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Germline Targeting and Antibody Maturation in HIV-1 Vaccine Studies in Human-VRC01 Class

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Abstract. While germline-targeting vaccines have activated human precursors of broadly neutralizing antibodies, the steps from precursor activation to long-lived serum neutralization are incomplete. The aim of this narrative synthesis is to review the studies of VRC01-class HIV-1 vaccines and determine which maturation steps are supported and which functional transitions are not. Primary clinical reports, structural analysis reports and selected mechanistic reports were identified from a focused search of PubMed and through reference tracing. Immunisation sequence, antibody sampling, envelope glycosylation, neutralisation assay and follow up were used for comparison of evidence. Preclinical trials were successful in demonstrating precursor recruitment and early maturation in humans. Heterologous boosting generated antibodies with desired mutations, but this was most prominent for certain antibody clones and serum neutralization was not detected under the conditions tested. Germline-targeting trimer vaccination resulted in some weakly active monoclonal antibodies against fully glycosylated virus, but failed to achieve broad serum neutralization upon repeated vaccination. In macaques, serum breadth was seen with extended sequential boosting, but does not prove the same in humans. The two studies together indicate two future hurdles: improving the ability to induce recognition of a variety of viral envelopes from engineered antigen, and maximizing the magnitude and persistence of circulating neutralizing antibody responses from mature memory B cells. This distinction connects future vaccine assessments with matching glycan-sensitive assays, antibody sample representative sampling and serum assessments over time. Further evaluation for human protective efficacy, response consistency and tolerability to complete regimens is required.

Keywords: HIV-1 vaccines, germline targeting, sequential immunization, VRC01-class antibodies, antibody maturation

1. Introduction

Generating broadly neutralizing antibodies (bnAbs) is a key goal of HIV-1 prevention studies. Passive administration of VRC01 was shown to reduce acquisition of antibody-sensitive isolates, but did not significantly reduce acquisition of HIV-1 overall in the Antibody Mediated Prevention trials [1]. This discovery provides further evidence for the clinical need to try to induce antibodies of appropriate breadth and concentration, but not proof that vaccination can do this. An early barrier is

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overcome by creating immunogens to bind rare naive B cells with receptors that are similar to VRC01 class precursors. Initial protein-engineering efforts yielded outer-domain immunogens of gp120 that could bind VRC01-class receptors, but the wild-type gp120 did not have detectable binding ability to those receptors [2]. Later clinical studies revealed that target B-cell populations were recruited in human volunteers [3].

Germline-targeted vaccination triggers maturation of VRC01-class antibodies and phase 1 human trials have started to map this process. IAVI G001 was able to show precursor recruitment, somatic hypermutation and improved affinity but not VRC01-class serum neutralization [3]. Structural characterization of G001-derived antibodies showed VRC01-like epitope recognition and partial tolerance to N276 on engineered outer domains but not equivalent recognition of intact viral envelopes [4]. IAVI G002 evaluated heterologous boosting with the aim of switching the antigen after priming to target further maturation. Increased key heavy-chain residues in the boosted immunizations and the selected mAbs neutralized the N276-deficeient viruses, while the serum assays still showed negative results [5]. IAVI C101 was immunized multiple times with the GT1.1 trimer and some of these monoclonal antibodies demonstrated weak neutralization against the fully glycosylated BG505 but failed to induce broad serum neutralization [6]. The serum breadth was achieved in complementary macaque studies by sequential immunization for a long period targeting BG18, which is a V3-glycan-directed antibody class. Differences in antibody target, species and regimen limit extrapolation to human VRC01-class responses [7]. Taken as a whole, these results indicate why it is necessary to assess separately the recruitment of precursors, the monoclonality of the cells and the circulating neutralization.

The question remains as to what will drive partially matured VRC01-class lineages past these intermediate results. This narrative synthesis is structured around two questions: (I) Can recognition be expanded from engineered immunogens to a variety of naturally glycosylated viral envelopes? (II) Do favorable memory B cell responses lead to sustained circulating neutralization? The primary clinical reports, associated structural analysis and selected mechanistic studies were qualitatively compared following a focused search of PubMed and reference tracing. Human trials, animal studies with mice and macaques are separated to differentiate clinical results and supporting animal studies. Antibody sampling, glycan status, assay conditions, and follow-up and tolerability in the context of interpreting maturation are compared. It is intended to serve as an informative assay for use in later evaluation of vaccine and not to be interpreted as proof of protection serum immunity.

2. Basis for mechanism of germline targeting and sequential immunization

2.1. Boosting to include priming and selection

A germ-line targeting prime sets up B-cell lineages capable of responding to subsequent immunogens. Homologous boosting means that the same antigen is boosted while heterologous boosting means that the antigen is changed and other properties are selected. Initial sequential-immunization studies showed that in transgenic mice, specific boosters could be used to direct partially matured VRC01-class antibodies to neutralize [8]. It's important to differentiate between antigen and delivery: G002 made a switch from eOD-GT8 to core-g28v2, but still retained an mRNA-lipid nanoparticle platform [5].

This progression is aided by the Germinal Centers where repeated mutation and selection can occur. In mice, antigen capture and presentation has been correlated with the follicular helper T cells and their downstream effects on B-cell division and hypermutation [9]. A booster thus must involve existing precursors and must exert selection related to the desired viral target. The increase in

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binding to the booster does not demonstrate that the mutations generated improve their ability to recognise functional envelope trimers.

2.2. Glycan accommodation and functional assessment glycan accommodation and functional assessment

The N276 glycan, which is close to the CD4-binding site, is a major steric hindrance for VRC01-class antibodies. Cottrell et al. demonstrated that antibodies from eOD-GT8 priming and core-g28v2 boosting were able to induce key mutations and recognition of near-native trimers lacking N276 in humanized mice, which neutralized the corresponding glycan-deficient pseudoviruses [10]. This provided a mechanistic rationalisation for G002, with the recognition of more heavily glycosylated envelopes to be addressed at a later stage.

Accommodation of glycan is not an easy one-sequence feature. Agrawal et al. determined that decreases in the length of the first light-chain CDR were not enough to allow for broad neutralization for the intermediate VRC01-like antibodies tested [11]. The result favors the evaluation of combinations of mutations and of the functional consequences rather than using the number of mutations or a deletion as a universal maturation end point. These mouse results could help identify mechanisms, but they do not tell what the relative importance is in humans who have been vaccinated.

Assessment is then shifted from lineage recruitment to sequence change, to antigen binding, and then to neutralization. Monoclonal assays refer to individual antibodies and serum assays consider the mixture of antibodies that circulate in the blood. Breadth and potency must be interpreted with the virus panel, glycan modifications, assay threshold and sampling time specified. All of these measurements address similar questions but are different biological outcomes [5, 6, 10].

3. Based on studies in humans and complementary animal studies

3.1. Human precursor recruitment

G001 recruited 48 adults (36 of whom received vaccine) who were randomized to eOD-GT8 60mer with AS01B or placebo. When post-immunization samples were used, VRC01-class responses were seen in 35 of 36 recipients. After the second vaccination, there was a rise in mutations and affinity within the polyclonal response. Neutralizing activity of the VRC01 class was not detected by serum testing [3]. These results highlight recruitment as a goal accomplished by this priming regimen, and initial maturation.

A structural analysis of the derived antibodies from G001 provided further non-independent evidence. Lin et al. solved five complexes in which the VRC01 bound to its target epitope in a similar fashion. Of the 230 antibodies, 201 were able to recognize eOD-GT8 even in the presence of N276, but with a lower affinity [4]. This shows partial glycan accommodation on an engineered outer domain, but not a full viral envelope. The structural report is an extension of interpretation of G001 with no new vaccinated cohort.

3.2. Human heterologous boosting

G002 was an open label, randomized, phase 1 study of 60 adult subjects. It contrasted eOD-GT8 followed by core-g28v2 with that of repeated eOD-GT8 and core-g28v2 alone. The two eOD-GT8 administrations in a single arm were in the accompanying G003 trial, which involved 18 people in

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Rwanda and South Africa and evaluated priming, not heterologous boosting [5]. Heterologous boosting raised the numbers of total mutations at week 16 compared with homologous boosting in G002. As the total levels merged by week 24, enrichment for key VRC01-class heavy-chain residues was seen for the heterologous sequence at both time points. Thus, Mutation identity added to the amount of mutation. Additionally, affinity measurements relied on sampling: the median core-g28v2 dissociation constant at week 16 was 0.6 nM for antibodies that were selected for a desirable heavy-chain mutation and 1167 nM for those boosted without a mutation. For randomly sampled antibodies, there was no appreciable difference in the median affinity [5]. Selected set included two receptors (per participant and time point) with the most key VRC01-class heavy-chain mutations, and random set included two receptors from a subset of participants. The contrast is used to describe the variation in different components of the response distribution and not contradictory measurements of the same antibody sample.

14 pseudoviruses were created from 5 isolates and tested against the selected antibodies. Significant activity was observed towards N276-omitted viruses, but not against N276-containing viruses tested. No neutralization was seen at 1:10 dilution of sera at week 16 and 24 for viruses susceptible to the antibodies isolated. This study therefore relates directed maturation with partial monoclonal function, but not circulating neutralization. There were no serious adverse events, but 11 of 60 experienced skin adverse events that were considered to be possibly or probably vaccine-related [5].

3.3. Trimer priming and human neutralization

In a randomized double blind placebo controlled study, C101 assessed three GT1.1 trimer immunizations in 47 adults with 39 randomized to receive vaccination with AS01B. Unlike G002 it was repeated administration of the same immunogen. A subpopulation of the isolated VRC01 class mAbs neutralized fully glycosylated BG505. It shows only weak activity against T332N with a reported median half maximal inhibitory concentration of 133 μg/mL. Activity against further wild-type pseudoviruses was observed, especially those viruses which naturally do not contain N276 [6].

Engineered GT1.1 was still detectable in the serum of all but one of those who responded to the third vaccination at least 6 months after the last shot [6]. The persistence result is for the engineered target and not for durability of breadth against circulating HIV-1. C101 thus provides human evidence of monoclonal recognition of more native envelope contexts and it is a serum endpoint different from broad neutralization.

3.4. Supporting data from macaques

24 rhesus macaques were included in a BG18-directed study, 18 of which were boosted in a heterologous manner. Eight animals were chosen for favourable monoclonal responses and after a seventh boost sera were tested for breadth against BG18 on a 63-pseudovirus panel on BG18-sensitive cells. There was relatively little change in activity from 2 to 6 weeks [7]. Limited Generalization due to Selection and Panel Composition. The study provides proof of serum breadth following a much longer sequence, but due to the different antibody target, species and schedule, it cannot be used as a validation of a human VRC01 class regimen.

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4. Integrated interpretation and research priorities are established by the Unit

4.1. Including viral envelope contexts in the realm of recognition

Glycan tolerance depends on the context of the associated antigen, as is illustrated by reading together G001, G002 and C101. G001-derived antibodies were able to neutralize outer domains restored with N276, while G002 antibodies were only able to neutralize the N276 deficient viruses tested, and some C101 antibodies weakly neutralized fully glycosylated BG505 [3-6]. The results are consistent: attachment of a glycan to an engineered domain does not mimic the constraints imposed by the complete trimer. This distinction would be masked by binary classification of N276-tolerant or N276-intolerant.

Wild-type" must be used with care as well. There are some naturally occurring viruses lacking N276, as do susceptible pseudoviruses in C101 [6]. Biological activity against these viruses is biologically relevant, but does not prove that the antibody has surmounted an N276 associated barrier. Weak activity against one glycan-containing isolate on the other hand, cannot provide breadth. It is therefore more informative to describe the origin of the viral sequence, as well as the glycan sites, than simply to group all the results from the wild-type.

It is therefore helpful to make a comparison between each maturation claim and the most demanding antigen/virus actually recognized. Increased numbers of mutations, increased booster binding and native-virus neutralization should not be considered equivalent accomplishments. The Agrawal experiments also warn that what looks like a good structural change can lead to better binding but not breadth [11]. Subsequent boosters should be tested with the same glycan-containing and glycan-defective viruses as well as a prespecified panel covering all the diversity of viruses. This would separate progress towards accommodating a specific obstacle from progress towards overall antiviral coverage.

4.2. Relating memory B cells with serum function

G002 shows that there is one more transition that cannot be resolved by antigen recognition alone. However, the functional activity of the selected memory-derived antibodies was found in the functional serum assays, which were negative [5]. The two assays analyze different amounts: a purified monoclonal antibody can be tested amount the known concentration while the serum activity is dependent on the quantity, strength and type of antibodies secreted. To find a promising clone is not enough to assure a sufficient level of circulation.

There is no specific evidence to suggest that this difference is attributable to one particular factor. It cannot conclude which of the two mechanisms—more maturation of the antibodies and/or more secretion of appropriate antibodies—will be the main drivers of subsequent improvement. These possibilities could be distinguished by paired longitudinal measurements of lineage development, antibody-secreting responses and serum neutralization. In intermediate boosts, circulating-function persistence is also an informative endpoint for the assessment of a completed regimen; memory-lineage advancement can continue to be an endpoint for this type of boost. This latter endpoint in the other antibody class is illustrated by the macaque serum results [7].

4.3. Comparability and practical development of regimen

The best comparisons are within trials that have an explicit control. Due to the differences between the antigen structure, delivery, immunization number and analytical sampling between G002 and

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C101, the results of these monoclonals do not indicate which is better. The selected-versus-random affinity results in G002 also indicate the need to report the affinity of an outstanding subset separately from the affinity of the entire responding repertoire [5, 6]. Multiple clones from a participant improve molecular resolution – but are not additional participants who have been vaccinated.

There is a difference in meaning between binding and neutralization measurements. Dissociation constant (binding affinity) is measured at the assay conditions, inhibitory concentration (IC) is measured as the effect on viral entry. They cannot be measured against a standard measure of vaccine performance. Even in the neutralization assays themselves, the number of different denominators for a proportion of selected antibodies active against one virus, and for a proportion of a virus panel neutralized by one serum sample are different [5, 6].

Future research needs to maintain sample size of participants, describe the selection process of antibodies and evaluate the results for recipients. Host variation should also be considered: the alleles IGHV1-2 were associated to C101 associated VRC01-class responses. G003 expanded the spatial data for priming, and did not examine a full sequential regimen [5]. These observations can help assess comprehensive schedules in populations and can help distinguish genetic explanations from causal ones.

The pathway to practical development should balance immune progression, tolerability and dose burden. G002 skin events warrant safety assessment in the prospective setting and controlled dose study; no cross-trial differences can distinguish a general mRNA-platform effect [5]. The long macaque program provides an incentive for assessing the efficacy of fewer treatments [7]. Such studies should measure gains in the light of the burden of administration, and should not take it for granted that the more doses that are administered, the better the eventual response will be.

The results of the additional-prime comparison in G002 show why the dose number is an incomplete design variable. The additional eOD-GT8 immunization resulted in a boost to serum binding to the subsequent core immunization, and the later core boost resulted in modest increases in a few measures of B-cell response. The investigators thought this was due to antibody interference [5]. It offers an explanation, but the comparison did not take any action to independently manipulate the antibody feedback while keeping the schedule of vaccinations the same. Therefore, in sequential regimens, booster timing and pre-existing antibody levels should be considered in concert, and it does not necessarily mean that in all sequential regimens the dominant mechanism is as described here.

4.4. The synthesis can only be carried out at a low rate

This targeted selection of narratives may not include pertinent studies and instead may prioritize programs that have been well characterized. It is not a comprehensive evaluation of all HIV-1 vaccine targets and there was no formal risk of bias score. The human data come from small early phase studies, heterogeneous immunogens, antibody analyses limited to endpoints and short periods of follow-up for the functional endpoints. The outcomes of any negative assays are reported as such and unevaluated outcomes are unknown. The proposed comparison is not a validated predictor of vaccine efficacy, but rather a method of organizing evidence. However, intermediate endpoints mentioned here do not address the question of protection against acquisition [1, 3, 5-7].

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5. Conclusion

Human VRC01 class vaccine studies demonstrate precursor recruitment and early maturation, with some monoclonal activity against more natural viral targets upon multiple trimer immunizations and a directed change following a first heterologous vaccine boost. They jointly contribute to their effects in two transitions: the extension of recognition to non-engineered antigens and the conversion of beneficial memory responses to sustained serum neutralization. One transition does not provide evidence for another. This separation is important to determine what further immunizations should show, and what metrics are required for gauging progress. It is therefore important that a vaccine for this next stage is evaluated in terms of the functional gap that it closes — and that improvements are shown among vaccine recipients and against well-defined viral targets. Small cohorts, literature selection to suit comparisons, antibody sampling and heterogeneous assays are still limiting. Future testing should be combined with representative lineage analysis and glycan-sensitive viral panels with longitudinal serum analyses and evaluation of complete-regimen tolerability. The ability to target the germ line has thus provided a plausible basis for further vaccine development, and wide and persistent human serum neutralization and HIV-1 acquisition resistance have yet to be demonstrated for the regimens evaluated.

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