EBioMedicine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Cell-Mediated Immune Predictors of Vaccine Effect on Viral Load and CD4 Count in a Phase 2 Therapeutic HIV-1 Vaccine Clinical Trial

Yunda Huang [a,b], Giuseppe Pantaleo [c], Gonzalo Tapia [c], Brittany Sanchez [a], Lily Zhang [a], Monica Trondsen [d], Arnt-Ove Hovden [d], Richard Pollard [e], Jürgen Rockstroh [f], Mats Ökvist [d], Maja A. Sommerfelt [d,*]

[a] Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, M2-C200, WA, USA
[b] Department of Global Health, University of Washington, Seattle, WA, USA
[c] Centre Hospitalier Universitaire Vaudois, Rue du Bugnon 46, BH10-527, CH-1011 Lausanne, Switzerland
[d] Bionor Pharma AS, P.O. Box 1477 Vika, NO-0116 Oslo, Norway
[e] University of California, Davis School of Medicine, 4150 V Street, Suite G500 PSSB, 95817 Sacramento, CA, USA
[f] Oberarzt an der Medizinischen Universitätsklinik, Innere-Rheuma-Tropen Ambulanz, Sigmund-Freud-Str. 25, 53105 Bonn, Venusberg, Germany

ARTICLE INFO

Article history:
Received 10 June 2017
Received in revised form 8 September 2017
Accepted 21 September 2017
Available online xxxx

Keywords:
Analytical treatment interruption (ATI)
HIV
Therapeutic vaccine
Viral load
Immune predictors
CD4

ABSTRACT

Background: In a placebo-controlled trial of the peptide-based therapeutic HIV-1 $p24^{Gag}$ vaccine candidate Vacc-4x, participants on combination antiretroviral therapy (cART) received six immunizations over 18 weeks, followed by analytical treatment interruption (ATI) between weeks 28 and 52. Cell-mediated immune responses were investigated as predictors of Vacc-4x effect (VE) on viral load (VL) and CD4 count during ATI.
Methods: All analyses of week 28 responses and fold-changes relative to baseline considered per-protocol participants (Vacc-4x:placebo = 72:32) resuming cART after week 40. Linear regression models with interaction tests were used. VE was estimated as the Vacc-4x–placebo difference in $\log_{10}$-transformed VL (VE^{VL}) or CD4 count (VE^{CD4}).
Findings: A lower fold-change of CD4+ T-cell proliferation was associated with VE^{CD4} at week 48 ($p = 0.036$, multiplicity adjusted q = 0.036) and week 52 ($p = 0.040$, q = 0.080). A higher fold-change of IFN-γ in proliferation supernatants was associated with VE^{VL} at week 44 ($p = 0.047$, q = 0.07). A higher fold-change of TNF-α was associated with VE^{VL} at week 44 ($p = 0.045$, q = 0.070), week 48 ($p = 0.028$, q = 0.070), and week 52 ($p = 0.037$, q = 0.074). A higher fold-change of IL-6 was associated with VE^{VL} at week 48 ($p = 0.017$, q = 0.036). TNF-α levels (>median) were associated with VE^{CD4} at week 48 ($p = 0.009$, q = 0.009).
Interpretation: These exploratory analyses highlight the potential value of investigating biomarkers in T-cell proliferation supernatants for VE in clinical studies.
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1. Introduction

Vacc-4x is a peptide-based therapeutic human immunodeficiency virus (HIV)-1 vaccine candidate, that aims to induce cell-mediated immune responses to conserved regions of $p24^{Gag}$. Vacc-4x is administered intradermally for uptake by dendritic cells in the presence of granulocyte-macrophage colony stimulating factor (GM-CSF). Dendritic cells are potent antigen presenting cells that have the capacity for cross presentation allowing for epitope presentation on human leukocyte antigen (HLA) classes I and II. Following peptide uptake and intracellular proteolytic processing within dendritic cells, presentation of conserved epitopes on HLA class II stimulates CD4+ T cells. Moreover, presentation of epitopes on HLA class I stimulates CD8+ T cells, which can then kill infected cells presenting these conserved epitopes. Since sustained cytolytic effects of CD8+ T-cells require immune help from CD4+ T-cells, Vacc-4x carries both HLA class I and class II epitopes. Effective cell-mediated immune responses that can control and/or reduce virus levels, may maintain CD4+ T-cell counts and/or slow their decline during analytical treatment interruption (ATI).

Vacc-4x was shown to be safe and immunogenic in a randomized, double-blind, placebo-controlled phase 2 clinical study enrolling 137 HIV-1-infected participants who were virologically suppressed on combination antiretroviral therapy (cART) (Pollard et al., 2014). Six immunizations were given between week 1 (baseline) and week 18 while participants continued cART. At week 28 eligible participants underwent ATI and were removed from cART for up to six months until week 52. Although no differences between the treatment groups

* Corresponding author at: Bionor Pharma AS, P.O. Box 1477 Vika, NO-0116, Oslo, Norway.
E-mail addresses: yunda@scharp.org (Y. Huang), Giuseppe.Pantaleo@chuv.ch (G. Pantaleo), Gonzalo.Tapia@chuv.ch (G. Tapia), bprigmore@scharp.org (B. Sanchez), yzhang2@scharp.org (L. Zhang), rbpollard@ucdavis.edu (R. Pollard), Juergen.Rockstroh@ukb.uni-bonn.de (J. Rockstroh), mo@bionorpharma.com (M. Ökvist), ms@bionorpharma.com (M.A. Sommerfelt).

https://doi.org/10.1016/j.ebiom.2017.09.028
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Please cite this article as: Huang, Y., et al., Cell-Mediated Immune Predictors of Vaccine Effect on Viral Load and CD4 Count in a Phase 2 Therapeutic HIV-1 Vaccine Clinical Tria..., EBioMedicine (2017), https://doi.org/10.1016/j.ebiom.2017.09.028

for the pre-specified efficacy endpoints (cART resumption and changes in CD4 count change during ATI) were observed, there was a significant reduction in viral load (VL) set-point (Pollard et al., 2014) and better preserved CD4 counts (Huang et al., 2016a) in the Vacc-4x group compared to placebo. In addition, both groups demonstrated increases from baseline in HIV-1-specific IFN-γ-secreting peripheral blood mononuclear cells (PBMCs) against p24 antigens by Enzyme-linked ImmunoSpot (ELISPOT) assay at week 52; however, only in the Vacc-4x group was this increase associated with a statistically significant reduction in VL set-point compared to the placebo group during ATI (Pollard et al., 2014). This finding suggests the quality of cell-mediated immune responses (CMIs) during ATI may have differed between the two treatment groups and that specific CMIs elicited by Vacc-4x contributed to the beneficial Vacc-4x effect (VE) on VL and CD4 count.

The objective of this study was to follow up on the prior observations by systematically assessing CMIs at week 28 relative to baseline (wk28/wk1) or on their own (week 28) as predictors of VE during ATI. We hypothesized that T-cell immunity in terms of the magnitude and/or the presence (yes or no) of immune biomarkers (with or without reference to baseline values), especially those related to immune stimulation, T-cell proliferation and inflammation would predict the effect of Vacc-4x (vs. placebo) on controlling VL and/or maintaining CD4 count during ATI. To address these hypotheses, we considered immune biomarkers including interferon-gamma (IFN-γ) secreting T-cells measured by ELISPOT, CD4+ and CD8+ and Total (CD3+) T-cell proliferation measured by a carboxyfluorescein succinimidyl ester (CFSE) assay, and cytokines released from T-cell proliferation supernatants ex vivo measured by the Luminex assay.

2. Materials and Methods

2.1. Study Procedures

The Vacc-4x study took place between July 2008 and June 2010 in Spain, Italy, the USA, the UK, and Germany (Pollard et al., 2014). Participants aged 18–55 years and virologically-suppressed on cART prior to enrollment were randomized to receive four weekly Vacc-4x or placebo priming immunizations and two boosting immunizations at weeks 16 and 18, followed by ATI by week 28. During ATI, participants returned to cART if their CD4 count reached 350 cells/μl or fell by 50%. Pre-ART VL and CD4 count were also available for most participants. All participants in the study had provided informed consent, and the study protocol had been approved by all participating regional ethics committees. Full inclusion criteria can be found at www.clinicaltrials.gov under the identifier NCT00659789.

2.2. Immunological and Safety Measurements

CMIs to p24 antigens were measured at weeks 1 and 28 in PBMCs using ELISPOT and T-cell proliferation assays [CFSE staining]. Cytokine and chemokine concentrations were assessed in proliferation supernatants using a Bioplex 200 Luminex machine (Bio-Rad, Hercules, CA). A total of 22 continuous and 30 binary immune variables based on immune responses measured at week 28 relative to baseline (wk28/wk1 fold-change) or on their own (week 28) were evaluated.

Safety measurements, e.g. lactose dehydrogenase (LDH), white blood cell (WBC) and anti-C5/gp41$^{732–744}$ antibody levels which target a region on HIV-1 envelope glycoporteins, were also determined at baseline and over time. Antibodies to the 5th constant (C5) domain of HIV-1 envelope glycoproteins and a part of the transmembrane glycoprotein gp41, (gp41$^{732–744}$), were tested because they have been shown to be associated with moderate viral load and slower disease progression, suggesting they may have an impact upon HIV-associated immune activation (Sørensen et al., 2017).

2.2.1. Peptides and Protein Antigens

Peptides were synthesized at Schafer-N (Copenhagen, Denmark). All peptides used for in vitro immunological analyses were first resuspended in DMSO and then water. Recombinant p24 core protein, HTLV IIIB was obtained from Bioprocess Pty Ltd. Staphylococcal enterotoxin B (SEB) (200 ng/mL) and media containing DMSO concentrations equivalent to those in the peptide pool wells were used as positive and negative controls, respectively. The p24 antigens used in these analyses were overlapping 15-mer peptides offset by 2 amino acids corresponding to the region on p24 covered by Vacc-4x. In addition, full length recombinant p24 protein clade B was used.

2.2.2. Preparation of Peripheral Blood Mononuclear Cells (PBMCs)

T-cell responses were evaluated from PBMCs prepared at each participating site. After collection, all PBMCs were frozen at −80 °C and shipped to the central laboratory at CHUV, Switzerland within 21 days for storage in liquid nitrogen. The same batch of fetal bovine serum (FBS) was used for all sites to reduce inter-laboratory variation. All sites preparing PBMCs were accredited prior to the study start and their ability to prepare PBMCs with recovery >70% and viability >80% on thawing was confirmed (Boaz et al., 2009).

Ex vivo interferon-γ (IFN-γ) secretion was examined in thawed PBMCs following stimulation with p24 antigens using an ELISPOT assay, T-cell proliferation was examined using a CFSE assay as described in Pollard et al. (2014), and cytokine/chemokine production was assessed in proliferation supernatants using a Luminex assay at weeks 1 and 28.

2.2.3. ELISPOT Assay Criteria

ELISPOT assays were considered valid if the mean of triplicate wells did not exceed 50 spot forming units (SFUs)/10^6 PBMCs in the negative control (medium) and >500 SFUs/10^6 PBMCs in the positive control (SEB). Three ELISPOT assay immune variables were assessed as predictors of vaccine effect: fold-change of the mean SFU in the stimulated over the negative control as well as a continuous biomarker, fold-change of the mean SFU above or below the median value, and the protocol–defined response positivity as dichotomized variables. Assay responses were defined positive if the mean SFU in stimulated was four times or greater than the negative control and at least 55 SFUs per 10^6 PBMCs (Bart et al., 2008; Harari et al., 2008).

2.2.4. T-Cell Proliferation Assays (CFSE)

T-cell proliferation was assessed ex vivo in CD4+ and CD8+ T-cells, as well as in the total T-cell population (CD3+), using a CFSE assay (Cellerai et al., 2010; Perreau et al., 2011). Staphylococcal enterotoxin B (SEB) stimulation and medium alone served as positive and negative controls, respectively (Harari et al., 2008). Three CFSE assay immune variables were assessed as predictors of vaccine effect: fold-change of percent CFSE low dividing cells (CFSELo) in the stimulated over the negative control wells as a continuous biomarker, fold-change of percent CFSElo above or below the median value, and the protocol-defined response positivity as dichotomized variables. Assay responses were defined as positive if the percentage CFSElo dividing cells reactive to p24 antigens was three times or greater than the percentage CFSE low dividing cells in the negative (medium) control (Harari et al., 2008).

2.2.5. Cytokine Measurements by Luminex

In addition, supernatants from proliferation assays were frozen for subsequent Luminex assays. Upon thawing, the concentrations of 27 chemokines/cytokines (pg/mL levels) in the thawed supernatants were determined upon recombinant p24 stimulation using the Luminex platform (BioRad Inc.; Hercules, CA). Among these markers, the following had an appropriate dynamic range and were evaluated as predictors of vaccine effect: IFN-γ, tumor necrosis factor (TNF)-α, T-helper (Th)1, interleukin (IL)-14, IL-13, and Th2; the immune modulators IL-10 and IL-17; and the inflammatory markers IL-6 and macrophage

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