Multicistronic vaccines with immune-stimulatory proteins can boost the efficacy of antigen specific T and B cell responses

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nykode therapeutics

Introduction

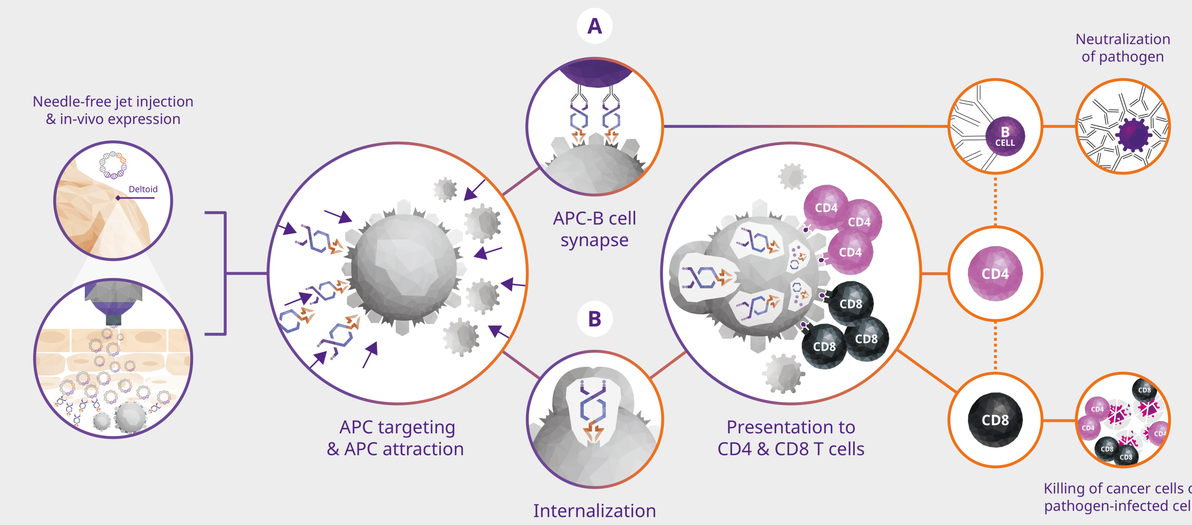
The recent pandemic has highlighted the need for efficacious vaccines to tackle the diseases of tomorrow. Moreover, in the field of cancer, the failure of classical immunotherapies is often linked to the absence of an anti-tumor immune response that can be boosted or revitalized. Both examples reinvigorate the need for potent and versatile vaccine platforms. Recent advances in delivery technologies combined with the intrinsic qualities of the DNA matrix have positioned DNA vaccines as a safe and flexible alternative to other vaccine technologies. Nykode Therapeutics is developing DNA vaccines to allow specific targeting of antigens to antigen presenting cells (APCs), thus inducing a fast, strong and long-lasting specific immune response. The Vaccibody™ (VB) molecule consists of three functional units; a targeting unit that binds to surface receptors on APCs, a dimerization unit, and an antigenic unit that is derived from the disease-causing agent. The Vaccibody platform is versatile in its modularity and allows for tailoring of the different modules to induce disease-specific responses.

Most recently, Nykode Therapeutics has developed a second-generation version of the Nykode DNA vaccine platform in which a VB molecule can be co-expressed with one or more immune-stimulatory proteins using a multicistronic design.

Here, we show co-expression of the VB protein with the immune-stimulatory protein GM-CSF (granulocyte-macrophage colony-stimulating factor). Co-expression of different VB proteins and GM-CSF elicited 6-fold elevated antigen-specific T cell reponse and 10-fold elevated total IgG titer in vaccinated mice compared to VB alone.

These data demonstrate the flexibility and potential of DNA vaccines as well as the advantages of combining an APC targeted delivery of disease-specific antigens together with a local production of immune stimulatory proteins.

Mechanism of action



In-house bioinformatic tools for optimal vaccine design

OncoSHARED

Identification of shared epitopes across patient population/cancer indication for off-the-shelf vaccines

epicPATH

Identification of conserved epitopes in pathogens

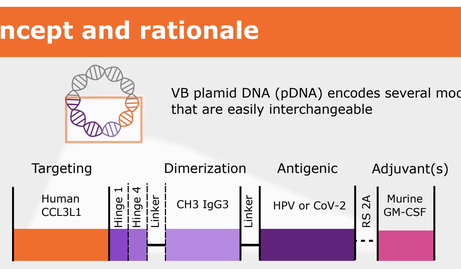
- Hotspots with epitopes predicted to bind across population scale MHC I/II
- Proteosomal processing
- Self similarity
- TCR reactivity
- Patient safety

Optimised vaccine construct design

A multicistronic design to facilitate co-expression from a single plasmid DNA

Concept and rationale

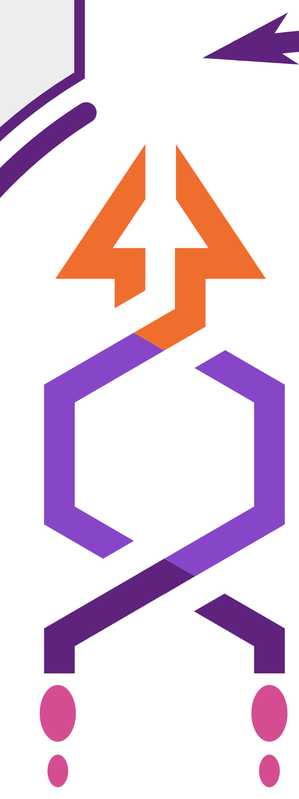
The second-generation of Vaccibody platform consists of an optimized multicistronic format resulting in a high expression and secretion of the different modules. Each of the modules can be replaced to tailor a desired immune response.



Secretion of VB and GM-CSF proteins

Secretion from transient transfected Expi293F cells determined by sandwich ELISA. Data from two VB constructs with HPV or CoV-2 (Wuhan RBD) as antigenic unit are presented.

Vaccibody™: A modular vaccine platform



Targeting — **Attract and bind Antigen Presenting Cells (APCs)**
Molecules that bind surface receptors on APCs:
- Natural ligands, including cytokines and chemokines.
- Bacterial proteins
- scFv from mAb binding

Dimerization — **Crosslinking targeted receptor on the surface of the APC**
Favorize essential vaccine mechanisms:
- Molecule internalization
- Endosome escape for optimal HLA-I loading

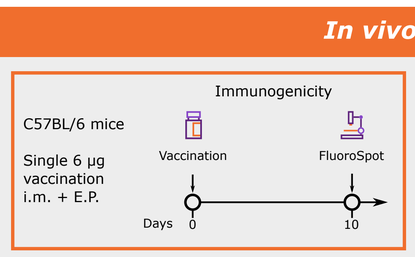
Antigen(s) — **Mount a target-specific immune response**
Full-length antigens
- Cancer, viral, bacterial, parasitic etc.

Multiple T cell epitopes
- Individualized and shared cancer products
- T cell epitopes for infectious disease
- T cell epitopes for autoimmunity

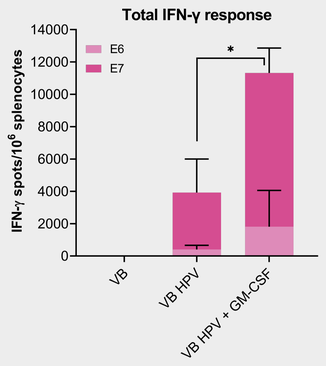
Adjuvant(s) — **Enhance the immune response**
- Cytokines
- Chemokines
- Growth factors
- Immune modulators

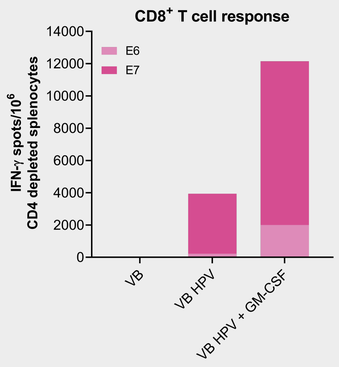
VB HPV+GM-CSF vaccination in mice induced significantly higher T cell reponse compared to VB HPV

In vivo efficacy



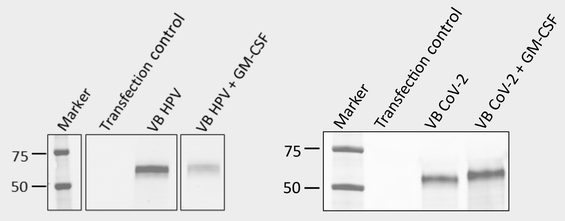
The total IFN-γ response upon HPV peptide re-stimulation was higher in splenocytes from mice vaccinated with VB HPV+GM-CSF compared to VB HPV. Moreover, the total T cell response was majorly CD8⁺ T cell response, as was observed upon re-stimulation with HPV peptide in CD4⁺ depleted splenocytes.





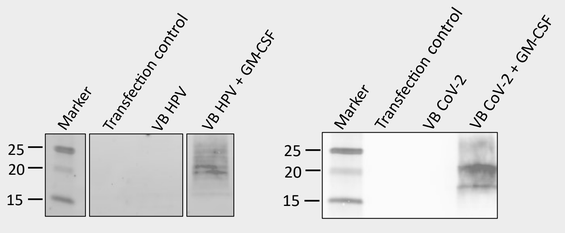
Western blot data confirms proper ribosome skipping between the VB molecule and GM-CSF protein

VB protein detection by anti-MIP-1α antibody



The VB HPV and VB CoV-2 proteins were detected at expected sizes under reducing condition by anti-MIP-1α antibody.

GM-CSF protein detection by anti-GM-CSF antibody

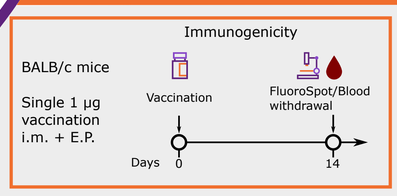


Staining with anti-GM-CSF antibody under reducing condition resulted in several bands close to each other indicating heterogenously glycosylated GM-CSF.

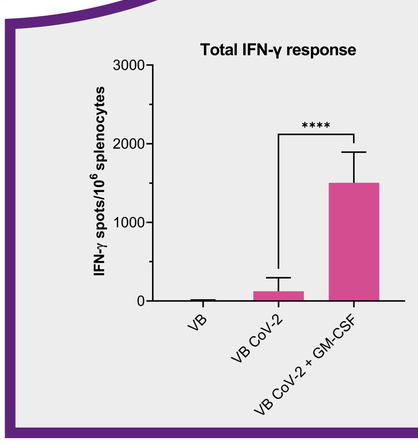
No bands at higher molecular weights were detected which confirms individual expression and secretion of VB protein and GM-CSF indicating proper ribosome skipping.

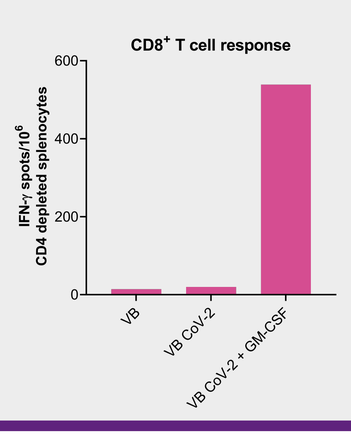
VB CoV-2 (Wuhan RBD)+GM-CSF showed higher T cell response and total IgG titer in vaccinated mice

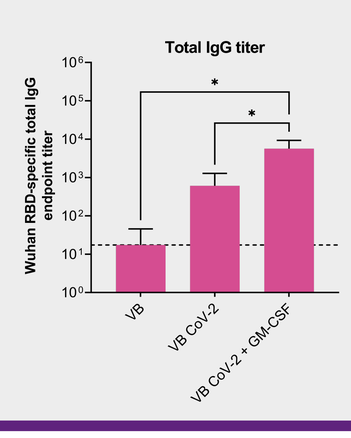
In vivo efficacy



The total IFN-γ response upon RBD (Wuhan) peptide re-stimulation was higher in splenocytes from mice vaccinated with VB CoV-2+GM-CSF compared to VB CoV-2. The anti-RBD (Wuhan)-specific IgG titer in mouse sera was 10-fold higher in mice vaccinated with VB CoV-2+GM-CSF compared to VB CoV-2.







Take-home message

- **Multicistronic vaccine deisgn has the potential to enhance T cell response and increase total IgG titer.**
- **Multi-cistronic vaccine design is fully flexible with the opportunity to integrate one or more immune stimulatory proteins and polypeptides.**

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Statistical analysis:
Statistically significant differences were determined using Tukey's multiple comparisons test. Significant differences are indicated as follows: *, $p \leq 0.05$; ****, $p \leq 0.0001$.

Abbreviations:
VB = Vaccibody
GM-CSF = Granulocyte-Macrophage Colony-Stimulating Factor
RS = ribosome skipping
HPV = Human Papilloma Virus
CoV-2 = SARS-CoV-2
RBD = Receptor Binding Domain
IFN-γ = Interferon gamma
i.m. = intramuscular
E.P. = electroporation

Learn more about Nykode Therapeutics