1 **Prospects for a Globally Effective HIV-1 Vaccine**

2

3 **Running title:** Globally Effective HIV-1 Vaccines

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23 **Abstract**

24 A globally effective vaccine strategy must cope with the broad genetic diversity of HIV
25 and contend with multiple transmission modalities. Understanding correlates of protection
26 and the role of diversity in limiting protective vaccines with those correlates is key. RV144
27 was the first HIV-1 vaccine trial to demonstrate efficacy against HIV-1 infection. A correlates
28 analysis compared vaccine-induced immune responses in vaccinated-infected and vaccinated-
29 uninfected volunteers suggested that IgG specific for the V1V2 region of gp120 was
30 associated with reduced risk of HIV-1 infection and that plasma Env IgA was directly
31 correlated with infection risk. RV144 and recent NHP challenge studies suggest that Env is
32 essential and perhaps sufficient to induce protective antibody responses against mucosally
33 acquired HIV-1. Whether RV144 immune correlates can apply to different HIV vaccines, to
34 populations with different modes and intensity of transmission, or to divergent HIV-1
35 subtypes remains unknown. Newer prime-boost mosaic and conserved sequence
36 immunization strategies aiming at inducing immune responses of greater breadth and depth as
37 well as the development of immunogens inducing broadly neutralizing antibodies should be
38 actively pursued. Efficacy trials are now planned in heterosexual populations in southern
39 Africa and MSM in Thailand. Although NHP challenge studies may guide vaccine
40 development, human efficacy trials remain key to answer the critical questions leading to the
41 development of a global HIV-1 vaccine for licensure.

42

43 **Keywords:** HIV-1, vaccine, correlates, clinical trial, efficacy, public health, licensure

44

45 **Introduction**

46 According to the UNAIDS, 35 million people were living with HIV-1 at the end of 2013,
47 the vast majority being in Sub-Saharan Africa, with however dynamic epidemics in Asia [1].
48 Worldwide the number of people including children with new HIV-1 infections fell by 38%
49 since 2001 [2]. Success in prevention programs can be ascribed to the strengthening and
50 scaling-up of antiretroviral treatment along with existing and new prevention methods [3-6].
51 New prevention strategies include pre-exposure prophylaxis (PrEP) [7-10], antiretroviral
52 treatment for prevention [10, 11], and topical microbicides [12, 13].

53 Waning benefits of behavioral risk reduction for HIV-1, cultural opposition to male
54 circumcision, still limited coverage of antiretroviral treatment, low adherence to PrEP [14],
55 absence of PrEP efficacy in African women [15], and legal sanctions for sex worker and
56 homosexuals may counterbalance the benefits achieved so far [16]. Although new HIV-1
57 infections fell worldwide without HIV-1 vaccine intervention, critical exceptions to these
58 trends are HIV epidemics among key populations such as people who inject drugs, young
59 women and adolescents in southern sub-Saharan Africa, and young men who have sex in men
60 (MSM) [17-19], and the increasingly complex HIV-1 genetic diversity [20, 21]. Taken
61 together, these considerations justify aggressive pursue development of a globally cost-
62 effective HIV-1 vaccine against several HIV-1 strains and routes of transmission. The
63 different HIV-1 vaccine approaches [22] and lessons learned from clinical trials [23] have
64 recently been reviewed.

65

66 **What works and what doesn't for protection against HIV-1 acquisition**

67 Both antibodies and cell-mediated immune responses are thought to be important to
68 prevent HIV-1 infection [24] in the mucosal compartment, the entry point for sexual
69 transmission [25]. **Table 1** summarizes the key animal challenge studies conducted with HIV-
70 1 and SIV vaccines. Data from three non-human primate (NHP) studies [26-28] suggest that
71 Env is a necessary component for successful protection from SIV acquisition. Two

72 approaches are currently being pursued to elicit Env-specific antibody-mediated protection:
73 vaccines that induce potent and broadly reactive neutralizing antibodies (bNAbs) with activity
74 against viruses which are common in human transmission (tier 2 viruses) or vaccines that
75 induce antibodies which neutralize only lab adapted and less commonly transmitted HIV
76 strains (Tier-1) but may block HIV-1 infection by non-neutralizing mechanisms [29, 30].

77

78 **Vaccines that induce non-neutralizing antibodies**

79 The renewed interest in nonneutralizing antibodies (nNAbs) was prompted by the results
80 of several HIV-1 vaccine efficacy trials and plausible mechanisms of action of nNAbs in
81 protection against HIV-1 acquisition have recently been reviewed [31, 32]. Neutralization is
82 defined here in the standard TZM-bl pseudovirus assay [33].

83 Monomeric gp120 HIV-1 envelope proteins alone failed to protect high-risk volunteers
84 against infection or disease progression in two previous efficacy trials, Vax003 [34] and
85 Vax004 [35-37]. Vax004 tested AIDSVAX® B/B, a bivalent recombinant HIV-1 subtype B
86 (GNE8 and MN) gp120 envelope glycoprotein subunit vaccine in US MSM and women at
87 high risk for heterosexual transmission of HIV-1 [35]. High NAb levels against the easy-to-
88 neutralize MN strain were however significantly inversely correlated with HIV-1 incidence
89 while low levels against more-difficult-to-neutralize viruses did not, suggesting that level and
90 breadth of elicited NAb were not sufficient for protection [36]. Interestingly, the level of
91 vaccine-induced antibody-dependent cellular virus inhibition activity (ADCVI) correlated
92 inversely with the rate of acquiring HIV-1 infection following vaccination. Moreover,
93 ADCVI activity correlated poorly with NAb or CD4-gp120-blocking antibody activity
94 measured.

95 Vax003 tested AIDSVAX® B/E, a bivalent recombinant HIV-1 subtype B/E (A244
96 CRF01_AE and MN subtype B) gp120 envelope glycoprotein subunit vaccine in injecting
97 drug users in Bangkok, Thailand. Antibody levels for gp120, A244 V2, A244 V3, blocking of
98 A244 binding to CD4, and MN neutralization were not associated with infection risk [34].

99 In RV144 (reviewed in [38]), consisting of ALVAC-HIV (vCP1521) prime and
 100 AIDSVAX® gp120 B/E boost the modified intent-to-treat analysis showed an estimated
 101 31.2% efficacy after 42 months [39, 40], 60% at 12 months and 44% at 18 months post first
 102 vaccination, suggesting an early, but nondurable, vaccine effect [41]. NAb were observed
 103 against Tier-1 laboratory strains and was modulated by FcR polymorphisms [42]. CD4+ T
 104 cell-mediated, IFN- γ ELISPOT positive responses were detected in 41% of the vaccinees and
 105 targeted the HIV-1 Env region, with 60% of vaccinees recognizing peptides derived from the
 106 Env V2 region, which includes the $\alpha 4\beta 7$ integrin binding site [43, 44]. MN and A244 Env
 107 binding antibodies were present in nearly all vaccinees, but dropped 15 fold after 6 months.
 108 Antibody-dependent cell-mediated cytotoxicity (ADCC) in vaccine recipients and ADCC-
 109 mediating monoclonal (mAb) antibodies from vaccinees have been described [45, 46].

111 The RV144 trial permitted a case-control study of correlates of risk for HIV acquisition.
 112 Plasma IgG binding antibody to scaffolded gp70 V1V2 CaseA2 envelope protein (HIV-1
 113 subtype B) correlated inversely with risk, while Env plasma IgA correlated directly with risk.
 114 Neither low levels of V1V2 antibodies nor high levels of Env-specific IgA antibodies were
 115 associated with higher rates of infection than in the placebo group, ruling out the possibility
 116 of vaccine-induced enhancement. In vaccinees with low levels of Env-specific IgA
 117 antibodies, IgG avidity, ADCC, NAb to easy-to-neutralize viruses, and Env-specific CD4+ T
 118 cells, were inversely correlated with risk of infection [47-49]. RV144 antibodies to subtype A,
 119 C and CRF01_AE gp70 V1V2 scaffolded proteins also correlated inversely with risk [50],
 120 suggesting that the RV144 regimen might protect against heterosexual transmission of HIV
 121 strains heterologous (A and C) to the vaccine components. Two weeks post last vaccination
 122 97% of RV144 studied plasma samples from vaccine recipients contained antibodies to V2
 123 region synthetic peptides, falling to 19% at 48 weeks, suggesting that waning vaccine efficacy
 124 may be correlated to waning V2 antibody response.

125

126 The response to V3 CRF01_AE also inversely correlated with the risk of HIV infection in
127 vaccine recipients with lower levels of Env-specific plasma IgA and neutralizing antibodies.
128 In Vax003 and Vax004 (no protection), serum IgG responses targeted the same epitopes as in
129 RV144 with the exception of an additional C1 reactivity in Vax003 and infrequent V2
130 reactivity in Vax004. These results along with a recent sieve analysis [51] generate the
131 hypothesis that IgG to linear epitopes in the V2 and V3 regions of gp120 are part of a
132 complex interplay of immune responses that contributed to protection in RV144 [52].
133 Correspondingly, in a SIVm251 low dose intrarectal challenge study of rhesus macaques,
134 protection by an Ad26-MVA Gag-Pol-Env vaccine combination was associated with
135 requirement for Env immunogen, anti-Env binding antibody, and V2 specific antibody
136 responses [26]. In the Step and Phambili HIV-1 vaccine trials [53, 54], the MRKAd5 HIV-1
137 Gag/Pol/Nef vaccine did not induce Env-specific antibody, and in HVTN 505 [55], the
138 DNA/rAd5 Gag-Pol-Env HIV-1 vaccine regimen induced significantly lower levels of anti-
139 V1V2 antibody, comparatively to RV144.

140
141 It was hypothesized that RV144 vaccine-induced antibodies to Env-V1V2 could
142 selectively prevent HIV-1 infections by certain variants, and that this effect would be evident
143 in the V1V2 region of breakthrough viruses. The sieve analysis examined the relationship
144 between vaccine status and V1V2 sequence characteristics using HIV-1 sequences from
145 breakthrough infections. Two vaccine-associated genetic signatures were identified in V2
146 corresponding to sites 169 and 181, further supporting the hypothesis that vaccination-
147 induced immune responses directed against the V2 loop were associated with protection [56].
148 Monoclonal antibodies from RV144 vaccine recipients contact the V2 K169 residue,
149 providing additional evidence that vaccine-induced antibodies correspond to the observed
150 sieve effect. These V2-specific antibodies can mediate ADCC, neutralization of easy-to-
151 neutralize viruses, and low-level virus capture [57, 58]. In RV144, Env IgG3 was correlated
152 with decreased risk of HIV infection, a response that declined rapidly compared to overall
153 IgG responses [59, 60]. A comparison of RV144 and Vax003 showed that Env-specific IgG3

154 and V1/V2 IgG3 response rates were higher in recipients of the RV144 vaccine compared to
155 Vax003 vaccinees and conversely that IgG4 were considerably lower in RV144. These
156 findings generate the hypothesis that V2 IgG plays a role in protection against HIV-1
157 acquisition [61] but do not distinguish between mechanistic or non-mechanistic mechanisms
158 of protection [62].

159

160 The RV144 correlates of risk (CoR) analysis suggests that an increase in magnitude,
161 affinity, breadth, and importantly in frequency and durability of V2- and V3-specific
162 antibodies of IgG3 and IgG1 subclasses may confer a higher and more durable rate of
163 protection against HIV-1 infection. The induction of cross-reactive V1V2 specific IgG raises
164 the hypothesis of cross-clade protection. However, issues of magnitude and durability
165 attendant the original RV144 regimen must be addressed to improve responses in order to
166 optimize hypothesis testing in future trials.

167 Additional booster vaccinations may increase antibody levels. Residual antibody
168 responses against gp120 were detected 6-8 years post vaccination in RV144 vaccinees.
169 Additional boosts increased plasma IgG gp120 and gp70 V1/V2 antibodies at titers higher
170 than the RV144 peak time point while weak gp120 IgA responses were induced [63]. These
171 HIV-specific IgG antibodies were also detected in rectal secretions while IgA were
172 undetectable [64]. Surprisingly, the late boosts given to RV144 volunteers increased VH
173 mutation frequency from that seen following the initial RV144 vaccine regimen (RV305
174 mean 5.50%; RV144 mean 2.60%) and expanded a population of antibodies with HCDR3 >
175 22 amino acids. Similar to V1V2 bNAbs and other NAbs with long HCDR3s, these mAbs
176 principally used D2/D3 and JH6 and are being characterized for neutralization capacity [65].

177 The use of potent adjuvants may also augment and shape antigen-specific antibody
178 responses and contribute to antigen dose sparing. Several adjuvants have been tested in NHP
179 and humans [66] showing a significant benefit of HIV envelope proteins formulated with
180 either MF59 [67] and AS01 [68, 69]. In macaques, alum protected macaques from
181 SIVmac251 acquisition while MF59 did not despite its ability to elicit higher systemic T-cell

182 and antibodies responses. Adjuvant-associated differences in the homing of plasmablasts and
183 induction of key cellular signaling pathways may explain the acquisition effects [70]. The
184 formulation of HIV-1 gp120 with L(MPLA) and alum induced significantly higher levels of
185 neutralizing antibodies and T-cell lymphoproliferation compared to alum, MF59 or MPLA
186 alone [71]; importantly antibodies to gp70 V1V2 (subtypes B, C and CRF01_AE) were
187 induced more rapidly, to higher magnitude and with greater durability than alum-adjuvanted
188 gp120 [71, 72]. Formulation of antigens with solid nanoparticles may prolong the duration of
189 antibody responses by increasing antigen deposition/retention locally in the tissues driving B-
190 cell responses, enhancing dendritic cell antigen presentation [73], and development of CD4+
191 Tfh cells [74] that provide cytokines and signals required to initiate somatic hypermutation
192 and affinity maturation for effective B-cell memory [75].

193

194 **Broadly neutralizing antibodies**

195 Unlike classical infectious diseases, such as mumps or rubella, natural HIV-1 infection
196 does not induce protective immunity that eradicates (sterilizing) the virus or prevents
197 progression to disease. Extending older studies [76-80], Hraber et al. showed that cross-clade
198 NAb responses are commonly induced in response to infection by any virus clade.
199 Nonetheless, neutralization was significantly greater when the plasma clade matched the
200 clade of the virus tested. Features of the gp120 V1V2 loop, in particular, length, net charge,
201 and number of N-linked glycans, were associated with Env susceptibility and plasma
202 neutralization potency in a manner consistent with neutralization escape being a force that
203 drives viral diversification and plasma neutralization breadth [81]. The trimeric HIV-1
204 envelope glycoprotein is composed of variable regions that are immunodominant and induce
205 type-specific NAbs of limited breadth, while the conserved regions such as the CD4 binding
206 site are cryptic and poorly accessible to the immune system [82]. bNAbs develop in roughly
207 20% of HIV-infected after 2-3 years, but these bNAbs do not limit disease progression [83-
208 87]. HIV-1 Env is covered with glycans that shield conserved epitopes from antibody

209 recognition and evade the neutralizing antibody response [88]. An effective HIV-1 vaccine
210 should therefore induce responses that differ qualitatively and quantitatively from that
211 induced by natural infection, and able to cross-protect against various HIV-1 clades [89].

212 As a proof of concept, first and second generation bNAbs protect against intravaginal
213 SHIV challenge [90-92], requiring high concentrations (25-50mg/kg). Enhanced bNAbs
214 confer protection at lower concentration (5 to <1mg/kg) [93, 94]. While the epitope
215 specificity and putative germline sequences of the B-cell receptors of these antibodies have
216 been defined, the challenge for epitope-based vaccine design is that only broadly conserved
217 and exposed epitopes are suitable for vaccine targeting, but these epitopes, in their natural
218 context, tend to elicit poor antibody responses [95]. When bNAb responses are elicited in the
219 context of HIV infection, the corresponding broadly neutralizing monoclonal antibodies
220 (bnMAbs) tend to have uncommon features such as high levels of somatic mutations,
221 insertions/deletions, long HCDR3 loops, post-translational modifications, polyreactivity, and
222 rare structural motifs such as domain exchange [96-101]. Long HCDR3, though low in
223 frequency, are present in the human mature naïve B-cell repertoire and generated by
224 recombination during B-cell development [102]. A first step of the approach aims at fully
225 defining the antibodies and epitopes associated with broadly HIV neutralization. This may
226 dictate that immunogens and/or immunization protocols should be designed to increase
227 antibody affinity maturation [103]. Alternatively, the high levels of somatic hypermutation
228 may simply reflect the outcome of chronic antigen stimulation resulting from long-term HIV
229 infection. Anti-Env antibodies from chronic infection in general, whether neutralizing or not,
230 tend to have high levels of somatic hypermutation [104, 105].

231

232 *Designing trimeric HIV-1 envelopes*

233 The challenge is to design, engineer and produce a pure stable envelope immunogen that
234 mimics the antigenic profile of the functional envelope spike [95, 106, 107]. The first
235 generation of HIV-1 gp120 immunogens designed were not, in general, well recognized by
236 germline sequences of the PG9, PG16, and CH01 lineage bNAbs and failed to elicit bNAb in

237 animals as most of the unmutated germline precursors of the bNAb do not bind to HIV-1
238 spike proteins from most viral isolates. However, B cells producing bNAb precursors could
239 not be recruited into immune responses by these immunogens (reviewed in [108]). Initially
240 engineered trimeric envelope was unable to induce bNAb in animals [109]. Modification of
241 the trimers including removal of individual glycans proximal to CD4-binding region [110],
242 elimination of the glycosylation site near the gp41 loop [111], linker-stabilized gp140 trimeric
243 envelopes [112] have resulted in improved immunogenicity but have not yielded the desired
244 bNAb. The use of multivalent mixtures of natural HIV-1 subtype C envelope uncleaved
245 trimers elicited higher magnitude NAb against Tier 1 viruses than individual trimers, but not
246 against Tier 2 viruses [113]. A combination of mosaic envelopes tested increased the
247 magnitude of NAb but not the breadth the response in macaques [114]. Ad26/MVA and
248 Ad26/Ad35 regimens expressing mosaic Env immunogens were able to protect macaques
249 against SHIV-SF162P3 challenge. Protection was correlated with binding nNAb (mosaic
250 Env binding antibody, ADCP, and trend for antibody-dependent complement deposition), and
251 with NAb titers against SF162 [27]. So far, no trimeric envelope induces bNAb in humans
252 [83].

253

254 *B-cell lineage vaccine design*

255 Another approach is called B-cell lineage vaccine design and aims at engaging the naive
256 B-cell repertoire residing in bone marrow and secondary lymphoid tissues. Specifically, one
257 or more clonally related bNAb must be isolated and an antibody lineage constructed through
258 inference that links the mutated bNAb-producing cell to its naive, germline ancestor.
259 Recombinant antibody technology would express members of that bNAb lineage in order to
260 select HIV-1 envelope constructs that optimally bind them. Those envelope constructs would
261 be used as immunogens in a prime–boost to engage the naive B cell in vivo and iteratively
262 stimulate B-cell “evolution” until bNAb-producing cells are elicited [115]. Liao et al. reported
263 the isolation, evolution and structure of a bNAb from an African donor followed from the
264 time of infection. The mature antibody, CH103, neutralized approximately 55% of HIV-1

265 isolates, and its co-crystal structure with the HIV-1 envelope protein gp120 revealed a new
266 loop-based mechanism of CD4-binding-site recognition. Virus and antibody gene sequencing
267 revealed concomitant virus evolution and antibody maturation. Notably, the unmutated
268 common ancestor of the CH103 lineage avidly bound the transmitted/founder HIV-1
269 envelope glycoprotein, and evolution of antibody neutralization breadth was preceded by
270 extensive viral diversification in and near the CH103 epitope. These data describe the viral
271 and antibody evolution leading to induction of a lineage of HIV-1 bNAbs, and provide
272 insights into strategies to elicit similar antibodies by vaccination [116]. Doria-Rose et al.
273 demonstrated that HIV-1 V1V2-directed neutralizing antibodies can develop relatively
274 rapidly through initial selection of B cells with a long CDR H3, and limited subsequent
275 somatic hypermutation. Identifying features of antigens able to engage naive B cells with
276 such CDRH3 is a critical step in design of vaccines targeting V1V2. Such antigens could be
277 screened for binding to the unmutated common ancestor versions of neutralizing antibodies as
278 an indicator of the ability to engage an appropriate naive B cell receptor. This work also
279 suggests that although an appropriate trimeric V1V2 construct may elicit neutralizing V1V2
280 antibodies, sequential immunogens that mirror viral evolution may be needed to drive the
281 development of breadth [117].

282 Wild-type gp120 proteins lack detectable affinity for predicted germline precursors of
283 VRC01-class bNAbs, making them poor immunogens to prime a VRC01-class response.
284 Jardine et al. used computation-guided, in vitro screening to engineer a germline-targeting
285 gp120 outer domain immunogen that binds to multiple VRC01-class bNAbs and their
286 germline precursors. When multimerized on nanoparticles, this immunogen activates both
287 germline and mature VRC01-class B cells [118]. Only nNAbs are elicited by Env
288 immunization. In contrast to germline-reverted bNAbs, germline-reverted nNAbs recognize
289 diverse recombinant Envs. In addition, nNAb B-cell progenitors become activated and
290 internalize Env compared with bNAb B-cell progenitors. McGuire et al. show that rational
291 immunogen modifications can reduce (and in certain cases eliminate) the activation of naïve

292 B cells that give rise to such nNAbs, while promoting the activation of naïve B cells that give
 293 rise to germline-reverted VRC01-class bNAbs [103].

294
 295

296 *Will broadly neutralizing antibodies protect against HIV-1 in humans?*

297 Whether bNAb will effectively confer protection against HIV acquisition in humans
 298 remains unknown. An alternative to inducing bNAb by vaccination with immunogens is to
 299 deliver these bnMAbs with viral vectors administered intramuscularly such as an adeno-
 300 associated virus (AAV) gene transfer vector expressing antibodies or antibody-like
 301 immunoadhesins having predetermined SIV specificity. SIV-specific molecules are
 302 endogenously synthesized in myofibers and passively distributed to the circulatory system.
 303 This approach generated long-lasting neutralizing activity in serum of macaques that
 304 observed complete protection against intravenous challenge with virulent SIVmac316 [119,
 305 120]. Similarly, full protection against intravenous HIV-1 challenge was observed in
 306 humanized mice receiving AAV carrying b12, while those receiving AAV carrying 2G12,
 307 4E10 and 2F5 were partially protected [121]. Moreover, humanized mice receiving AAV
 308 carrying VRC07 were protected against repeated vaginal challenge with diverse HIV-1 strains
 309 [122]. An AAV vector carrying PG9 is now tested in a Phase I trial (clinicaltrials.gov:
 310 NCT01937455).

311

312 **Cell-mediated immune responses**

313 The rationale for T-cell based vaccines has been recently reviewed [123-125]. T cell-
 314 based vaccine approaches have focused on three strategies: increasing the breadth of vaccine-
 315 induced responses [126] or increasing responses targeting only conserved regions of the virus
 316 [125] or using replication-competent viral vectors [127, 128]. Cytotoxic T lymphocyte (CTL)
 317 responses targeting specific HIV proteins, in particular Gag, have been associated with
 318 relative control of viral replication and elite control of viremia in vivo (reviewed in [124,

129]. In a SIVmac251 intravenous challenge model, breadth of Gag CTL epitope recognition correlated with control of peak and setpoint viremia [130].

321

322 *Natural HIV-1 sequences*

323 The Step (HVTN 502/Merck 023) [53, 131] and Phambili (HVTN 503) [54] vaccine trials
 324 explored for the first time whether cell-mediated immune response-inducing vaccines could
 325 prevent infection or reduce post-infection plasma viral load. The Merck vaccine (MRKAd5
 326 HIV-1) used in these two trials was a mixture of replication-defective Ad5 vectors expressing
 327 HIV-1 *gag*, *pol*, and *nef* subtype B genes. The vaccine did not confer protection against HIV-
 328 1 acquisition or disease progression measured by post-infection setpoint viral load (reviewed
 329 in [22, 23]. There were excess HIV-1 infections in the vaccine group but statistical
 330 significance was not seen in the primary study; post-hoc follow-up of data from both Step and
 331 Phambili rAd5 trials has suggested increased risk of HIV-1 infection in vaccine recipients. A
 332 sieve analysis showed evidence of vaccine-elicited immune pressure on the founder virus
 333 though no specific CD8⁺ CTL recognizing that epitope could be identified [132]. Vaccinees
 334 with HLA alleles associated with HIV-1 control had a significantly lower mean viral load
 335 over time [133]. Interestingly, the most highly conserved epitopes were detected at a lower
 336 frequency, suggesting that stronger responses to conserved sequences may be as important as
 337 breadth for protection [134]. The outcome of the Step trial was recapitulated in an Indian
 338 rhesus macaque study where animals vaccinated with a regimen similar to that employed in
 339 the Step trial were not protected against a heterologous SIVsmE660 challenge [135]. Rhesus
 340 macaques chronically infected with a host-range mutant Ad5 and immunized with a rAd5
 341 SIVmac239 Gag/Pol/Nef vaccine were challenged with a series of escalating dose penile
 342 exposures to SIVmac251. Despite inducing CD8⁺ T-cell responses in 70% of the monkeys
 343 the vaccine did not protect vaccinated animals from penile SIV challenge [136].

344 Aiming at inducing and improving breadth of both functional antibodies and cell-
 345 mediated responses [24] against 3 subtypes, a regimen with DNA vaccine prime composed of
 346 DNA plasmids encoding Gag, Pol, and Nef from HIV-1 subtype B and Env from subtypes A,

347 B, and C and replication-defective rAd5-HIV-1 vaccine boost containing a mixture of 4 rAd5
348 vectors encoding the HIV-1 subtype B Gag-Pol and Env matching the DNA Env components
349 was tested in the HVTN 505 vaccine efficacy trial. In contrast with MRKAd5 that did not
350 contain an *env* gene, the vaccine contained 3 envelope genes. The vaccine regimen induced
351 polyfunctional CD4+ and CD8+ T-cells, multi-clade anti-Env binding antibodies, and Nab
352 against easy to neutralize Tier 1 viruses. The Phase IIB trial (HVTN 505) was stopped for
353 futility, showing no efficacy and no statistically significant effect on viral load and a non-
354 significant excess of HIV infection in the vaccinated group [55]. This prime-boost vaccine
355 regimen failed to protect NHP against SIVmac251 infection, but 50% of vaccinated monkeys
356 were protected from infection with SIVsmE660 with about a one-log reduction in peak
357 plasma virus RNA in Mamu-A*01-positive animals, suggesting a role of CTL in the control
358 of SIV replication. However, low levels of NAb and envelope-specific CD4+ T-cell responses
359 were associated with animal protection [137].

360 Other approaches with DNA prime and MVA boost have elicited broad and potent T-cell
361 responses and deserve further development [138]. Interestingly, heterologous vector prime-
362 boost regimens enhance immunity by increasing the magnitude, onset and multi-functionality
363 of the insert-specific cell-mediated immune responses compared to homologous regimens
364 [139].

365

366 *Mosaic HIV-1 immunogens*

367 Polyvalent mosaic immunogens derived by recombination of natural HIV-1 strains are
368 designed to induce cellular immune responses that recognize genetically diverse circulating
369 virus isolates. Increasing the breadth and depth of epitope recognition may contribute both to
370 protection against infection by genetically diverse viruses and to the control of variant viruses
371 that emerge as they mutate away from recognition by cytotoxic T lymphocytes [140].

372 Mosaic HIV-1 Gag, Pol and Env antigens expressed by Ad26 vectors markedly
373 augmented both the breadth and depth without compromising the magnitude of antigen-
374 specific T lymphocyte responses as compared with consensus or natural sequence HIV-1

375 antigens in rhesus monkeys [141]. As described above, Ad26/MVA and Ad26/Ad35 vector-
376 based vaccines expressing HIV-1 mosaic Env, Gag, and Pol afforded protection against
377 intrarectal SHIV-SF162P3 challenge, correlated with antibody responses, the vaccine
378 regimens had only a modest effect on viral setpoint post-challenge [27]. In contrast, similar
379 vector regimens expressing SIVsmE543 antigens afforded >2 log reductions of setpoint viral
380 loads following heterologous SIVmac251 challenges [26]. However, a head-to-head
381 comparison in humans of mosaic and natural sequences should deserve consideration.

382

383 *HIV-1 conserved sequences*

384 An alternative to multivalent wild-type or mosaic vaccines is the use of conserved
385 element immunogens as a novel and effective strategy to broaden responses against highly
386 diverse pathogens by avoiding decoy epitopes, while focusing responses to critical viral
387 elements for which few escape pathways exist [126, 142-144]. Priming with conserved
388 elements boosted with the complete immunogen induced broad cellular and humoral
389 immunity focused on the conserved regions of the virus [145]. In contrast, full-length HIV-1
390 immunogens induced greater magnitude and comparable breadth of T-lymphocyte responses
391 to conserved HIV-1 regions compared with conserved-region-only HIV-1 immunogens in
392 rhesus monkeys [146].

393 Conserved sequences and immune responses have been characterized in animals [147,
394 148], conferring partial protection against SIVmac251 in macaques [149]. In humans, a
395 combination of DNA, ChAd63 and MVA vectors was found safe [150] and immunogenic,
396 inducing high levels of effector T cells that recognized virus-infected autologous CD4+ cells.
397 In vitro inhibition of HIV-1 replication was mediated by Gag- and Pol-specific effector CD8+
398 T cells targeting epitopes that are subdominant in natural infection [151].

399

400 *Use of replicating vector*

401 A replication-competent rhesus cytomegalovirus vaccine expressing SIV proteins induced
402 and maintained high frequency of SIV-specific CD4+ and CD8+ T-cell effector memory

(TEM) responses at extra-lymphoid sites without measurable antibody responses to SIV. Half of vaccinated monkeys showed a stringent control of three routes of transmission: intrarectal, intravaginal, or intravenous SIVmac239. The outcome of challenge was predicted by peak SIV-specific CD8⁺ TEM frequencies in peripheral blood pre-challenge [152, 153].

Whether these new T cell-based vaccines (Table 1) will be transitioned to human efficacy trials is unknown, though the conservation of particular cytotoxic epitopes does make them good candidates for a global HIV-1 vaccine.

Conclusions

RV144 was the first HIV-1 vaccine trial to demonstrate efficacy against HIV-1 infection. Put in perspective, RV144 and recent NHP challenge studies suggest that Env is essential and perhaps sufficient to induce protective antibody responses against HIV-1 acquisition at the mucosal site of entry. Whether the CoR identified in RV144 can be extended to other populations with different modes and intensity of transmission and against increasing complex HIV-1 strain recombinations remains to be demonstrated. While the RV144 immunization strategy remains a priority for future efficacy trials, newer prime-boost mosaic and conserved sequence immunization strategies aiming at inducing immune responses or greater breadth and depth as well as the development of immunogens inducing broadly neutralizing antibodies should be actively pursued and tested in humans as soon as available. Efficacy trials are now planned in heterosexual populations in southern Africa and MSM in Thailand and possibly in China. Although NHP challenge studies may guide vaccine development, human efficacy trials remain key to answer the critical questions leading to the development of a global HIV-1 vaccine for licensure [154].

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435

436 **Disclaimer**

437 The opinions herein are those of the authors and should not be construed as official or
438 representing the views of the U.S. Department of Defense or Department of the Army.

439 **Table 1.** Key non-human primate and humanized mouse challenge studies of HIV-1 and SIV vaccines
 440

Vaccines	Challenge	Immune responses and Efficacy	References
ALVAC-SIV/gp120 in alum or MF59	Intrarectal SIVmac251	<ul style="list-style-type: none"> • Alum protected macaques from SIVmac251 acquisition while MF59 did not despite its ability to elicit higher systemic T-cell and antibodies responses. • Alum, in contrast, increased the frequency of plasmablasts expressing the mucosal integrin $\alpha 4\beta 7$ that positively correlated with IgA responses to cyclic V2 in rectal mucosa. • In the alum group mucosal IgG to cyclic V2 correlated with lower risk SIVmac251 acquisition. 	[70]
DNA/Ad5 mosaic Gag or mosaic heterologous envelope (Env) or heterologous Env based on a natural SIVmac239	Intrarectal SIVsmE660	<ul style="list-style-type: none"> • SIVmac239 Env provided significant protection against acquisition, whereas mosaic Env immunization did not achieve significance. • No difference in acquisition between Gag-immunized animals and control animals. • For protection against acquisition, vaccine efficacy was 69% for SIVmac239 Env. • Best control of acute VL in the mosaic Env arm, whereas mosaic Gag arm showed the best long-term control. • Antibodies to the SIV envelope are necessary and sufficient to prevent infection. 	[28]
AAV carrying b12, or 2G12, or 4E10 or 2F5 in humanized mice	Intravenous HIV-1	<ul style="list-style-type: none"> • Full protection with AAV carrying b12 • Partial protection with AAV carrying 2G12, or 4E10 or 2F5 	[121]
AAV carrying VRC07 in humanized mice	Intravaginal HIV-1	<ul style="list-style-type: none"> • Full protection 	[122]
Ad25/Ad5, Ad35/Ad5, Ad5/Ad5 expressing SIVmac239 Gag	Intravenous SIVmac251	<ul style="list-style-type: none"> • Ad26/Ad5 regimen elicited a mean of 8.6 Gag epitopes per animal, whereas Ad35/Ad5 elicited a mean of 4.5 epitopes per animal and Ad5/Ad5 a mean of only 2.2 epitopes per animal. • Ad25/Ad5 reduced peak and setpoint viral load by 1.43 and 2.6 log 	

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		<p>and AIDS-related mortality as compared with controls.</p> <ul style="list-style-type: none"> • Breadth and magnitude of Gag-specific cellular responses correlated with control of setpoint viral loads. 	
Trivalent replication-incompetent rAd5 vaccine expressing SIVmac239 Gag, Pol, and Nef	Intrarectal SIVsmE660	<ul style="list-style-type: none"> • SIV-specific CD4+ and CD8+ responses by IFN-γ and ICS • No efficacy on SIV acquisition or viral load 	[135]
Chronically infected macaques with rAd5 SIVmac239 Gag, Pol, Nef	Penile SIVmac251	<ul style="list-style-type: none"> • CD8+ T-cell responses in 70% of the animals • No protection 	[136]
SIVmac239 Env and Gag-Pol DNA prime/rAd5 boost	Intrarectal SIVmac251 and SIVsmE660	<ul style="list-style-type: none"> • No protection against SIVmac251 infection, 50% of vaccinated monkeys protected from SIVsmE660. • One-log reduction in peak plasma virus RNA in Mamu-A*01 monkeys. • In Mamu-A*01-negative monkeys challenged with SIVsmE660, no CD8+ T cell response or innate immune response was associated with protection against virus acquisition. • Low levels of neutralizing antibodies and an envelope-specific CD4+ T cell response were associated with vaccine protection in these monkeys. 	[137]
Ad26/MVA and Ad26/Ad35 expressing mosaic HIV-1 Env, Gag, and Pol	Intrarectal SHIV-SF162P3	<ul style="list-style-type: none"> • Protection against SHIV-SF162P3 acquisition correlated with NAb titers against SF162 and ADCP, and trend with ADCD C3b complement deposition. • No correlation with cyclic V2 peptides or gp70 V1V2 antibodies or any measure of CD8+ T lymphocytes. • Modest effect on viral set point post-challenge likely linked to Env-specific immune responses 	[27]
DNA/MVA, MVA/MVA, Ad26/MVA, and MVA/Ad26 SIVsme543 Gag-Pol and Env	Intrarectal SIVmac251	<ul style="list-style-type: none"> • Ad26/MVA and the MVA/Ad26 required three challenges to infect 50% of animals in each group, whereas only one challenge was required to infect 50% of animals in the control group. • The regimens resulted in at least 2.32 and 1.08 log reductions of 	[26]

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		<p>mean set point viral load, respectively.</p> <ul style="list-style-type: none"> • Protection against acquisition of infection correlated with Env-specific binding ELISA antibodies, Tier-1 neutralizing, and V2-specific binding antibodies. • Virological control was correlated with Gag IFN-γ ELISPOT breadth. 	
SIVconsv synthetic long peptides adjuvanted (S) alone or DNA (D): SSS or DDSS regimens	Intrarectal SIVmac251	<ul style="list-style-type: none"> • Strong polyfunctional CD4+ T-cell and modest CD8+ T-cell responses of central memory T-cell phenotype. • SIVconsv-specific antibody responses were induced capable of recognizing the Env glycoprotein. • 2/6 DDSS animals protected against infection, while all 14 animals in the SSS and 2 control groups infected. • Vaccine induced SIV-specific IgG responses in mucosal washes pre-challenge were highest in the two protected animals. 	[149]
Replication-competent rhesus cytomegalovirus expression SIV Gag, Rev/Tat/Nef, Env and Pol	Intrarectal, intravaginal, and intravenous SIVmac239	<ul style="list-style-type: none"> • Protection of 50% of vaccinated animals • Complete clearance of SIV in protected animals • Effector memory SIV-specific CD4+ and CD8+ T-cell responses to all SIV inserts, but little to no SIV Env-specific antibody responses 	[152, 153]

441

442 Ad5, Ad26 and Ad35: replication-incompetent adenovirus type 5, 26, 35; AAV: adeno-associated virus; MVA: Modified Vaccinia Ankara;

443 SIVcons: SIV conserved sequences; SHIV: chimeric simian-human immunodeficiency virus

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