First clinical study of germline-targeting strategy: One step closer to a successful bnAb-based HIV vaccine

Caijun Sun,[1,3,*] Teng Zuo,[2,*] and Ziyu Wen[1]

[1]School of Public Health (Shenzhen), Shenzhen Campus of Sun Yat-sen University, Shenzhen 518107, China

[2]Laboratory of Immunoengineering, Institute of Health and Medical Technology, Hefei Institutes of Physical Science, Chinese Academy of Sciences, Hefei 230031, China

[3]Key Laboratory of Tropical Disease Control (Sun Yat-sen University), Ministry of Education, Guangzhou 511400, China

*Correspondence: suncaijun@mail.sysu.edu.cn (C.S.); zuot@cmpt.ac.cn (T.Z.)

Received: December 19, 2022; Accepted: January 1, 2023; Published Online: January 2, 2023; https://doi.org/10.1016/j.xinn.2023.100374

Citation: Sun C., Zuo T., and Wen Z. (2023). First clinical study of germline-targeting strategy: One step closer to a successful bnAb-based HIV vaccine. The Innovation **4(1)**, 100374.

The epidemic of human immunodeficiency virus type 1 (HIV-1) remains a major threat to global public health, with approximately 38 million people living with HIV-1 worldwide. However, a prophylactic vaccine against HIV-1 is not available yet. In a recent study,[1] Leggat and colleagues reported the first-in-human test of a germline-targeting HIV-1 vaccine candidate, eOD–GT8 60-mer adjuvanted with $AS01_B$. Encouragingly, this strategy effectively activated the B cell precursors (germlines) of VRC01-class broadly neutralizing antibodies (bnAbs) in 97% (35 of 36) of vaccine recipients. Moreover, these activated precursors shared properties with mature bnAbs and acquired substantial somatic hypermutation (SHM) and affinity maturation. This study not only establishes the clinical proof

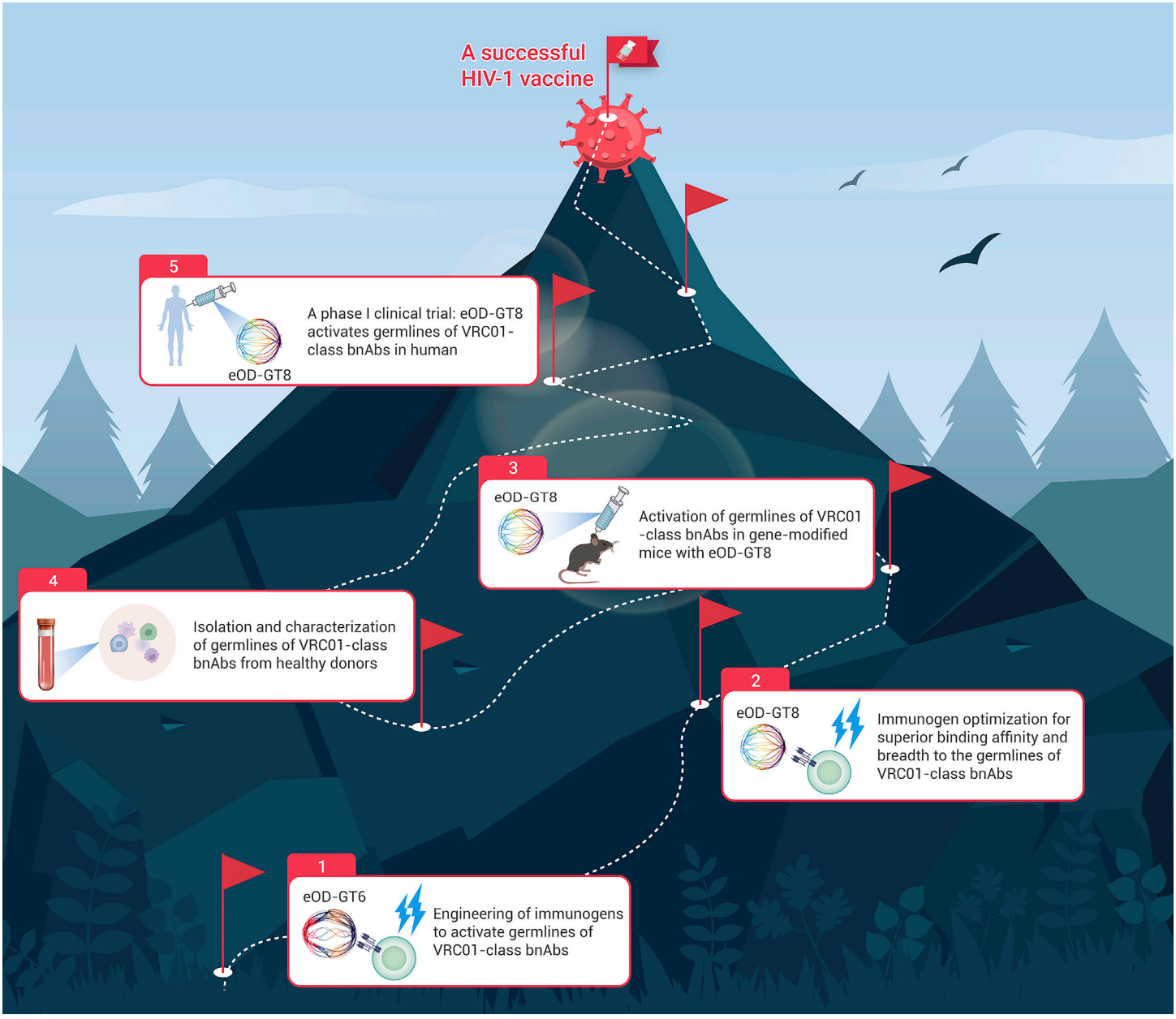

Figure 1. The challenging road leading to a successful HIV vaccine Scientists are making efforts to a successful HIV-1 vaccine in various ways, although the road ahead is endless and far away. Encouragingly, a recent study[1] reported the first-in-human test of a germline-targeting HIV-1 vaccine candidate, eOD–GT8, which is an essential step toward an effective HIV-1 vaccine aiming to induce bnAbs. Let us pay our respects to these brave climbers to the peak of science. bhAbs, broadly neutralizing antibodies

of concept for a germline-targeting strategy but also accomplishes one essential step toward an effective HIV-1 vaccine aiming to induce bnAbs.

Despite efforts over the past 40 years, an HIV-1 vaccine is still far from a success, owing at least to the exceptional genetic diversity of HIV-1. More critically, an ideal HIV-1 vaccine has to induce sterilizing immunity to completely block the entry of any HIV-1 virion into host cells, due to the ability of HIV-1 to integrate into the host genome and form long-lived viral reservoirs within 3 days of initial infection. Therefore, the mission of the HIV-1 vaccine is indeed extraordinary.

With advancements in antibody cloning techniques, a new generation of bnAbs against HIV-1 has been isolated since 2009. These bnAbs can neutralize 50%–100% of HIV-1 strains, and passive bnAb infusion can protect macaques from simian HIV (SHIV) challenge. Although the recent trials of VRC01 infusion did not show overall protection from HIV-1 variants, it did protect against HIV-1 isolates sensitive to VRC01. The discovery of HIV-1 bnAbs and validation of their protection efficacy lead to the consensus that an effective HIV-1 vaccine should elicit bnAbs.[2]

So far, it is a daunting mission to effectively induce the production of long-lasting bnAbs in humans, owing to the following roadblocks. (1) As the sole target for bnAbs, HIV-1 envelope (Env) glycoprotein is heavily glycosylated, and the epitopes of bnAbs are subdominant compared with dominant non-neutralizing epitopes. (2) HIV-1 bnAbs have unusual features including restricted germline usage, polyreactivity, and autoreactivity, and thus B cell precursors of bnAbs may be extremely rare. (3) HIV-1 bnAbs usually have high levels of SHM and intrinsically improbable mutations, and different configurations of Evns are needed to activate the B cell precursors of bnAbs.[2]

With longitudinal analysis of the "arms race" between the evolving virus and the B cell lineages of HIV bnAbs, a co-evolution model is proposed. Envs from transmitted/founder virus can activate the rare B cell precursors of bnAbs, which then undergo expansion and SHM. Subsequently, Envs from the variants virus bind to the B cell lineages with desired mutations for further diversification, which are then selected by new variants. After repeated selections, B cell lineages gradually accumulate high levels of SHM, including these rare mutations, and eventually mature to produce bnAbs. Following this model, a sequential vaccination with different immunogens is essential to induce HIV bnAbs. Currently, some strategies are extensively exploited, including (1) a germline-targeting strategy, in which a priming immunogen is engineered to activate the B cell precursors of bnAbs, and then a series of boost immunogens are designed to guide the evolution of B cell lineages; (2) a B cell lineage vaccine strategy, in which a series of immunogens are selected from the longitudinal studies of co-evolution to recapitulate the development of bnAbs; and (3) structure-guided immunogen design, in which a series of immunogens are rationally designed to converge antibody responses to one or more particular structural epitopes on Envs. Among them, the germline-targeting strategy is designed to activate the rare B cell precursors of bnAbs to recognize the CD4-binding site (CD4bs), the V3 glycan, and the V2 apex.

Impressively, Professor William R. Schief has proposed the concept of germline-targeting strategy and then validated it step by step in the last decade (Figure 1). The priming of "right" B cells (precursors) is the initial key step of bnAb production, but most Envs usually have no detectable affinity for the germlines of bnAbs. To address this issue, in the first step,[3] they engineered a germline-targeting gp120 outer domain, eOD–GT6, that is capable of binding and activating the B cells germlines of multiple VRC01-class bnAbs via computation-guided *in vitro* screening. In the second step,[4] they further developed eOD–GT8 using deep mutational scanning and multi-targeted optimization. Compared with eOD–GT6, eOD–GT8 demonstrated superior binding affinity and breadth to the germlines of VRC01-class bnAbs, indicating that eOD–GT8 has potential as a priming immunogen. Then, the third step was to test whether eOD–GT8 could activate the B cell precursors of VRC01-class bnAbs *in vivo*. To this end, eOD–GT8 was used to immunize mice expressing knockin germlines of VRC01-class bnAbs or mice transgenic for human immunoglobulin loci.[5] Characterization of B cell responses revealed that eOD–GT8 efficiently activated the B cell precursors of VRC01-class bnAbs in mice, and these B cell precursors could undergo clonal expansion and SHM and differentiate into memory B cells. Considering the gaps between mice and humans, the fourth step was to determine whether eOD–GT8 could bind to human B cell precursors of VRC01-class bnAbs and then activate a sufficient immune response. To address these questions, they used eOD–GT8 to successfully isolate naive B cell precursors of VRC01-class bnAbs from HIV-uninfected donors. These findings further support eOD–GT8 as a priming immunogen.[4]

Now, this strategy has moved to the critical fifth step**.** A randomized, double-blind, placebo-controlled phase I clinical trial (IAVI G001) was conducted to evaluate the safety and immunogenicity of eOD–GT8 60-mer adjuvanted with $AS01_B$ in humans.[1] The eOD–GT8 60-mer is a self-assembling nanoparticle presenting 60 copies of eOD–GT8, and $AS01_B$ is an adjuvant system composed of two immune enhancers combined in a liposomal formulation. In this study, 48 participants received two administrations of placebo (n = 12), low-dose vaccine (n = 18), or high-dose vaccine (n = 18) at 8-week intervals. This regiment had a favorable safety and tolerability profile. Consistent with pre-clinical experiments, serological analysis showed that all vaccine recipients, but no placebo recipients, produced immunoglobulin G (IgG) antibodies targeting the CD4bs epitope, though no neutralizing activity was found. Further analysis showed that this regiment induced substantial frequencies of CD4bs-specific IgG memory B cells in peripheral blood and germinal center B cells in lymph nodes. Then, CD4bs-specific single B cells were sorted, and their B cell receptor (BCR) sequences were analyzed, which showed that 35 of 36 vaccine recipients produced VRC01-class IgG B cell responses. Further, BCR sequence analysis showed that the activated B cell precursors of VRC01-class bnAbs were genetically diverse, shared multiple properties with mature bnAbs, and gained substantial SHM and affinity maturation, implying that B cell responses primed by this regiment might be guided to mature toward VRC01-class bnAbs by further sequential immunization with other boosting immunogens.

Overall, this study provides persuasive human data supporting the germline-targeting strategy for the development of a bnAb-based HIV vaccine. Moreover, this strategy has the potential for rational immunogen design to induce bnAbs against other pathogens with high genetic diversity, such as influenza, hepatitis C virus, and coronaviruses. Nevertheless, to further evaluate the success of this germline-targeting strategy, some crucial issues should be clarified in the future. For example, (1) they did not test the neutralizing activities of cloned BCR in this study, and (2) why did eOD–GT8-reactive BCR fail to bind to native HIV-1 trimer?

From the proposal of concept to this first clinical validation, the germline-targeting vaccine strategy has accomplished several essential milestones toward the ultimate goal. To date, another two follow-up clinical trials are ongoing. One is to evaluate the safety and immunogenicity of the eOD–GT8 60-mer mRNA vaccine (mRNA-1644) in HIV-1-uninfected adults (IAVI G003). The other is to evaluate the safety and immunogenicity of the eOD–GT8 60-mer mRNA vaccine (mRNA-1644) and the Core-g28v2 60-mer mRNA vaccine (mRNA-1644v2-Core) in HIV-1-uninfected adults (IAVI G002), in which priming and boost immunogens are administrated sequentially into humans. In sum, complete proof of the germline-targeting vaccine strategy will only be achieved once potent bnAbs are safely and effectively induced in humans. From this aspect, the validation of a germline-targeting strategy for an effective HIV-1 vaccine is still at the beginning but is already one step closer to the end.

REFERENCES

1. Leggat, D.J., Cohen, K.W., Willis, J.R., et al. (2022). Vaccination induces HIV broadly neutralizing antibody precursors in humans. Science ***378***, eadd6502.
2. Haynes, B.F., Wiehe, K., Borrrow, P., et al. (2022). Strategies for HIV-1 vaccines that induce broadly neutralizing antibodies. Nat. Rev. Immunol. 1–17.
3. Jardine, J., Julien, J.P., Menis, S., et al. (2013). Rational HIV immunogen design to target specific germline B cell receptors. Science ***340***, 711–716.
4. Jardine, J.G., Kulp, D.W., Havenar-Daughton, C., et al. (2016). HIV-1 broadly neutralizing antibody precursor B cells revealed by germline-targeting immunogen. Science ***351***, 1458–1463.
5. Verkoczy, L., Alt, F.W., and Tian, M. (2017). Human Ig knockin mice to study the development and regulation of HIV-1 broadly neutralizing antibodies. Immunol. Rev. ***275***, 89–107.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (81971927, 82271786, and 32200765), the Science and Technology Planning Project of Shenzhen City (JCYJ20200109142601702 and 20190804095916056), and Youth Program of Chinese Academy of Sciences (E1BDEDF7241).

DECLARATION OF INTERESTS

No conflict of interest is declared.