Immune Checkpoint Inhibition in Sepsis: A Phase 1b Randomized, Placebo-Controlled, Single Ascending Dose Study of Antiprogrammed Cell Death-Ligand 1 (BMS-936559)

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Objectives: To assess for the first time the safety and pharmacokinetics of an antiprogrammed cell death-ligand 1 immune checkpoint inhibitor (BMS-936559, Bristol-Myers Squibb, Princeton, NJ) and its effect on immune biomarkers in participants with sepsis-associated immunosuppression.

Design: Randomized, placebo-controlled, dose-escalation.

Setting: Seven U.S. hospital ICUs.

Study Population: Twenty-four participants with sepsis, organ dysfunction (hypotension, acute respiratory failure, and/or acute renal injury), and absolute lymphocyte count less than or equal to 1,100 cells/μL.

Interventions: Participants received single-dose BMS-936559 (10–900 mg; $n = 20$) or placebo ($n = 4$) infusions. Primary endpoints were death and adverse events; key secondary endpoints included receptor occupancy and monocyte human leukocyte antigen-DR levels.

Measurements and Main Results: The treated group was older (median 62 yr treated pooled vs 46 yr placebo), and a greater percentage had more than 2 organ dysfunctions (55% treated pooled vs 25% placebo); other baseline characteristics were comparable. Overall mortality was 25% (10 mg dose: 2/4; 30 mg: 2/4; 100 mg: 1/4; 300 mg: 1/4; 900 mg: 0/4; placebo: 0/4). All participants had adverse events (75% grade 1–2). Seventeen percent had a serious adverse event (3/20 treated pooled, 1/4 placebo), with none deemed drug-related. Adverse events that were potentially immune-related occurred in 54% of participants; most were grade 1–2, none required corticosteroids, and none were deemed drug-related. No significant changes in cytokine levels were observed. Full receptor occupancy was achieved for 28

days after BMS-936559 (900 mg). At the two highest doses, an apparent increase in monocyte human leukocyte antigen-DR expression (> 5,000 monoclonal antibodies/cell) was observed and persisted beyond 28 days.

Conclusions: In this first clinical evaluation of programmed cell death protein-1/programmed cell death-ligand 1 pathway inhibition in sepsis, BMS-936559 was well tolerated, with no evidence of drug-induced hypercytokinemia or cytokine storm, and at higher doses, some indication of restored immune status over 28 days. Further randomized trials on programmed cell death protein-1/programmed cell death-ligand 1 pathway inhibition are needed to evaluate its clinical safety and efficacy in patients with sepsis. (*Crit Care Med* 2019; XX:00–00)

Key Words: antiprogrammed cell death-ligand 1 BMS-936559; immune checkpoint inhibition; immunotherapy; sepsis; sepsis-associated immunosuppression

Sepsis is life-threatening organ dysfunction caused by a dysregulated host response to infection (1, 2), with high mortality rates worldwide (3, 4).

For years it was presumed that sepsis morbidity and mortality were secondary to an exaggerated systemic inflammatory response, but therapies intended to dampen this response failed to improve survival (5, 6). Although the causes of these failures are multifactorial, sepsis-associated immune dysregulation may play an important role (7, 8). Studies suggest that sepsis-associated immune dysregulation increases the risk of secondary infections and mortality (8–11).

Immune checkpoint pathways are endogenous components of the immune system that keep the immune response "in check" under normal physiologic conditions; tumor cells exploit these pathways to avoid recognition by the host. One of these immune checkpoint pathways is the programmed cell death protein-1 (PD-1)/programmed cell death-ligand 1 (PD-L1) pathway (12). PD-1 is a receptor that is inducibly expressed on T cells and functions as a negative regulator of T-cell function (12). Tumor cells express its primary ligand, PD-L1, which binds to PD-1 and triggers T-cell "inactivation"; PD-L1 expression on tumor cells is associated with poor prognosis (12, 13). Monoclonal antibodies (mAb) that block PD-1 and PD-L1 activity have proved highly successful at reducing tumor burden and are licensed for therapeutic use in patients with certain cancer types (14–18).

Immune dysregulation in sepsis bears similarities to that seen in certain cancers, particularly the up-regulation of the PD-1/PD-L1 pathway (19–21), and PD-1 and PD-L1 may be important in sepsis-associated immunosuppression. PD-1 and PD-L1 are also key mediators of T-cell exhaustion in infections (12, 22, 23); blocking their interaction prevents T-cell death, modulates cytokine production, and is associated with reduced organ dysfunction and fewer deaths in mice with cecal ligation and puncture-induced sepsis (24–27). PD-1 knockout mice also have marked protection against sepsis lethality versus wild-type mice (28). PD-1 and PD-L1 are also up-regulated on immune cells of patients with sepsis, and higher expression of these proteins is associated with increased mortality (9, 27–34). Furthermore, ex vivo studies using blood samples from patients with sepsis have reported decreased apoptosis and improved immune cell function with antibodies against PD-1 and PD-L1 (31, 33, 34). Therefore, anti–PD-L1 could be a promising approach for patients with sepsis-associated immunosuppression.

As with any agent that inhibits immune checkpoint pathways, there is a theoretical risk that this approach could induce an unbridled pro-inflammatory response or "cytokine storm." Therefore, any novel agent under investigation in the oncology or sepsis fields should be monitored carefully for such a response. However, although animal and ex vivo sepsis studies have reported some cytokine changes with anti–PD-1 and anti–PD-L1 antibodies (e.g., increased interferon [INF]–γ, interleukin [IL]–6, and tumor necrosis factor-α), no excessive pro-inflammatory changes were reported (24–26, 31, 33). Furthermore, to the authors' knowledge, no clinical finding of cytokine storm in patients with cancer receiving anti–PD-1 or anti–PD-L1 therapy has been reported.

BMS-936559 (Bristol-Myers Squibb, Princeton, NJ), an investigational, fully human immunoglobulin G4 mAb that inhibits binding of PD-L1 to both PD-1 and CD80, has been explored in phase 1 studies in individuals with cancer (35) and those with HIV-1 infection on suppressive antiviral therapy (36). Anti–PD-L1 was effective in augmenting host immunity, as indicated by its ability to induce tumor regression and prolong disease stabilization in patients with cancer, and to enhance HIV-1 Gag-specific $CD8^+$ T-cell function, respectively (35, 36). The present study is the first clinical trial of checkpoint inhibitors in sepsis and represents a unique new immunotherapeutic approach to sepsis by targeting T-cell exhaustion and defective host adaptive immunity.

This study was undertaken primarily to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of anti–PD-L1 (BMS-936559) in patients with sepsis-associated immunosuppression. The study also explored biologic efficacy by examining the effects on immune system biomarkers.

MATERIALS AND METHODS

Ethics Statement

Written informed consent was obtained from all participants. Institutional Review Boards/Independent Ethics Committees approved the protocol and amendments. The study was conducted in accordance with Good Clinical Practice, as defined by the International Conference on Harmonisation, and in accordance with the ethical principles underlying European Union Directive 2001/20/EC and the United States Code of Federal Regulations, Title 21, Part 50 (21CFR50).

Study Design and Population

This was a phase 1b, prospective, randomized, double-blind, placebo-controlled, multicenter study of BMS-936559 in adults with sepsis-associated immunosuppression (ClinicalTrials.gov

identifier: NCT02576457), and was a sequential, single ascending-dose assessment at seven U.S. sites (December 2015–March 2017; **supplementary information**, Supplemental Digital Content 1, http://links.lww.com/CCM/E415).

Inclusion Criteria. Eligible participants were at least 18 years old with documented/suspected infection and sepsis onset at least 24 hours prior to study treatment administration based on one of three organ dysfunction criteria: hypotension (defined as treatment with any vasopressor[s] for at least 6 hr to maintain systolic pressure ≥ 90 mm Hg or mean arterial pressure ≥ 70 mm Hg), acute respiratory failure (mechanical ventilation for ≥ 24 hr), or acute kidney injury (creatinine > 2.0 mg/dL [from a normal pre-sepsis value] or urine output < 0.5 mL/kg/hr for > 2 hr despite adequate fluid resuscitation). Preexisting renal impairment required the participant to meet another organ dysfunction criterion. Participants were required to have sepsis-associated immunosuppression (in this study, operationally defined as absolute lymphocyte count [ALC] ≤ 1,100 cells/μL within the 96 hr before study treatment administration) (8, 11), and needed to be receiving treatment in an ICU.

Exclusion Criteria. Key criteria were a previous episode of sepsis with ICU admission during the current hospitalization, an advanced directive for withholding/withdrawing life-sustaining treatment/do not resuscitate order/comfort measures-only order, active autoimmune disease, history of transplantation, or cancer diagnosis or treatment in the preceding 6 months (supplementary information, Supplemental Digital Content 1, http://links.lww.com/CCM/E415).

Treatment Assignment and Study Procedures. Participants were assigned to a single dose of BMS-936559 or placebo; study treatment assignment was to one of five sequential groups (BMS-936559 10, 30, 100, 300, 900 mg). BMS-936559 was given as an IV infusion on day 1 (maximum infusion time: 180 min) (**Fig. 1**). The decision to wait at least 24 hours after the onset of organ dysfunction before starting treatment was taken in order to account for resolution of the peak pro-inflammatory response associated with sepsis (37).

All participants received standard-of-care therapy (38) and were followed for 90 days after dosing, unless they died or were lost to follow-up, or access to the participant was denied. After completing dosing in any group, subsequent group dosing was not initiated until blinded safety data through day 14 (or earlier for dosed participants who discontinued or died before day 14) in the earlier dose group(s) were reviewed and deemed acceptable by the sponsor's Medical Monitor in consultation with the investigators. Doses in groups 4 (300 mg) and 5 (900 mg) were selected based on a review of safety, receptor occupancy (RO), and pharmacokinetic data available through day 14 from earlier groups. Study stopping rules are described in the supplementary information (Supplemental Digital Content 1, http://links.lww.com/CCM/E415).

Participants were randomized 4:1 (BMS-936559 to placebo) using a computer-generated randomization scheme, provided by the sponsor. The site pharmacist (who prepared the infusion) was notified of a participant's treatment assignment. All other site staff and the sponsor remained blinded.

Endpoints and Assessments

Key Objectives and Endpoints. The primary objective was to assess safety and tolerability over 90 days following single-dose administration of BMS-936559 10–900 mg to participants with sepsis. Safety was assessed based on review of physical examination findings, vital sign measurements, electrocardiogram, adverse event (AE) reports, ophthalmoscopic examinations, and laboratory tests (for definitions of AEs and serious AEs [SAEs], see supplementary information, Supplemental Digital Content 1, http://links.lww.com/CCM/E415).

Key secondary objectives were to assess pharmacokinetics and RO of BMS-936559 following single-dose administration, and the effect of a single dose of BMS-936559 on immune function.

Assessments. Serial blood samples for pharmacokinetics/pharmacodynamics and biomarker analyses were collected at pre-dose and selected post-dose time points.

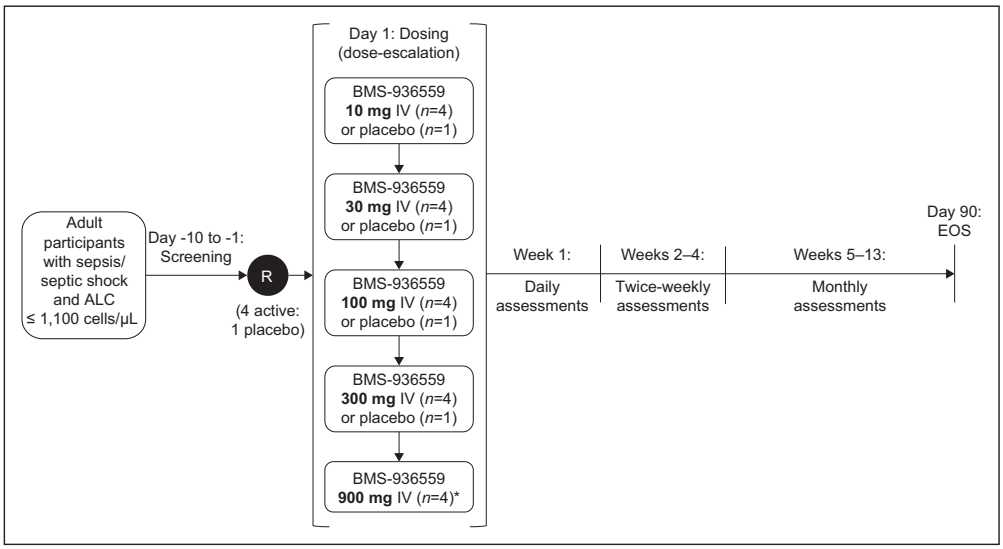


Figure 1. Study design. *A total of five participants were planned, but only four were dosed (all received BMS-936559). There was no intra-participant dose escalation. ALC = absolute lymphocyte count, EOS = end of study, R = randomization.

Pharmacokinetics. BMS-936559 serum concentrations were measured using a validated enzyme-linked immunosorbent assay method (39). Pharmacokinetic variables (maximum observed serum concentration [C_{max}], time of maximum observed serum concentration, area under the curve [AUC] from time 0 to time of the last measurable concentration after drug administration/extrapolated to infinity [$AUC_{(0-T)}/AUC_{(INF)}$], total body clearance, volume

of distribution, and terminal half-life were derived by noncompartmental analysis using Phoenix WinNonlin, v6.3 or higher [Pharsight Corporation, Phoenix, Mountain View, CA]). Dose proportionality, based on C_{max}, $AUC_{(0–T)}$, and $AUC_{(INF)}$, was assessed using the power model approach (40).

BMS-936559 RO and Immune System Status. PD-L1 RO on $CD3^+$ T cells and immune system status (monocyte human leukocyte antigen [mHLA]-DR expression and ALC) up to day 90 were measured. In vitro data suggest that T-cell function (as assessed by INF-γ production [BMS data on file] or T-cell proliferation [41]), is dose-dependently enhanced by PD-1/PD-L1 blockade. PD-L1 RO on T cells and T-cell activity saturate/plateau similarly; at saturating RO, there is a plateau in INF-γ production (BMS data on file). BMS-936559 doses that achieved at least 80% RO were expected to restore or enhance T-cell function, and greater than or equal to 80% was thus regarded as a relevant RO level.

The mHLA-DR levels were assessed using whole blood in flow cytometry-based assays; an mHLA-DR level of less than 5,000 mAb/cell has been generally considered indicative of immunoparalysis (42). ALC was determined using a standard hematology analyzer. Cytokine levels (IL-6, IL-8 [CXCL8], and IL-10) were also measured up to day 90. IL-6 and IL-8 are markers of generalized immune system activation and inflammation, and IL-10 expression is considered an appropriate anti-inflammatory marker.

Statistical Methods

The study employed a single ascending-dose design of four participants dosed with BMS-936559 and one with placebo at each dose level. Sample size was not based on statistical power considerations. Rather, if the incidence of an AE was 10%, then the sample size provided a 34.4% probability to observe at least one event in a given dose group.

Safety, pharmacokinetics, RO, and immunologic outcomes analyses were conducted on a modified intent-to-treat (ITT) population (ITT-exposed), comprising all participants who received at least a partial dose of study treatment. Descriptive statistics were used to summarize safety, pharmacokinetics, RO, and immunologic data.

RESULTS

Participant Disposition and Baseline Characteristics

Thirty-five participants were enrolled, of whom 25 were randomized. Ten participants were excluded for the following reasons (in some cases, participants were excluded for more than one reason): one participant did not have documented or suspected infection; one participant had active autoimmune disease or documented history of autoimmune disease; three participants did not meet organ dysfunction criteria; six participants did not have ALC less than or equal to 1,100 cells/μL; five participants were not in the ICU at the time of study drug administration; and for one participant, the enrollment milestone had already been reached. One randomized participant's status changed to "do not resuscitate" after randomization but prior to dosing, and so did not receive study treatment. Therefore, 24 participants received BMS-936559 (n = 20 [n = 4 per dose group]) or placebo (n = 4). Fourteen participants (58.3%) completed the 90-day study period (**Supplementary Fig. S1**, Supplemental Digital Content 1, http://links.lww.com/CCM/E415).

Baseline characteristics were comparable across groups, except for age and number of organ dysfunctions (**Table 1**).

Safety

Six deaths occurred 2–52 days following BMS-936559 administration (**Table 2**); these were considered unrelated to study treatment by the investigator. Deaths occurred in two of four participants (50%) receiving BMS-936559 10 mg, two of four (50%) receiving 30 mg, one of four (25%) receiving 100 mg, and one of four (25%) receiving 300 mg (Table 2). The causes of death were not unexpected for these severely ill patients (**Supplementary Table S1**, Supplemental Digital Content 1, http://links.lww.com/CCM/E415).

SAEs occurred in four participants (16.7%; BMS-936559, n = 3/20 [15.0%]; placebo, n = 1/4 [25.0%]); all were considered unrelated to study treatment (Table 2; and **Supplementary Table S2**, Supplemental Digital Content 1, http://links.lww.com/CCM/E415). The most frequent (≥ 20%) on-treatment AEs (pooled BMS-936559 doses) were hypotension (n = 11; 55%), diarrhea and delirium (n = 7; 35% each), anemia (n = 6; 30%), increased lipase and pleural effusion (n = 5; 25% each), and decreased weight, hypokalemia, and malnutrition (n = 4; 20% each) (Table 2). Most AEs were mild to moderate (grade 1–2), with similar frequency and intensity across dose groups. The observed AEs were not unexpected for this population. One participant (BMS-936559 30 mg) had AEs considered related to study treatment: increased amylase (grade 2) and lipase (grade 1), and increased blood lactate dehydrogenase (LDH) (grade 1). The increased amylase and lipase events began approximately 24 hours after infusion and resolved after 5 days, with neither event requiring treatment. The increased blood LDH event began 6 days after infusion, resolved after approximately 14 hours, did not require treatment, and was not associated with hemolysis or anemia. No AEs led to discontinuation from the study.

AEs of special interest (AEOSIs, i.e., AEs with potential immune-related causes as identified in the anti–PD-L1 study in cancer patients [35]) occurred in 13 of 24 participants (54.2%; BMS-936559, n = 11/20 [55.0%]; placebo, n = 2/4 [50.0%]) (**Table 3**). Most were grade 1–2; none were deemed related to study treatment or were suggestive of a drug-induced exaggerated inflammatory response. Two participants (10%) had grade 3 AEOSIs of diarrhea: in one, the event began on day 7, resolved on day 12, was treated with loperamide, and did not require corticosteroids; in the other, the event began on day 6, resolved on day 23, was treated with diphenoxylate atropine, and did not require corticosteroids. One participant (5%) had a grade 3 AEOSI of lung infiltration, beginning 18 hours after study treatment infusion and resolving by 33 hours. It was treated with antibiotics and did not require corticosteroids. No cases of pneumonitis were reported.

TABLE 1. Baseline Demographics and Disease Variables

Variables	BMS-936559 Treatment						Placebo (*n* = 4)
	10 mg (*n* = 4)	30 mg (*n* = 4)	100 mg (*n* = 4)	300 mg (*n* = 4)	900 mg (*n* = 4)	Pooled (*n* = 20)	
Age, yr, median (range)	57 (55–61)	68 (59–76)	56 (24–76)	60 (54–69)	66 (45–76)	62 (24–76)	46 (39–64)
Age categorization, yr, *n* (%)							
< 65	4 (100)	2 (50)	2 (50)	3 (75)	2 (50)	13 (65)	4 (100)
≥ 65	0	2 (50)	2 (50)	1 (25)	2 (50)	7 (35)	0
Sex, *n* (%)							
Male	2 (50)	0	2 (50)	2 (50)	3 (75)	9 (45)	3 (75)
Female	2 (50)	4 (100)	2 (50)	2 (50)	1 (25)	11 (55)	1 (25)
Race, *n* (%)							
White	2 (50)	4 (100)	3 (75)	3 (75)	4 (100)	16 (80)	2 (50)
Black/African American	2 (50)	0	1 (25)	0	0	3 (15)	2 (50)
Asian	0	0	0	1 (25)	0	1 (5)	0
Absolute lymphocyte count, $\times 10^9$ cells/L, mean (± SD)[a]	1.50 (1.05)	1.04 (0.63)	0.93 (0.40)	0.70 (0.27)	0.84 (0.32)	1.00 (0.61)	1.31 (1.05)
Site of infection, *n* (%)							
Abdomen	1 (25)	1 (25)	0	1 (25)	2 (50)	5 (25)	1 (25)
Blood	0	1 (25)	0	0	1 (25)	2 (10)	1 (25)
Lung	1 (25)	1 (25)	3 (75)	2 (50)	0	7 (35)	0
Skin/soft tissue	0	0	1 (25)	0	0	1 (5)	0
Urinary tract	2 (50)	0	0	0	0	2 (10)	1 (25)
Other	0	0	0	1 (25)	1 (25)	2 (10)	1 (25)
Systemic inflammatory response syndrome criteria, *n* (%)							
≤ 1	0	0	0	2 (50)	1 (25)	3 (15)	0
2	0	0	0	1 (25)	1 (25)	2 (10)	1 (25)
3	2 (50)	2 (50)	0	0	0	4 (20)	2 (50)
4	2 (50)	2 (50)	4 (100)	1 (25)	2 (50)	11 (55)	1 (25)
Organ dysfunctions, *n* (%)							
1	1 (25)	0	1 (25)	1 (25)	0	3 (15)	0
2	2 (50)	2 (50)	1 (25)	1 (25)	0	6 (30)	3 (75)
3	1 (25)	2 (50)	2 (50)	2 (50)	4 (100)	11 (55)	1 (25)
Sequential Organ Failure Assessment score, mean (± SD)	7.5 (2.7)	7.8 (4.6)	7.0 (2.9)	9.0 (5.4)	6.8 (3.6)	7.6 (3.6)	7.5 (1.7)

n = number of participants.

[a]Absolute lymphocyte count values shown represent the lowest recorded in the 48 hr prior to dosing.

Organ dysfunctions: hypotension, acute respiratory failure, and acute kidney injury.

Systemic inflammatory response syndrome criteria: hyperthermia/hypothermia, tachycardia, tachypnea, leukocytosis/leukopenia/immature neutrophils increased.

Post-dose ophthalmoscopic data (at index hospitalization discharge and/or day 90) were available for 15 participants. No cases of focal retinal lesions were reported.

Pharmacokinetics

The pharmacokinetic findings are presented and discussed in more detail in the **supplementary information** (see

TABLE 2. Safety Results–All Treated Participants

Variables	BMS-936559 Treatment						
	10 mg (*n* = 4)	30 mg (*n* = 4)	100 mg (*n* = 4)	300 mg (*n* = 4)	900 mg (*n* = 4)	Pooled (*n* = 20)	Placebo (*n* = 4)
Deaths, *n* (%)	2 (50)	2 (50)	1 (25)	1 (25)	0	6 (30)	0
On-treatment serious AE, *n* (%)	1 (25)	1 (25)	0	0	1 (25)	3 (15)	1 (25)
On-treatment grade 3–4 AE, incidence ≥ 20%, *n* (%)	1 (25)	3 (75)	1 (25)	1 (25)	1 (25)	7 (35)	0
On-treatment AE of any grade, incidence ≥ 20%, *n* (%)	4 (100)	4 (100)	4 (100)	4 (100)	3 (75)	19 (95)	4 (100)
Vascular disorders, *n* (%)							
Hypotension	1 (25)	3 (75)	1 (25)	4 (100)	2 (50)	11 (55)	0
Investigations, *n* (%)							
Lipase increased	0	1 (25)	3 (75)	1 (25)	0	5 (25)	2 (50)
Weight decreased	2 (50)	0	2 (50)	0	0	4 (20)	2 (50)
Metabolism and nutrition disorders, *n* (%)							
Hypokalemia	1 (25)	1 (25)	1 (25)	1 (25)	0	4 (20)	1 (25)
Malnutrition	1 (25)	0	2 (50)	1 (25)	0	4 (20)	1 (25)
Blood and lymphatic system disorders, *n* (%)							
Anemia	0	0	1 (25)	2 (50)	3 (75)	6 (30)	2 (50)
Gastrointestinal disorders, *n* (%)							
Diarrhea	2 (50)	0	3 (75)	1 (25)	1 (25)	7 (35)	1 (25)
Psychiatric disorders, *n* (%)							
Delirium	2 (50)	1 (25)	2 (50)	1 (25)	1 (25)	7 (35)	0
Respiratory, thoracic, and mediastinal disorders, *n* (%)							
Pleural effusion	1 (25)	0	2 (50)	2 (50)	0	5 (25)	0

AE = adverse event, *n* = number of participants.

All recorded AEs were summarized by system organ class and preferred term using Medical Dictionary for Regulatory Activities Version 20.0 (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use). AEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events Version 4.03.

The on-treatment phase of the study represents the 90-d period following single-dose administration of study treatment.

Supplementary Tables S3–S5 and **Supplementary Fig. S2**, Supplemental Digital Content 1, http://links.lww.com/CCM/E415). BMS-936559 mean terminal half-life ranged from 29 hours (10 mg) to 189 hours (300 mg). BMS-936559 exhibited nonlinear pharmacokinetics and target-mediated drug disposition kinetics, with faster elimination rates and higher volumes of distribution observed compared with patients with cancer. After dose-normalization, anti–PD-L1 observed concentrations were generally higher and decreased more slowly in participants with solid tumors than in participants with sepsis.

Biomarkers

RO. A dose-dependent increase in RO duration was observed (**Fig. 2*A***). There was also a dose-dependent increase in the time period over which greater than or equal to 80% RO was achieved; full RO was achieved for 28 days following the 900-mg single dose (Fig. 2*A*).

mHLA-DR. Increased mHLA-DR expression over time was demonstrated, with the greatest increase observed at the two highest doses (**Fig. 2*B***). From days 15 to 90, median mHLA-DR levels with BMS-936559 300 and 900 mg were greater than 5,000 mAb/cell (between ~6,000 and ~18,000 mAb/cell) (Fig. 2*B*).

A post hoc analysis combined the 10, 30, and 100 mg dose groups ("low-dose") and 300 and 900 mg groups ("high-dose") for RO and mHLA-DR to day 29 (**Fig. 2*C***). Full RO was maintained until day 8 (low-dose) and until day 29 (high-dose). mHLA-DR expression rose above 5,000 mAb/cell at day 8 in the low-dose group and at day 4 in the high-dose group; beyond day 8, mHLA-DR expression was consistently higher at each time point in the high-dose versus low-dose group.

TABLE 3. Adverse Events of Special Interest–All Treated Participants

Variables	BMS-936559 Treatment						
	10 mg (*n* = 4)	30 mg (*n* = 4)	100 mg (*n* = 4)	300 mg (*n* = 4)	900 mg (*n* = 4)	Pooled (*n* = 20)	Placebo (*n* = 4)
AEs of special interest, grade 3–4, *n* (%)	0	1 (25)	0	1 (25)	1 (25)	3 (15)	0
Gastrointestinal disorders, *n* (%)							
Diarrhea	0	0	0	1 (25)	1 (25)	2 (10)	0
Respiratory, thoracic, and mediastinal disorders, *n* (%)							
Lung infiltration	0	1 (25)	0	0	0	1 (5)	0
AE of special interest, any grade, *n* (%)	3 (75)	1 (25)	3 (75)	2 (50)	2 (50)	11 (55)	2 (50)
Gastrointestinal disorders, *n* (%)							
Diarrhea	2 (50)	0	3 (75)	1 (25)	1 (25)	7 (35)	1 (25)
Endocrine disorders, *n* (%)							
Hypothyroidism	1 (25)	0	1 (25)	0	0	2 (10)	0
Investigations, *n* (%)							
Aspartate aminotransferase increased	0	0	0	1 (25)	0	1 (5)	1 (25)
Alanine aminotransferase increased	0	0	0	1 (25)	0	1 (5)	0
Respiratory, thoracic, and mediastinal disorders, *n* (%)							
Lung infiltration	0	1 (25)	0	0	0	1 (5)	0
Skin and subcutaneous tissue disorders, *n* (%)							
Rash	0	0	0	0	1 (25)	1 (5)	0

AE = adverse event, *n* = number of participants.

All recorded AEs of special interest were summarized by preferred term using Medical Dictionary for Regulatory Activities Version 20.0 (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use). AEs of special interest were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events Version 4.03.

AEs of special interest are AEs with potential immune-related causes as identified in the antiprogrammed cell death-ligand 1 study in patients with cancer (35).

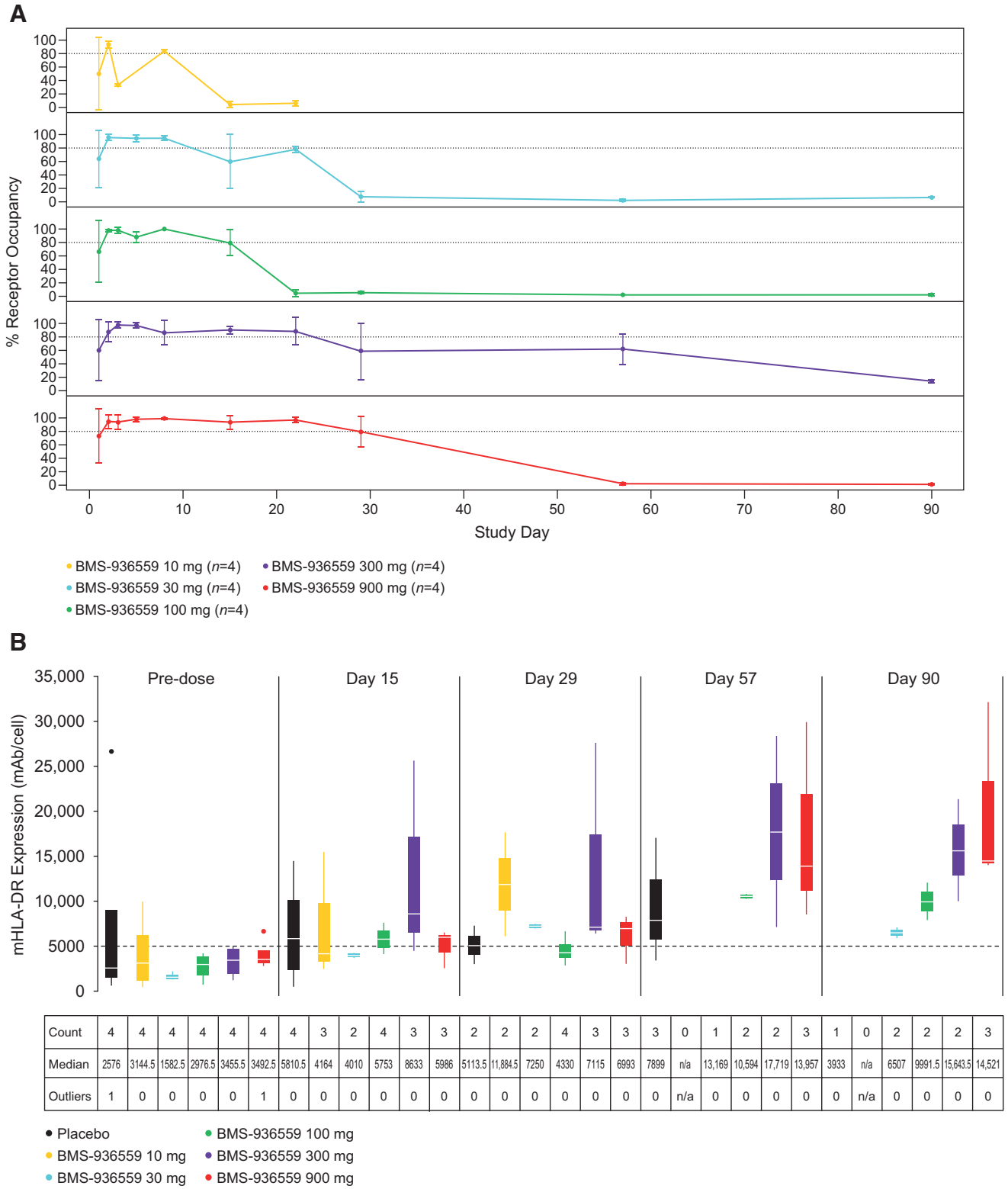
Other Biomarkers. No clear dose-related changes or trends were observed in ALC or cytokine levels (**Supplementary Figs. S3–S5**, Supplemental Digital Content 1, http://links.lww.com/CCM/E415).

DISCUSSION

These data describe the first clinical evaluation of an anti–PD-L1 mAb in patients with sepsis-associated immunosuppression. Single doses of BMS-936559 (10–900 mg) were well tolerated. Most AEOSIs were of mild-to-moderate intensity, consistent with BMS-936559 mechanism of action, and generally similar to those reported in patients with cancer (rash, hypothyroidism, and diarrhea) (35). No cases of pneumonitis (a potentially life-threatening condition that has been recorded with anti–PD-1/PD-L1 mAb in the oncology setting [14–18]) were reported. As documented in the AE profile and cytokine measurements, there was no clinical or biomarker evidence of a drug-induced cytokine release syndrome. Specifically, there was no indication that administration of anti–PD-L1 was temporally associated with worsening fever or hemodynamic instability, and no observed clinically significant changes in cytokine concentrations. This is important because, although PD-1/PD-L1 pathway inhibition is effective (14–18), studies of therapies to boost immune activity in sepsis have not been performed, partly because of the theoretical risk of an excessive pro-inflammatory response (see introduction).

In BMS-936559 multiple-dose, 3-month primate toxicology studies, focal retinal lesions were reported, and monitoring was performed in the current clinical study. No focal retinal lesions were detected in any participant. This is consistent with the phase 1 BMS-936559 HIV-1 study, which did not reveal focal retinal findings similar to those seen in monkeys (36).

The pharmacokinetic findings of this study are interesting because the faster elimination rates and higher volumes of distribution seen compared with patients with cancer suggest that higher doses or more frequent dosing might be warranted for patients with sepsis. The duration of full RO increased dose-dependently, and the prolonged (28 d) RO at the 900-mg dose



Count	4	4	4	4	4	4	4	3	2	4	3	3	2	2	2	4	3	3	3	0	1	2	2	3	1	0	2	2	2	3
Median	2576	3144.5	1582.5	2976.5	3455.5	3492.5	5810.5	4164	4010	5753	8633	5986	5113.5	11,884.5	7250	4330	7115	6993	7899	n/a	13,169	10,594	17,719	13,957	3933	n/a	6507	9991.5	15,643.5	14,521
Outliers	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	n/a	0	0	0	0	0	n/a	0	0	0	0

Figure 2. Changes in receptor occupancy (RO) and monocyte human leukocyte antigen (mHLA)-DR expression with BMS-936559. **A**, Change in programmed cell death-ligand 1 RO with BMS-936559 (10–900 mg) on CD3+ T cells over the 90-d study treatment period. *Dashed lines* indicate 80% RO. **B**, Change in mHLA-DR expression with BMS-936559 (10–900 mg) over the 90-d study period (*box plots* are shown for greater clarity and distinction). *Dashed line* indicates mHLA-DR 5,000 monoclonal antibodies (mAb) per cell. Visit windows: day 15 ± 3, day 29 ± 7, days 57 and 90 ± 14. *Box plot*: *rectangle spans* interquartile range; *horizontal line* is median. **C**, Changes in RO and mHLA-DR expression in participants receiving placebo, or 10, 30, and 100 mg dose combined ("low-dose group"), or 300 and 900 mg dose combined ("high-dose group") up to day 29 post-infusion. *Green dashed line* represents 80% RO; *blue dashed line* represents mHLA-DR 5,000 mAb per cell. n/a = not applicable.

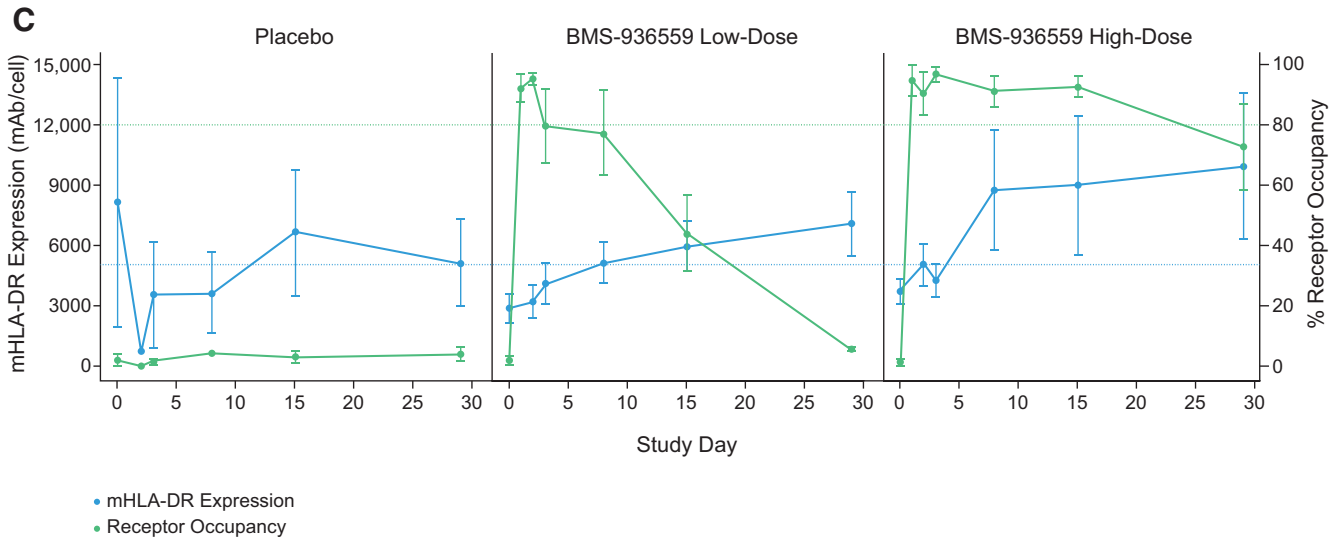


Figure 2. *(Continued)*

level could be advantageous in preventing new secondary infections that cause additional morbidity and mortality.

The mHLA-DR expression also appeared to increase over time, consistent with a restoration of immune function. The post hoc analysis highlighted the sustained, high degree of PD-L1 target engagement (RO) and implied a more rapid recovery of immune function (mHLA-DR expression) at the two higher doses than at the three lower doses. Due to small participant numbers, these data should be interpreted with caution. However, similar associations between immunotherapy and increased mHLA-DR expression (and improved outcome) have been reported elsewhere. Two INF-γ (multiple-dose) studies in patients with sepsis/trauma and immunoparalysis reported improved mHLA-DR expression and associated clinical improvements (43, 44). A study of granulocyte-macrophage colony-stimulating factor (multiple doses) versus placebo in patients with sepsis-associated immunosuppression reported mHLA-DR normalization, along with improved patient outcomes (45).

There was no clear trend in ALC levels after dosing with BMS-936559. Reduced ALC is a frequent and easily measured feature of sepsis-associated immunosuppression (8, 21), but may not be the optimal pharmacodynamic marker to assess the effect of PD-1/PD-L1 pathway inhibition in a short-term study. Lymphocyte counts may increase through homeostatic proliferation or de novo lymphocyte production; however, both processes would be expected to take a significant amount of time (46, 47). Thus, ALC may not fully recover over 90 days.

There were also no clear or dose-related trends in IL-6, IL-8, and IL-10 levels observed. Although animal/ex vivo studies have shown some changes in cytokine levels (24–26, 31, 33), there may be several reasons for a lack of a detectable change in markers in the current study. For example, the sampling times chosen may not have detected a rapid or transient change in cytokine level. Another reason may be that any substantial cytokine change was highly localized and not detectable systemically. The small sample size may also be a factor. However, there was an absence of any systemic change in cytokine levels indicative of a cytokine storm, which is an important "goal" from a first-in-human study perspective. For comparison, it is worth noting one study from the literature in which 39 human cytokines and chemokines (including IL-6, IL-8, and IL-10) were simultaneously quantified in pre- and post-dose plasma samples from 24 patients with cancer who were undergoing anti–PD-1 therapy. Only significant increases in IL-1 alpha and CXCL10 plasma levels were reported with anti–PD-1 alone post- versus pre-dose (48).

There are some limitations to the study that should be discussed. As already alluded to, the small sample size results in a lack of power to detect issues associated with a pro-inflammatory response. Larger studies may reveal other AEs not reported here; however, the broad similarity between the AEs reported here and those in the larger study in patients with cancer (35) is encouraging. There is also the question of the definition of immunosuppression used in the study; for practical purposes, an ALC of less than or equal to 1,100 cells/μL within the 96 hours before study treatment administration was employed. Reduced ALC correlates with worse outcomes in patients with sepsis (8, 10, 11). However, it is not a definitive marker of sepsis-associated immunosuppression. It is important to note that, in the current study, pre-dose mHLA-DR levels were very low (< 5,000 mAb/cell) (Fig. 2*B*), which would indicate that these participants were immunosuppressed and at increased risk of mortality (42, 49). Recently, an integrated genomics study of patients with sepsis admitted to the ICU also identified two distinct sepsis phenotypes: Sepsis Response Signature (SRS) 1 and SRS2, with SRS1 being an immunosuppressed phenotype (50). This immunosuppressed phenotype had lower ALC levels than the SRS2 phenotype (J. C. Knight, personal communication, 2017). Together, these findings provide a strong indication that lower ALC levels are indeed associated with immunosuppression in patients with sepsis.

These data suggest that PD-1/PD-L1 pathway inhibition in patients with sepsis-associated immunosuppression is well

tolerated. BMS-936559, particularly with the higher doses studied, results in an apparent increase in mHLA-DR expression; further studies are needed to determine whether it safely restores immune functional status and protects against secondary infections and related clinical consequences (readmissions, morbidity, and mortality).

CONCLUSIONS

This was the first clinical evaluation of an anti–PD-L1 (BMS-936559) in patients with sepsis-associated immunosuppression.

PD-1/PD-L1 pathway inhibition represents an evolution in conceptual understanding of approaches to sepsis treatment. Rather than seeking to inhibit the host response, checkpoint inhibitor therapies that enhance host immunity may represent a new way forward against this highly lethal syndrome. Further study of PD-1/PD-L1 pathway inhibition is warranted.

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