mol Therapy* 2021

BACKGROUND AND METHODS

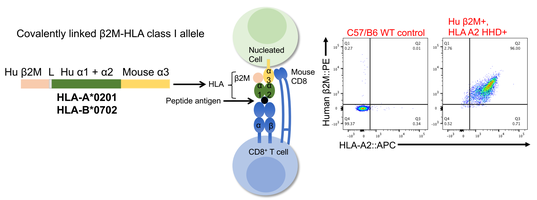
GRAd vector induces robust CD8⁺ T cells in humans



Capone et al., *Cell Reports Medicine* 2023

GRAd vaccination in humans induces robust, antigen-specific CD8+ T cell responses with sustained magnitude and polyfunctionality, as assessed by IFN-γ ELISpot and intracellular cytokine staining (ICS).

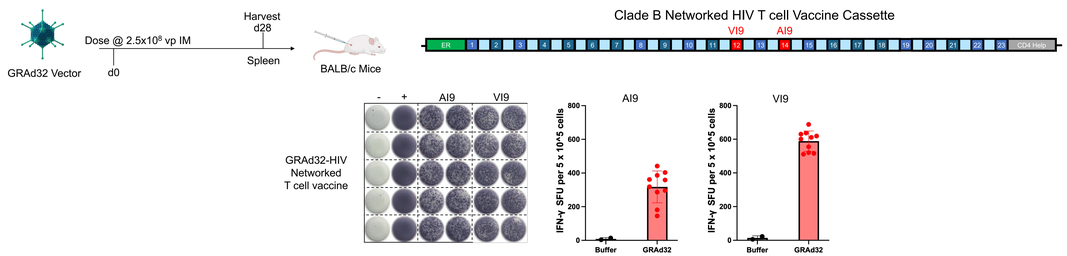
Novel HLA knock-in mice enable human translation



To make the HLA-A*02:01 and HLA-B*07:02 knock-in mouse lines, CRISPR/Cas9 was used to knock in a monochain of human β2m domain linked to the human α1 and α2 domains, as well as the mouse α3 domain, into the Rosa26 locus of a CD45.2 H-2K(b)/D(b)-knockout mouse line. Expression of the HLA allele in the mouse was measured by flow cytometry.

RESULTS

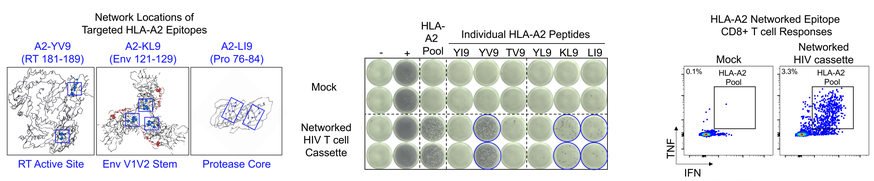
GRAd32 vector encoding novel networked HIV T cell vaccine cassette generates T cell responses



BALB/c mice were immunized intramuscularly with GRAd32 encoding the networked HIV T cell vaccine cassette (2.5 × 10⁸ vp) on day 0. Splenocytes were harvested on day 28 post-vaccination, and antigen-specific T cell responses against Gag AI9 and protease VI9 were quantified by IFN-γ ELISpot, demonstrating robust vaccine-induced cellular immunity.

GRAd32 networked HIV T cell vaccine cassette generates CD8⁺ T cell responses in HLA-KI mice

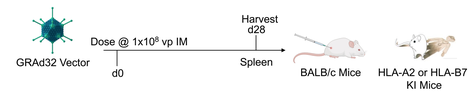


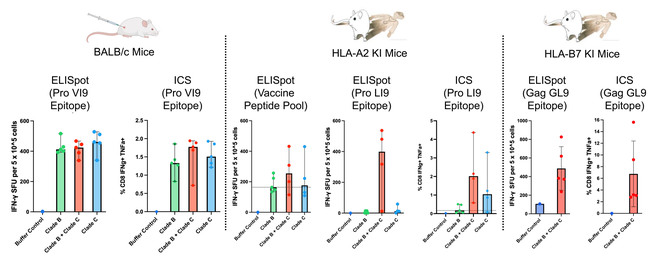


HLA-A*02:01 knock-in mice were immunized intramuscularly with GRAd32 encoding the networked HIV T cell vaccine cassette (1 × 10⁹ vp) on day 0. Splenocytes were harvested on day 28 post-vaccination, and antigen-specific CD8⁺ T cell responses were quantified by IFN-γ ELISpot and ICS. Robust responses were detected against HLA-restricted networked epitopes, including RT YV9, Env KL9, and Pro LI9.

RESULTS

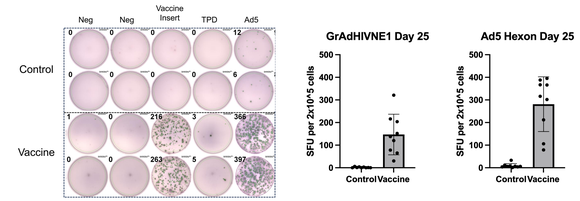
GRAd32 vector expressing the Clade B + Clade C networked epitopes enhances breadth and magnitude of T cell responses





BALB/c, HLA-A*02:01, and HLA-B*07:02 knock-in mice were immunized intramuscularly with GRAd32 encoding the combined Clade B + Clade C networked HIV T cell vaccine cassette (1 × 10⁸ vp) on day 0. Splenocytes were harvested on day 28 post-vaccination, and antigen-specific T cell responses were quantified by IFN-γ ELISpot and ICS. Vaccination elicited robust and broadened CD8⁺ T cell responses across MHC backgrounds, including responses to Pro VI9 (BALB/c), Pro LI9 (HLA-A*02:01 KI), and Gag GL9 (HLA-B*07:02 KI), demonstrating enhanced breadth and magnitude of immunity.

New Zealand White rabbits immunized with GRAdHIVNE1 vector generate T cell responses to the vaccine insert



New Zealand White rabbits were immunized intramuscularly with GRAdHIVNE1 encoding the combined Clade B + Clade C networked HIV T cell vaccine cassette (2 × 10¹¹ vp) on day 0. Peripheral blood mononuclear cells were collected on day 25 post-vaccination, and antigen-specific T cell responses were quantified by IFN-γ ELISpot. Vaccination elicited robust T cell responses directed against the vaccine insert, supporting cross-species immunogenicity of the GRAdHIVNE1 construct.

REFERENCES

1. Gaiha GD, Rossin EJ, et al. Structural topology defines protective CD8+ T cell epitopes in the HIV proteome. *Science*. 2019 May 3;364(6439):480-484. doi: 10.1126/science.aav5095.

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PLAIN LANGUAGE SUMMARY

We developed an HIV vaccine that directs immune cells (T cells) to target vulnerable regions of the virus that are less likely to mutate and escape immune responses. In preclinical studies, the vaccine generated strong immune responses across several animal models, paving the way for a clinical trial now underway in Africa.