# Identification of bNAb-Initiating HIV-1 Envelopes to Inform the Design of V3-Glycan-Specific Germline Targeting Immunogens.

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## Abstract

Broadly neutralising antibodies (bNAbs) are able to neutralise diverse HIV-1 strains and are therefore important to elicit by an effective HIV-1 vaccine. bNAbs that target the V3-glycan epitope on the HIV-1 envelope (Env) are among the most common and broad bNAbs elicited during infection, and predominantly use a common IGHV4 germline gene, making this a particularly promising vaccine target. This study aimed to identify Envs which triggered broad V3-glycan-specific responses (bNAb-initiating Envs) in HIV-1 infected donors, which could be used to inform the design of immunogens. Putative bNAb-initiating Envs were identified through PacBio deep sequencing of the *env* genes for two HIV-infected participants (CAP255 and CAP314) who developed V3-glycan bNAbs. The timepoints sequenced were selected based on the emergence of the V3-glycan bNAb precursor, the unmutated common ancestor (UCA). The CAP255 and CAP314 bNAb-initiating Envs were selected based on the absence of two key glycans and the shortening of the V1 loop, respectively, both features are known enhance accessibility for V3-glycan binding. These two Envs were used as the basis for the design of 11 Env trimer immunogens, each incorporating various mutations to increase stability and affinity for V3-glycan bNAbs. We expressed the trimers in mammalian cells and purified them using affinity and size exclusion chromatography. Although CAP314 did not express stable trimers, even with the introduction of additional stabilising mutations, CAP255-derived trimers were more successful, and the CAP255.GT0 and CAP255.GT2 immunogens produced the highest yields. Binding and functional assays showed CAP255.GT2 to be the most stable and well-formed trimer. No UCA interaction were observed with the CAP255.GT2 trimer but early CAP255 intermediate antibodies neutralised the CAP255.GT2 pseudovirus. This study resulted in the design of a successful subtype C trimer that can be used as a basis for future immunisation studies.

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