Clinical Trial of the Anti-PD-L1 Antibody BMS-936559 in HIV-1 Infected Participants on Suppressive Antiretroviral Therapy

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Background. Reversing immune exhaustion with an anti-PD-L1 antibody may improve human immunodeficiency virus type 1 (HIV-1)–specific immunity and increase clearance of HIV-1–expressing cells.

Methods. We conducted a phase I, randomized, double-blind, placebo-controlled, dose-escalating study of BMS-936559, including HIV-1–infected adults aged ≥18 to ≤70 years on suppressive antiretroviral therapy with $CD4^+$ counts ≥350 cells/μL and detectable plasma HIV-1 RNA by single-copy assay. Data on single infusions of BMS-936559 (0.3 mg/kg) versus placebo are described. The primary outcomes were safety defined as any grade 3 or greater or immune-related adverse event (AE) and the change in HIV-1 Gag-specific $CD8^+$ T cell responses from baseline to day 28 after infusion.

Results. Eight men enrolled: 6 received 0.3 mg/kg of BMS-936559, and 2 received placebo infusions. There were no BMS-936559-related grade 3 or greater AEs. In 1 participant, asymptomatic hypophysitis (a protocol-defined immune-related AE) was identified 266 days after BMS-936559 infusion; it resolved over time. The mean percentage of HIV-1 Gag-specific $CD8^+$ T cells expressing interferon γ increased from baseline (0.09%) through day 28 (0.20%; $P = .14$), driven by substantial increases in 2 participants who received BMS-936559.

Conclusions. In this first evaluation of an immunologic checkpoint inhibitor in healthy HIV-1–infected persons, single low-dose BMS-936559 infusions appeared to enhance HIV-1–specific immunity in a subset of participants.

Clinical Trials Registration. NCT02028403.

Keywords. human immunodeficiency virus type 1 (HIV-1); anti-PD-L1; checkpoint inhibitors; HIV eradication; HIV cure; immune response; BMS-936559.

Low-level human immunodeficiency virus type 1 (HIV-1) antigen expression and viremia persist in HIV-1–infected patients on clinically effective combination antiretroviral therapy (cART) [1]. Chronic HIV-1 antigen stimulation upregulates inhibitory coreceptors such as PD-1 and CTLA-4 on T cells [2–4], resulting in "immune exhaustion" [5] and downregulation of HIV-specific cellular immune responses [6]. These inhibitory coreceptors, called immune checkpoints, dampen immune responses and provide protection from autoimmunity.

Received 7 February 2017; editorial decision 11 April 2017; accepted 17 April 2017; published online April 18, 2017.

Presented in part: Conference on Retroviruses and Opportunistic Infections, Boston, Massachusetts, 22–25 February 2016. Abstract 25.

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Increased expression of PD-1 and CTLA-4 on $CD4^+$ and/or $CD8^+$ T cells is associated with disease progression in untreated HIV-1 infection [7–11]. Although cART reduces PD-1 expression on HIV-1–specific $CD8^+$ and $CD4^+$ T cells [7, 12], PD-1 expression remains elevated compared with uninfected participants [7, 13, 14]. Expression of PD-L1, a ligand for PD-1, is also upregulated on antigen-presenting cells [15] and $CD4^+$ and $CD8^+$ HIV-1–specific T cells despite cART [13, 16].

Antibodies against PD-1 and PD-L1 have revolutionized cancer immunotherapy [17]. They have been studied in patients with hepatitis C virus [18] and in animal models of viral infection [19, 20]. In untreated simian immunodeficiency virus (SIV)–infected macaques [21, 22], anti-PD-1 antibody administration expanded and increased functionality of virus-specific $CD8^+$ T cells [21], significantly reduced plasma SIV RNA, prolonged survival [21], and reduced markers of immune activation [22]. In a subsequent study, 4 of 8 SIV-infected macaques on suppressive ART administered an anti-PD-L1 monoclonal antibody (BMS-936559) had delayed SIV rebound with ART

discontinuation, and 2 maintained SIV RNA below detectable limits intermittently for >8 weeks [20]. Simian immunodeficiency virus RNA levels were significantly lower in BMS-936559–treated macaques versus isotype control animals ($P = .02$). Anti-PD-L1 antibody administration reduced HIV-1 replication and increased $CD4^+$ T cells in untreated, HIV-infected humanized mice [23].

While promoting antitumor activity in patients with advanced malignancies [24, 25], anti-PD-1 and anti-PD-L1 and other checkpoint inhibitors [26] revealed immune-mediated adverse events (AEs) likely related to disrupted self-tolerance. In 1 study of BMS-936559 [24], 39% of 207 cancer patients receiving multiple doses (0.3, 1, 3, or 10 mg/kg) had possible immune-related AEs, including predominantly grade 1 or 2 rash, hypothyroidism, adrenal insufficiency, hepatitis, and single cases of sarcoidosis, endophthalmitis, diabetes mellitus, pneumonitis, and myasthenia gravis. Most of the possibly immune-related AEs were managed with BMS-936559 interruption or discontinuation and/or corticosteroids. Among treatment-related AEs, including possibly immune-related AEs, none showed a clear dose-related pattern, with the exception of an increase in infusion-related reactions at higher doses. As clinical experience with immune checkpoint inhibitors increased, the scope of immune-mediated AEs has been better described [27, 28].

Reversing immune exhaustion may improve HIV-1–specific immunity and increase clearance of HIV-1–expressing cells but may increase the risk of immune-related AEs. This dose-escalation study sought to evaluate the safety of single infusions of BMS-936559 in HIV-infected participants with viremia suppressed by cART to below the limit of detection (LOD) of standard clinical assays but with detectable plasma HIV-1 RNA by single-copy assay (SCA). We evaluated whether blocking the PD-1/PD-L1 axis could improve HIV-1–specific cellular immune responses in chronic HIV-1 and impact persistent viremia.

METHODS

This was a phase I, randomized, double-blind, placebo-controlled study of single infusions of anti-PD-L1 antibody (BMS-936559) with sequential, dose-escalating cohorts of 0.3, 1, 3, and 10 mg/kg. In cohort 1, 8 participants were centrally randomized 3:1 by permuted block method to receive BMS-936559 or placebo (normal saline), resulting in 6 participants who received single BMS-936559 0.3-mg/kg infusions and 2 participants who received placebo.

Eligible participants were aged ≥18 to ≤70 years and were on cART with $CD4^+$ counts ≥350 cells/μL and plasma HIV-1 RNA <40 copies/mL by Abbott RealTime HIV-1 Assay (Abbott m2000) or <20 copies/mL by Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Test (Roche Taqman v2.0). Eligibility included detectable plasma HIV-1 RNA ≥0.4 copies/mL by SCA [29]. Exclusion criteria included history of active hepatitis B or C, positive test for tuberculosis, history of any autoimmune disorder including hypothyroidism or hyperthyroidism, adrenal insufficiency, and history or evidence of uveitis. Site institutional review boards approved the study. All study participants provided written informed consent. This study is registered with ClinicalTrials.gov (NCT02028403).

The primary clinical outcome was safety, defined as any grade 3 or greater AE or immune-related AE of any grade definitely, probably, or possibly related to study treatment, as judged by the core study team blinded to treatment arm. Because of eye findings in a 3-month BMS-936559 toxicity study in cynomolgus macaques with higher doses of BMS-936559, only the initial 0.3-mg/kg cohort was enrolled. All participants subsequently underwent ophthalmologic exams, retinal photography, and ocular coherence tomography at 3 time points, which were reviewed by a single retinal specialist prior to unblinding.

The primary immunologic outcome was the change in magnitude of HIV-1 Gag-specific $CD8^+$ T cells by intracellular staining (ICS) for IFN-γ from baseline to 28 days after treatment. Plasma HIV-1 RNA levels were measured by the Abbott RealTime HIV-1 assay (limit of quantification = 40 copies/mL). Low-level viremia was assessed by SCA (LOD of 0.4 copies/mL) [29]. Secondary outcomes included change in cell-associated (CA) HIV-1 RNA, HIV-1 DNA, HIV-1 RNA/DNA ratio and total $CD4^+$ T-cell number from baseline to day 28. The CA-HIV-1 RNA and DNA were quantified as described [30]. In HIV-1–infected patients on suppressive antiretroviral therapy, HIV-specific immune responses are downregulated. A single infusion of an anti-PD-L1 antibody (BMS-936559) increased HIV-1 Gag-specific $CD8^+$ T-cell responses in 2 of 6 participants. One nonresponder had a potentially immune-mediated AE.

PD-L1 receptor occupancy (RO) [31] was measured in whole blood collected before treatment and at 2 hours (±15 minutes), day 28 (±3 days), and weeks 10, 16, 24, 36, and 48 (±5 days) after infusion. Samples were incubated with phosphate-buffered saline (PBS; tubes 1–2; "bound PD-L1") or saturating concentrations (5 μg/mL) of BMS-936559 (tubes 3–4; "total PD-L1") at 4°C for 30 minutes. Bound antibodies were stained at 4°C (dark) for 30 minutes with 5 μg/mL of biotin-labeled murine immunoglobulin G3 (isotype control; tubes 1 and 3; Ancell) or mouse antihuman IgG4 (tubes 2 and 4; Invitrogen) and detected with streptavidin-PE (BD Biosciences) using flow cytometry (FACSCanto, BD Biosciences). Post-treatment PD-L1 RO was calculated as a ratio of the differences in bound PD-L1 mean fluorescence intensity (MFI) (tube 2 – tube 1) and total PD-L1 MFI (tube 4 – tube 3) on $CD3^+$, $CD4^+$, and $CD8^+$ T-cell subsets.

Expression of PD-1, PD-L1, and other immune exhaustion markers on total and HIV-1 Gag-specific memory (exclusion of $CD27^+CD45RO^-$ naive cells) $CD8^+$ T cells ($CD3^+$) was assessed by multiparameter flow cytometry. HIV-1 Gag specificity was defined by positive ICS of IFN-γ, CD107a, or tumor necrosis factor (TNF) after 6 hours of incubation of thawed peripheral

blood mononuclear cells with a subtype B Gag-peptide pool (NIH AIDS Reagent Program) plus costimulation with antibodies against CD28 and CD49d as described [32] with an unstimulated control for each assay. Assays were performed on the same modified and calibrated LSR-II flow cytometer at the Vaccine Research Center. Changes in immune activation of $CD8^+$ T cells were assessed by quantifying $CD38^+$ $HLA\text{-}DR^+$ expression before and after BMS-936559 administration by flow cytometry. Poly-functionality of HIV-1–specific $CD8^+$ T-cell responses was defined as the absolute percentage of memory $CD8^+$ T cells expressing IFN-γ, CD107a, and TNF.

Proliferative responses to antigenic stimulation with HIV-1 Gag peptide pools were evaluated in a 6-day ex vivo assay. Peripheral blood mononuclear cells (approximately 10–15 million cells) derived from fresh whole blood from each participant at pre-entry were labeled with 2 μM of CellTrace Violet (CTV; Life Tech C34557) in PBS at a density of 2×10^7 cells/mL for 20 minutes at 37°C. Labeling reactions were quenched with a 10-fold excess of media (Roswell Park Memorial Institute 1640 media + 10% fetal bovine serum), cells were washed and resuspended at density of 2×10^6 cells/mL in medium supplemented with 10 U/mL recombinant interleukin-1 (Peprotech 200–02). Antibodies (hu anti-hPDL1 [BMS-936559], 10 mg/mL or isotype control hIgG4, Zymogenetics DT-1D12-g4P, 2.7 mg/mL) and peptides (HIV-1 Gag overlapping 15-mer library [JPT custom order]: peptide pool A [peptides 1–61] or pool B [peptides 62–122] or CEF peptide pool [JPT CEF pool], each at 500 μg/mL in dimethyl sulfoxide) were mixed with aliquots of 1 million cells to final concentrations of 10 μg/mL for antibodies and 2.5 μg/mL for peptides, respectively. After 6 days at 37°C, 5% carbon dioxide, cells were washed, transferred to a 5-mL fluorescence-activated cell sorting tube and, incubated for 10 minutes with 1:1000 dilution of Live-Dead solution (Molecular Probes [Life Tech] Live/Dead Fixable Near-IR Dead Cell Stain Kit reference L10119) in PBS. Cells were washed again, resuspended in fresh FACS buffer (PBS + 0.5% fetal bovine serum [Gibco 01-4020DJ]), incubated with normal mouse immunoglobulin G (Invitrogen reference 10400C) and then with a mix of Horizon BV510 mouse antihuman CD3 (clone UCHT1; BD reference 563109), PerCP mouse antihuman CD4 (BD Pharmingen reference 550631), and fluorescein isothiocyanate mouse antihuman CD8 (BD Pharmingen reference 555366) in a final volume of 100 μL, incubated for 20 minutes at room temperature, washed, and the cell pellet fixed with 200 μL of 2% paraformaldehyde (16% w/v aqueous solution, methanol free; Alfa Aesar 43368). This cell preparation was read on a Cytek DxP8 flow cytometer and analyzed using Flow Jo V.10 software.

Whole blood samples were collected before infusion and 15 minutes, 2, 6, and 12 hours (±15–60 minutes), and days 3, 7, 14, 28 (±1–3 days), and weeks 10, 16, 25, 36, and 48 (±5 days) after BMS-936559 intravenous administration over 1 hour. An enzyme-linked immunosorbent assay quantitated BMS-936559 in human serum. Mouse anti-BMS-936559 was immobilized onto a 96-well microtiter plate blocked with bovine serum albumin buffer. Serum samples were diluted 1:100 before loading into wells and incubated at ambient temperature. To detect bound BMS-936559, mouse antihuman IgG4-HRP conjugate was added. After the final wash step, tetramethylbenzidine peroxidase substrate solution was added, and the enzymatic reaction was stopped with phosphoric acid. Results were read on a plate reader using 450 nm/650 nm wavelengths, and concentrations were interpolated from a standard curve plotted using a 4 parameter logistic curve-fitting program with $1/y^2$ weighting. The calibration curve range was 100–5000 ng/mL, with an anchor point at 50 ng/mL. Pharmacokinetic parameters were estimated by noncompartmental analysis using Phoenix WinNonlin 6.4 (Certara, Princeton, NJ).

The activity of BMS936559 was assessed by a paired *t* test within the treated group, comparing the average pretreatment (pre-entry and day 0) and post-treatment (day 7, 14, and day 28) measurements. Statistical tests were limited to 4 outcomes chosen before analysis: HIV-1 RNA by SCA, CA-RNA, the proportion of HIV-1 Gag-specific $CD8^+$ T cells expressing IFN-γ, and the proportion expressing CD107a. Results below the LOD for SCA and CA-RNA were set to half the lower limit (0.2 copies/mL and 2.48 copies/million CD4, respectively). All virologic measures were $\log_{10}$ transformed before analysis. Additional analyses were descriptive.

RESULTS

Twenty-four individuals across 5 sites were screened with 16 individuals determined ineligible, most commonly due to low SCA result of <0.4 copies/mL (n = 9). Of 8 men enrolled in cohort 1, 6 received single 0.3-mg/kg BMS-936559 infusions, and 2 received placebo infusions. All 8 participants completed 48 weeks of follow-up. No further cohorts were enrolled due to retinal findings in a toxicity study in macaques.

The median age of participants was 46 years (range = 26–53) (Table 1). Five participants (62.5%) were white non-Hispanic, 2 (25%) were black non-Hispanic, and 1 (12.5%) was Hispanic. The median baseline $CD4^+$ cell counts was 864 cells/mm^3 (range = 385–1162). The median baseline $CD8^+$ cell count and CD4/CD8 ratio were 909 cells/mm^3 (range = 476–1499) and 0.86 (range = 0.52–1.56), respectively. The median cART duration was 13.2 years (range = 3.4–21.2), and the median duration of suppression below the limits of standard HIV-1 RNA assays was 7.4 years (range = 3.2–15.8). Median baseline SCA was 0.7 copies/mL (range = 0.2–4.9), and the median percentage of memory $CD8^+$ cells expressing IFN-γ to HIV-1 Gag was 0.09% (range = 0.01%–0.36%). Additional baseline immunologic and virologic parameters, including the percentage of memory $CD8^+$ cells with HIV-1 Gag-specific responses by CD107a, TNF expression, and expressing all 3 response measures, as well as

Table 1. Baseline Demographic, Clinical, and Laboratory Characteristics of Participants Who Received BMS-936559 or Placebo

Treatment	PID	Age, y	Sex	Race/ ethnicity	Nadir CD4, cells/mm^3	Entry CD4, cells/mm^3	Entry CD4/ CD8 ratio	ART duration, y	Duration HIV viral suppression, y	ARV regimen	SCA, copies/mL	% CD8$^+$ with HIV-1 Gag-specific response by IFN-γ
Active	1	40	M	W	640	799	0.86	NA	NA	FTC,TDF,EVG,COBI	3.8	0.134
Active	2	44	M	H	825	1156	0.77	17.6	15.1	FTC,TDF,NVP	0.2	0.078
Active	**3**	**46**	**M**	**B**	**364**	**920**	**1.03**	**5.5**	**5.2**	**FTC,TDF,EVG,COBI**	**0.8**	**0.167**
Active	**4**	**51**	**M**	**W**	**373**	**385**	**0.52**	**21.2**	**5.2**	**FTC,TDF,RTV,DRV,RAL,MVC**	**0.4**	**0.065**
Active	5	52	M	W	212	849	0.86	7.8	7.4	ABC,3TC,RTV,ATV	4.9	0.094
Active	6	53	M	W	23	879	0.82	13.2	13.0	FTC,TDF,EVG,COBI	1.7	0.007
Placebo	7	26	M	B	473	1162	1.56	3.4	3.2	FTC,TDF,ETR	0.4	0.009
Placebo	8	45	M	W	9	462	0.97	15.9	15.8	FTC,TDF,EFV	0.7	0.355
	Median	46	.	.	369	864	0.86	13.2	7.4		0.7	0.086

Baseline single-copy assay (SCA) values and human immunodeficienvy virus type 1 (HIV-1) Gag-specific responses are the mean of entry and pre-entry values. The two possible responders are in bold.

Abbreviations: 3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; ARV, antiretroviral; ATV, atazanavir; B, black Non-Hispanic; COBI, cobicistat; DRV, darunavir; EFV, efavirenz; ETR, etravirine; EVG, elvitegravir; FTC, emtricitabine; H, Hispanic (regardless of race); HIV-1, human immunodeficiency virus type 1; IFN-γ, interferon γ; M, male; MVC, maraviroc; NA, data not available; NVP, nevirapine; PID, patient identification; RAL, raltegravir; RTV, ritonavir; SCA, single-copy assay; TDF, tenofovir disoproxil fumarate; W, white non-Hispanic.

CA-DNA and CA-RNA levels and CA-RNA/DNA ratios, are presented (Supplementary Table 1). Baseline levels of PD1 and PD-L1 expression on memory CD8$^+$ cells and CD14$^+$ cells are presented (Supplementary Table 2).

The median maximum concentration (C_{max}) following 0.3-mg/kg single BMS-936559 infusions was 6156 ng/mL (range = 5401–8243), and the median half-life was 3.7 days (range = 1.5–5.1). Median RO on CD8$^+$ T cells was 94% (range = 70%–97%) 2 hours after infusion and decreased to a median of 7% (range = 0%–48%) 28 days after infusion (Supplementary Figure 1). A representative flow cytometry plot for RO for a BMS-936559 and placebo recipient are shown (Supplementary Figure 2). The RO on CD4$^+$ and CD3$^+$ T cells was similar (data not shown).

Among participants receiving BMS-936559 infusions, antidrug antibodies were detected at week 4 in 3 participants and in all 6 by week 10, with no differences in anti-PD-L1 pharmacokinetics or RO in participants based on timing of antidrug antibody development.

After 0.3-mg/kg BMS-936559 infusions, the mean percentage of HIV-1 Gag-specific CD8$^+$ T cells expressing IFN-γ increased from baseline (0.09%) through day 28 (0.20%; P = .14) in the active group (Table 2). This positive, nonsignificant increase was driven by responses in 2 participants, shown as green lines

Table 2. Immunologic and Virologic Primary Outcomes Through Day 28 After Infusion

Outcome		Baseline	Mean through day 28	Change	P value[a]
% HIV-1 Gag-specific CD8$^+$ IFN-γ	Active	0.09	0.20	0.11	.14
	Placebo	0.18	0.20	0.02	…
% HIV-1 Gag-specific CD8$^+$ CD107a	Active	0.09	0.21	0.12	.09
	Placebo	0.18	0.21	0.04	…
% HIV-1 Gag-specific CD8$^+$ TNF	Active	0.07	0.14	0.07	…
	Placebo	0.16	0.17	0.01	…
% HIV-1 Gag-specific CD8$^+$ IFN-γ and CD107a and TNF	Active	0.05	0.12	0.07	…
	Placebo	0.14	0.16	0.02	…
SCA, copies/mL	Active	1.1	1.3	1.12	.69
	Placebo	0.5	0.7	1.40	…
CA-RNA, copies/10^6 CD4$^+$ cells	Active	201	194	0.96	.77
	Placebo	36	44	1.23	…
CA-DNA, copies/10^6 CD4$^+$ cells	Active	435	513	1.18	…
	Placebo	90	100	1.11	…
CA-RNA/DNA ratio	Active	0.46	0.38	0.82	…
	Placebo	0.40	0.44	1.10	…

For immunologic assay results, baseline and mean through day 28 are arithmetic means, and change is presented as the difference. For single-copy assay (SCA), cell-associated (CA)–RNA, CA-DNA, and CA-RNA/DNA ratio, baseline and mean through day 28 are geometric means, and change presented is the fold change (mean through day 28/baseline).

Abbreviations: CA, cell-associated; HIV-1, human immunodeficiency virus type 1; IFN-γ, interferon γ; SCA, single-copy assay; TNF, tumor necrosis factor.

[a] P values were calculated for the 2 primary immunologic and virologic outcomes through day 28

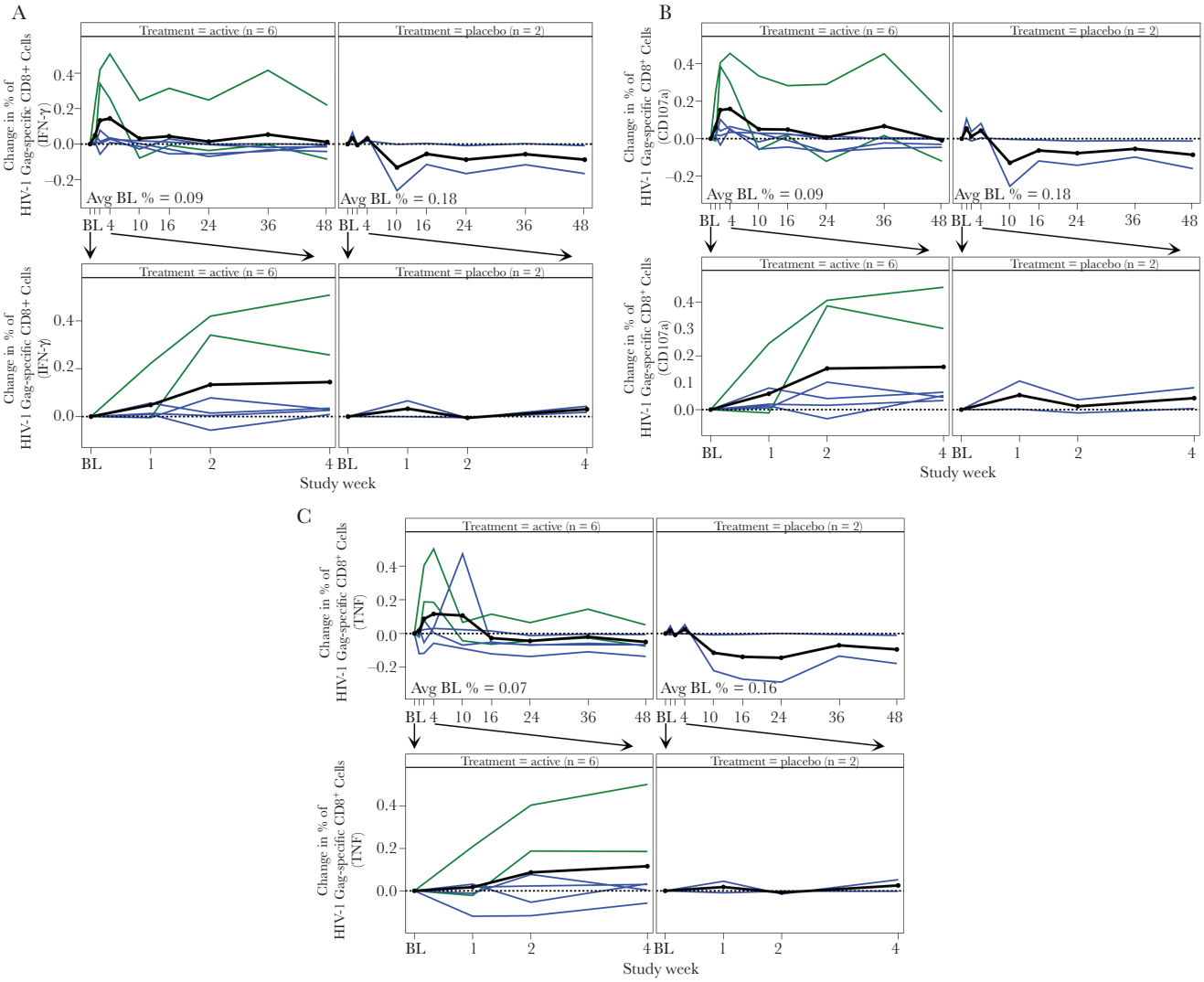

Figure 1. Change from baseline over time in the absolute percentage of human immunodeficiency virus type 1 (HIV-1) Gag-specific CD8+ T cells. Gag-specific CD8+ T cell responses from baseline (average of pre-entry and entry) through week 48 are shown for interferon γ (IFN-γ) (*A*), CD107a (*B*), and tumor necrosis factor (TNF) (*C*). In each panel, the upper 2 graphs show the change in the percentage of HIV Gag-specific responses over 48 weeks of the study. The lower 2 graphs in highlight the first 28 days of the study, which defined the primary immunologic endpoint. Two participants who received treatment with BMS-936559 and appeared to show an increase in HIV-1 Gag-specific CD8+ responses are shown as green lines. The black line with circular markers represents the mean change over time. *A*, Change in HIV-1 Gag-specific CD8+ T cells by intracellular cytokine staining for IFN-γ. *B*, Change in HIV-1 Gag-specific CD8+ T cells by mobilization of CD107a. *C*, Change in HIV-1 Gag-specific CD8+ T cells by mobilization of TNF. Abbreviations: BL, baseline; HIV-1, human immunodeficiency virus type 1; IFN-γ, interferon γ; TNF, tumor necrosis factor.

in Figure 1A–C, with substantial increases in the percentage of HIV-1 Gag-specific CD8+ IFN-γ responses (Figure 1A). HIV-1 Gag-specific CD8+ response in participant 3 (P3) increased from 0.17% at baseline to 0.43% at day 28 and in participant 4 (P4) from 0.07% at baseline to 0.57% at day 28. No other participant (active or placebo) increased by more than 0.1% between baseline and day 28. Representative flow cytometry plots of the CD8+ T-cell responses to TNF and IFN-γ for the highest responder (P4) before and after infusion of BMS-936559 are shown (Supplementary Figure 3). Mean percentage change by HIV-1 Gag-specific CD107a responses also increased from 0.09% at baseline to 0.21% though day 28 (P = .09) (Table 2), driven by the 2 participants with IFN-γ responses, with increases from 0.18% to 0.48% in P3 and 0.05% to 0.51% in P4. The change in HIV-1 Gag-specific CD107a responses was <0.1% in all other participants (Figure 1B). In both responders, HIV-1 Gag-specific CD8+ T-cell responses declined after day 28. Responses as measured by TNF expression were similar though more variable (Figure 1C). The 1 placebo recipient with a decline in all 3 response measures at week 10 had substantially higher baseline levels than other participants: approximately 4 times the median level (Table 1 and Supplementary Table 1).

Notably, pre-entry CD8+ T cells from the 2 apparent responders demonstrated proliferative responses to Gag peptides after

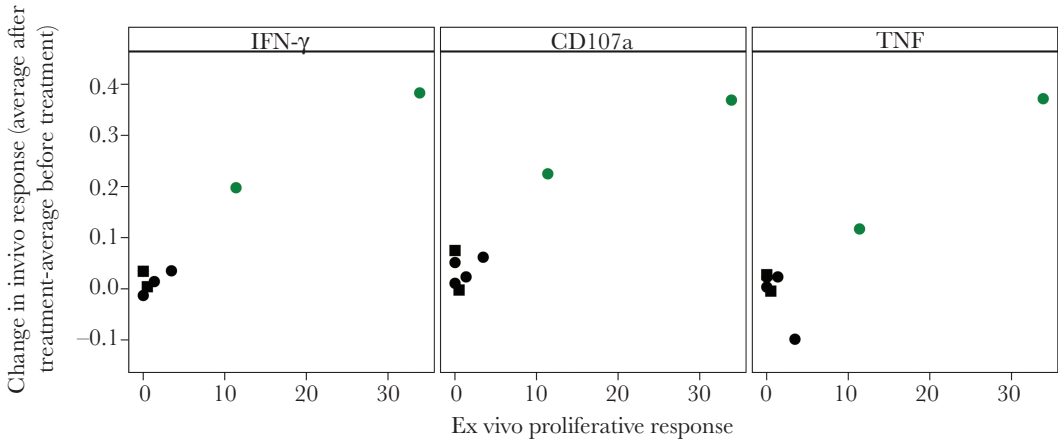

Figure 2. Correlation between pre-entry ex vivo proliferation to Gag and in vivo Gag-specific CD8+ T-cell responses in each participant. The predefined primary endpoint for in vivo response was the change in magnitude of human immunodeficiency virus type 1 (HIV-1) Gag-specific CD8+ T cells by intracellular staining for interferon γ from baseline to 28 days after treatment (the average of day 7, 14, and 28 measurements). Shown are the average differences in pretreatment ex vivo proliferative responses to Gag peptides after BMS-936559 exposure compared with isotype control versus the average difference in pre- and post-treatment in vivo HIV-1 Gag-specific CD8+ responses for each of the 3 measures of response. Results from the 2 participants with apparent in vivo responses are shown in green. Abbreviations: IFN-γ, interferon γ; TNF, tumor necrosis factor.

anti-PD-L1 exposure ex vivo compared with isotype antibody exposure. This proliferation corresponded to their in vivo response as assessed by IFN-γ, CD107a, and TNF. CD8+ cells from no other participant demonstrated a similar response (Figure 2). There were no apparent differences between the 2 potential responders compared with the nonresponders in either BMS-936559 pharmacokinetics or RO (Supplementary Figure 1).

The poly-functionality of responses was assessed by calculating the percentage of total CD8+ memory cells that were

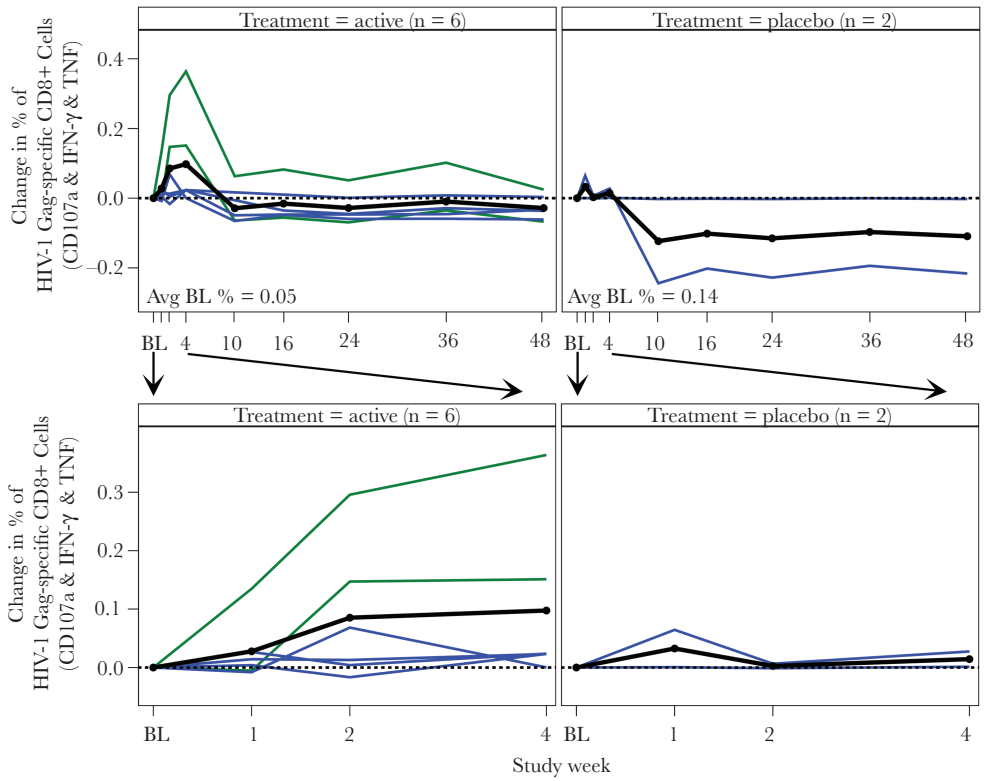

Figure 3. Change from baseline over time in the absolute percentage of human immunodeficiency virus type 1 Gag-specific CD8+ T cells that were poly-functional defined by cells staining for interferon γ, CD107a mobilization, and tumor necrosis factor from baseline through week 48. Abbreviations: BL, baseline; HIV-1, human immunodeficiency virus type 1; IFN-γ, interferon γ; TNF, tumor necrosis factor.

HIV-1 Gag-specific and had responses to IFN-γ, CD107a, and TNF. Mean changes over 28 days (Table 2) and individual changes (Figure 3) are presented. The percentage of poly-functional HIV-1 Gag-specific cells increased from 0.08% to 0.23% in P3 and from 0.01% to 0.37% in P4. No other participant had a change >0.03%. The relative percentage for each of 7 possible Boolean combinations of the 3 response markers was determined for each participant, and mean relative percentages over time are displayed (Supplementary Figure 4). There were no apparent changes in the median $CD4^+$ cell count, $CD4^+$ percentage, or CD4/CD8 ratio from baseline through day 28 after infusion. CD4 responses were examined using ICS for IFN-γ and TNF, and no obvious responses were observed in any participant over the 28-day primary or 48-week endpoints (data not shown).

All participants' standard HIV-1 RNA levels remained <40 copies/mL except for an HIV-1 RNA of 98 copies/mL in 1 placebo recipient at week 48. There was no significant change in the average pretreatment SCA (1.1 copies/mL) versus mean post-treatment SCA (1.3 copies/mL; $P = .69$) or in CA-HIV-1 RNA ($P = .77$) through day 28 (Table 2). In post hoc analyses, low-level viremia via SCA appeared to decline from a pretreatment average of 1.1 copies/mL to 0.5 copies/mL ($P = .06$) at day 3 after infusion among the 6 participants administered anti-PD-L1 but was not observed at days 7 through 28 (Supplementary Figure 5). There was no consistent change from baseline in mean HIV-1 DNA or RNA/DNA ratio (Supplementary Figure 6).

There were no BMS-936559 related grade 3 or greater AEs. One participant had grade 1 chest tightness and shortness of breath, and a second participant had grade 1 headache, blurry vision, and fatigue after infusion. One participant had grade 2 diarrhea and leg pain 1 week after infusion. These AEs, considered possibly related to treatment, resolved without intervention. A single participant had grade 3 aspartate aminotransferase elevation at week 24 associated with trauma and elevated creatine phosphokinase assessed as not treatment related. No serious AEs occurred.

Thirty-six weeks after infusion, an initially asymptomatic participant with a previously normal cortisol, who did not have a HIV-Gag response, had a low morning cortisol level and was diagnosed with hypoadrenalism and subsequently hypogonadism, which led to clinical diagnosis of hypophysitis. A brain MRI was normal, and the participant was treated with replacement corticosteroids and testosterone for approximately 6 months. Approximately 11 months after the initial low cortisol, his adrenocorticotropic hormone stimulation test normalized, and corticosteroid and testosterone replacement were discontinued (see Clinical Summary Supplement).

Review of the full ophthalmological evaluations, including retinal photographs and ocular coherence tomography, by a retinal specialist before unblinding did not reveal any retinal findings consistent with those observed in macaques.

DISCUSSION

This is the first prospective study of an immunologic checkpoint inhibitor in healthy HIV-1–infected persons on suppressive cART. In this small cohort, a single, low-dose infusion of BMS-936559 appeared to enhance HIV-1–specific responses in 2 of 6 participants receiving active treatment. These responses correlated with pretreatment ex vivo proliferative responses of HIV-1–specific $CD8^+$ T cells to Gag peptides in the presence of BMS-936559. The proportion of HIV-1–specific $CD8^+$ T cells that expressed all 3 response measures (IFN-γ, CD107a, and TNF) in 2 apparent responders also increased and then declined after infusion. Adrenal insufficiency and hypogonadism occurred in 1 participant, potentially consistent with autoimmunity.

The 0.3-mg/kg dose was anticipated to have little biological effect but chosen to assess safety in HIV-infected individuals. The prespecified immunologic responses (change in mean HIV-1 Gag-specific $CD8^+$ T cells as measured by IFN-γ and CD107a) demonstrated a nonsignificant trend. Closer review of the data revealed potential responses in 2 participants and essentially no change in IFN-γ or CD107a responses in the other participants administered BMS-936559 or placebo. The finding that ex vivo proliferative responses to Gag peptides corresponded to in vivo poly-functional responses in the 2 responsive participants provides additional support for an effect of BMS-936559. The correlation of ex vivo and in vivo response suggests that ex vivo response could be used as a predictor of checkpoint inhibitor response in future trials. The observation that only a subset of treated individuals showed a response is similar to that observed for antitumor responses to BMS-936559 and nivolumab [24, 25], for SIV control following BMS-936559 treatment of rhesus macaques [20], and for hepatitis C virus response in a pilot study of nivolumab [18].

This study found no change in plasma HIV-1 RNA, CA-HIV-1 RNA, and CA-HIV-1 DNA after treatment with anti-PD-L1 through day 28, contrasting with studies of BMS-936559 and another anti-PD-L1 antibody (avelumab) in SIV-infected macaques showing transient HIV RNA control following multiple doses [20, 33]. One potential reason for the difference in our study is that, although single low doses of anti-PD-L1 resulted in high RO immediately after infusion, the duration of RO was short lived with median RO only 7% by day 28. Future studies with checkpoint inhibitors should incorporate higher doses, multiple dose administration, and multiple early assessments of RO to fully characterize cellular target binding.

Before our study, there were no data on AEs following single BMS-936559 infusions in any population. In our study, there were no treatment-related grade 3 or greater AEs. In patients with advanced malignancy who received multiple infusions of

BMS-936559, most receiving 10 mg/kg every 14 days in 6-week cycles, adverse events with potential immune-related causes were reported at all doses, including 0.1-mg/kg and 0.3-mg/kg doses, with 1 reported case of adrenal insufficiency at the 0.3-mg/kg dose. In our study, a participant (without an HIV-1–specific response) was found to have hypoadrenalism and hypogonadism consistent with autoimmune hypophysitis. Despite occurring 36 weeks after infusion and the fact that adrenal insufficiency and hypogonadism have been reported in HIV-infected individuals [34–37], the study team could not rule out relatedness to study treatment. Our study was stopped after the first cohort due to retinal toxicity observed in 3-month toxicology studies in macaques. In response, all participants subsequently underwent serial ophthalmologic evaluations, and no participant developed retinal lesions similar to those observed in the animal toxicity study.

The finding that a subgroup of individuals demonstrated improvements in HIV-1–specific $CD8^+$ responses predicted by ex vivo response suggests blockade of the PD1-PD-L1 pathway has the potential to improve HIV-1–specific immunity. Several other monoclonal antibodies blocking the PD-1-PD-L1 axis are in clinical use or late-stage clinical development as cancer immunotherapy. Given the clear risk of immune-mediated toxicity, such antibodies should be tested cautiously in otherwise healthy HIV-infected individuals, but our results suggest PD1-PD-L1 blockade is a potential means for reversing immune exhaustion and could be a component of HIV remission or cure strategies, perhaps in combination with latency-reversing agents or as an adjuvant to a therapeutic vaccine.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Role of Authors. Study design was done by J. J. E., C. L. G., J. W. M., R. L. T., R. J. B., R. A. K., E. P. A., J. M. C., S. W. M., and C. K. H. Data generation/assay performance was done by J. M. H., J. R., E. A., J. E., C. L. G., J. W. M., J. C. C., R. J. B., R. A. K., E. P. A., S. W. M., C. K. H., and N. R. Drafting or editing of the paper was done by J. J. E., C. L. G., J. W. M., J. M. H., J. R., J. C. C., R. L. T., R. J. B., R. A. K., E. P. A., J. M. C., S. W. M., C. K. H., D. M. G., and N. R. ACTG A5326 Team Members other than the coauthors are as follows: Mwenda Kudumu, BS, PMP (clinical trials specialist); Susan Pedersen, RN, BSN (field representative); Cheryl L. Jennings, BS (laboratory technologist); Bernadette Jarocki, BS (data manager); Thucuma Sise, PharmD, BCPS (DAIDS pharmacist); Andrew Kaytes and Aaron Laxton, BA (Community Scientific Subcommittee representatives); Alex Benns, BS, and Sarah Strabino, BS (laboratory data managers); Odette Houghton, MD (ophthalmologist); and Kelly Misar, BS (industry representative). The coauthors would also like to acknowledge assistance from the following individuals: Becky Straub, RN, BSN, and Susan Pedersen RN, BSN—CTU: University of North Carolina at Chapel Hill CRS (site 3201) grants UM1 AI069423 CTSA: 1UL1TR001111 CFAR: P30 AI50410; Christine Griesmer and Graham Ray—University of Colorado Hospital CRS (Site 6101) grant 2UM1AI069432, UL1 TR001082; Michelle Saemann, RN, BSN, and Carl J. Fichtenbaum, MD—University of Cincinnati (site 2401) grant UM1AI068636; Becky Basham CCRP and Vicki Bailey, BSN, RN—Vanderbilt Therapeutics CRS (site 3652) grant UM1AI 069439; and Michael Klebert and Debra DeMarco Shaw—Washington University in St Louis CRS (site 2101) grant NIH 66522. The authors sincerely thank all the participants who participated in this study.

Financial support. Research reported in this publication was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (award no. UM1 AI068634, UM1 AI068636, UM1 AI106701, and UM1 AI069423 and contract no. HHSN272200800014C and HHSN271201500007I). This work was also supported by University of North Carolina at Chapel Hill Center for AIDS Research (P30 AI50410) National Center for Advancing Translational Sciences (UL1TR001111). BMS-936559 was provided by Bristol-Myers Squibb. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Potential conflicts of interest. C. L. G. has received research support from Bristol-Myers Squibb, Gilead Sciences, Abbott, Tibotec Therapeutics, Janssen, and ViiV Healthcare. J. J. E. receives research support from ViiV Healthcare, Gilead Sciences, and Janssen and is a consultant to Bristol-Myers Squibb, Merck, Gilead Sciences, Janssen, and ViiV Healthcare. J. W. M. is a consultant for Gilead Sciences, owns shares in Cocrystal Pharma, Inc, and has received research support from Bristol-Myers Squibb, Janssen, and Gilead Sciences. J. C. C. has received research support from Janssen and Gilead Sciences. E. P. A. is a consultant to Viiv. D. M. G., C. K. H., S. W. M., and N. R. are/were full-time employees and shareholders of Bristol-Myers Squibb during the study. R. J. B., J. R., J. M. H., E. A., R. L. T., J. M. C., and R. A. K. report no conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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