Development of an HIV Vaccine Component Based on Carbohydrate Mimicry

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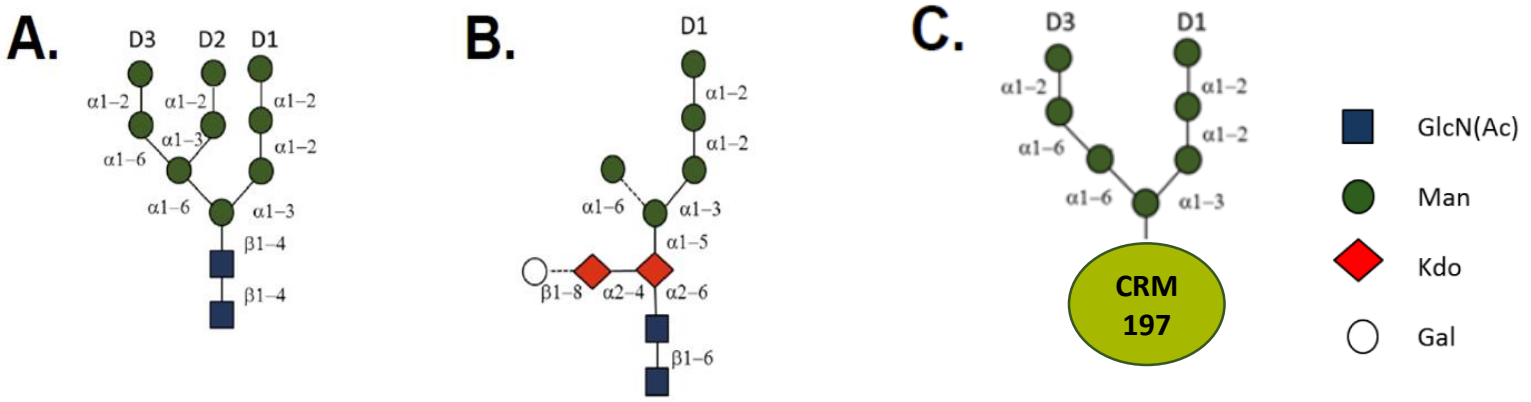
Introduction: The existence of several broadly neutralizing antibodies (bnAbs) specific for a patch of oligomannose-type glycans on the HIV spike has highlighted it as a vaccine target. We are exploring antigenic mimicry to develop an immunogen susceptible to elicit bnAbs to HIV-1 oligomannose-type glycan in transgenic mice expressing a full human antibody repertoire. Our immunogen, dubbed NIT211, is composed of a previously published oligomannose mimetic[1] derived from a bacterial oligosaccharide that we conjugated to the Cross Reactive Material CRM197. Here we report the antigenicity and the immunogenicity of this new conjugate.



Mammalian Oligomannose

Bacterial oligosaccharide

NIT211
Oligomannose mimetic conjugated to CRM197

Fig1. Schematic representation of the chemical structures of A) mammalian oligomannose, B) the bacterial oligosaccharide with the D1 arm analog of oligomannose, and C) its D3-elongated derivatives without Kdo unit conjugated to CRM197, NIT211.

Methods :

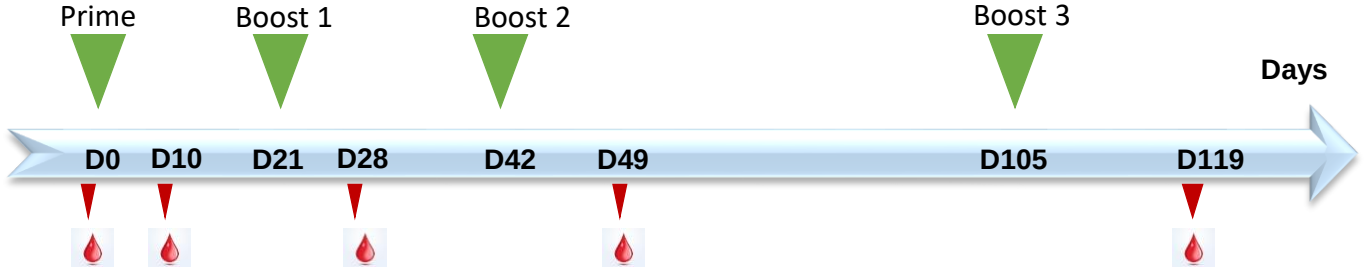
1) Antigenicity study:

- First, we report the antigenicity of NIT211, our lead CRM197 glycoconjugate, by evaluating the binding interaction of oligomannose-specific bnAbs (PGT128/130 family, BG18, BF520, PGDM21, PGDM12, VRC41, PCDN33A) and their inferred germline precursors by ELISA.
- Then, we assessed the role of the glycoside density on NIT211 antigenicity using PGT128 antibody and the Fab fragment by ELISA and competitive ELISA.
  - Coating: NIT211 (4.1 ligands) at 3.5-3.9 pmol per well
  - Secondary antibody: anti-human IgG (Fc)-HRP or anti-human CH1-biotin
  - Substrate: 3.3', 5,5'-tetramethylbenzidine (TMB), or streptavidn-HRP followed by TMB

2) Immunogenicity study:

- Then we evaluated the immnunogenicity of NIT211. To identify an adjuvant that best elicits an antibody response, we immunized human-antibody transgenic Trianni mice with NIT211 formulated with three different adjuvants: Alhydrogel (Th2-inducing), glucopyranosyl lipid adjuvant GLA-SE (Th1-inducing), or AddaVax (Th1+2-inducing).
- Finally, we assessed the binding of immune sera to the oligomannose mimetic and HIV-1 Env by ELISA. We also assessed the neutralizing activity against a panel of HIV-1 strains by Monogram PhenoSense assay.

Subcutaneous immunization at day 0 (D0), D21, D42 and D105



Serum sampling at D0, D10, D28, D49 and D119

Fig 1. Immunization schedule. Trianni mice were immunized subcutaneously with or without NIT211 (30 ug per mouse).

- Unimmunized (n=3)
- NIT211 + Alhydrogel (n=5)
- NIT211 + AddaVax (n=5)
- NIT211 + GLA-SE (n=5)

I) Antigenicity of NIT211, an oligomannose mimetic conjugated on CRM197:

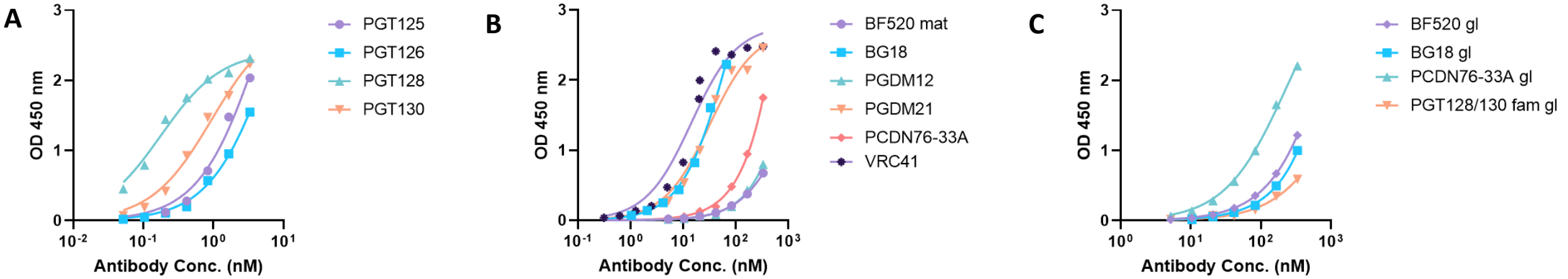


Fig 3. NIT211 is recognized by anti-oligomannose bnAbs and their germline precursors. (A) Antibodies of the PGT128/130 family binds NIT211 with an estimated Kd ranging from 6.5 to 0.2 nM. (B) Apparent Kd of VRC41, BG18, and PGDM21 range from 14 to 70 nM. PCDN73-33A, BF520 and PGDM12 1 – 5 µM. (C) Germline precursor (gl) of PCDN76-33A 200 nM, BG18 BF520 and PGT128/130 family (fam) 1 – 5 µM.

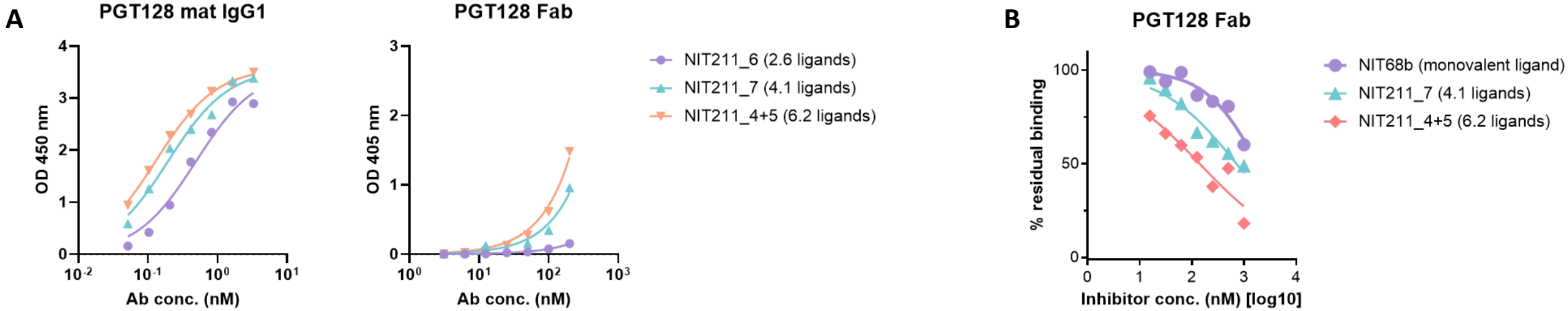


Fig 4. Oligomannose mimetics on NIT211 are bound multivalently by PGT128 IgG1 and its Fab fragment. (A) Binding avidity of PGT128 IgG1 and its Fab fragment to CRM197-conjugate increases with the ligand density. (B) Inhibition of PGT128 Fab binding to NIT211 by CRM197-conjugates increases with the ligand density. The inhibitor concentration corresponds to the concentration of oligomannose mimetic.

II) Immunogenicity of NIT211 in human transgenic mice expressing a full human antibody repertoire (Trianni mice):

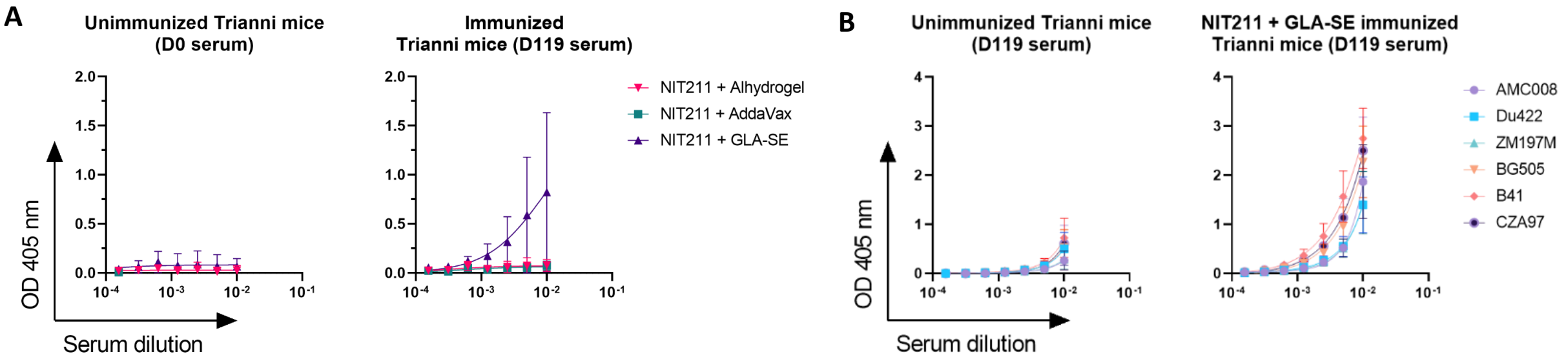


Fig 5. Subcutaneous immunizations of Trianni mice with NIT211 and GLA-SE elicit an antibody response to the oligomannose mimetic and to HIV-1 Env trimers. (A) Binding of total IgG antibody in pre-immune (at D0) and immune sera (D119) to the oligomannose mimetic on a BSA heterologous conjugate (5 µg/ml) assessed by ELISA. (B) Binding of total IgG antibody in unimmunized and immunized mice with NIT211 + GLA-SE as adjuvant to HIV-1 SOSIP trimers. Graphs depict mean values for the five serum samples from each group.

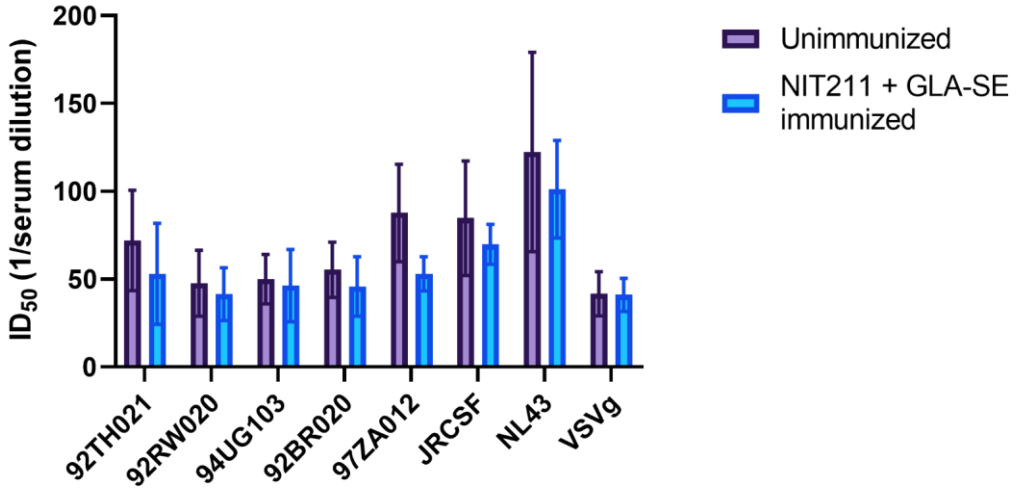


Fig 6. The antibody response elicited in Trianni mice immunized with NIT211 and GLA-SE does not neutralize HIV-1. Neutralization ID50s were measured by the Monogram PhenoSense assay using unimmunized and NIT211+GLA-SE immune serum (D119) against a panel of Tiers 1 (clade A 92RW020, and clade C 97ZA012) and Tiers 2 pseudoviruses (clade A 94UG103, clade B JRCSF and 92BR020, clade CRF01-AE 92TH021). The drug-sensitive HIV-1 strains, NL43, and the vesicular stomatitis virus, VSVg, were used as reference.

Conclusion :

1) Antigenicity of NIT211:

- Recognition of the oligomannose mimetic conjugated on CRM197 by various oligomannose bnAbs and most importantly some of their respective germline precursors shows promising immunogenic properties of NIT211.
- Oligomannose specific bnAb binding to NIT211 correlate with glycoside loading density.

2) Immunogenicity study

- NIT211 is highly immunogenic in Trianni mice, as shown by a strong antibody response elicited to the CRM197 carrier protein (data not shown).
- NIT211 elicit an antibody response to the oligomannose mimetic when formulated with Glucopyranosyl lipid adjuvant (GLA-SE), a TLR4 agonist and Th1 inducer but not with Alhydrogel (Alum-like adjuvant) or Addavax (MF59-like adjuvant).
- Antibodies elicited by NIT211 + GLA-SE immunization binds HIV-1 Env but are not neutralizing.
- It was suggested that oligomannose-conjugate could be sensitive to mannosidase trimming in serum[2]. We recently showed that NIT211 could also be sensitive to serum mannosidase trimming *in vitro* and *in vivo*[3]. Mannosidase trimming could lessen the likelihood of eliciting antibodies with capacity to bind full-sized oligomannose, which typifies the binding mode of existing bnAbs to the oligomannose patch on HIV-1.

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References :

1. Pantophlet R*, et al.* Nat Commun. 17;8(1):1601, (2017).
2. Nguyen, D. N. *et al.* ACS Central Science. 5, 237–249, (2019).
3. Bruxelle J-F., et al. bioRxiv 2020.02.24.962233

I have no conflict of interest