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REVIEW

The path towards an HIV vaccine

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SUMMARY

Since the beginning of the HIV/AIDS epidemy in the eighties, hundreds of phase I human immunization studies were performed, however, only nine tested efficacy in phase IIb/III clinical trials. While immunogens for SARS-CoV-2 did move along the development and clinical trial pipeline at unprecedent speed, two HIV immunization vaccine trials, started in 2016 and 2017, did meet non-efficacy criteria at the interim analysis and were thus, halted by the Data and Safety Monitoring Boards. The challenges in the quest to develop a safe, effective and durable HIV vaccine are unchanged. However, as research on HIV vaccine discovery moves forward there are many new tools and platform technologies to iterate vaccine strategies faster. Among these, there is a growing interest to conduct experimental medicine approaches where product development is directly informed by human data at an early stage of product development.

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INTRODUCTION

The challenges for the development of an effective and safe HIV vaccine are manifold and unchanged. HIV has a vast genetic diversity, is transmitted via multiple routes, disseminates in a short time frame widely in immune-privileged reservoirs and ultimately integrates into the human genome seeding long-lived immune cells (Deeks *et al*., 2015). Along this, during the last 40 years since the first cases of HIV/AIDS were described, different vaccination approaches have been tested based on the induction of non-neutralizing antibodies or antibodies that neutralize the virus, or on the activation of cell-mediated immunity, though with little success and many disappointments (*Table 1*).

HIV efficacy vaccine trials: history and present

Among the various phase III, efficacy, clinical studies, only one, RV144, conducted in Thailand and published in 2009 induced protection from infection in approximately 30% during the first three years of exposure to the virus (Rerks-Ngarm *et al*., 2009) (*Table 1*). Since then, two efficacy trials, Uhambo/HVTN 702 and Imbokodo/HVTN 705, were interrupted after the results showed that the tested vaccine candidates did not significantly reduce the risk of HIV infection.

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More in detail, the phase IIb/III clinical trial, HVTN702, which aimed to confirm in Africa the results of the RV144 study, was unfortunately suspended in 2020 for futility (Gray *et al*., 2021). The ALVAC-HIV regimen given twice and followed by four booster of the same vaccine associated with MF59-adjuvanted subtype C gp120 protein was given to 2704 participants while 2700 received placebo. During 24 months of follow-up HIV-1 infection was diagnosed in 138 vaccinees and 133 placebo recipients, and thus, showed that this regimen did not prevent from HIV-1 infection. Zolla-Pazner and colleagues thoroughly compared the differences of HVTN702 and RV144 to understand what could have impacted and contributed to the different outcome (Zolla-Pazner, Michael and Kim, 2021). Though HVTN702 was modeled on RV144, it came with substantial difference. Indeed, both used a recombinant canarypox vaccine-based platform but with a different env insert covering subtype A/E *vs* C. Analogously the gp120 protein was selected on the most frequently circulating viral clades in the two countries, Thailand and South Africa, but came with some other additional modifications, one of these the adjuvant used, Alum *vs* MF59. Beyond these, other differences may have contributed to the lack of any efficacy signal of HVTN 702, such as the higher number of booster doses used which may have impacted on immune responses, the ten times higher HIV incidence rate and the higher prevalence of genital inflammatory diseases in the South African population. Thus, it is expected that, as soon as available, virological and immunological data of HVTN702 will help us to understand and possibly reveal immune correlates useful for further vaccine development.

Shortly thereafter, in August 2021, the IMBOKOKO trial, which was ongoing in five sub-Saharan African countries and aimed at testing the efficacy of two vaccine approaches administered simultaneously, was stopped. Participants received a total of four doses over 12 months of either a prime-boost vaccine regimen of a mosaic viral-vectored vaccine, Adeno26.Mos4.HIV (Ad26 prime) and an aluminum phosphate-adjuvanted clade C gp140 protein (boost), or a placebo. The Adenovirus 26-based HIV-Mosaic vaccine was found to be safe but did not significantly reduce risk of HIV infection among over 2600 wom-

Table 1 - *HIV-1 efficacy clinical trials.*

Trial Acronym	*Immunogen*	*Vaccine*	*Duration*	*Cohort*	*Country*	*Comment*
VAX 003	Bivalent gp120	AIDSVAX B/B	1999-2003	Intravenous drug users	Thailand	No protection nor modification of infection
VAX 004	Bivalent gp120	AIDSVAX® B/E	1998-2003	Discordant couples	US	No protection nor modification of infection
HVTN 502/504 (STEP)	Ad5 vectored (Gag, Pol and Nef) + protein		2004-2007	MSM High risk heterosexual men and woman	US	Interrupted in 2007. Risk of infection appeared to be higher in Ad5 sero-positive or uncircumcised males
HVTN 503 (Phambili)	Ad5 vectored (Gag, Pol and Nef) + protein		2007	Heterosexual men and women	South Africa	Interrupted in September 2007 due to stop of STEP trial
RV 144	Prime: canarypox vector Boost: bivalent gp120	ALVAC-HIV vCP1521 AIDSVAX® B/E	2003-2009	Men and women	Thailand	31,2% protection
HVTN 505	Prime: DNA encoding Gag, Pol, Nef and Env Boost: Ad5 encoding Gag-Pol and Env		2009-2017	MSM, Transgender MSM	US	No protection no reduction of viral load
HVTN 702 (Uhambo)	Prime: Canaripox vector Boost: bivalent gp120	ALVAC-HIV vCP2438 gp120 C/MF59	2016-2012	Men and women	South Africa	Interrupted February 2020 for non - efficacy
HVTN 705/ HPX2008 (Imbokodo)	Prime doses 1 to 4: Ad26.Mos.HIV Boost doses 5 and 6: trimeric gp140		2017-2022	Women	South Africa	Interrupted August 2021 for non - efficacy
HVTN 706/ HPX3002 (Mosaico)	Prime doses 1 and 2: Ad26.Mos4.HIV Boost doses 3 and 4: Ad26.Mos4.HIV + Mosaic and clade C gp140		2019-2023	MSM Transgender men	Argentina, Brazil, Italy, Mexico, Perù, Poland, Spain and US	Ongoing
PrEPVacc	*First randomization* Group A: DNA-HIV-PT123 + AIDSVAX®/BE Group B: DNA-HIV-PT123 + CN54gp140/MPLA + MVA-CMDR+CN54gp140/ MPLA *Second randomization* Drug: TAF/FTC or TDF/FTC		2020-2023	Men and women	Mozambique, South Africa, Tanzania and Uganda	Ongoing

en. Johnson & Johnson reported the primary analysis of the data, which showed an efficacy estimate of 25.2%, but with a wide confidence interval that crossed zero (-10.5% to 49.3%). It has to be reminded that the same Adenovirus 26-based platform with specific inserts for Ebola and COVID-19 was proven effective (Pollard *et al*., 2021; Ramasamy *et al*., 2020), which again shows that HIV is a particularly challenging virus.

While analysis in both these trials is ongoing and will help inform future research, the MOSAICO/HVTN706, phase III clinical trial continues in the Americas and Europe. Although it uses a regimen similar to IMBOKOKO with the same Ad26 platform for the prime immunogen, continuation was granted due to the different form of protein boost, and a different target population. It is currently enrolling 3,800 men and transgender people and should provide results by 2023.

At the same time the PrEPVacc trial is ongoing and testing experimental HIV vaccines and oral pre-exposure prophylaxis (PrEP) against a placebo, in 1668 women and men of four African countries (*Https://Clinicaltrials.Gov/Ct2/Show/NCT04066881*, n.d.). Participants are randomized to receive one of two experimental vaccines, which have both been tested before but did not advance to late-stage trials on their own. In addition, participants are also randomized to receive two types of daily oral PrEP, F/TDF (Truvada) or F/TAF (Descovy). This phase IIb study, which should provide results next year, is particularly relevant in view of combining different strategies of prevention in a multimodal approach.

How to proceed in the future?

Many collaborations have been established over the years to advance HIV vaccine research. A common strategic focus has enabled coordination and sharing of data and technical capabilities across the continuum of vaccine development, from basic research to translational science and to clinical trials. Indeed, lessons from HIV vaccine development efforts helped to accelerate the development of highly efficacious SARS-CoV-2 vaccines (Fauci, 2021; McMahon *et al*., 2020).

Due to the numerous complexities that make HIV such a difficult target for vaccine development and roll-out, in general, pharmaceutical companies did not make substantial high-risk investments. The field needs to improve through-put of early-stage clinical candidates to diversify the pipeline and to implement selection processes that accelerate different scientific approaches. In this regard, experimental medical studies are designed to accelerate HIV vaccine development, increasing the probability of success for products moving into clinical evaluation. While investigating critical scientific questions with a rigorous ethical approach these studies do not provide benefit to the participant. These clinical investigations provide opportunities for early iteration between preclinical and clinical research, evaluation of novel concepts prior to formal product development and thus, de-risking drug development and possibly reducing high level of late-stage clinical trial failures.

Examples of experimental medical trial design

The experimental medical trial approach is used by the EAVI2020 consortium, which evaluated B cell lineage design through iterative trials and tested the hypothesis that different prime-boost combinations with newly developed HIV-1 envelope proteins would influence and increase breadth and potency of neutralizing antibody responses. Native-like gp140 envelope trimers based on a consensus or mosaic design were manufactured in small batches in 48 months time supported through a single pivotal toxicology package for multiple products (Sliepen *et al*., 2021). During the last two years several trials were started in UK with multiple parallel arms of small number of participants. To mention one: EAVI001, a single-blind study with HIV-uninfected volunteers randomized into five groups of 10 participants each, has completed enrollment (*Https://Clinicaltrials.Gov/Ct2/Show/NCT03816137*, n.d.). Participants were vaccinated first with the consensus sequence-based trimer (ConM or ConS) designed to prime B cell responses to epitopes common to all HIV-1 subtypes, and then boosted with a cocktail of the mosaic gp140 trimers to overcall the immunodominance of hypervariable regions with the final goal to focus antibody responses towards conserved epitopes. This approach has shown to provide considerable savings in time and will accelerate insight in in-depth immunological evaluation to generate new hypothesis.

Trial G001 was the first in human test of a germline targeting approach with self-assembling nanoparticle eOD-GT8 60mer at two different concentrations, with a strong adjuvant (AS01B) used already for other licensed vaccines, administered twice 8 weeks apart (*Https://Clinicaltrials.Gov/Ct2/Show/NCT03547245*, n.d.). The study conducted in the US, was started in 2018 and concluded vaccinations in March 2020, proved to be safe and well tolerated, and induced increasing CD4-binding site specific IgG memory B cell responses in approximately 97% of vaccinees. This approach tested the scientific concept that a certain immunogen design can stimulate relevant germline B cells in a significant number of individuals (Jardine *et al*., 2016), and the vaccine is now ready to proceed in the clinical pipeline.

The path to mRNA vaccines

A long chain of struggles and advances during several decades constructed the path to success of mRNA (Dolgin, 2021). The first clinical trial with mRNA vaccine for an infectious disease, rabies, was test-

ed less than 10 years ago. Proof of the potential of mRNA vaccines comes from the recent COVID-19 pandemic, when two companies developed rapidly SARS-CoV-2 RNA based vaccines and progressed with success to efficacy trials (Baden *et al*., 2021a; Polack *et al*., 2020a). Indeed, synthetically produced nucleic acid vaccines bypass hurdles of costly and lengthy procedures by utilizing a standardized manufacturing procedure. While the mRNA technology is versatile and comes with several advantages, there are still a few technical considerations for mRNA vaccine development. In specific on the mRNA construct format that encodes the immunogen, and the delivery vehicle that facilitates cellular entry and expression (Kumar *et al*., 2022). Modified nucleosides are included in the two licensed COVID-19 mRNA, while self-amplifying RNAs are in development with promise of greater potency and persistence though yet to be demonstrated in clinical trials (Kumar *et al.*, 2022). Most RNAs are delivered with lipid nanoparticle formulation, which was clinically validated. The nanoparticle formulation can be a limiting step due to its complexity and availability of some components, therefore alternative delivery vehicles such as cationic nanoemulsions, lipidoids and polymers are rapidly advancing into the clinic (Blakney *et al*., 2021).

mRNA vaccines for HIV: a reality?

RNA based immunogens are not new to HIV research and already showed to induce cellular and humoral immune responses in pre-clinical studies (Blakney *et al*., 2019; Melo *et al*., 2019). Only to mention a few, Moyo *et al*. showed that a single immunization with a self-amplifying mRNA encoding six highly conserved regions of the HIV genome through Gag and Pol induced specific CD4+ and CD8+ T cells in mice (Moyo *et al*., 2019). More recently, Aldon *et al*. explored the potential of stabilized native-like envelope trimers delivered by self-amplifying RNA polyplexes (Aldon *et al*., 2021). They showed in mice that the choice of the self-amplifying RNA platform can make the difference in inducing a humoral response to the encoded transgene, and that low doses multiple administrations of vaccine were sufficient to induce high HIV env-specific IgG responses in non-human primates.

Zhang *et al*. showed that mRNA platform technology may overcome some challenges that HIV poses (Zhang *et al*., 2021). They encapsulated the viral envelope with the structural protein gag into a lipid nanoparticle to produce virus like particles *in vivo*. This mRNA construct administered to non-human primates elicited antibodies able to broadly neutralize a panel of HIV-1 isolates. Though extremely promising results, the protocol used to immunize the animals was complex with multiple administrations, and long time, one year, was needed to develop low level broadly neutralizing antibodies. This result is at variance to the swift success of the mRNA-based SARS-CoV-2 vaccines, which induced antibodies and cellular responses in a short time (Baden *et al*., 2021b; Polack *et al*., 2020b).

The mRNA platform is now tested also for HIV immunization approaches in human, and promising products, which induce neutralizing antibody, were converted into mRNA. In the pipeline are now three new phase 1 trials testing Moderna mRNA for HIV vaccine development. One trial (HVTN302) is testing in adults in US mRNA delivery of native gp140 envelope trimers for safety and induction of immune responses, while other two clinical trials use the mRNA vaccine of eOD-GT8, the nanoparticles coated with HIV env gp120 proteins that were previously shown to bind those pre-existing B cells specific for HIV CD4-binding site in macaques and humans (Jardine *et al*., 2016). mRNA eOD-GT8 60mers are administered in HIV-uninfected adults in US and Africa (trials IAVI G002 and G003) to test safety and immunogenicity, and to confirm that the nucleic-acid based vaccine triggers similar B cell precursors to the nanoparticles. Parallel performance of these two trials will narrow the gap between proof-of-concept and testing of the vaccine in relevant populations, provide know-how on differences of immune responses in different populations, and accelerate the inclusion of African sites early in the development pipeline.

CONCLUSION

As of today, global anti-retroviral therapy (ART) coverage was estimated to be one-third below the target, and thus, approximately only 26 million people living with HIV (PLWH) are under treatment. While it is indisputable that U = U, i.e. "undetectable equals untransmissible", makes ART the most potent approach to control viremia and transmission today, we also know that currently only 59% of PLWH fully adhere to therapy. Thus, new approaches such as long-acting formulations and injectables, drugs or broadly neutralizing antibodies, are in development and moving forward in the evaluation. PrEP with long-acting injectable drugs, that increase the interval between administrations, may gain in adherence, while becoming more affordable world-wide. Said this, there is no doubt that an effective vaccine would provide significant improvements and less burden on the health system. Historically, vaccination represents the most economical public health intervention with a large diffusion and persistence as needed to eradicate infectious diseases.

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