Global Foot-and-Mouth Disease
Research Alliance
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Full protection of swine against foot-and-mouth disease by a bivalent B-cell epitope dendrimer peptide vaccine

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Conventional FMD vaccines

CONVENTIONAL VACCINES
(chemically inactivated viruses)

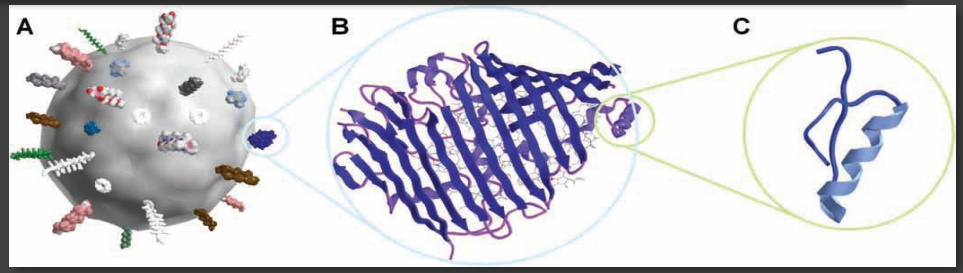
+ Good protection against antigenically related viruses.
- Its use is instrumental for disease control

- ***Risk of viral escapes from vaccine production plants***
- ***A cold-chain is required***
- ***Need for differentiation between infected and vaccinated animals***
- ***Antigenic match between field viruses and vaccine strains***

"Vaccination to live": recently gained acceptance

- **World Organization for Animal Health (OIE) Code:**
  - **recognized a new category "FMD-free country/zone where vaccination is practised".**
  - **Reduced the waiting periods to recover the status when vaccination is applied during emergencies.**
- **Vaccination minimizes the dependence on large-scale culling of animals to control the disease.**

Peptide-based vaccines



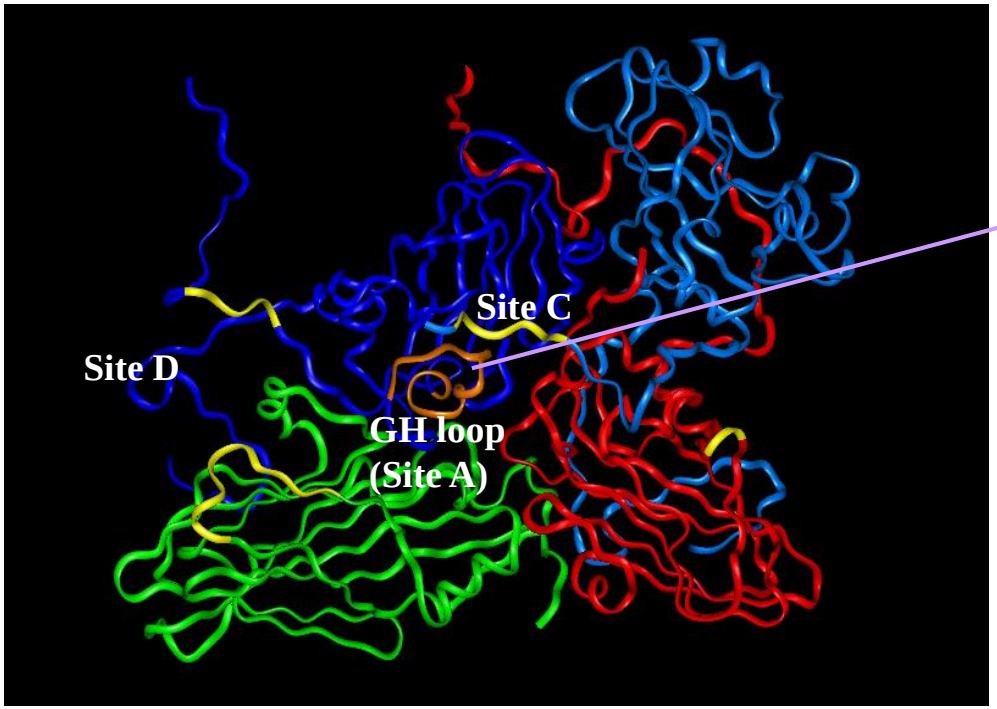
pros

- No infectious agent involved
- High structural flexibility. Easily adaptable to new, different strains
- Combination of B and T cell epitopes
- Total differentiation between infected and vaccinated animals (DIVA)
- Safe storage and transport (no cold-chain required)
- Fast, affordable large scale production

cons

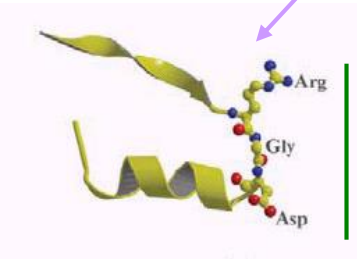
- Most epitopes are conformational (discontinuous)
- conformational epitopes difficult to reproduce by chemical means
- immunogenicity usually needs to be enhanced by: adjuvants
  multimerization

VP1 GH loop: a B cell antigenic site in FMDV capsid used for peptide vaccines design



VP1 GH loop
(site A)

A continuous B cell site that can be mimicked using synthetic peptides



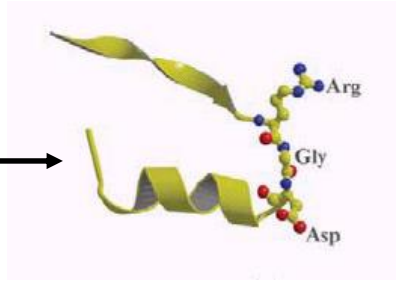
Integrin-Binding Motif (RGD)

Highly variable in sequence: serotype specific

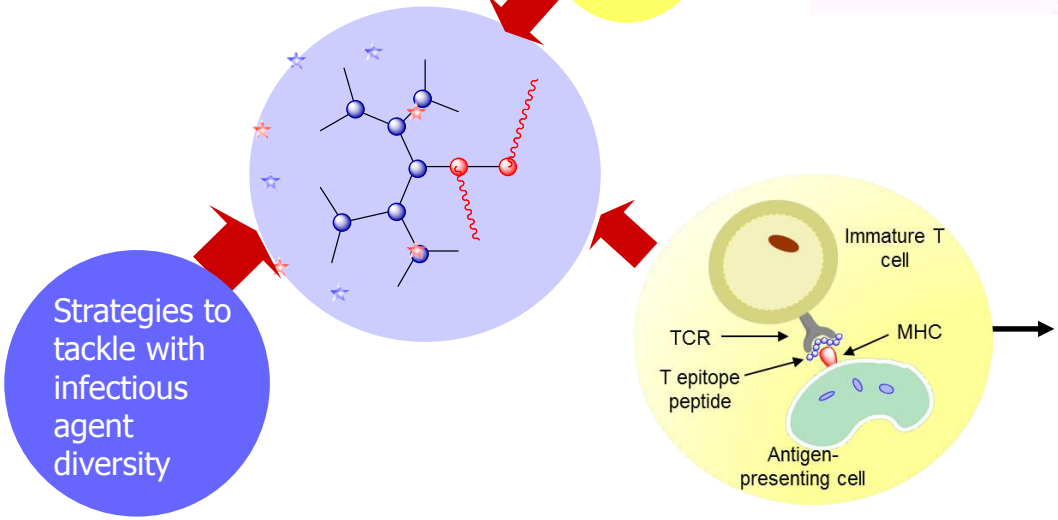
Limited immunogenicity of GH loop linear peptides

An improved synthetic peptide vaccine: pig as a model

Optimized replicas of continuous B cell epitopes



GH loop



T-cell epitopes for Helper/CTL activation and memory

T helper (CD4+) lymphocytes

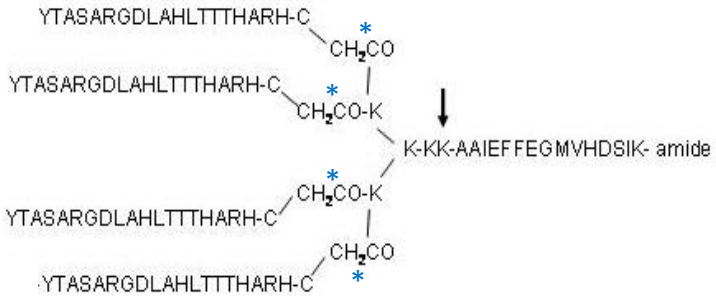
- **Sequence conservation among serotypes**
- **Widely recognised by porcine MHC class II**

3A 21-35

<u>Multivalent, dendrimeric scaffolds (dendrimers)</u>

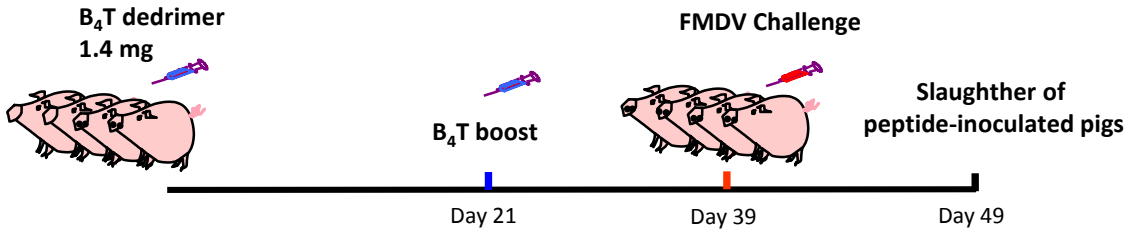
Esther Blanco et al. J. Virol (2000)
Esther Blanco et al. J. Virol (2001)
Mercedes García-Briones et al. Virology (2004)

The dendrimeric peptide of type C FMDV

Peptide	FMDV protein residues	Sequence
B	VP1 [136-154]	YTASARGDLAHLTTTHARH-amide
T	3A [21-35]	AAIEFFEGMVHDSIK-amide
B_4T^*	VP1 [136-154] 3A [21-35]	

** Thioether linkage(C-terminal)*

Arrow indicates a putative cathepsin D cleavage site



Solid, Sterilizing Protection

Neutralizing Ab

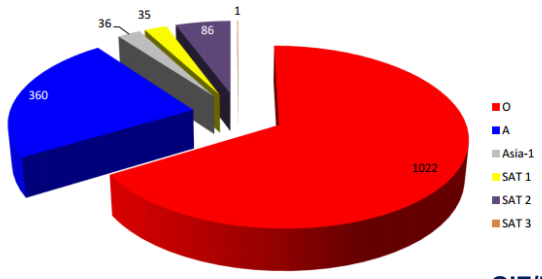
Specific IgA in serum and mucosae

FMDV-specific T cell responses

Carolina Cubillos et al. J. Virol (2008)

Peptides as vaccines against foot-and-mouth disease virus

Extension to other FMDVs epidemiologically relevant: type O (O UK 2001)



OIE/FAO FMD Lab Network 2014

Dissecting the B_4T components responsible for the immunity observed: Improved, cost- effective dendrimeric constructions

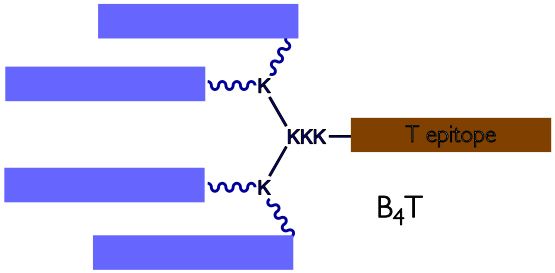
- Mouse (screening model)
- Swine

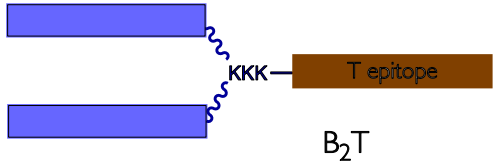
Patents P20130101063, P20130101063, 2013800289123

Is tetravalency essential?
B_4T vs. B_2T constructs (O UK 2001)

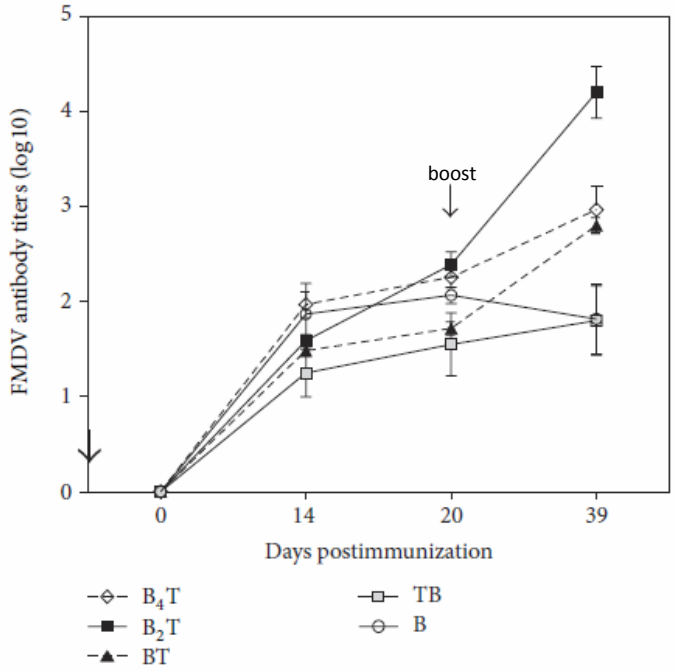
Swiss mice "outbred"
(100 μg peptide/mouse)

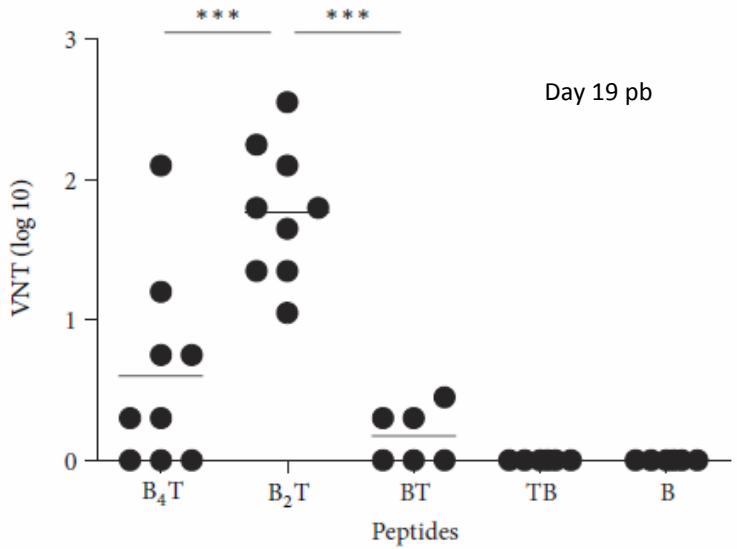
B epitope: VP1 140-160
T epitope: 3A 21-35





thioether linkage (C-terminal)

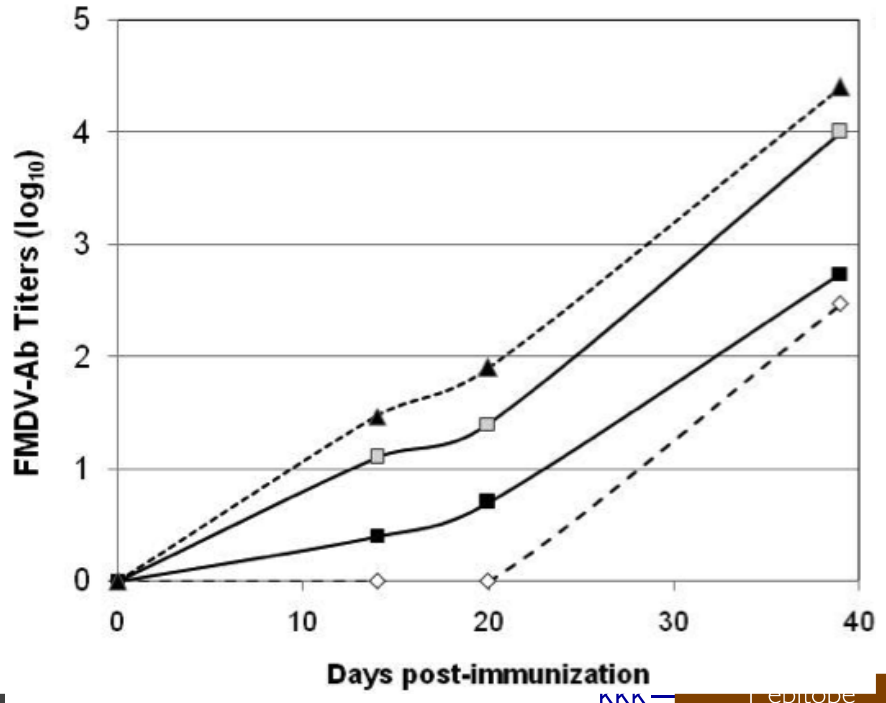


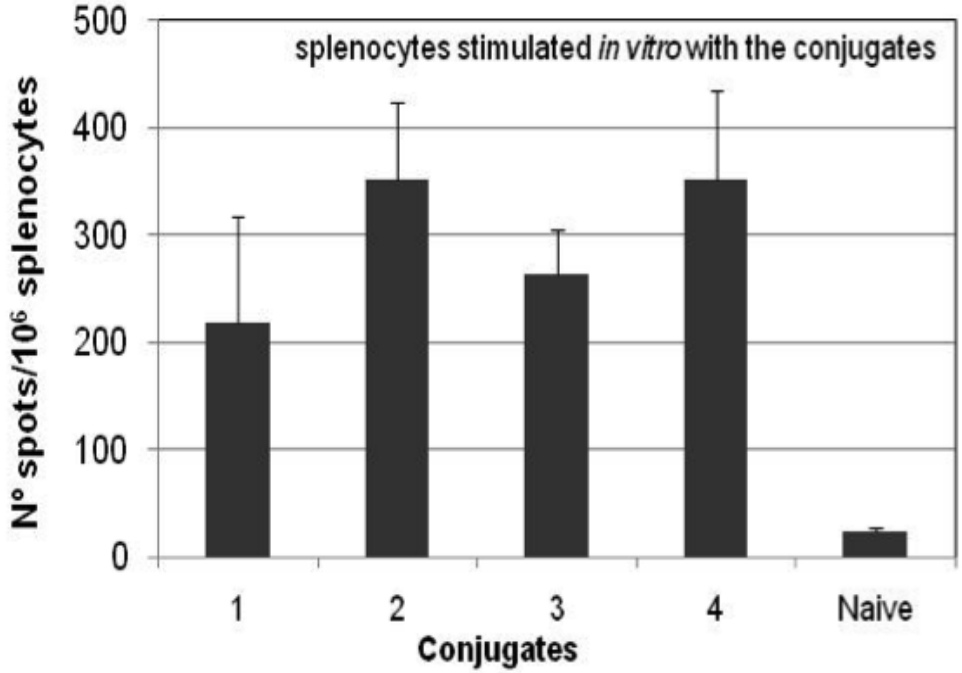


Esther Blanco et al. Clin. Dev. Immunol. (2013)

Swiss mice

2. Orientation and attachment mode also matter!





A

1 C-terminal attachment

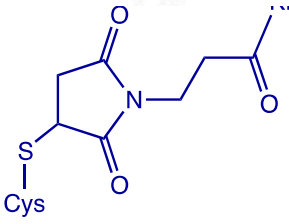
2 N-terminal attachment

3 "Reverse" thioether attachment

4 Maleimide attachment

Ease of production

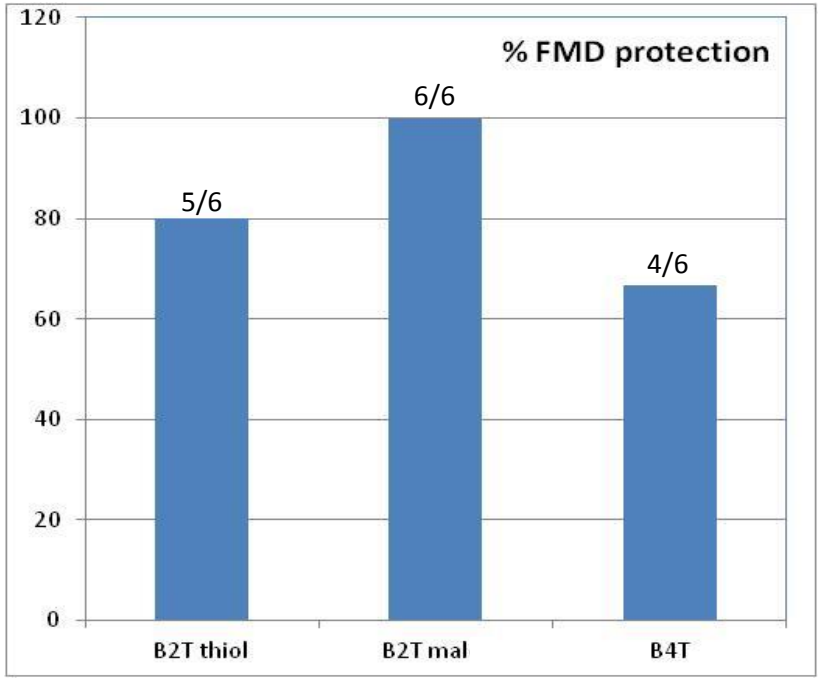
Lower cost



Maleimide attachment

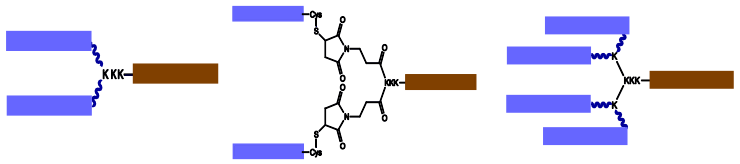
Bioconjug. Chem. (2013)

Protection conferred in pigs by type O tetra - and bivalent dendrimers (O UK 2001)



n = 6 pigs

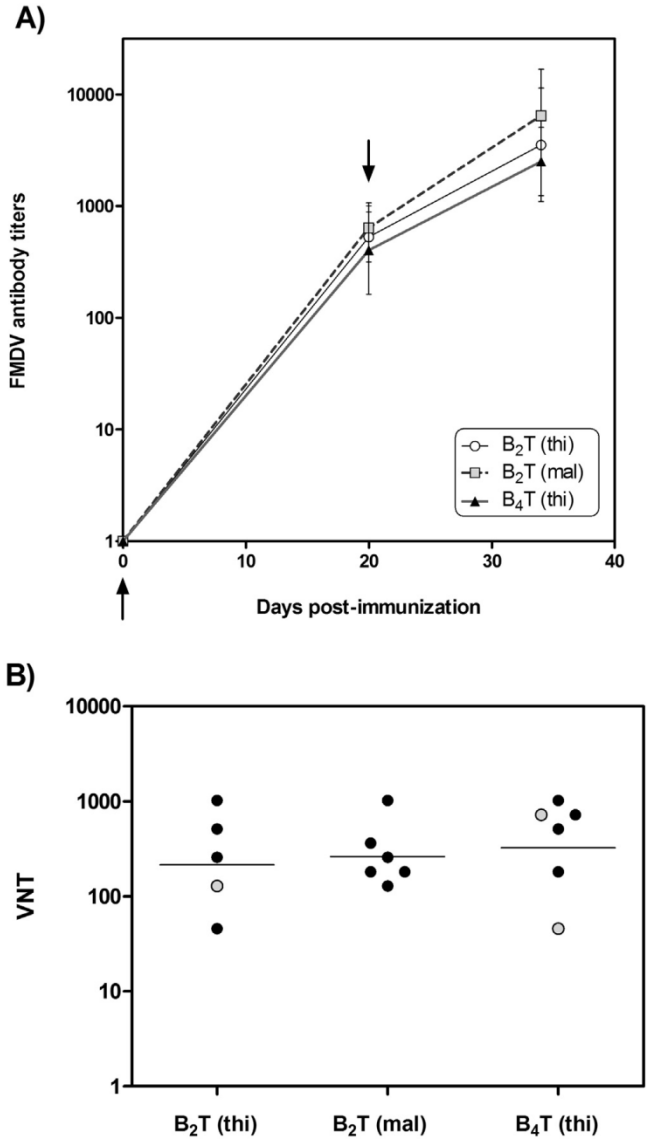
2 mg peptide/ 2 doses
Montanide ISA 50V2
(commercial oil adjuvant)



thioether linkage (C-terminal)

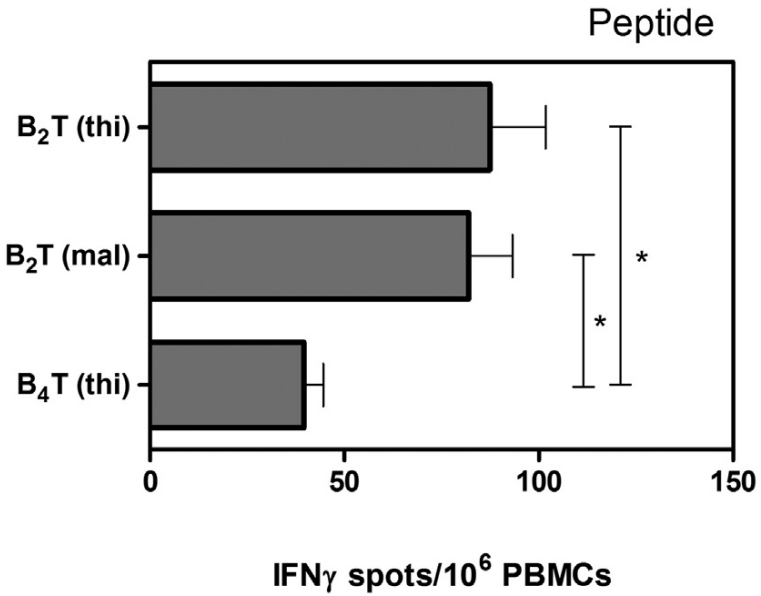
Esther Blanco et al. Antiviral Res. (2016)

Total and neutralizing Ab elicited by type O tetra - and bivalent dendrimers



Bivalent dendrimers elicit higher levels of IFNγ-producing activated T cells

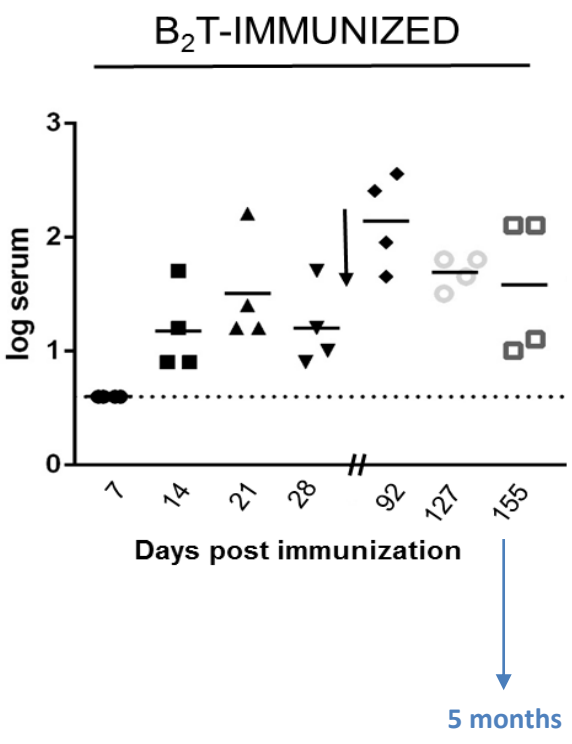
ELISPOT IFNγ



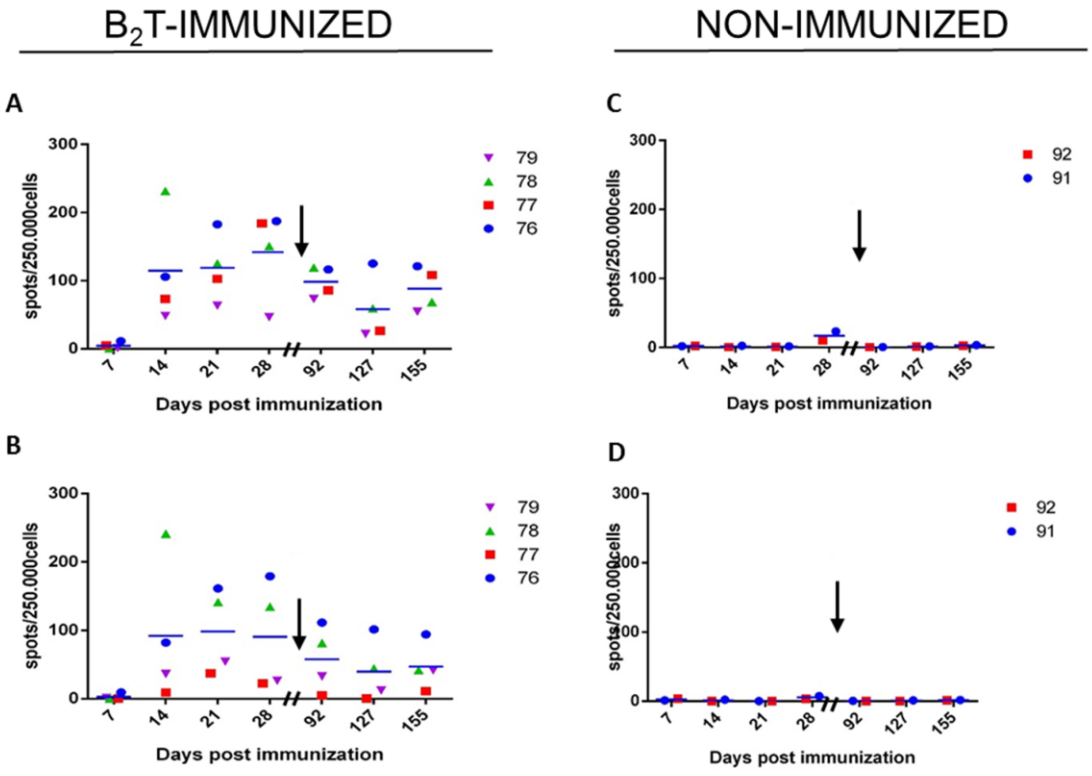
Esther Blanco et al., et al. Antiviral Res. (2016)

Bivalent dendrimer B_2T (mal) elicits long lasting neutralizing antibodies associated with the induction of IFNy-producing T cells

VNT



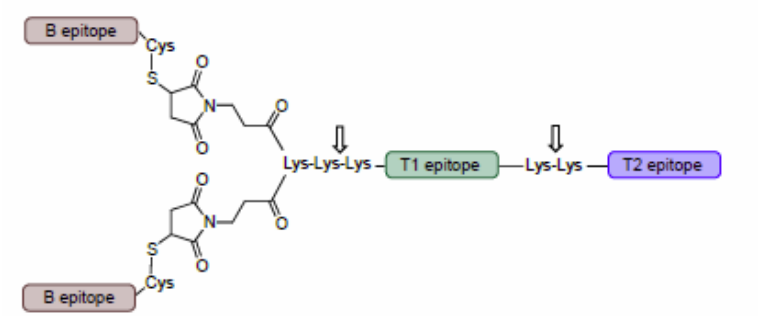
ELISPOT IFNy

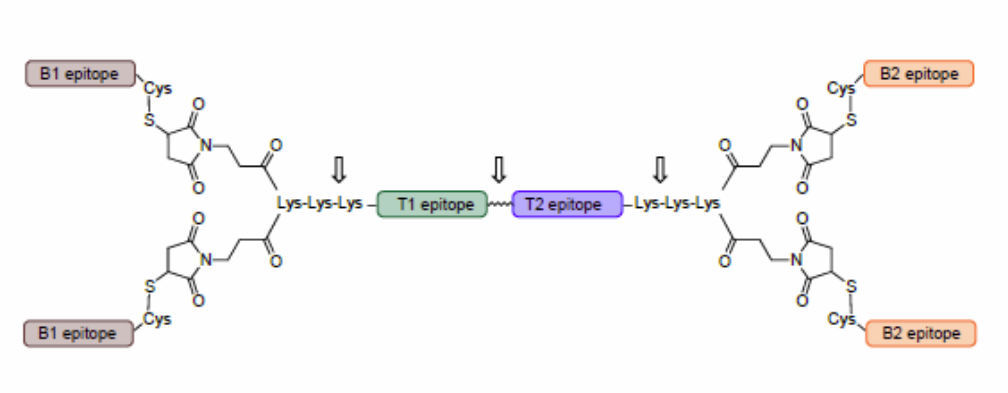


Dendrimers can afford solid FMD protection: ongoing work with this modular approach

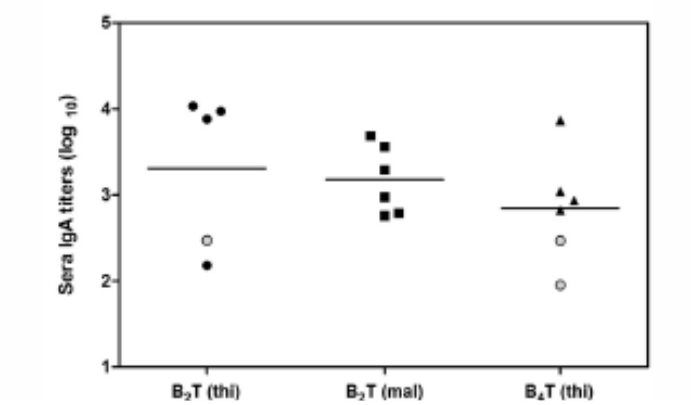
- Optimizing conjugation chemistry and inclusion of new T cell epitopes taking advantage of the versatility of the dendrimeric modular approach.

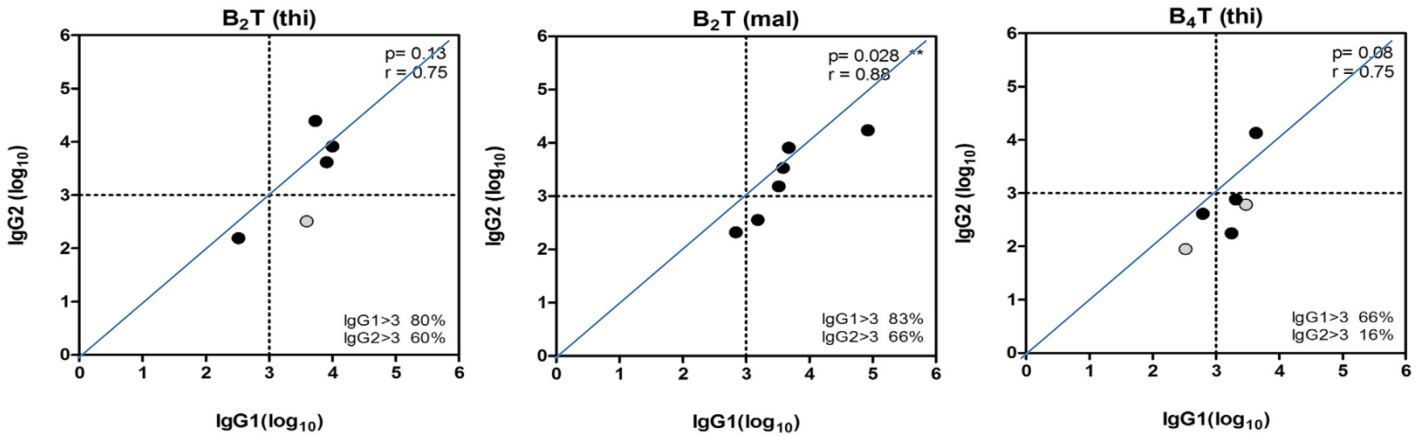
- Incorporation of different B cell peptide sequences in single dendrimers/combination of different dendrimeric molecules





Isotypes of the Ab elicited by type O tetra - and bivalent dendrimers





- This results suggests association between a Th-1 biased isotype balance (lower IgG1/IGg2 ratio) and improved protection

- IgG2 and IgA can enhance T cell responses via an FcR-mediated mechanism