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Authors

Rodriguez, Benigno
Asmuth, David M
Matining, Roy M
et al.

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Safety, Tolerability and Immunogenicity of Repeated Doses of DermaVir, a Candidate Therapeutic HIV Vaccine, in HIV Infected Patients Receiving Combination Antiretroviral Therapy. Results of the ACTG 5176 Trial

Benigno Rodriguez[1,*], **David M. Asmuth**[2], **Roy M. Matining**[3], **John Spritzler**[3], **Jeffrey Jacobson**[4], **Robbie Mailliard**[5], **Xiao-Dong Li**[2], **Ana I. Martinez**[6], **Allan Tenorio**[7], **Franco Lori**[8], **Julianna Lisziewicz**[9], **Suria Yesmin**[10], **Charles R. Rinaldo**[5], and **Richard B. Pollard**[2]

[1]Division of Infectious Diseases and Center for AIDS Research, Case Western Reserve, University/University Hospitals of Cleveland, Cleveland, OH

[2]University of California Davis Medical Center, Sacramento, CA

[3]Harvard School of Public Health, Boston, MA

[4]Drexel University College of Medicine, Philadelphia, PA

[5]University of Pittsburgh, Pittsburgh, PA

[6]National Institute of Allergy and Infectious Diseases, Bethesda, MD

[7]Rush University School of Medicine, Chicago, IL

[8]ViroStatics srl, Sassari, Italy

[9]Genetic Immunity, Budapest, Hungary, and McLean, VA

[10]Social & Scientific Systems, Inc., Silver Spring, MD

Abstract

Background—HIV-specific cellular immune responses are associated with control of viremia and delayed disease progression. An effective therapeutic vaccine could mimic these effects and reduce the need for continued antiretroviral therapy. DermaVir, a topically administered pDNA-nanomedicine expressing HIV (CladeB) virus-like particles consisting of 15 antigens, induces predominantly central memory T-cell responses.

Methods—Treated HIV-infected adults (HIV RNA<50, CD4>350) were randomized to placebo or escalating DermaVir doses (0.1 or 0.4 mg pDNA at weeks 1/7/13 in the low and intermediate-dose groups, 0.8 mg at weeks 0/1/6/7/12/13 in the high-dose group), n=5–6 evaluable subjects/group. Immunogenicity was assessed by a 12-day cultured IFN-γ ELISPOT assay, at baseline and at 9/17/37 weeks, using one Tat/Rev and three overlapping Gag peptide pools (p17/p24/p15).

[*]Address all correspondence and reprint requests to: Benigno Rodriguez, MD, MSc, FIDSA, 2061 Cornell Road, Suite 401, Cleveland OH, 44106, Ph: 216-844-2057, Fax: 216-844-5523, rodriguez.benigno@clevelandactu.org.

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Results—Groups were comparable at baseline. The study intervention was well tolerated, without dose-limiting toxicities. Most responses were highest at week 17 (4 weeks after last vaccination), when Gag p24 responses were significantly greater among intermediate-dose compared to control subjects (median [IQR]: 67,600 [5,633 – 74,368] vs. 1,194 [9 – 1,667] net spot-forming units/million cells, p = 0.032. In the intermediate-dose group, there was also a marginal Gag p15 response increase from baseline to week 17 (2859 [1867 – 56933], p=0.06), and this change was significantly greater than in the placebo group (0 [−713 – 297], p=0.016).

Conclusions—DermaVIr administration was associated with a trend toward greater HIV-specific, predominantly central memory T-cell responses. The intermediate DermaVir dose tended to show the greatest immunogenicity, consistent with previous studies in different HIV-infected patient populations.

Keywords

DermaVir; CTL responses; HIV therapeutic vaccines; HIV-specific immune response

BACKGROUND

Combination antiretroviral therapy (cART) decreases HIV-related morbidity and mortality[1,2] and in some settings, reduces HIV transmission[3–5]. Nonetheless, even during successful cART-mediated suppression of HIV viremia, replication-competent viruses persist in certain tissues and long-lived cells[6], and exaggerated systemic inflammation can be readily demonstrated[7–11]. This in turn is related to a host of end-organ damage syndromes and to accelerated mortality, particularly in the context of limited CD4+ T cell reconstitution. Development of HIV-specific cellular immune reactivity, either spontaneously or in response to therapeutic immunization, is accompanied by amelioration of this cascade of events in humans and in non-human primate SIV infection models[12–24]. Thus, induction of such immune responses may confer physiological and clinical benefits in chronic HIV infection, including potential reductions of antiretroviral exposure with its attendant toxicities. Furthermore, identification of an effective strategy to elicit robust HIV-specific immune responses may improve understanding of the biological correlates of protection against HIV acquisition.

DermaVir is the first *in vivo* dendritic cell-targeting therapeutic vaccine developed for treatment of HIV-infected persons. The vaccine employs a plasmid DNA (pDNA) immunogen to express 15 HIV antigens, in a synthetic pDNA nanomedicine formulation shown to deliver antigen effectively to lymph node DCs and to induce significant expansions of the HIV-specific precursor/memory T cell pool[25–32]. The effects of DermaVir in humans have been analyzed in two previous studies. In a phase I study, nine HIV-infected subjects on suppressive cART who received a single dose (0.1, 0.4 or 0.8 mg pDNA) of DermaVir demonstrated significant increases in HIV-specific memory T cell responses after immunization, and these responses were detectable up to a year after vaccine administration[33]. In a subsequent phase I-II study, 36 cART-naïve HIV-infected patients were assigned to three different doses of DermaVir or matching placebo. Subjects receiving the intermediate dose of 0.4 mg showed a median plasma HIV decrease from baseline, and compared to placebo, of 0.5 $\log_{10}$ copies/mL[34]. Thus, DermaVir appears to induce immune responses in humans reminiscent of those seen in preclinical non-human primate models[35]. A5176 was designed to extend these observations to a larger group of HIV-infected subjects receiving suppressive cART.

METHODS

Study design and study population

A5176 was a multicenter, randomized, placebo-controlled clinical trial comparing three doses of DermaVir and a placebo arm among chronically HIV-infected subjects of both sexes, aged 18–50 years, receiving stable cART, with confirmed plasma HIV RNA <50 copies/mL, CD4+ T-cell count >350 cells/μL, nadir CD4+ T-cell count >250 cells/μL, and negative hepatitis B surface antigen and hepatitis C antibody. Other exclusion criteria are listed in supplemental table 1. Participants were recruited from: University of California Davis Medical Center (Sacramento, CA); Chicago Children's Hospital (Chicago, IL); Metro Health Medical Center (Cleveland, OH); University Hospitals of Cleveland Case Medical Center (Cleveland, OH); and University of Pittsburgh (Pittsburgh, PA). The protocol was approved by the the participating institutions' IRBs of. All subjects provided written informed consent.

Study intervention

Subjects were sequentially enrolled into one of three dosing cohorts and randomized to receive either DermaVir (six subjects per cohort) or placebo (two subjects per cohort). DermaVir or placebo were administered over either two or four skin sites on the left and right upper back and both upper ventral thighs using the DermaPrep device[33]. The selected area was disinfected and exfoliated to disrupt the stratum corneum of the skin and induce mild erythema. This procedure enhances DermaVir nanoparticle endocytosis s by activated Langerhans cells and delivery of pDNA-encoded antigens to naïve T-lymphocytes in the regional lymph nodes[26]. After skin preparation, a patch covering approximately 80 cm^2 was applied over the area, and the vaccine solution was placed on the skin underneath the patch with a needleless syringe. The patch was kept for 3 hours after vaccination. Subjects assigned to the active arm received the following doses of DermaVir: In the low-dose cohort, three vaccinations (0.1 mg DNA/subject, 0.8 mL total over two skin sites of 80 cm^2 each, 0.4 mL/site) on weeks 1, 7, and 13; in the intermediate-dose cohort, three vaccinations (0.4 mg DNA/subject, 3.2 mL total over four skin sites of 80 cm^2 each, 0.8 mL/site) on study weeks 1, 7, and 13; in the high-dose cohort E, six vaccinations (0.4 mg DNA/subject, 3.2 mL total over four skin sites of 80 cm^2 each, 0.8 mL/site) on study weeks 0, 1, 6, 7, 12, and 13. Subjects in the placebo arm received a matching glucose placebo, administered as described above. Each cohort was enrolled sequentially, from low to high dose. Enrollment into each higher dose cohort required that at least 6 of the 8 subjects in the previous dose cohort had been followed on study for ≥14 days after their second study vaccination, and that no subject had experienced a primary safety endpoint, as defined below.

Assessment of safety study endpoints

The primary endpoint was the occurrence of grade 3 or higher clinical or laboratory adverse events possibly or definitely related to study treatment from first day of study treatment to 28 days after last study vaccination. Events were defined and graded according to the current DAIDS AE Grading Table (found at: http://rsc.tech-res.com/safetyandpharmacovigilance). Relationship to study treatment was determined by the protocol core team, including site clinicians, blinded to treatment arm. Reactions felt to be solely due to adhesive and not the vaccine itself were not used to determine the primary safety endpoint. Anti-double stranded DNA was measured by ELISA to exclude potential autoimmune reactions to the immunization.

Assessment of immunogenicity endpoints

We report the results of a previously described[36] extended culture IFN-γ ELISPOT assay as the main immunogenicity readout. The ELISPOT readout was chosen over other immunogenicity endpoints because of its reproducibility and sensitivity across HIV vaccine studies[37]. This assay detects predominantly T-cell precursors with high proliferating capacity (PHPC), a memory T-cell population that has been shown to be induced by DermaVir immunization and to correlate inversely with plasma viremia in untreated HIV infection[36].

Briefly, cryopreserved peripheral blood mononuclear cells (PBMCs) were thawed, rested overnight, and suspended in R10 complete culture medium to a final concentration of 5×10^5 cells/mL. Cases in which cell viability at the beginning of the culture was below 80% were excluded from the analysis. The cut-off of 80% was chosen to remove the artifactual effect of poor viability at the start of the PHPC culture (24 hours after thawing) on the PHPC readout. Cells were plated in 48-well tissue culture plates with the HIV peptide pools, phytohemaglutinin (PHA)/staphylococcal enterotoxin B (SEB) as positive control, or medium alone as negative control, and cultured at 37°C in 5% CO_2 for 12 days. On days 3 and 7, 500 μL of supernatant per well were removed and replaced with fresh complete culture medium supplemented with 10 IU/mL recombinant human IL-2. The HIV peptides were obtained from the NIH AIDS Research and Reference Reagent Program, and were 15 amino acids in length overlapping by 11, representing the entire HIV-1 subtype B sequence of Gag (divided into 3 pools, p17, p24, and p15), Tat, and Rev. At the end of the 12-day culture, cells were harvested, and a standard overnight ELISPOT assay was performed as previously described with minor modifications[38]. Briefly, cells were plated in triplicate into 96-well anti-human INF-γ antibody pre-coated ELISPOT plates (BD Biosciences, San Diego CA) at 2.5×10^4 cells/well and stimulated with each of the HIV peptide pools (5μg/ml), PHA/SEB (5μg/ml), or medium alone. After incubation for 24 hours at 37°C in 5% CO_2, plates were washed and developed following the manufacturer's protocol (BD Biosciences, San Diego CA). Spots were counted using an automated ELISPOT reader. Results were expressed as net IFNγ spot-forming units(SFU)/million PBMCs (the difference between mean SFU/million PBMCs in stimulated wells and mean SFU/million PBMCs in medium-only wells) multiplied by the proliferation index (number of stimulated cells/number of control-stimulated cells at the end of the culture), to account for the expected expansion of T cells during prolonged culture.

Because HIV-specific T-cell responses were frequently detected at baseline, we defined development of a new response in two ways: 1) As any post-vaccination response that was >1 $\log_{10}$ above the $\log_{10}$ baseline response, regardless of study arm; and 2) as an area under the response curve (AUC) to a given peptide antigen pool that was greater than the maximum AUC to the same stimulant observed in the placebo arm, which meant that this definition applied only to those subjects assigned to a DermaVir dosing group. All timepoints were considered, and the point of greatest response magnitude was derived from the data.

Other immunogenicity assays included HIV-specific CD4+ and CD8+ T-cell proliferation by CFSE dye dilution, HIV-specific intracellular cytokine (IFN-γ and IL-2) staining by flow cytometry, and conventional overnight ELISPOT. As previously reported[36,39], these assays were less sensitive than the cultured ELISPOT assay, and will not be reported here.

Statistical analysis

Vaccine site reactions, clinical events, signs and symptoms, laboratory toxicities and adverse events grade 2 or higher from first day of study treatment to 28 days after the last study

vaccination were tabulated by study group considering all subjects who started study treatment. Time-averaged area under the curve was computed, using the linear trapezoidal method, only for subjects who started study treatment with non-missing cultured ELISPOT data until week 37 (i.e. AUC/37). Differences among study groups were analyzed by Fisher's exact test or Wilcoxon's rank sum test, as appropriate. Dose-response trends were assessed by the Jonckheere-Terpstra test. Changes from baseline were assessed by the sign test. All p-values presented were two-sided and nominal, unadjusted for multiple comparisons. Analyses were done using SAS, version 9.2 (SAS Institute, Cary, North Carolina) and StatXact 8 PROCs (Cytel, Cambridge, Massachusetts).

RESULTS

Twenty-six subjects were enrolled. One subject in the intermediate-dose group withdrew voluntarily before receiving any study vaccination and was excluded from all analyses. Another subject was non-compliant with study visits and was discontinued at week 8. Both were replaced. Groups were well balanced with respect to baseline characteristics, except for a slightly younger age in the placebo group. Detailed demographic characteristics are shown in table 1. Median age was 39 years, median baseline CD4 count was 645 cells/μL, 92% of participants were men, and 64% were white. All had plasma HIV RNA <50 copies/mL at study entry.

Safety endpoints

Overall, study interventions were well tolerated. No subject experienced a grade ≥3 treatment-related adverse event (primary endpoint). There were no deaths. The overall proportions of subjects experiencing any adverse event between the first treatment date and 28 days after the last dose were 57% in the placebo arm and 67%, 100%, and 50%, in each of the low-, intermediate- and high-dose DermaVir groups, respectively (p = 0.31). Differences were also not significant comparing the placebo to the combined Dermavir arms (p=0.64). General body complaints (aches/pain/discomfort, asthenia/fatigue/malaise, fever) were most common and occurred in 7 subjects, followed by cutaneous symptoms (pruritus, rash) and blood chemistry abnormalities in 5 subjects each. A summary of adverse events is shown in table 2. The proportion of treatment-related adverse events was comparable between the DermaVir and placebo arms (0/7, 3/6, 1/6 and 0/6 in the placebo arm and the low-, intermediate- and high-dose DermaVir groups, respectively, p = 0.063). All adverse events felt to be possibly, probably or definitely related to study agent and all abnormal blood chemistries were mild (grade 1–2) and no subject had to modify or discontinue the study treatment due to an adverse event. None of the subjects with available data developed detectable anti-double stranded DNA antibodies.

Changes in CD4+ and CD8+ T-cells

CD4+ T and CD8+ T-cell counts were measured at weeks 0, 3, 9, 15, 17, 24, 37, and 61, and did not change significantly during the study in any of the dosing groups (p ≥0.688 and ≥0.219, respectively). Median (IQR) change in CD4+ T-cell count from baseline to week 61 was +13 (−132, +229) cells/μL in the combined DermaVir groups vs. +151 (−126, +237) in the placebo group. Differences between the DermaVir arm (either combined or each group separately) and the placebo arm were not statistically significant at any of the measured timepoints, and there was no evidence for a dose-related trend in CD4+ T-cell change across the dosing groups of Dermavir (Jonckheere-Terpstra test p-values for all timepoints >0.49).

Changes in plasma HIV RNA

All subjects had plasma HIV RNA below the limit of detection at baseline, and all maintained virologic suppression through week 24, except for three subjects who had

episodes of transient viremia (one subject in each of the DermaVir groups, with 65 copies at week 9, 274 copies at week 24, and 933 copies at week 61, respectively).

Extended culture ELISPOT (PHPC) responses

Cultured ELISPOT responses are shown in Figure 1. There was considerable baseline variability in all groups. Overall, there was a trend toward higher responses in the DermaVir groups, particularly the low- and intermediate-dose groups, compared to placebo. Responses in the high-dose group were not significantly different than the placebo group at any of the studied timepoints in response to any of the peptide pools used (all P>0.05), and responses in this group did not correlate significantly with baseline CD4+ T cell count. At week 17 (four weeks after last study vaccination), responses to Gag p24 were significantly greater among subjects in the intermediate-dose group compared to control subjects (median [IQR]: 67,600 [5,633 – 74,368] vs. 1,194 [9 – 1,667] net SFU/million cells, p = 0.032. Responses to both Gag p24 and to Gag p15 among intermediate-dose subjects were also significantly greater than among high-dose subjects at week 17 (67,600 [5,633 – 74,368] vs. 485 [172 – 1,440], p = 0.017 and 17,067 [5,495 – 67,600] vs. 642 [74 – 4,086] net SFU/million cells, p = 0.03.

The increased anti-HIV T cell response frequency in the intermediate-dose group was consistent across stimulus conditions (figure 1): with the exception of responses to Gag p15 at week 37, responses in the intermediate-dose group were higher than those in the placebo group and the other two DermaVir dosing groups at all post-vaccine timepoints (figure 1), although the difference was only significant at week 17.

Because baseline reactivity showed substantial variation, we tested whether there was evidence for a change from baseline in cultured ELISPOT responses in any of the groups (not shown). Response to Gag p15 increased significantly among participants in the high-dose group from baseline to week 17 (median [IQR] change 149 [44 – 3049] net SFU/million, p=0.031), but this increase was not significantly different from placebo (p=0.247). Participants in the intermediate-dose group experienced a marginally significant increase from baseline at the same time point in response to Gag p15 (2859 [1867 – 56933], p=0.06), and this change was significantly greater than in the placebo group (0 [−713 – 297], p=0.016). We observed a similar trend toward a greater absolute week 0–37 AUC in the intermediate-dose group compared to placebo in response to Gag p24, Gag p15 and Tat-Rev (figure 1), although these differences were marginal (all p = 0.057). In this analysis, the intermediate-dose group also showed significantly greater responses to each of those peptide pools compared to the high-dose group (p = 0.019, 0.019 and 0.038, respectively).

Finally, we compared the proportions of subjects who achieved a response to the study intervention in each of the groups. When a response was defined as a 1-$\log_{10}$ net SFU increase from baseline to each of the Gag peptide pools, 20 to 60% of the DermaVir recipients were classified as responders, compared to 20% of the placebo recipients, although the differences were not statistically significant (table 3). A similar trend was observed when we defined a DermaVir-induced response as an area under the curve in any DermaVir dosing group that was higher than the AUC in the placebo group. In the latter analysis, 100% of subjects in the low- and intermediate-dose groups achieved a response, compared to only 33% of subjects in the high-dose group (table 3).

DISCUSSION

An effective therapeutic vaccine capable of inducing durable HIV-specific CD8+ T-cell responses with potent cytotoxic activity and reducing viremia could theoretically confer many of the benefits of cART without some of its limitations. Evidence in favor of this

approach comes from observations in non-human primate models indicating that cytotoxic T-cell responses are crucial in maintaining spontaneous and DermaVir-induced control of viremia[15,35], by the recognized persistence of vigorous CD8+ T-cell responses in human long-term non-progressors[40–42], and by the over representation of protective HLA alleles thought to be capable of presenting highly conserved HIV epitopes among spontaneous controllers of HIV disease progression[43]. Previous human studies have shown trends toward enhanced immune responses and virologic control after administration of various CTL-targeting therapeutic vaccination strategies[33,44–52]. Further, *in vitro* studies indicate that Gag peptide stimulation of CD8+ T-cells results in efficient killing of autologous HIV-infected CD4+ targets reactivated from latency, suggesting that induction of CTL responses may be a component in a successful reservoir eradication strategy[53].

In A5176, we administered escalating doses of DermaVir, a therapeutic vaccine candidate, or matched placebo, to 25 HIV-infected patients with chronic HIV infection receiving suppressive cART. The study interventions, including repeated doses of DermaVir, were well tolerated, without dose-limiting toxicities. Adverse events were generally mild, and there was no significant difference in adverse event frequency among study groups (table 2).

DermaVir administration tended to be associated with greater cultured ELISPOT (PHPC) responses to all three Gag peptide pools compared to placebo, particularly among patients who received the intermediate dose of DermaVir. These DermaVir-induced T-cell responses were greatest at week 17, 4 weeks after the last immunization dose. Responses to Tat/Rev, on the other hand, were infrequently observed and of low magnitude (figure 1), perhaps due to the smaller number of potential high-affinity epitopes in these small proteins[30]. While the large number of comparisons included in the analysis could conceivably have led to spurious results, these findings are consistent with a previous study in treatment-naïve patients, in which the 0.4 mg dose was associated with a 0.5 log reduction in plasma HIV RNA and with the most robust cultured ELISPOT responses at 24 weeks[34]. The greater immunogenicity of the intermediate dose may reflect high-dose antigen-induced hyporesponsiveness, as shown in animal models[54].

The nature of the T-cell responses elicited in this study deserves separate comment. Although T cell subsets were not sorted or characterized in this study, the cultured ELISPOT assay detects predominantly antigen-specific, long-lived central memory T-cells[55,56], and robust central memory T-cell responses have been associated with spontaneous control of HIV viremia[57]. Thus, DermaVir could enhance the type of cellular immune responses needed for control of plasma viremia, which are almost universally lost early in the course of HIV infection.

Nonetheless, the magnitude of DermaVir-induced responses in this study was variable and reached statistical significance relative to placebo only at week 17 for the intermediate-dose group and only in response to certain peptide pools. A number of limitations in the study design may have contributed to these findings. First, the small sample sizes in each of the dosing groups hindered detection of significant differences, despite responses in the DermaVir groups that were thousands of net SFUs above those in the placebo group (figure 1). Second, in this as in most HIV vaccine studies, predicting and monitoring the breadth and magnitude of meaningful HIV-specific responses remains a major challenge, because the range of immunodominant epitopes varies dramatically from person to person as a result of the complex sequence of mechanisms that enable the establishment of immunodominance[58]. Indeed, an analysis of the high-affinity epitopes present in Dermavir predicted that this immunogen can potentially present up to 933 high-affinity MHCI-restricted epitopes and up to 2330 high-affinity MHCII-restricted epitopes[30]. By comparison, the peptide pools used in our monitoring assay cover only approximately 25%

of the entire repertoire potentially presented by DermaVir. Therefore, a more closely matched set of peptides may be needed to test the full range of immunogenicity induced by this candidate preparation. Equally important will be to assess the HLA background of future study participants, and to map in greater detail the epitopes predicted to be bound with highest affinity.

This short-term study was not designed to assess efficacy, as neither clinical nor virological endpoints were included in the study design, but possible roles for this therapeutic vaccine strategy can nonetheless be envisaged. If, as shown previously[34], DermaVir is capable of reducing plasma HIV RNA durably and safely in the absence of cART, this approach could allow delayed initiation or even interruption of antiretroviral therapy in some individuals, or it could be used as a bridge strategy for patients awaiting new antiretroviral options in the setting of multidrug resistance or intractable toxicity. Therapeutic immunization could also be a component of a future multi-pronged approach to eradicate HIV infection.

In summary, DermaVir is a safe candidate therapeutic vaccine that may induce HIV-specific central memory T-cell responses in a some HIV-infected persons receiving suppressive cART. Larger studies, potentially including DermaVir immune intensification on cART prior to activation of latently infected cells followed by an analytic treatment interruption, are necessary to quantify the entire repertoire of potential responses more accurately, to explore the clinical, virological, and immunological effects of repeated administration, and to define the potential therapeutic role of DermaVir in the HIV armamentarium.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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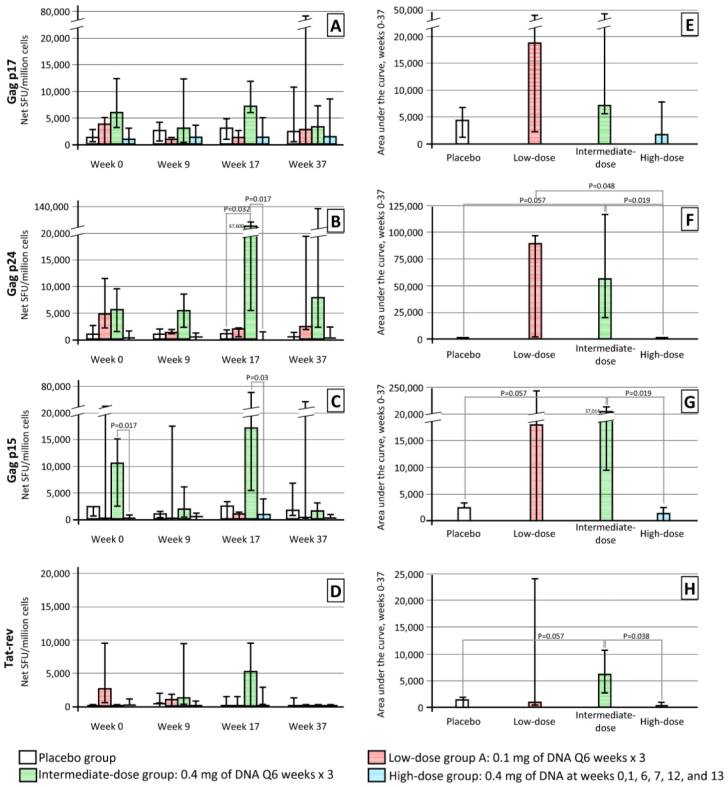

Figure 1.

PHPC responses to escalating doses of Dermavir in A5176, shown as spot-forming units (SFU)/million cells (A, B, C, D) or as the area under the curve of responses from week to week 37 (E, F, G, H). Bars represent the median values and error bars the interquartile range.

Table 1

Characteristics of study participants. Data are shown as n (%), unless otherwise specified

	Study group				
	Placebo, n = 7 (Placebo × 3 or 6 doses)	**Low-dose, n=6) (DermaVir, 0.1 mg × 3 doses)**	**Intermediate- dose, n=6 (DermaVir, 0.4 mg × 3 doses)**	**High-dose, n = 6 (DermaVir, 0.4 mg × 6 doses)**	**Overall**
Age, years, median (IQR)	34 (30, 35)	43.5 (32, 48)	41 (38, 43)	45.5 (34, 47)	39 (32, 45)
Male gender	6 (86)	5 (83)	6 (100)	6 (100)	23 (92)
Race/Ethnicity					
White, non-hispanic	3 (43)	4 (67)	4 (67)	5 (83)	16 (64)
Black, non-hispanic	4 (57)	1 (17)	1 (17)	1 (17)	7 (28)
Hispanic, any race	0 (0)	1 (17)	1 (17)	0 (0)	2 (8)
History of IV drug abuse	1 (14)	0 (0)	0 (0)	0 (0)	1 (4)
CD4+ T cell count, cells/μL, median (IQR)	674 (611, 762)	664 (608, 718)	799 (592, 1123)	551 (517, 704)	645 (565, 762)

Table 2

Numbers of subjects experiencing adverse events of grade 2 severity occurring from first day of study treatment to 28 days after the last study vaccination. Data are shown as number of subjects (percent). All events were included in the categories shown. Some subjects had more than one event. The highest severity of a given event for each subject was recorded

	Study group					
	Placebo, n = 7 (Placebo × 3 or 6 doses)	**Low-dose, n=6) (DermaVir, 0.1 mg × 3 doses)**	**Intermediate- dose, n=6 (DermaVir, 0.4 mg × 3 doses)**	**High-dose, n = 6 (DermaVir, 0.4 mg × 6 doses)**	**Overall**	**P- value**
Any event	4 (57.1)	4 (66.7)	6 (100)	3 (50)	17 (68)	0.306†
General/systemic	1 (14.3)	2 (33.3)	2 (33.3)	2 (33.3)	7 (28)	
Ache/pain/discomfort	0 (0)	1 (16.7)	1 (16.7)	2 (33.3)	4 (16)	
Asthenia/fatigue/malaise	1 (14.3)	1 (16.7)	1 (16.7)	0 (0)	3 (12)	
Fever	0 (0)	0 (0)	1 (16.7)	0 (0)	1 (4)	
Skin abnormalities	2 (28.6)	3 (50)	0 (0)	0 (0)	5 (20)	
Pruritus	1 (14.3)	2 (33.3)	0 (0)	0 (0)	3 (12)	
Rash	1 (14.3)	1 (16.7)	0 (0)	0 (0)	2 (8)	
Laboratory abnormalities*	2 (28.6)	2 (33.3)	4 (66.7)	2 (33.3)	10 (40)	
Neurologic abnormalities**	1 (14.3)	1 (16.7)	1 (16.7)	1 (16.7)	4 (16)	
Lymphadenopathy/edema	0 (0)	1 (16.7)	1 (16.7)	0 (0)	2 (8)	

*Events included hypoglycemia, hypo/hyperphosphatemia, increased creatinine, increased SGPT, and hyperbilirubinemia

**Events included sleep disturbances, headache, and memory loss

†Testing for a difference among all groups. Pairwise comparisons between groups were also not statistically significant

Table 3

Summary of new HIV-specific T cell responses on study. Two definitions of a new response were used. In definition 1, subjects were considered responders if they had any post-vaccination cultured ELISPOT titer that was >1 $\log_{10}$ above the $\log_{10}$ ELISPOT response at baseline, regardless of study arm. In definition 2, a response was an area under the response curve (AUC) to a given peptide antigen pool that was greater than the maximum AUC to the same stimulant observed in the placebo arm. Because the latter definition was intended to account only for those responses that exceeded the greatest background responses seen in the absence of DermaVir exposure, this meant that subjects in the placebo group could not be classified as responders under this approach. Similarly, this definition required no missing data up the 37-week timepoint. Data are shown as number (percent) of responders per group

	Peptide pool					
	Gag p17	**Gag p24**	**Gag p15**	**Tat-Rev**	**Any peptide pool**	**P-value**
Responder definition 1						
Study group						
Placebo (N =5)	1 (20)	1 (20)	1 (20)	4 (80)	4 (80)	
Low-dose (N = 5)	2 (40)	3 (60)	2 (40)	1 (20)	4 (80)	1*
Intermediate-dose (N = 5)	2 (40)	2 (40)	1 (20)	3 (60)	4 (80)	1*
High-dose (N = 6)	3 (50)	2 (33.3)	2 (33.3)	1 (16.7)	3 (50)	0.545*
Combined DermaVir (N = 16)	7 (43.8)	7 (43.8)	5 (31.3)	5 (31.3)	11 (68.8)	1*
Responder definition 2						
Study group						
Low-dose (N = 3)	2 (66.7)	3 (100)	2 (66.7)	1 (33.3)	3 (100)	0.167**
Intermediate-dose (N = 4)	2 (50)	4 (100)	4 (100)	4 (100)	4 (100)	0.076**
High-dose (N = 6)	2 (33.3)	1 (16.7)	1 (16.7)	1 (16.7)	2 (33.3)	
Combined DermaVir (N = 13)	6 (46.2)	8 (61.5)	7 (53.8)	6 (46.2)	9 (69.2)	

* Comparing each group to the placebo group, Fisher's exact test

** Comparing to the high-dose group, Fisher's exact test. P value is not defined for the comparison of the low- and intermediate-dose groups (both 100%).