Supplementary Materials and Methods

Subjects and samples

All the five drug products were formulated in water for injection (WFI) and aliquoted in single dose vials and stored at -20ºC. The drug products correspond to the active substance TriMix or combination of TriMix and HTI. The presentation was vials of 200 µl and during aseptic vialing an extra volume of 10% was aliquoted per single dose. Prior to injection the vialed IMP (investigational medical product) was reconstituted in 880 µl of Hartmann solution. Only 1 ml of the IMPs was administered by ultrasound-guided intranodal injection.

The different doses of TriMix or iHIVARNA were generated by eTheRNA BVBA, Brussels, Belgium. The production of the mRNA follows GMP guidelines and has been certified by the Belgian competent regulatory agency (Federal Agency for Medicines and Health Products, FAMHP). All materials used in the production of the mRNA comply with the requirements of the Note for Guidance EMEA/410/01 Rev. 3 on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products, and are endotoxin and RNase free. In brief, the starting material for in vitro synthesis of mRNA was the plasmid pETheRNA (Figure 2). The different TriMix components CD40L, CD70 and caTLR4 as well as the functional "HTI" open reading frame gene were cloned into different pETheRNA vectors downstream from a T7 promotor sequence and a 5' translation enhancer sequence. The production process started with the linearization of the plasmid DNA that was purified, controlled and subsequently used as template for the in vitro transcription of mRNA. Therefore, the linearized plasmid DNA was incubated with the appropriate T7 reaction buffer, Anti Reverse Cap Analogue/NTP mix and T7 enzyme mix at 37°C and subsequently the plasmid DNA was degraded by addition of DNase1. The mRNA was purified by precipitation with LiCl. After a second precipitation using NaCl to remove traces of lithium ions, the mRNA was resuspended in water for injection. Further analyses included determination of concentration, integrity, RNA identity assay, plasmid DNA contamination, protein contamination, appearance, pH, osmolality, potency, endotoxin level and sterility. The study was approved by the institutional ethical review board and by the Spanish Regulatory Authorities (AEMPS).

The primary endpoints included grade 3-4 local, systemic or other clinical or laboratory adverse events (AE) or any event attributable to vaccination leading to discontinuation of the immunisation regimen. Data on local and systemic events were solicited with specific diary cards for a minimum of 7 days following each immunization. Data on other clinical and laboratory events were collected with an open questionnaire at each visit and through scheduled routine analyses, respectively. The severity of each adverse event was graded by the investigator. Criteria for grading clinical and laboratory events were based on systems in use at NIH Division of AIDS. The investigator stated the relationship of each adverse event to the study medication by using pre-specified terms including: unrelated, unlikely to be, possibly, probably and definitely related to vaccination. Secondary-exploratory endpoints were immunogenicity (as measured by ELISPOT), changes in reservoir [cell-associated HIV-DNA (caHIV-DNA) and cell-associated HIV-RNA (caHIV-RNA)], ultrasensitive plasma RNA (usVL) and transcriptomic profiling (using limma).

Supplementary Fig. S1.- Plots from groups 4 and 5 depicting correlations between the increment of spot-forming cells per 10^6 PBMC at week 6 (respect to their basal) and the usVL (copies/ml) at a similar time are shown. The relationship between both parameters showed a

significant and positive correlation against peptides included in the HTI immunogen (A) whereas correlation was not significant against peptides outside the insert (B).

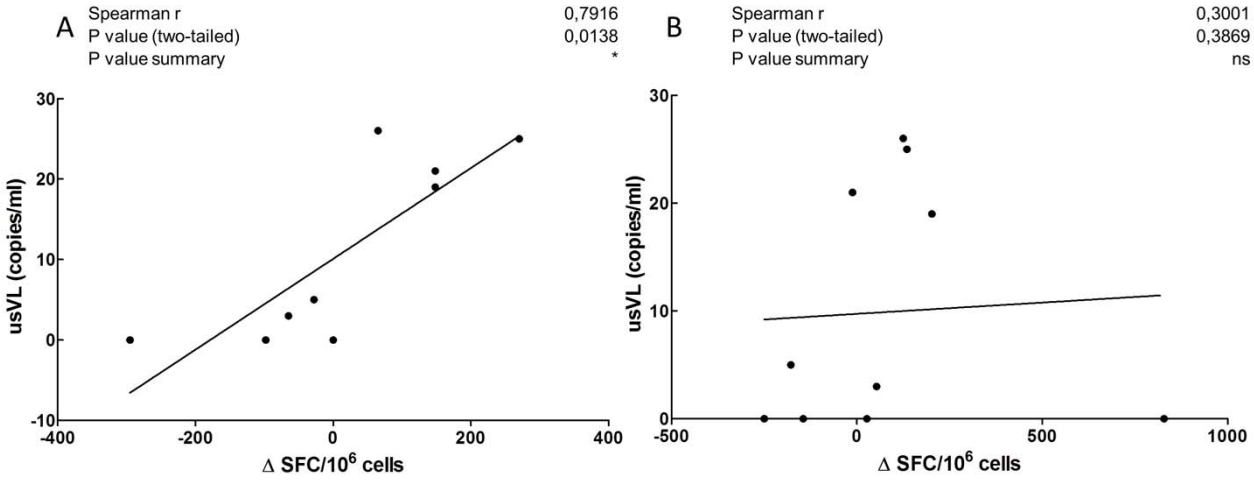

Table S1.- iHIVARNA Phase I – Cytometry activation template

4 color template	Immunophenotype	Tube
T lymphocytes	**Isotype control** (F-PE-PC-APC)	1
	CD4-APC/ **DR-F/ CD38-PE** / CD8-PC	2
	CD4-APC/**CD57-F/ CD28-PE** / CD8-PC	3
	CD4-APC/**CD45RA-F/ CD45RO-PE** / CD8-PC	4
	CD4-APC/**PD-1-F/ CD69-PE** / CD8-PC	5
NK lymphocytes	**CD16-APC***/ HLADR-F/ **CD56-PE / CD3-PC**	6
B lymphocytes	**CD27-APC*/ CD95-F** / **CD19-PE** / HLA-DR-PC	7
Monocytes	**CD16-APC/ CD11b-F** / **CX3CR1-PE*** / CD14-PC	8

- All the antiobodies were purchased from BD Biosciences (San Diego, CA, USA).