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Phase I clinical trial of an intranodally administered mRNA-based therapeutic vaccine against HIV-1 infection

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Objective: The efficacy of therapeutic vaccines against HIV-1 infection has been modest. New inerts to redirect responses to vulnerable sites are urgently needed to improve these results.

Design: We performed the first-in-human clinical trial with naked mRNA (iHIVARNA) combining a dendritic cell activation strategy (TriMix:CD40L+CD70+caTLR4 RNA) with a novel HIV immunogen sequences (HTI immunogen).

Methods: A dose escalation, phase I clinical trial was performed in 21 chronic HIV-1-infected patients under ART who received three intranodal doses of mRNA (weeks 0, 2 and 4) as follow: TriMix-100 g, TriMix-300 g, TriMix-300 g with HTI-300 g, TriMix-300 g with HTI-600 g, TriMix-300 g with HTI-900 g. Primary end-point was safety and secondary-exploratory end-points were immunogenicity, changes in viral reservoir and transcriptome.

Results: Overall, the vaccine was secure and well tolerated. There were 31 grade 1/2 and 1 grade 3 adverse events, mostly unrelated to the vaccination. Patients who received the highest dose showed a moderate increase in T-cell responses spanning HTI sequence at week 8. In addition, the proportion of responders receiving any dose of HTI increased from 31% at w0 to 80% postvaccination. The intervention had no impact on caHIV-DNA levels, however, caHIV-RNA expression and usVL were transiently increased at weeks 5 and 6 in the highest dose of iHIVARNA, and these changes were positively correlated with HIV-1-specific-induced immune responses.

Conclusion: This phase I dose-escalating trial showed that iHIVARNA administration was safe and well tolerated, induced moderate HIV-specific T-cell responses and transiently increased different viral replication readouts. These data support further exploration of iHIVARNA in a phase II study.

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Introduction

Antiretroviral therapy (ART) has proven to be highly effective to prevent HIV-associated clinical progression and death [1] and to influence the AIDS pandemic as part of HIV prevention strategies [2,3]. Despite these successes, current ART has a number of public health, economical and clinical limitations. First, although 80% of HIV-infected individuals in United States and Europe know that they are HIV-infected, only 30 and 50%, respectively, are virally suppressed (hence with low probability of sexual transmission) [4,5]. Second, ART is unable to cure or eradicate the infection [6]. The high cost of lifelong treatment remains an important issue in the implementation of the the Joint United Nations Programme on HIV/AIDS (UNAIDS) strategy of universal treatment. Third, suboptimal treatment adherence can lead to the development of viral resistance [7]. Finally, the potential medium–long term adverse effects of ART have important clinical limitations.

A well tolerated, affordable and scalable cure could address both the individual and public health limitations that are associated with lifelong ART. The scientific community has acknowledged this position and there is a growing interest in developing curative strategies to tackle HIV persistence [8,9], among, which therapeutic vaccines represent one of the most promising approaches [10,11]. However, although, most immunogens have been able to induce HIV-specific immune responses in clinical trials, they have shown very limited efficacy to control viral replication [12]. The cytolytic T-lymphocyte (CTL) escape mutations and a poor antigen presentation by dendritic cells are some of the major hurdles that need to be addressed by rationally designed therapeutic vaccine candidates to improve their effectiveness. New inserts to redirect responses to vulnerable sites of HIV and vectors targeting dendritic-cell pathways could be necessary to achieve remission of HIV-1 infection [13].

Recently, direct administration of mRNA targeting dendritic cells has been proposed as an alternative to the classic immunogens [14–19]. Van Lint *et al.* [20] have designed mRNAs encoding a mixture of activation molecules functional in antigen-presenting cells (APC), including CD40L, a constitutively active variant of Toll-like receptor (TLR) 4 and CD70 (jointly referred as TriMix). The intention behind this strategy is to induce dendritic-cell maturation with CD40L and caTLR4 and to support activated T-cell survival and proliferation with CD70. dendritic cells modified *in vitro* or *in vivo* with TriMix mRNA have been shown to be significantly more potent and immunogenic than unmodified dendritic cell [20,21]. Complementing these advances in vector design, Mothe *et al.* [13] proposed a rational design for the selection of HIV antigens based on the viral targets of protective HIV-1 specific T-cell responses observed in three large cohorts of HIV-infected individuals [13]. This approach resulted in the design of the HIVACAT T-cell Immunogen (HTI) sequence constituting 16 joined fragments of 10–70 amino acids each, encoding critical HIV-1 target epitopes in Gag, Pol, Vif and Nef. These particular HIV-1 regions are more conserved and elicited responses of higher functional avidity and broader cross-reactivity than other segments in the HIV proteome [22].

Mice immunized with mRNA-encoding HTI in combination with TriMix [21] and mice and rhesus macaques immunized with DNA/MVA expressing HTI [22] showed broad and balanced T-cell responses to several segments within Gag, Pol, and Vif. These data demonstrate that it is possible to redirect responses to vulnerable sites of HIV-1 while avoiding the induction of responses to potential decoy targets that may divert effective T-cell responses towards variable and less protective viral determinants.

We have performed the first-in-human phase I dose-escalating clinical trial with naked mRNA containing dendritic cell activation signals (TriMix) and encoding a novel HIV immunogen sequence (HTI) to redirect T-cell immunity in HIV-infected individuals to the most vulnerable viral targets. The primary endpoint of this study included feasibility and safety of the immune intervention, while the secondary exploratory endpoints were immunogenicity, changes in the viral reservoir and in transcriptomic variations.

Patients and methods

Patients and samples

From June 2015 until October 2016 in Barcelona, we conducted a single-center, open-label, dose-escalating phase I clinical trial in 21 chronic HIV-1 infected patients under stable ART with plasma viral load (pVL) below 50 copies/ml and stable $CD4^+$ T-cell counts above 450 cells/μl. Patients received three inguinal intranodal doses of mRNA by ultrasound-guided injections performed by a radiologist at weeks 0, 2 and 4 according to the following dose escalation scheme (see Fig. 1a and b):

Group 1 (control): three patients received 100 μg of mRNA (i.e. 100 μg TriMix mRNA).

Group 2 (control): three patients received 300 μg of mRNA (i.e. 300 μg TriMix mRNA).

Group 3 (study): three patients received 600 μg of mRNA (i.e. 300 μg HTI mRNA and 300 μg TriMix mRNA).

In these three groups, if two or more of the three patients would have developed a dose-limiting toxicity (DLT), Data and Safety Monitoring Board (DSMB) would have been consulted and study could potentially have been

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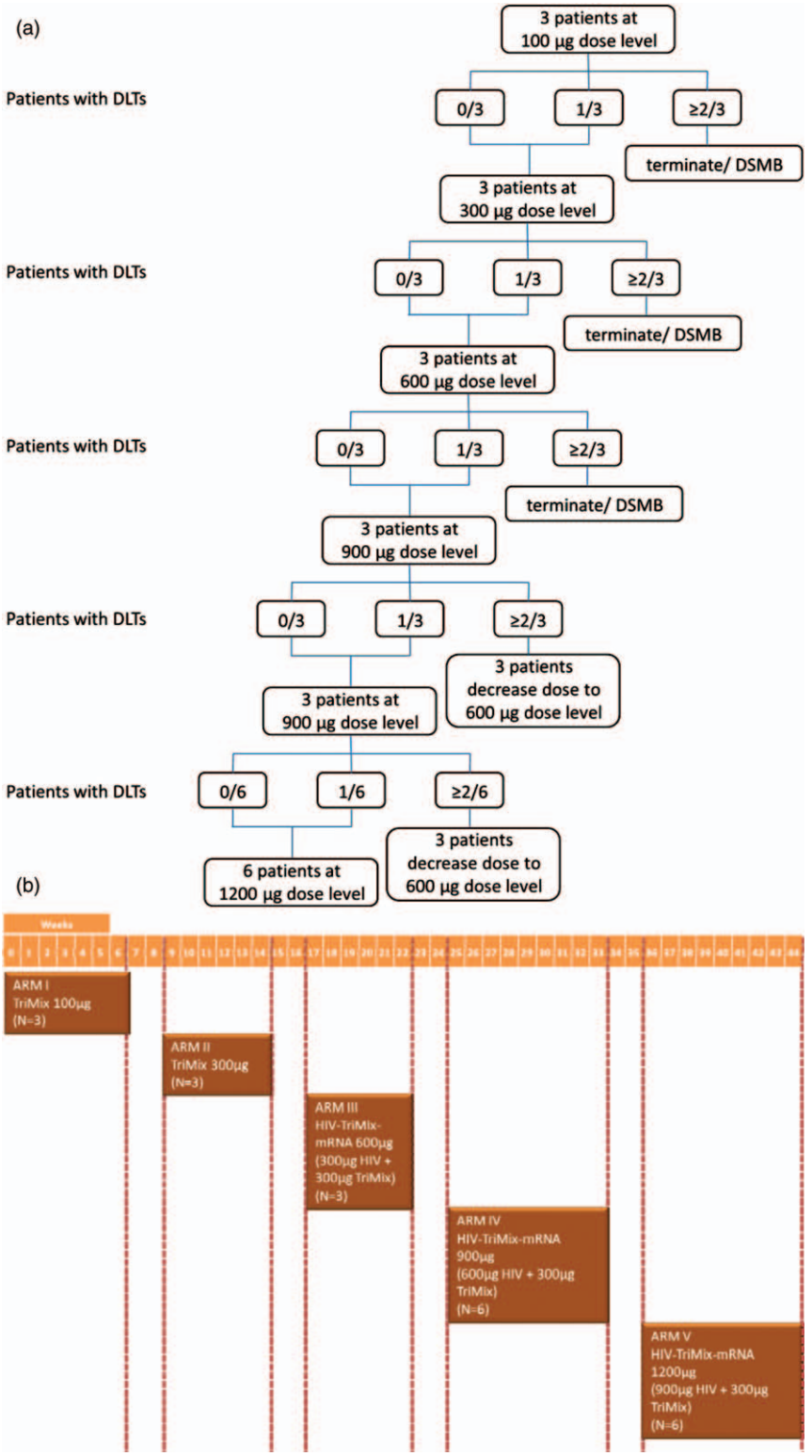

Fig. 1. (a) Dose escalation flow chart. (b) Assignation of cohorts. DSMB, Data and Safety Monitoring Board; DLT, dose-limiting toxicity.

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terminated. If one or no patients would have had a DLT, three patients would be enrolled at the next dose level.

Group 4 (study): three patients received 900 μg of mRNA (i.e. 600 μg HTI mRNA and 300 μg TriMix mRNA). If two or more of the three first patients would have shown DLT, then three additional patients would have been enrolled at the previous dose level (600 μg of mRNA per vaccination). If one or no patients would have had a DLT, three additional patients would have been enrolled at the 900 μg of mRNA dose level. If two or more of the six patients receiving 900 μg of mRNA would have had a DLT, then three additional patients would have been enrolled at the previous level dose (600 μg of mRNA per vaccination). If one or no patients of the six patients would have had a DLT, six patients would be enrolled at the next dose level.

Group 5 (study): six patients received 1200 μg of mRNA (i.e. 900 μg HTI mRNA and 300 μg TriMix mRNA).

Within each group, each patient was observed for a minimum of 1 week before the next patient was treated. All patients within a group were observed for a minimum of 2 weeks after the third vaccination prior to administration of the vaccine to a patient at the next dose level (Fig. 1b). Patients could not change groups or dose assignments upon the initiation of the study protocol. The study treatment period was 4 weeks for each patient. All the patients were followed-up for 24 weeks (see supplementary M&M, http://links.lww.com/QAD/B364).

Immunogenicity evaluations

ELISPOT

Immunogenicity was assessed on cryopreserved peripheral blood mononuclear cells (PBMC) at baseline (i.e. day of first immunization) and weeks 4, 6, 8 and 24 of follow-up by the quantification of T-cell responses by a IFN-γ ELISPOT assay according to standardized operating procedures (SOP) in a single research laboratory. Briefly, cryopreserved PBMC were thawed and rested for overnight at 37 °C. Next, 100 000 PBMCs were stimulated with peptide pools (1 μg of each single peptide) in 100 μl of complete media (Roswell Park Memorial Institute with 10% fetal calf serum) in duplicate conditions. To evaluate the HIV-specific T-cell responses against the whole HIV proteome, we used sets of overlapping HIV peptide pools [10 different pools containing from 5 to 22 15-mer peptides, overlapping by 11-mer, which matched the HTI immunogen ('IN') and 8 pools ranging from 62 to 105 15-mer peptides covering NIH consB HIV sequences not located within HTI ('OUT' pools)]. Media alone in triplicate was used as negative control. Phytohemagglutinin PHA-P (1 μg/ml) and stimulation with CEF (cytomegalovirus, Epstein–Barr virus, and Flu) pool were used as positive controls. Results are expressed as the mean number of spot-forming cells (SFC)/10^6 cells from duplicate wells. The following criteria were used to define the technical validity and positive responses: PBMC viability had to be greater than 80% to be analyzed; the assay background (PBMCs with media alone) had to be less than 50 SFC/10^6 PBMC; positive responses against PHA-P had to be above 500 SFC/10^6 PBMC; and ELISPOT responses were considered positive in case of greater than 50 SFC/10^6 PBMC and number of SFC/10^6 PBMC at least two-fold over media control (Fig. 2).

Flow cytometry assays

We evaluated the activation (or senescence) of T ($CD4^+$ and $CD8^-$), B ($CD19^+$), natural killer cells ($CD56^+$), and monocytes ($CD14^+$) according to the expression of specific surface markers by flow cytometry (see Supplementary Table S1, http://links.lww.com/QAD/B364).

Viral reservoir and ultrasensitive plasma viral load assessment

To explore potential changes induced by the activation of HIV-1-specific latently infected $CD4^+$ T cells, proviral HIV-DNA levels and cell-associated HIV-RNA expression were quantified at baseline and at weeks 4, 6, 8, and 24 of follow-up in peripheral $CD4^+$ T cells. Additional determinations of caHIV-RNA at week 2 +1 day, week 3, week 4 +1 day, and week 5 were performed in groups 4 and 5.

Both the parameters were measured by digital droplet PCR (dd PCR) with two different sets of primers (5′ LTR and Gag loci) to avoid mismatching as previously described [23,24]. caHIV-RNA was calculated as HIV RNA copies relative to the housekeeping gene TATA-binding protein (TBP). Proviral HIV-DNA is expressed as copies of HIV-DNA per million of $CD4^+$ T cells measured by the housekeeping gene *RPP30*.

In addition, the effect of vaccination on plasma viral load (pVL) at baseline and week 2 + 1 day, week 3, week 4, week 4 + 1 day, week 5, week 6, week 8, and week 24 was assessed in groups 4 and 5. In short, 5 ml of EDTA plasma was ultracentrifuged at 170 000 × *g* for 1 h at 4 °C. Tubes were equilibrated with Tris-buffered saline (50 mM Tris-Cl, pH 7.6; 150 mmol/l NaCl) to a final volume of 12 ml. After centrifugation, 11.20 ml of supernatant was carefully aspirated and discarded. The pellet was thoroughly resuspended in the remaining 800 μl and tested for viral load with the Cobas HIV-1 test on the Roche Cobas 4800 system. To account for the concentration of the virus, the obtained result was multiplied by a factor 0.16 (0.8/5).

Transcriptome profiling

Transcriptome profiles were obtained from whole blood samples collected in Tempus blood RNA tubes (Thermo Fisher Scientific, Waltham, Massachusetts, USA), at baseline and week 6. RNA was isolated according to

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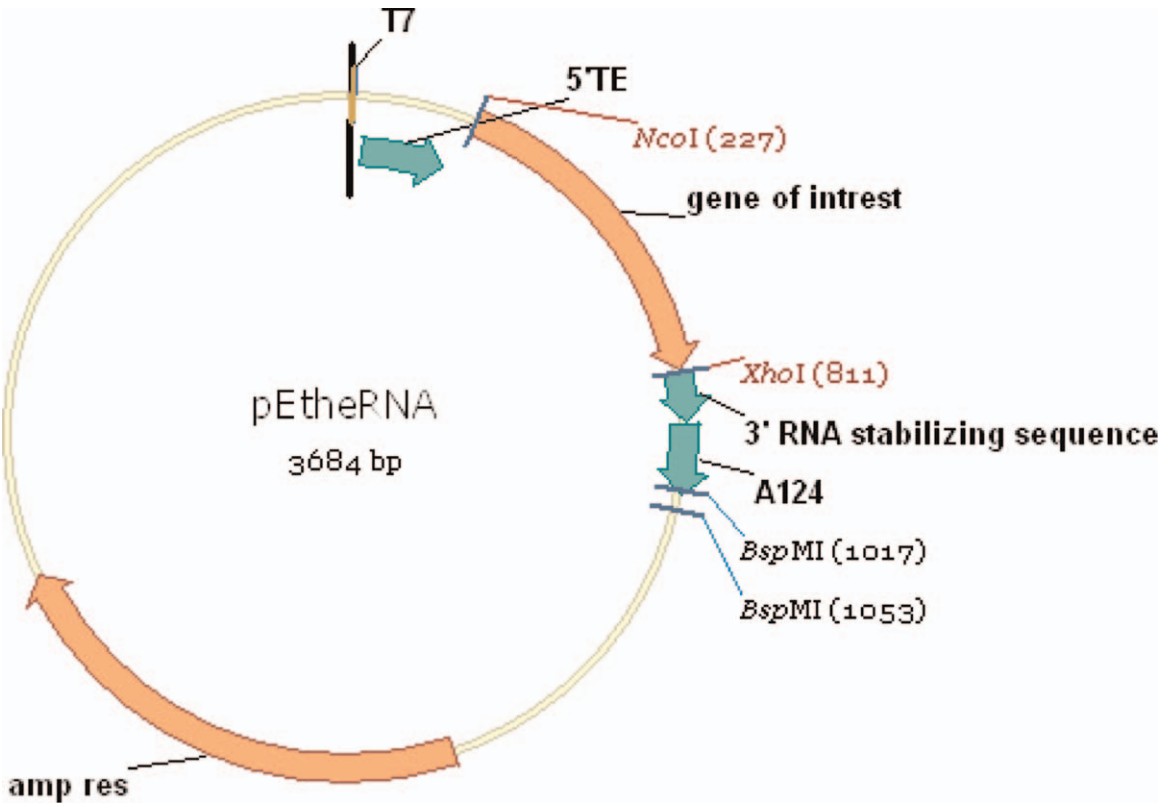

Fig. 2. pETheRNA mRNA vector.

the manufacturer's instructions and hybridized onto Affymetrix Human GenomeU133 Plus 2.0 microarray chips as previously described (http://dx.doi.org/10.1016/j.vaccine.2015.04.047). Samples were quantile normalized and summarized using median polish (i.e. the RMA method). During quality control, we had to remove a single sample as it had insufficient quality to be normalized. Batch effect removal, differential gene expression and gene set analysis were performed using limma. Principal component analysis (PCA) showed batch effects related to RNA processing and hybridization, but these could be effectively removed using limma. Gene set analysis was performed using limma and the REACTOME curated database gene set definitions [25,26].

Statistical analysis

The sample size is the minimum required to the study objectives as stated on Guideline on Requirements for First-in-man clinical trials for potential high-risk medicinal products (EMEA/CHMP/SWP/28367/2007). This was an exploratory study, and the safety analysis were descriptive. The safety endpoints were described and summarized by number and percentage of adverse events and grading. We also stratified the adverse events into related (possibly, probably and definitely related to vaccination) and unrelated to vaccination (unlikely to be related, unrelated). The total magnitude of HIV-1 specific IFN-γ T-cell responses was described as the sum of SFC/million input PBMC. Differences in breadth, magnitude of HIV-1 specific responses, caHIV-DNA, caHIV-RNA or usVL between two longitudinal determinations in the same individuals were assessed by Wilcoxon Signed-Rank Test.

Results

mRNA vaccination with TriMix or iHIVARNA in chronically antiretroviral-treated HIV-1-infected patients is well tolerated

All the 21 patients received the three doses of TriMix or the different doses of iHIVARNA (HTI with TriMix) given by inguinal intranodal route. All 21 participants completed the study as per protocol and were included in the safety analysis. Clinical characteristics of the patients are shown in Table 1. Overall, the vaccine was safe and well tolerated. No serious adverse events nor deaths were observed. A total of 32 adverse events were reported during follow-up (4, 7, 5, 11 and 5 in each group, respectively). Nineteen adverse events were classified as grade 1, twelve as grade 2 and one as grade 3. Half of them ($n = 16/32$) were not related to vaccination, 14 had a possible relationship and 2 had a definite relationship (Table 2). No laboratory abnormalities were observed during the follow-up.

Table 1. Clinical characteristics of participants.

$n=21$	Group 1 ($n=3$)	Group 2 ($n=3$)	Group 3 ($n=3$)	Group 4 ($n=6$)	Group 5 ($n=6$)
Median age (IQR)	48 (48–51)	51 (48–51)	45 (37–46)	55 (53–57)	47 (43–55)
Male	2	2	2	4	6
MSM	2	2	2	4	6
Heterosexual (HTSX)	1	1	1	0	0
Intravenous drug user (IDU)	0	0	0	2[a]	0
Hepatitis C virus (HCV) infection	0	0	0	2[b]	0
Median $CD4^+$ cell count at baseline (IQR)	762 (686–770)	726 (716–824)	821 (741–957)	829 (680–1124)	904 (868–1071)
Median $CD4^+$ cell count at week 6 (IQR)	672 (663–683)	938 (784–993)	643 (589–1040)	819 (640–928)	956 (805–1053)

IQR: Interquartile range.
[a]Former IDU.
[b]Both HCV infections cured: 1 spontaneously (2009), 1 treated with sofobusvir/daclatasvir (2015).

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Increased frequencies of HIV-1-specific T cells after iHIVARNA vaccination

At week 8, patients who had received the highest iHIVARNA dose (group 5) showed a moderate increase in T-cell responses spanning HTI sequence (IN) at week 8 whereas no changes were observed in responses against the rest of the HIV-1 proteome (OUT) compare to baseline (Fig. 3). In addition, the proportion of responders receiving any dose of iHIVARNA ($n=15$) increased from 31% ($n=5$) at week 0 to 80% ($n=12$) postvaccination. This increase was not observed in patients receiving TriMix alone ($n=6$) from 50% ($n=3$) to 67% ($n=4$). The HIV-specific T-cell responses were mainly directed against the following peptide pools: p2 (Gag p17), p4 (Gag p24), p5 (Gag p15), p7 (RT) and p8 (INT), in the IN-peptide pools, whereas in the HIV OUT-peptide pools, the responses were mainly towards: p1 (Gag), p2 (Pol), p4 (Pol), p6 (Env), p7 (Vif, Nef) and p8 (Tat, Vpu, Vpr, Rev; data not shown).

Concerning the analysis of cell subsets, we only observed statistically significant decreases between baseline and week 8 (fourth weeks after the last immunization) in percentages of $CD8^+$ $CD38^+$HLA-DR+ T cells and $CD8^+$ PD-1+ T cells in group 5 (data not shown).

Increased viral expression but stable proviral reservoir after iHIVARNA vaccination

Neither TriMix alone or different doses of iHIVARNA had an impact on proviral HIV-DNA in any of the studied arms (Fig. 4a). However, there was a transient increase in caHIV-RNA expression at higher doses of iHIVARNA (arms 4 and 5) during weeks 4–6 [whereas this was not observed with TriMix alone (groups 1 and 2) or with low doses of iHIVARNA (group 3)], and subsequently normalized at weeks 8 and 24 (Fig. 4b). Moreover, the ratio of ca-HIV RNA at week 6 as compared with week-4 levels was significantly higher in patients receiving any dose of iHIVARNA (groups 3–5 merged) vs. patients receiving TriMix alone ($P=0.0126$). Finally, usVL also significantly increased at weeks 6 and 8 ($P<0.05$) in groups 4 and 5 and returned to baseline values at week 24 (Fig. 4c). In fact, further analysis of these two groups, showed a positive and significant correlation between the increase of the elicited T-cell immune responses against HTI sequence (IN) and the usVL at week 6 ($P<0.05$).

Table 2. Total adverse events classified by severity and relationship with the vaccination.

Variable	Value	Val	I	II	III	IV	V	All
	Grade 1	N	3	4	1	9	2	19
		%	75	57	20	82	40	59
Severity	Grade 2	N	1	3	3	2	3	12
		%	25	43	60	18	60	38
	Grade 3	N	0	0	1	0	0	1
		%	0	0	20	0	0	3
	All	N	4	7	5	11	5	32
	Definite relationship	N	2	0	0	0	0	2
		%	50	0	0	0	0	6
	Probable relationship	N	0	0	0	0	0	0
		%	0	0	0	0	0	0
Casual relationship	Possible relationship	N	1	4	3	6	0	14
		%	75	57	60	55	0	44
	No related	N	1	3	2	5	5	16
		%	75	43	40	45	100	50
	Unknown	N	0	0	0	0	0	0
		%	0	0	0	0	0	0
	All	N	4	7	5	11	5	32

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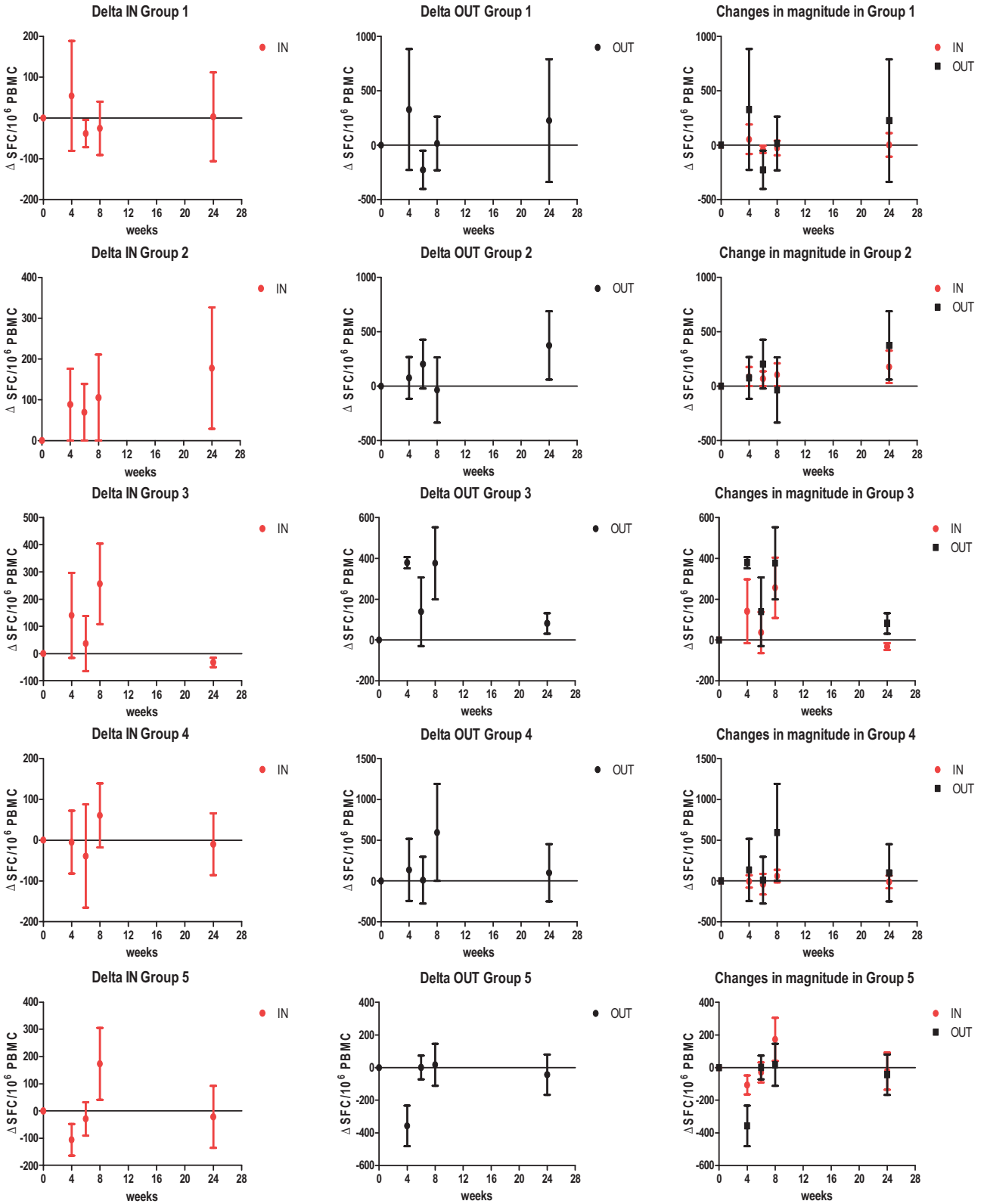

Fig. 3. Changes in the magnitude of total HIV-1-specific immune responses against IN and OUT peptide pools as measured by ELISPOT (at week 0, 4, 6, 8 and 24). Results were considered positive if the number of SFC/10^6 cells in stimulated wells was two-fold higher than that in unstimulated control wells, and if there were at least 50 SFC/10^6 cells after background subtraction. Mean differences from baseline (Δ+/− SEM SFC/10^6 PBMC) are represented on the graphs.

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No significant correlation was observed between T-cell responses against the rest of the HIV-1 proteome (OUT) and the usVL (See Supplementary Fig. S1, http://links.lww.com/QAD/B364).

iHIVARNA vaccination does not shift gene expression

We did not observe robust differentially expressed genes in any of the group-wise comparisons, although gene set analysis indicates some effect on pathways such as RNA metabolism and host response to viruses, but with very low significance levels (Fig. 5). There were no differentially expressed genes in any of the group-wise comparisons or in the immunologic responders vs. nonresponders, based on ELISPOT assays. Gene set analysis indicated some effect of the vaccine on pathways such as RNA metabolism and host response to viruses. These were related to the presence of a patient effect and an effect of the HIV reservoir size. However, these pathways had very low levels of significance (FDR >0.1), indicating that there was only a modest effect on most pathways.

Discussion

Recently, direct intranodal administration of naked mRNA has been proposed as an alternative to the immunogens used so far in HIV vaccination trials. As compared with plasmid DNA and viral vectors, mRNA has a better safety profile, can be easily obtained by commercially available kits and stored at room temperature. Additionally, mRNA-mediated gene transfer occurs in nondividing cells and offers the advantage of not being restricted to a subject-specific human leukocyte antigen (HLA) allele [27]. Promising preliminary results have been reported in other infectious diseases and cancer with mRNA vaccines. Indeed, an mRNA influenza vaccine candidate has demonstrated similar efficacy to licensed vaccines in animal models [14], a prophylactic mRNA-based candidate vaccine against rabies was well tolerated and induced boostable functional antibodies [17] and direct administration of mRNA has entered clinical testing in cancer [15,16,18,19]. To our knowledge, this is the first in human clinical trial using direct intranodal administration of naked mRNA as a therapeutic vaccine against HIV-1 infection. We have shown that intranodal injection of the iHIVARNA vaccine was feasible, safe and well tolerated. No severe adverse events were observed even with the highest dose of the vaccine, namely group 5 constituting a total of 1200 μg of mRNA (900 μg HTI mRNA and 300 μg TriMix mRNA). Therefore, this dose has been selected for a currently ongoing phase II clinical trial.

The vast majority (98%) of latent viruses in chronic HIV-1-infected patients carry CTL escape mutations that render infected cells insensitive to CTLs directed at standard (canonical) epitopes [28]. It is likely that many of the therapeutic vaccines currently under evaluation expand preexisting clones, which are exhausted and target escape variants. There is specific interest in approaches that stimulate responses against novel, nondominant epitopes [13,29,30]. Mothe *et al.* [13] proposed a rational design for the selection of the HIV antigens based on the viral targets of protective HIV-1 specific T-cell responses from three large cohorts of HIV-infected individuals [13]. In addition, two other groups have hypothesized that T-cell vaccines targeting the most conserved regions of the HIV-1 proteome will induce more efficient immune response than whole protein-based T-cell vaccines [31,32]. Letourneau *et al.* [33] designed the HIVconsv immunogen by assembling the 14 most conserved regions of the HIV-1 proteome into one chimeric protein. When delivered in vaccines vectored by MVA and chimpanzee adenovirus, these vaccines were able to shift preexisting immune responses towards conserved, vaccine-encoded regions of HIV in early-treated HIV-infected individuals [29–33]. Our iHIVARNA candidate supports this strategy. The data presented indicate that the vaccine was able to induce moderate HIV-specific immune responses (increase in

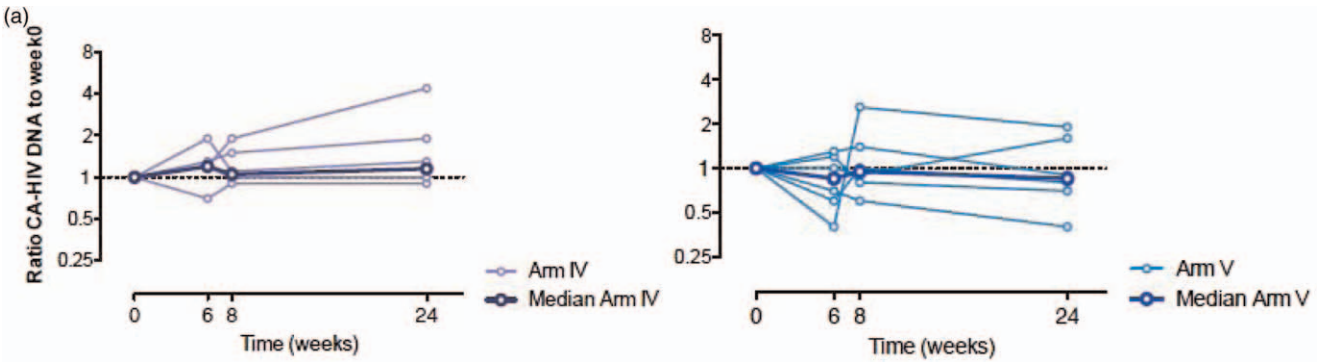

Fig. 4. (a) Impact of the highest doses of iHIVARNA (Groups 4 and 5) in HIV-1 cell-associated total DNA. (b) Impact of TriMix (Groups 1 and 2) or the different doses of iHIVARNA (Groups 3–5) in HIV-1 cell-associated RNA. (c) Impact of the highest doses of iHIVARNA (Groups 4 and 5) in HIV-1 ultrasensitive plasma RNA

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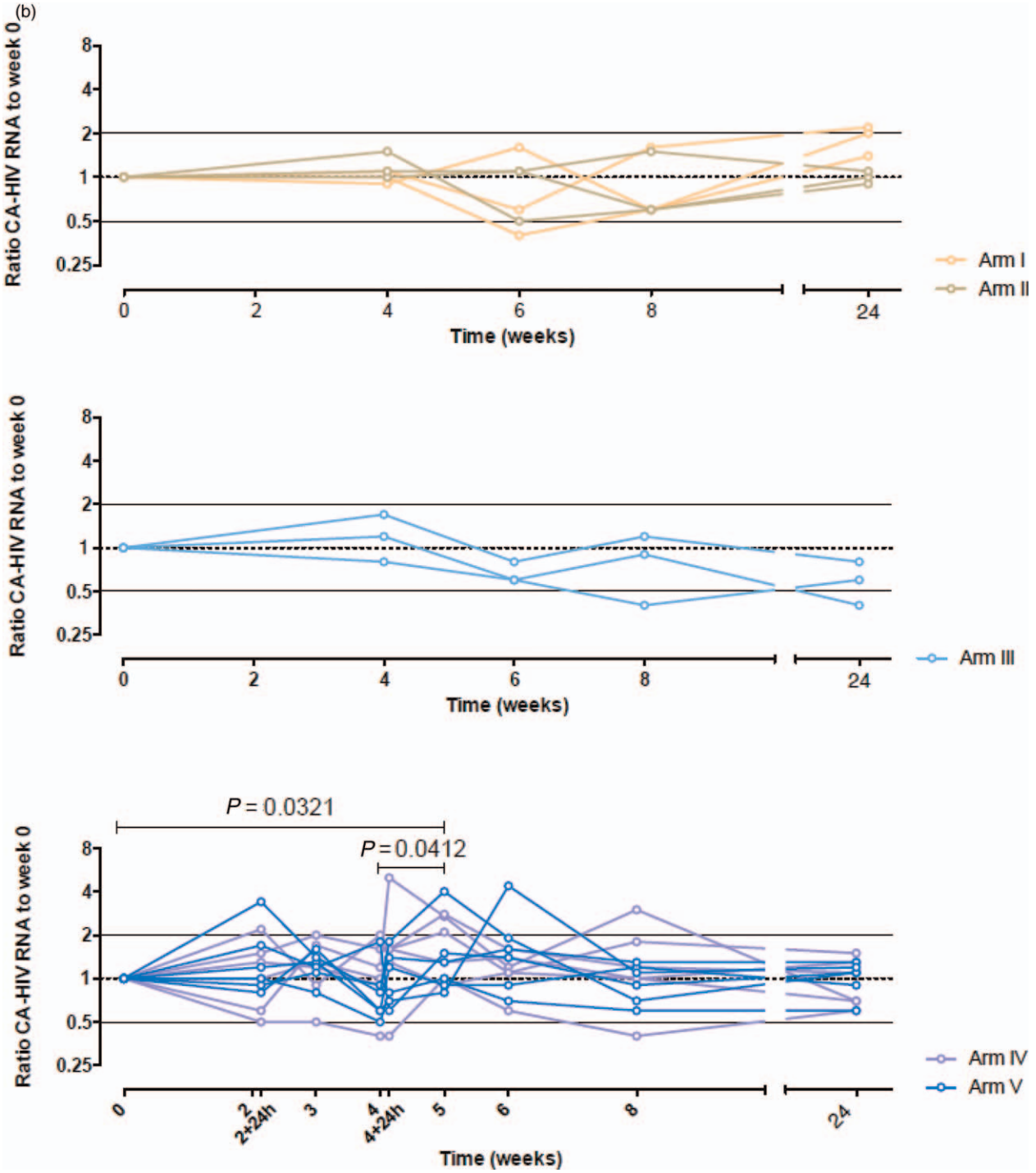

Fig. 4. (Continued).

magnitude and breadth as well as increase in percentage of responders) against overlapping HIV peptide pools, which matched the HTI sequence ('IN') whereas no augmented responses were observed against peptide pools covering HIV proteins not included in the HTI sequence ('OUT'). However, the Phase I results are not conclusive because of the limited number of patients included in each group.

The ELISPOT assay has been established for the direct ex-vivo quantification of peptide-reactive T lymphocytes from peripheral blood mononuclear cells (PBMC). However, it is true that the predictive power of this assay has been challenged because of the lack of efficacy of some HIV vaccine trials despite the induction of robust Elispot responses [34]. This finding and the emergence of new techniques that have the potential advantage of

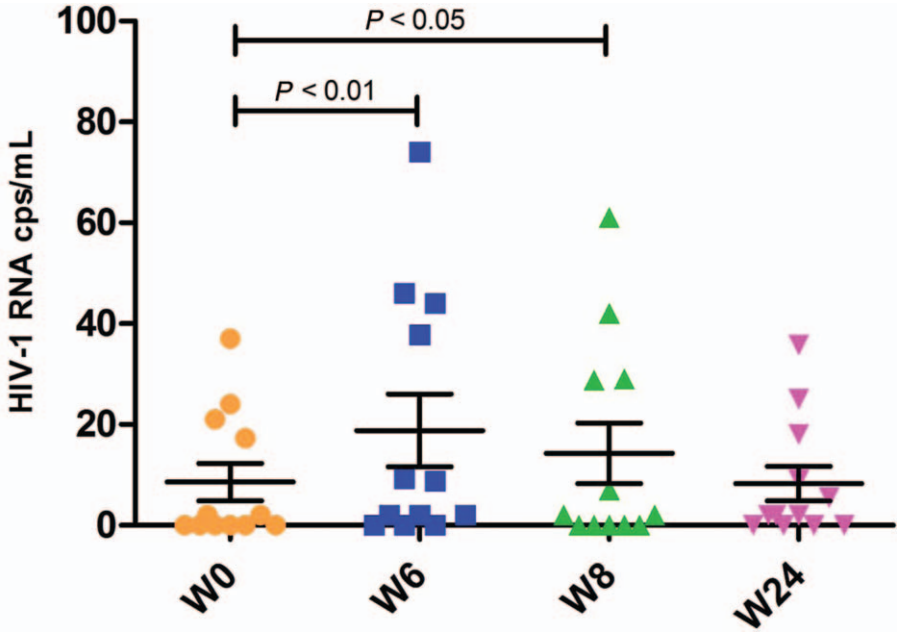

Fig. 4. (Continued).

simultaneously quantifying numerous parameters, raises questions regarding the future role of IFN-γ Elispot in clinical trials of candidate vaccines. Nevertheless, the IFN-γ Elispot assay has been, unlike other techniques, evaluated and validated in several proficiency panels and is advantageous in cost-effectively detecting and mapping T-cell responses [35]. All these benefits are particularly important in a Phase I clinical trial where safety and tolerability were the major end-points.

There is evidence that HIV-1 vaccines are by themselves insufficient to fully harness the stimulatory potential of dendritic cells. It has been suggested that targeting in-vivo dendritic cells by co-stimulatory molecules improves the effectiveness of the vaccines [20,36]. This type of strategy has already been tested in humans with a vaccine co-expressing immune activator molecules. A clinical trial testing a recombinant fowl pox virus vector co-expressing HIV1Gag/Pol and human interferon-γ has been reported [37,38]. In addition, Van Lint *et al.* [20] designed mRNAs encoding a mixture of APC activation molecules, referred as TriMix. Dendritic cells modified *in vitro* or *in vivo* with TriMix mRNA have been shown to be significantly more immunogenic than unmodified dendritic cells [20,21].

The higher doses of iHIVARNA mRNA might have increased HIV expression as a transient increment in caHIV-RNA expression and usVL were observed. It is likely to be triggered by activation of the immune system through recognition of TLRs. However, the existence of a direct and significant association between the elicited HIV-1 immune response against epitopes included in the vaccine (and not to the rest of the proteome) and the usVL (1 or 2 weeks after the last dose) suggest that it could be secondary to an specific stimulus rather than to an ambiguous and unspecific reaction because of the mere addition of mRNA. Given the limited number of patients, this association needs to be further explored in the ongoing phase IIa clinical trial to be confirmed.

Using whole blood-derived transcriptome analyses, we only observed modest effects on inflammatory pathways. These effects were related to intrinsic differences in the activity of inflammatory pathways between individual patients, rather than to the effect of any of the vaccine formulations. Therefore, the data suggest that any immune activation induced by this vaccine is modest and not detectable by comprehensive transcriptome profiling of whole blood samples.

In conclusion, this phase I exploratory dose-escalating trial showed that iHIVARNA vaccination was feasible, harmless and well tolerated, was able to induce moderate HIV-specific immune responses and transiently increased caHIV-RNA expression and ultrasensitive plasma viremia. These data support further exploration of iHIVARNA in the currently ongoing phase II clinical trial.

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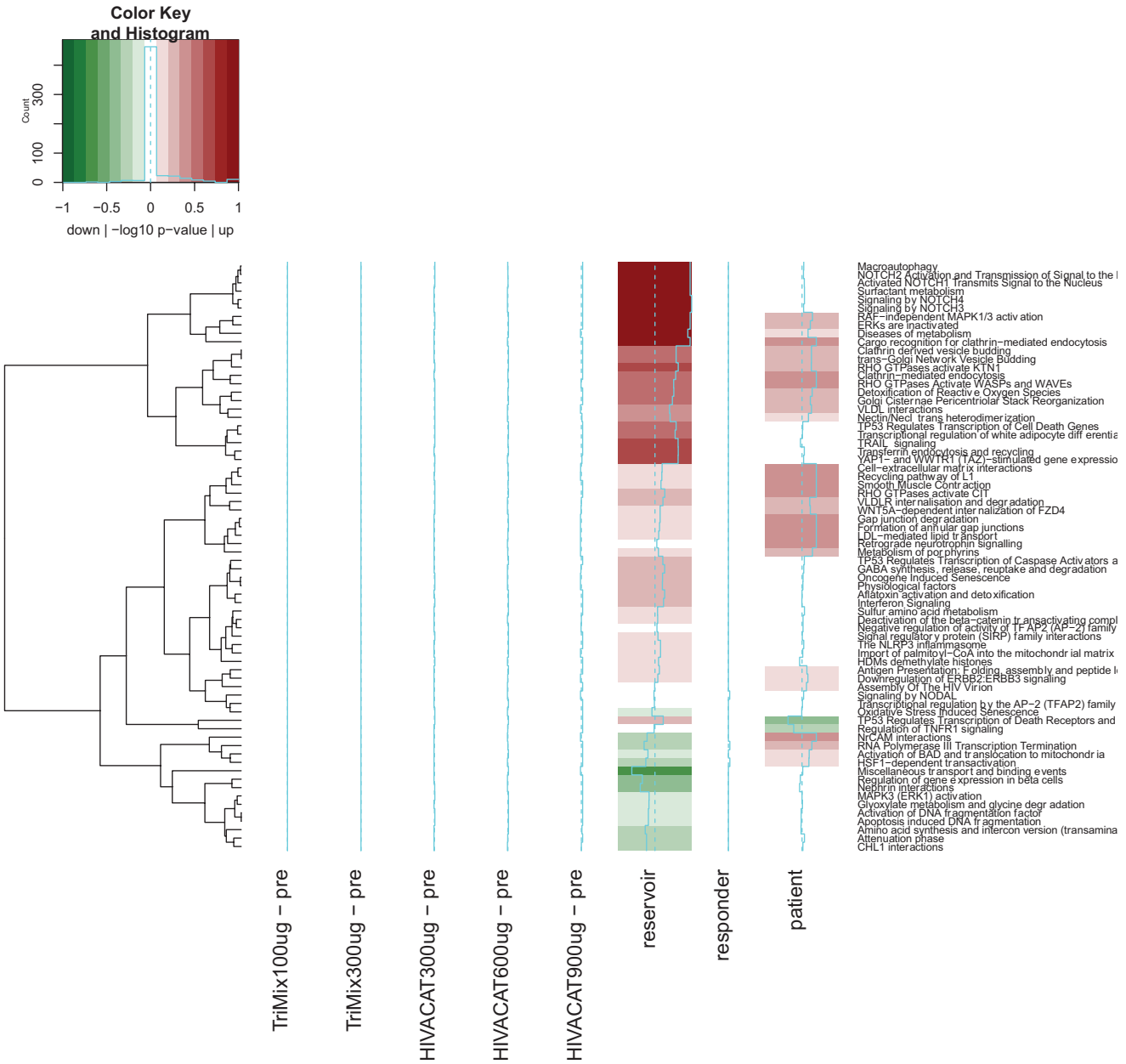

Fig. 5. Reactome gene set analysis.

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Authors contributions: L.L., J.P., J.A.A., J.M.G. and F.G. conducted the clinical trial. A.C.G. and M.P. conducted the immunogenicity analyses. B.M. and C.B. developed the HTI in the iHIVARNA vaccine. S.M.L., M.S. and J.M.P. were in charge of the reservoir study. C.H., S.V.M. and K.T. developed the Trimix in the iHIVARNA vaccine and quality control for the vaccine. G.V. and P.P. studied the changes in usVL. H.H., R.G. and A.A. were in charge of the transcriptome analyses. All authors contributed in writing and revising the manuscript.

Conflicts of interest

There are no conflicts of interest.

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