A Phase Ib/II Study of Pepinemab in Combination with Avelumab in Advanced Non–Small Cell Lung Cancer

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ABSTRACT

Purpose: The CLASSICAL-Lung clinical trial tested the combination of pepinemab, an IgG4 humanized mAb targeting semaphorin 4D, with the PD-L1 inhibitor avelumab to assess the effects of coupling increased T-cell infiltration and reversal of immune suppression via pepinemab with sustained T-cell activation via checkpoint inhibition.

Patients and Methods: This phase Ib/II, single-arm study was designed to evaluate the safety, tolerability, and efficacy of pepinemab in combination with avelumab in 62 patients with advanced non–small cell lung cancer (NSCLC), including immunotherapy-naïve (ION) patients and patients whose tumors progressed following anti-PD-1/L1 monotherapy (IOF). The main objectives were to evaluate safety/tolerability, establish a recommended phase 2 dose (RP2D), obtain a preliminary evaluation of antitumor activity, and investigate candidate biomarker activity.

Results: The combination was well tolerated with no major safety signals identified. Pepinemab, 10 mg/kg with avelumab, 10 mg/kg, every 2 weeks, was selected as the RP2D. Among 21 evaluable ION patients, 5 patients experienced partial responses (PR), 4 patients evidenced clinical benefit ≥1 year, and the disease control rate (DCR) was 81%. Notably, overall response rate with the combination therapy was higher than previously reported for single-agent avelumab in the PD-L1-negative/low population. Among 29 evaluable IOF patients, the combination resulted in a DCR of 59%, including 2 PR and 7 patients with durable clinical benefit of ≥23 weeks. Biomarker analysis of biopsies demonstrated increased CD8 T-cell density correlating with RECIST response criteria.

Conclusions: The combination of pepinemab with avelumab was well tolerated in NSCLC and showed signs of antitumor activity in immunotherapy-resistant and PD-L1-negative/low tumors.

Introduction

Immune checkpoint inhibition (ICI) has been validated as an efficacious strategy for the treatment of advanced non–small cell lung cancer (NSCLC; ref. 1), however most (>50%) subjects with NSCLC do not respond to single-agent ICI, and responses that do emerge are often not durable (2). Rational, safe combinations with ICI are needed to address this immunotherapy-resistant or refractory population, which take advantage of complimentary pathways to overcome the immunosuppressive tumor microenvironment (TME).

Pepinemab (VX15/2503;VX15) is a humanized IgG4 mAb, which binds specifically to semaphorin 4D (SEMA4D; CD100) and blocks the interaction with its receptors, plexinB1 (PLXNB1), plexin B2 (PLXNB2), and CD72 (3). SEMA4D is a member of the semaphorin family, which normally functions to regulate the motility and differentiation of multiple cell types. In the context of cancer, SEMA4D contributes to oncogenesis (4), cancer progression (5), restriction of immune infiltration (6), and promotion of immune suppression (7, 8). Blockade of SEMA4D signaling has been shown to reverse these effects and promote antitumor immunity in preclinical studies. IHC analysis of SEMA4D and PLXNB1 expression by several human tumor types revealed that SEMA4D is overexpressed in head and neck, prostate, colon, breast, and lung cancer (9). Elevated expression of SEMA4D and its receptor PLXNB1 correlates with invasive disease and poor prognosis (10).

Preclinical data demonstrated that SEMA4D alters the balance of the TME to favor immunosuppression, by forming a barrier that prevents efficient infiltration of dendritic cells (DC) and cytotoxic T lymphocytes (CTL) and by programming and recruiting immunosuppressive cells, including myeloid-derived suppressor cells (MDSC) and M2 tumor-associated macrophages (TAM; refs. 6, 7). Antibody blockade of SEMA4D promoted immune infiltration into the tumor that resulted in a marked redistribution of immune cells at the tumor invasive margin in multiple tumor models, including a significant increase in intratumoral $CD8^+$ T cells, increased T-effector to T-regulatory (T_{eff}:T_{reg}) cell ratios, and dispersion of M2-polarized TAMs and MDSCs. These changes promoted a proinflammatory profile with increased IFNγ and TNFα expression, and reduction in

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Translational Relevance

Pepinemab, a first-in-class mAb that binds to semaphorin 4D (SEMA4D), has demonstrated striking synergy with checkpoint inhibitors in preclinical models. The CLASSICAL-Lung study combined pepinemab with avelumab, an anti-PD-L1 antibody, in a phase Ib/II clinical trial enrolling patients with advanced non–small cell lung cancer (NSCLC). Presented here are the first clinical data showing that pepinemab is well tolerated when combined with immune checkpoint blockade and that the combination is clinically active. First, the combination of pepinemab plus avelumab appeared to halt or reverse tumor progression (partial response or stable disease) in select patients with primary or acquired resistance to anti-PD-1/L1 therapy. Furthermore, this combination appeared to provide clinical benefit in the difficult to treat PD-L1-low, immunotherapy-naïve population. Finally, exploratory biomarker analysis demonstrated improved tumoral infiltration of $CD8^+$ T cells and supported the proposed mechanism of action of pepinemab, namely, to shift the tumor microenvironment toward immunity and away from immunosuppression thus enhancing immune checkpoint inhibition.

MCP-1/CCL2 and CXCL1, immunosuppressive chemokines that act as MDSC chemoattractants and modulators of T_{eff}-to-T_{reg} cell ratios. Importantly, in mouse models of lung, colon, breast, and head and neck cancers, the combination of anti-SEMA4D with other immunotherapies, including a variety of IC blockade antibodies, resulted in durable and complete responses that were superior to IC treatment alone.

Two phase I trials using pepinemab have been completed in patients with solid tumors (11) or with multiple sclerosis (12), and pepinemab was found to be well tolerated in both studies. Avelumab is a PD-L1 blocking mAb that is approved for treatment of metastatic Merkel cell, locally advanced or metastatic urothelial, and in combination with axitinib for renal cell carcinoma (avelumab PI, revised November 2020; https://www.emdserono.com/us-en/pi/bavencio-pi.pdf) and is also being investigated for treatment of NSCLC (13, 14). Absence of durable responses to avelumab and other anti-PD-1/L1 therapies in the majority of patients with NSCLC indicates a need to improve activity. The CLASSICAL-Lung trial was designed to evaluate the safety and efficacy of pepinemab plus avelumab combination therapy in NSCLC, to confirm preclinical mechanism of action studies and, by demonstrating biologic activity of pepinemab in a clinical setting, to validate its potential as a broadly applicable strategy to enhance immunotherapies in oncology indications.

Patients and Methods

Study design and participants

This multicenter phase Ib/II trial of patients with histologically or cytologically proven, advanced stage IIIB/IV NSCLC (≥18 years) was designed to evaluate the safety and efficacy of pepinemab in combination with avelumab. All patients were required to have an Eastern Cooperative Oncology Group (ECOG) status of 0 or 1, measurable disease per RECIST v1.1, and adequate organ function. The complete list of inclusion and exclusion criteria are defined in the study protocol.

The phase Ib portion of the study enrolled immunotherapy-naïve (ION) patients that either declined or had progression of disease with first-line chemotherapy and was conducted utilizing a standard 3 + 3 dose-escalation design. Increasing doses of pepinemab (5, 10, or 20 mg/kg) were combined with a fixed dose of avelumab at 10 mg/kg. Both antibodies were successively administered, pepinemab followed by avelumab, intravenously at 2-week intervals. An additional 3 patients were permitted to be enrolled in the event of a dose-limiting toxicity (DLT) during a 28-day evaluation period, for a maximum of 18 patients.

The phase II portion of the study enrolled an additional 18 ION patients, as defined earlier, and 32 patients whose previous PD-1/LI immunotherapy treatment had failed (IOF), as defined as patients who have progressed on prior treatment with fewer than three lines of immunotherapy with or without chemotherapy. Patients in this expansion phase were treated with 10 mg/kg of pepinemab and 10 mg/kg of avelumab each every 2 weeks. The 10 mg/kg dose of pepinemab was selected on the basis of safety and tolerability of prior and ongoing single agent and combination pepinemab trials, prior clinical trials demonstrating complete target saturation at this dose (12), and preclinical studies demonstrating that target saturation correlates with minimal effective dose (6). Treatment in both phases continued until confirmed disease progression (PD), intolerable toxicity, protocol defined or patient consent withdrawal, or patient's death. Dose modifications or treatment interruption of one drug was permitted for clinically significant adverse events (AE), only if the investigator determined the patient was benefiting from treatment. The study was conducted at 11 investigator sites in North America and completed enrollment of 62 patients. All patients provided written informed consent. Institutional Review Board approvals for the study protocol, amendments, and informed consent documents were obtained prior to study initiation; study procedures were conducted in accordance with the Declaration of Helsinki. The ClinicalTrials.gov identifier was NCT03268057.

Study assessments

Safety

Safety assessments were performed on a regular basis by physical examination, scheduled and unscheduled laboratory assessments at a local and/or central laboratory, and other diagnostic evaluations. AEs were reported using the Common Terminology Criteria Adverse Events version 4.03 (CTCAE v4.03). During dose escalation, only patients who completed the 28-day observation period and were not discontinued for any reason other than study drug toxicity were included for safety assessment. Safety data were reviewed by the Safety Monitoring Committee (SMC) prior to opening the next cohort.

Efficacy

Tumor assessments via CT, MRI, or PET were conducted at baseline for all patients. Restaging scans were obtained every 6 weeks for the first 18 weeks and then every 12 weeks thereafter. Scans were obtained until confirmed disease progression, based on investigator assessment using RECIST v1.1.

Statistical analysis

Safety was defined by the number of patients who experienced a DLT during the 28-day observation period at each dose level of pepinemab ($n = 1$ at 10 mg/kg). Efficacy was evaluated in patients ($n = 50$) who received at least one dose of pepinemab and avelumab, who completed at least one cycle of treatment, and had a post-baseline response assessment. The overall response rate (ORR) was calculated as the number of patients that achieved a best response of complete response (CR) or partial response (PR), as defined by RECIST v1.1, divided by the total number of patients receiving at least one cycle of

treatment in the same cohort. Confidence intervals were calculated using the Clopper–Pearson method, and progression-free survival (PFS) and duration of response (DOR) were estimated using Kaplan–Meier methodology. Evaluation of PFS and censoring was performed as outlined in the Clinical Trial Endpoints for the Approval of Non-Small Cell Lung Cancer Drugs and Biologics guidance (April 2015). Weeks on study was defined as the time from first dose of study treatment to the end of treatment (EOT) visit, and if the EOT visit was not performed, the date the investigator withdrew study treatment was utilized. DOR was calculated from the first date a tumor response was observed per RECIST v1.1 to the first date of disease progression or censoring. Three patients ($n = 3$) were transferred to expanded access and were censored at their EOT scan.

For exploratory immunophenotyping, statistical analysis was performed using SPSS statistical software (Build 1.0.0.1406) and the data were expressed as the mean ± SEM for all continuous variables. Homogeneity of variances was assessed by Levene test for equality of variances ($P > 0.05$). Data normality distribution was assessed by Shapiro–Wilk test ($P > 0.05$). In instances where normality was violated, Wilcoxon–Mann–Whitney test was used to compare the groups. In addition, a multiple regression analysis was performed to correlate immune cell markers with clinical parameters, where the linearity was assessed by partial regression plots and a plot of studentized residuals against the predicted values. The independence of residuals was assessed by Durbin–Watson statistics. Cox proportional hazards model analysis was used to estimate the risk of each individual blood biomarker with PFS. The optimal cutoff point for absolute neutrophil count (ANC) and absolute monocyte count (AMC) was determined as described by Soyano and colleagues (15).

Pharmacokinetic/pharmacodynamic analysis

Blood samples for the analysis of pepinemab and avelumab drug levels were obtained on a weekly basis through their first three infusion cycles, then on a biweekly basis until they reached cycle 7 and every 6 weeks thereafter. Blood was collected on all dosing days prior to the first and after the last infusion. On days 1 and 43, an additional collection was completed at 0.5, 1, 2, 3, 4, 6, and 8 hours after dosing. Blood samples for pepinemab pharmacodynamic assays were collected at baseline and prior to each dose and included receptor occupancy, total soluble SEMA4D, and cellular levels of SEMA4D. Validated assays were utilized as described previously (12, 16).

PD-L1 expression

PD-L1 expression was assessed using a proprietary IHC assay (PD-L1 IHC 73–10 pharmDx; Dako). This 73–10 assay has been compared with the 22C3 assay used in pembrolizumab trials and the 73–10 assay showed greater sensitivity, with the >80% PD-L1 level for the 73–10 assay being comparable with the >50% PD-L1 level for the 22C3 assay (17). Therefore, in this study, expression will be presented in standard categories (<1%, 1–49%, 50–79%, >80%), but "low" PD-L1 expression will refer to expression of 1% to 79%.

Exploratory

Whole blood was collected for peripheral immunophenotyping from each patient every 2 weeks for their first seven cycles of treatment and then every 6 weeks thereafter. Peripheral lymphocytes were analyzed via flow cytometry at a central laboratory and T cells ($CD3^+$), B cells ($CD19^+$), NK cells ($CD3^-$, $CD16^+/56^+$), helper/inducer T cells ($CD3^+$, $CD4^+$), and cytotoxic T cells ($CD3^+$, $CD8^+$) were isolated and reported in absolute (cells/μL). MDSCs were analyzed using a qualified assay at a central laboratory using an extensive panel of MDSC markers (18, 19). MDSCs were defined as Lin-negative/$CD33^+$/HLA-DR-low/CD16dim (total MDSC). MDSC subsets were defined as polymorphonuclear (PMN-MDSC: $CD15^+/CD14^-$) and monocytic (M-MDSC: $CD15^-/CD14^+$); MDSC populations are presented as percent of live nucleated cells from peripheral blood. Complete blood count (CBC) was obtained at baseline and included: white blood cell count (WBC), ANC, absolute lymphocyte count (ALC), AMC, absolute eosinophil count (AEC), and platelet count.

Baseline pretreatment tumor biopsies were collected at time of enrollment or archival specimens obtained within prior 6 months were allowed. On-treatment biopsies were collected at end of cycle 2, approximately 29 days following the first treatment. Sample selection criteria included: both pre- and on-treatment biopsies collected from the same lesion and pathologist verification of tumor bed area; fine needle aspirates were excluded due to lack of tissue architecture to define tumor area. All samples that met these criteria are reported herein. Formalin-fixed, paraffin embedded sections (5 μm) were stained for CD8 and tumor content (cytokeratin) using a serial multiplex IHC staining method (20, 21). Single tumor slides were sequentially stained in a stain and strip multiplex assay with hematoxylin and the following immunostains: tumor mask (panCK, Thermo Fisher Scientific MA513203), CD8 (clone C8/114B), CD68 (D4B9C), CD11c (EP1347Y), CD163 (10D6). Whole slide scans were acquired at 40× magnification using the MoticEasyScan Pro digital slide scanner. Tissue histology was reviewed by an accredited pathologist for identification of various tissue regions of interest (tumor, stroma, necrotic, fibrosis, and normal). Images were imported into VISIOPHARM for virtual multiplex analysis and Tissuealign (VISIOPHARM) was used to coregister and align multiple immunostained images for virtual multiplex analysis. Tumor cells were masked with PanCK marker and regions of interest (ROI) for tumor bed were manually drawn based on pathology review. Multiplex IHC algorithms were written (VISIOPHARM) to determine immune cell density based on cell-specific markers. Algorithms were also written to identify individual cell nuclei based on hematoxylin stain. Thresholding was then set using immunostains to identify specific immune cell subsets. Immune cell densities were calculated as a function of tumor bed area. Tumor bed area was defined in biopsy samples as tumor, stroma, and fibrotic regions where tumor once resided. Normal and necrotic tissue areas were excluded in this analysis. Representative images were selected from each section by considering both T-cell density data and percent tumor, as determined by quantification across the entire tumor area.

Results

Study design and basic demographics

A total of 62 patients, whose baseline and clinical characteristics are listed in **Table 1**, were enrolled from October 2017 to August 2019 (Supplementary Fig. S1). Twelve ION patients (3 at 5 mg/kg, 6 at 10 mg/kg, and 3 at 20 mg/kg of pepinemab) were enrolled in phase Ib. Subsequently, 50 patients were enrolled into phase II; 18 ION patients and 32 IOF patients. Of the total ION patients enrolled, 17/30 (57%) had prior chemotherapy and 13/30 (43%) were treatment naïve. Of the IOF patients, most had progressed on either pembrolizumab or nivolumab. The majority of IOF patients (30/32, 94%) received at least one line of chemotherapy in addition to subsequent immunotherapy. Of the 29/32 (91%) evaluable IOF patients, 7 were considered to have experienced primary resistance (best response to prior therapy was PD) to ICI, whereas 18 IOF patients had acquired resistance to ICI [best response was PR or

Table 1. Baseline demographics and clinical characteristics.

	Phase Ib						Phase II			
	5 mg/kg (*n* = 3)		10 mg/kg (*n* = 6)		20 mg/kg (*n* = 3)		ION (*n* = 18)		IOF (*n* = 32)	
Characteristic	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**%**	***n***	**%**
Age, median (range)	37 (30–79)		65 (59–75)		61 (60–69)		64 (54–83)		67 (51–85)	
18 to <65	2	67%	3	50%	2	67%	9	50%	12	38%
65 and over	1	33%	3	50%	1	33%	9	50%	20	63%
Sex										
Men	1	33%	4	67%	2	67%	7	39%	23	72%
Women	2	67%	2	33%	1	33%	11	61%	9	28%
Race										
White	3	100%	5	83%	3	100%	18	100%	27	84%
Asian	0	0%	0	0%	0	0%	0	0%	1	3%
Black or African American	0	0%	0	0%	0	0%	0	0%	3	9%
NHOPI	0	0%	1	17%	0	0%	0	0%	0	0%
Other	0	0%	0	0%	0	0%	0	0%	1	3%
ECOG performance status										
0	1	33%	3	50%	0	0%	6	33%	5	16%
1	2	67%	3	50%	3	100%	12	67%	27	84%
Disease stage at screening										
IIIA	0	0%	0	0%	0	0%	0	0%	1	3%
IV	3	100%	6	100%	3	100%	18	100%	31	97%
Histology										
Adenocarcinoma	1	33%	4	67%	1	33%	13	72%	19	59%
Squamous cell	2	67%	2	33%	2	67%	5	28%	13	14%
Previous therapy										
Chemotherapy	3	100%	3	50%	3	100%	8	44%	30	94%
Immunotherapy	0	0%	0	0%	0	0%	0	0%	32	100%
None	0	0%	3	50%	0	0%	10	56%	0	0%
PD-L1 status (Dako PD-L1 73–10 pharmDx assay)										
No PD-L1 expression (negative)	0	0%	2	33%	1	33%	5	45%	11	38%
1–49% PD-L1 expression (low)	1	50%	3	50%	1	33%	5	45%	12	41%
50–79% PD-L1 expression (low)	1	50%	1	17%	0	0%	1	9%	6	21%
≥80% PD-L1 expression (high)	0	0%	0	0%	1	33%	0	0%	0	0%
Cancelled[a]	1		0		0		7		3	

Abbreviation: NHOPI, Native Hawaiian and other Pacific Islander.

[a]Testing was cancelled due to: sample past stability (1 test) sample not received (4 tests), no tumor present (4 tests), incorrect sample type (2 tests) and was not included in % calculation.

stable disease (SD)]; 4 had a treatment history that was partially unknown (**Fig. 1C**). Notably, only 1 patient (1/51 PD-L1 evaluable) enrolled in this study had a tumor with high PD-L1 expression (>80% by Dako PD-L1 IHC 73–10 pharmDx assay).

Patients received a median of five doses of avelumab and pepinemab given every 2 weeks for a median of 12 weeks. In addition, 3 patients who experienced long-term SD or PR on pepinemab plus avelemab therapy were subsequently enrolled into investigator-sponsored expanded access trials to continue the combination. Nine patients in the ION cohort were not evaluable for RECIST responses (6 withdrew prior to first scan and 3 patients died prior to first scan) and 3 patients in the IOF group were not evaluable (1 AE, 1 died prior to first scan, and 1 scan was concluded to be nonevaluable). Therefore, the total number of patients evaluable for clinical response in the study was 50.

The primary outcome measures were safety and tolerability of the combination and determination of recommended phase 2 dose (RP2D). Secondary outcomes included clinical response as determined by RECIST v1.1, pharmacokinetics, pharmacodynamics, and exploratory analysis of peripheral and tumoral biomarkers of immune-related antitumor activity.

Dose finding and safety

The combination therapy of pepinemab plus avelumab was well tolerated at all dose levels and no concerning safety signals beyond those previously associated with avelumab were identified. One DLT, a grade 3 pulmonary embolism, occurred in the 10 mg/kg pepinemab + 10 mg/kg avelumab escalation cohort, but resolved and did not recur in that same patient after further treatment or additional patients in any cohort. The 10 mg/kg pepinemab every 2 weeks (plus 10 mg/kg avelumab every 2 weeks) combination was selected by the SMC as the RP2D based on safety profile and complete receptor occupancy at that dose. The 20 mg/kg pepinemab dose level was also found to be safe but was not utilized in expansion due to the complete receptor occupancy at 10 mg/kg pepinemab. Overall, the most frequent related AEs were grade 1 or 2 fatigue and infusion-related reaction.

Three immune-related AEs (irAE) occurred independently in 3 patients during treatment (immune-mediated diabetic ketoacidosis, immune-related myositis, and immune-mediated pneumonitis). Immune-mediated diabetic ketoacidosis (grade 4) was attributed as possibly related to pepinemab and definitely related to avelumab, and although treatment was interrupted, this patient responded to insulin

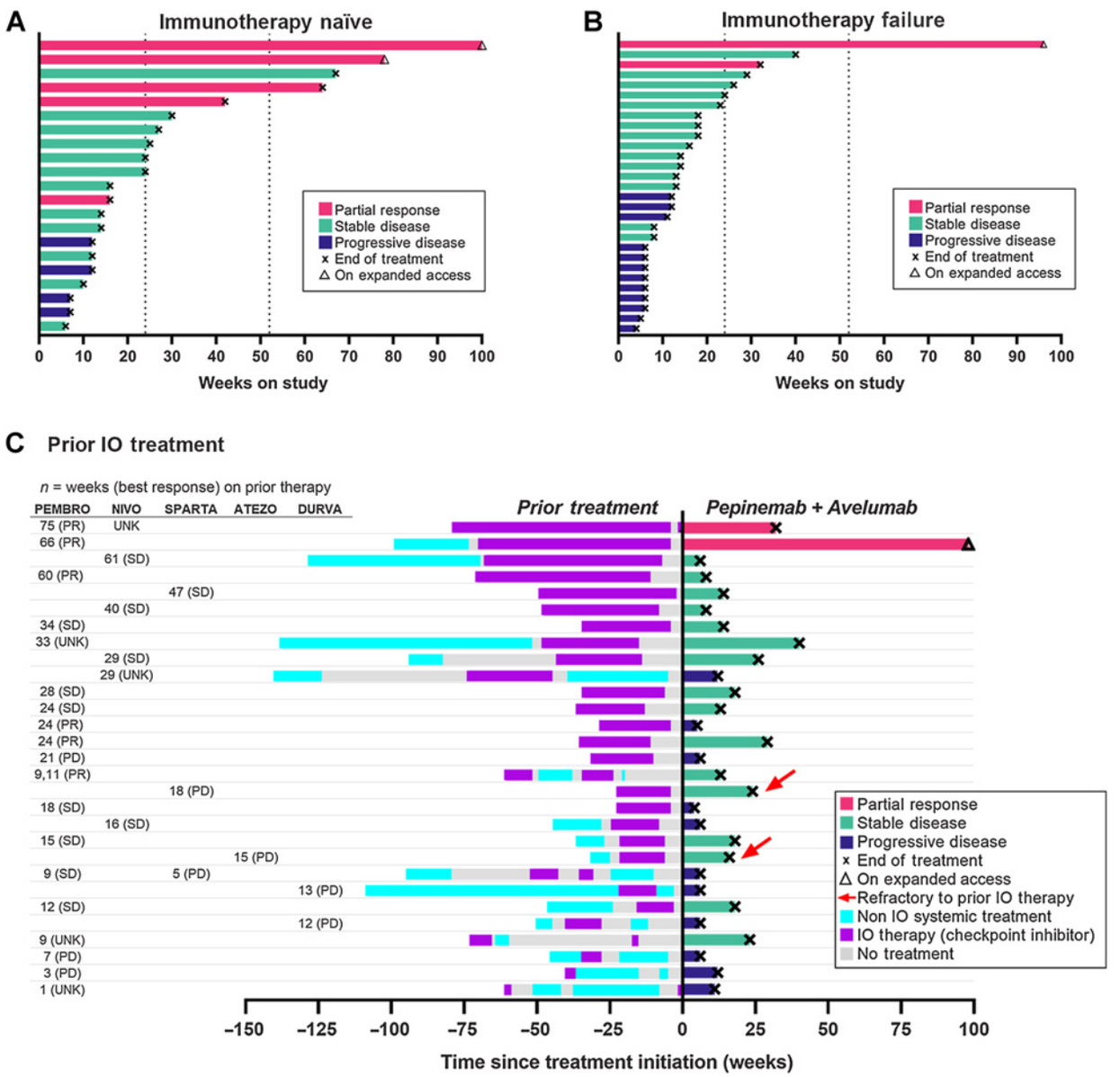

Figure 1.

Clinical response to combination therapy in patients naïve or resistant to prior immunotherapy. **A,** Duration of on-study treatment in patients that are naïve to immunotherapy. **B,** Duration of on-study treatment in patients whose prior immunotherapy failed. **C,** In patients who had progression of disease on prior IO therapy, duration and best response of prior therapy, along with subsequent on-study response is shown. ATEZO, atezolizumab; DURVA, durvalumab; IO, immunotherapy; NIVO, nivolumab; PEMBRO, pembrolizumab; SPARTA, spartalizumab; UNK, unknown.

treatment and resumed combination treatment. Immune-related myositis (grade 3) was considered possibly related to study treatment, with both avelumab and pepinemab withdrawn as a result, and the subject responded to high-dose steroid treatment. Immune-mediated pneumonitis (grade 3) was considered possibly related to study treatment, but then subsequently changed to a non-irAE of dyspnea, unrelated to study treatment and due to disease progression. Overall, pepinemab was not found to increase the amount or severity of irAEs normally seen with avelumab.

Table 2 lists the most common (>5%) AEs that were attributed to pepinemab, avelumab, and/or the combination study treatment for all patients on study. There were no grade 5 AEs related to study treatment. Two (3.2%) patients experienced AEs (grade 3 lipase, grade 2 rash) that required avelumab to be discontinued, however, AEs in these patients subsequently resolved on continued pepinemab monotherapy. Immunogenicity did not appear to be a concern with this combination and the majority of confirmed positive antidrug responses were transient and low titer (<20). Therefore,

Table 2. Commonly occurring (>5%) treatment-related AEs (TRAE) in the study ($N = 62$).

N (%)	All	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
All TRAEs	39 (62.9%)	13 (21.0%)	14 (22.6%)	9 (14.5%)	3 (4.8%)	0
Fatigue	17 (27.4%)	12 (19.4%)	5 (8.1%)	0	0	0
Infusion-related reaction	13 (21.0%)	7 (11.3%)	6 (9.7%)	0	0	0
ALT increased	6 (9.7%)	4 (6.5%)	1 (1.6%)	1 (1.6%)	0	0
Chills	6 (9.7%)	3 (4.8%)	3 (4.8%)	0	0	0
Lipase increased	6 (9.7%)	1 (1.6%)	0	4 (6.5%)	1 (1.6%)	0
Pyrexia	6 (9.7%)	4 (6.5%)	2 (3.2%)	0	0	0
Anaemia	5 (8.1%)	2 (3.2%)	3 (4.8%)	0	0	0
Decreased appetite	5 (8.1%)	2 (3.2%)	3 (4.8%)	0	0	0
Nausea	5 (8.1%)	3 (4.8%)	1 (1.6%)	1 (1.6%)	0	0
ALP increased	4 (6.5%)	3 (4.8%)	1 (1.6%)	0	0	0
AST increased	4 (6.5%)	2 (3.2%)	1 (1.6%)	1 (1.6%)	0	0
Hot flush	4 (6.5%)	3 (4.8%)	1 (1.6%)	0	0	0

Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase.

the primary endpoint of safety and tolerability of the combination treatment was met in this study.

Pharmacokinetics/pharmacodynamics

Supplementary Figure S2 shows the pepinemab serum drug levels versus pepinemab pharmacodynamic markers including levels of cellular SEMA4D (A), total soluble SEMA4D (B), and cellular saturation (C). Upon dosing with pepinemab, cellular levels of SEMA4D decrease, total soluble SEMA4D levels increase, and cellular saturation is complete after one dose and is maintained via subsequent doses. Additional pharmacokinetic analysis demonstrated that C_{max} (D) and exposure (AUC; E) increased proportionally with dose level over the range of 5 to 20 mg/kg and that steady state occurred at approximately cycle 4 (F).

Efficacy

ION

To investigate whether pepinemab enhanced single-agent PD-L1 blockade, we enrolled ION patients for first-line or second-line combination immunotherapy. Combination treatment with pepinemab plus avelumab in this ION cohort resulted in 5 PR, 12 SD, and 4 PD with a disease control rate (DCR) of 81% (17/21 evaluable patients; **Fig. 1A**; Supplementary Fig. S3A). Durable responses of ≥1 year were achieved in 4 patients, including 2 patients who transitioned to expanded access at data base lock (100 and 78 weeks). The ORR was 24% (5/21) in ION patients and the median PFS was 11.6 weeks. Interestingly, all five PR in the ION cohort were among patients with tumors determined to be either PD-L1 negative (<1%) or low (1–49%) and will be discussed further below.

IOF

Because resistance to immunotherapy represents an important unmet need and because the mechanism of action of pepinemab suggests that it may overcome important resistance factors including immune exclusion and myeloid suppression, we investigated activity of pepinemab plus avelumab combination treatment following progression on prior anti-PD-1/L1 treatments. Best overall response and time on study for the IOF population is shown in **Fig. 1B** and Supplementary Fig. S3B and duration of prior treatment and specific prior anti-PD antibody is also indicated for each patient in **Fig. 1C**.

Two IOF patients experienced PR on combination therapy, both of whom had progressed on prior treatment with pembrolizumab. The first of these patients had received prior treatment with pembrolizumab for 66 weeks and experienced a PR before progression. After a 30-day washout period, treatment with pepinemab and avelumab combination was initiated. This patient achieved a PR on CLASSICAL-Lung, with a maximum of −66.2% reduction of tumor burden, and remained on study for 96 weeks until database lock. The patient subsequently transitioned to receive investigator-sponsored expanded access treatment and continues to do well at time of publication. The second patient had previously received treatment with nivolumab for an unknown duration and experienced a PR during pembrolizumab treatment duration of 72 weeks, so this patient was pretreated first with nivolumab and then pembrolizumab. Following a 29-day washout period, this patient entered CLASSICAL-Lung and achieved a −51.9% reduction in tumor burden, with total study treatment duration of 32 weeks.

ORR was 7% (2/29) and the median PFS was 8.4 weeks for IOF patients. Seven patients in this cohort were immunotherapy refractory and of these, 2 experienced SD for >11 weeks upon treatment with pepinemab plus avelumab (**Fig. 1C**, red arrows). Overall, 59% (17/29) of IOF patients benefited when switched to the combination therapy, which appeared to induce a halt or reversal of tumor progression.

Correlative/biomarker analysis

Clinical responses observed in patients with PD-L1-negative/low tumors

Because pembrolizumab was approved for first-line therapy in NSCLC just before this study was initiated and patients with high PD-L1–expressing tumors were known to experience greatest benefit from pembrolizumab, most of the patients enrolled in this study had tumors with relatively low expression of PD-L1. Despite this paucity of expression of the specific target for avelumab, many of these patients derived clinical benefit from the combination therapy. For the entire study population, of those evaluable for PD-L1 expression, 50/51 (98%) were PD-L1 negative (<1%) or PD-L1 low (1–79% as determined by Dako PD-L1 IHC 73–10 pharmDx assay; see Materials and Methods). Strikingly, this included 28/29 of all subjects who responded with either PR or SD. All PR in the ION cohort were among patients with tumors determined to be either PD-L1 negative (2/5) or low (3/5). Among the IOF cohort, all 5 patients with disease control >6 months had PD-L1-low tumors (among PD-L1 evaluable patients). **Figure 2** further illustrates the length of time on study in relation to PD-L1 expression.

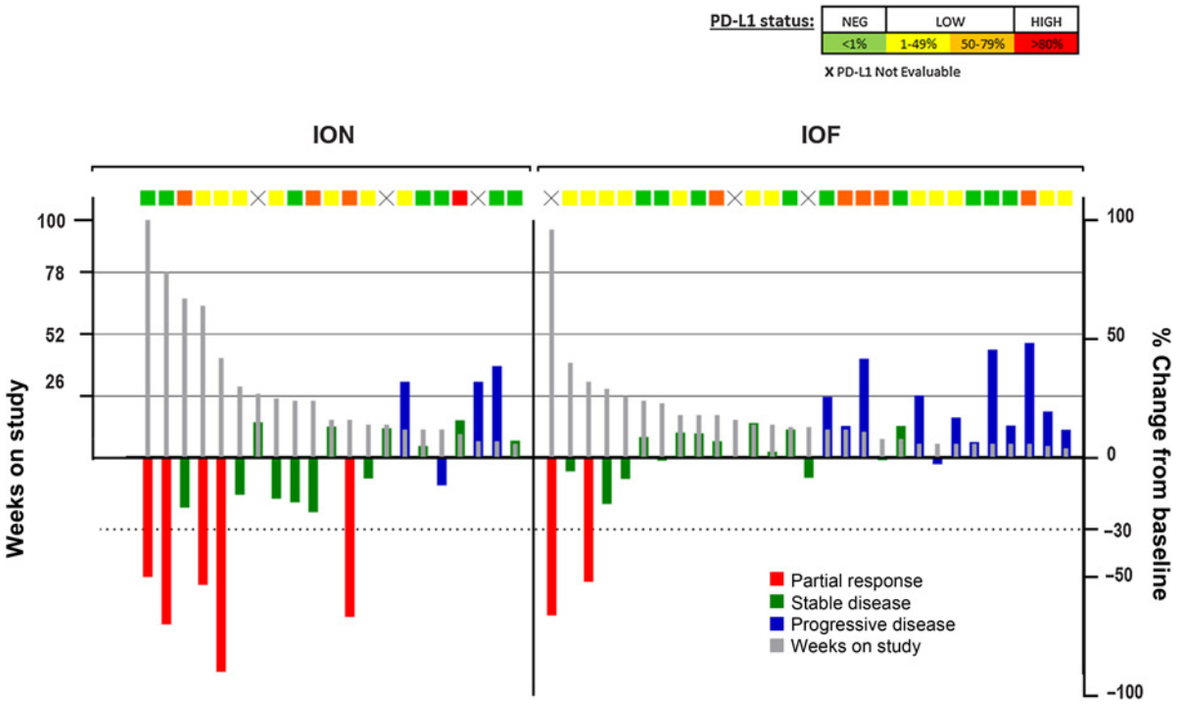

Figure 2.
Waterfall plot of best systemic response and time on study in immunotherapy naïve and failure patients in comparison to PD-L1 status.

Treatment-related increase in CD8$^+$ T-cell density in the tumors of patients with PR and SD

To investigate infiltration of CD8$^+$ T cells in the TME, pre- and on-treatment biopsies were evaluated by IHC. Baseline pre- and on-treatment biopsies derived from the same lesion were selected for analysis and stained for tumor content and immune cells using a multiplex IHC assay as described in the Materials and Methods.

Analysis of all available matched biopsies are shown in **Fig. 3A** and demonstrate increased CD8$^+$ T-cell infiltration in most tumors following treatment with pepinemab + avelumab. High tumoral CD8$^+$ T-cell density appeared to correspond with clinical response, as determined by RECIST and length of time on study. As expected, tumor content was reduced in biopsies of patients experiencing PR or SD. Interestingly, tumor was undetectable in three of five biopsies from IOF patients with stable disease. These observations of increased CD8 density and reduced tumor content corresponding with clinical response were consistent in both the IOF and ION cohorts.

In addition to increased infiltration and activity of tumoral CD8$^+$ T cells, we have previously reported an increase in pro-inflammatory antigen-presenting cells (APC), and a reduction in M2-type macrophage and MDSC in murine models treated with anti-SEMA4D antibodies (6, 7). These myeloid subsets were evaluated using the multiplex IHC methods described above. An increase in the ratio of pro-inflammatory APC (CD11c$^+$CD68$^+$) to suppressive APC (CD163$^+$) following treatment was observed, and this ratio appeared to be higher in patients who experienced PR or SD, as compared with those with PD (Supplementary Fig. S4A).

Association of peripheral immune cell subsets with response and resistance to ICI

Circulating levels of immune subsets in blood were also assessed to investigate biomarkers that may prospectively indicate response to treatment. The balance of tumoricidal to suppressive immune cells may be important predictors of response to ICI (22). Interestingly, flow cytometric analysis indicated higher circulating mMDSC at baseline in the patients with PD, compared with patients with PR or SD (**Fig. 3B**). Components of complete blood count (CBC) at baseline were also assessed as described in an independent evaluation of predictive biomarkers in patients with NSCLC treated with ICI (15). Higher levels of myeloid cells (AMC and ANC) significantly correlated with poor PFS and disease progression (Supplementary Figs. S4B and S4C). In contrast, no significant differences associated with clinical benefit were identified among circulating levels of CD8$^+$ and CD4$^+$ T cells, B cells, or NK cells.

We further investigated potential mechanisms of resistance by comparing the balance of immune subsets in the ION versus the IOF patients who had primary or acquired resistance to ICI. Interestingly, we observed an unfavorable balance of high circulating monocytic and granulocytic PMN MDSC but lower CD8$^+$ T cells (**Fig. 3C**; Supplementary Fig. S4D) among the IOF cohort as compared with the ION cohort. This unfavorable immune balance in the baseline blood samples of IOF patients may indicate mechanisms of resistance to prior anti-PD-1/L1 treatment.

Discussion

Although ICIs have changed the treatment landscape for many cancer subtypes, the occurrence of drug resistance results in poor

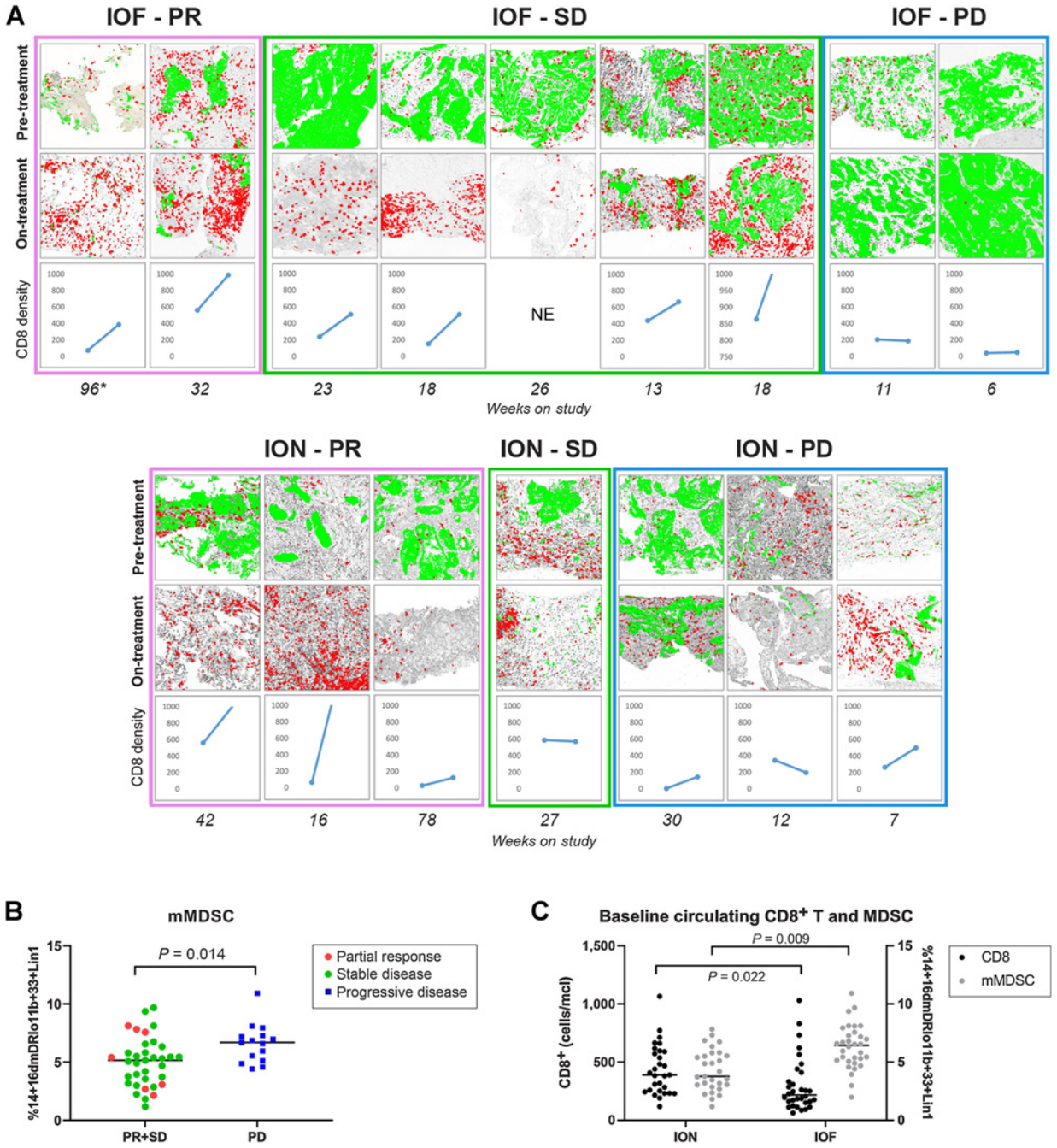

Figure 3.

Balance of lymphocytes and myeloid cells as biomarkers of response and resistance to ICI. **A,** CD8 density in the TME correlates with response in both immunotherapy naive and failure patients. Samples were stained for CD8$^+$ T cells (red) and tumor (green, cytokeratin+, and malignant tissue denoted by pathologist). *, patient was transferred to expanded access at the time of study closure; NE, CD8 density within tumor area was not evaluable because the on-treatment biopsy contained only necrotic tissue, therefore tumor area could not be drawn. **B,** Baseline peripheral mMDSC correlates with response. **C,** Baseline circulating CD8 and mMDSC are altered in immunotherapy-naïve patients in comparison to immunotherapy-failure patients.

prognosis, high incidence of recurrence, and low overall survival (OS) in patients with NSCLC. Combination therapies targeting independent mechanisms/molecules, such as PD-X/CTLA-4/chemotherapy, have demonstrated increased efficacy but also have increased toxicities. Safe, rational combinations are needed to overcome additional resistance mechanisms to ICIs, including immune exclusion and myeloid suppression. Antibody blockade of SEMA4D facilitates infiltration of APC and tumoricidal T cells, while also reducing myeloid suppression in preclinical models. This treatment was found to enhance immunomodulatory therapies including PD-1, PD-L1, and CTLA-4

blockade in preclinical studies. CLASSICAL-Lung is the first completed clinical trial to demonstrate safety and anti-tumor activity of the combination of pepinemab with a checkpoint inhibitor.

The primary outcomes of safety and determination of RP2D were achieved in this study and the combination was determined to be well tolerated. The TRAEs were generally low-grade and the frequency of grade 3 or 4 toxicities related to the combination (13%, 8/62) was similar to the frequency expected from avelumab only (10.2%, 117/1,738 patients; ref. 23). Pepinemab has been previously reported to be well tolerated as a single agent (11, 12). It was, therefore, not unexpected to find that the combination did not exacerbate the toxicity normally observed with avelumab. MTD was not reached, including a high dose of 20 mg/kg pepinemab in the dose-escalation cohort.

RP2D was selected as 10 mg/kg pepinemab in combination with 10 mg/kg avelumab because it had been demonstrated in previous trials (11, 12) that 10 mg/kg pepinemab exceeds by several fold the concentration of antibody needed to saturate the target in the periphery, and that peripheral saturation correlated with the minimum effective dose in preclinical studies (6). Receptor occupancy (peripheral saturation) was also confirmed in this trial, as all patients dosed with pepinemab were saturated immediately after dosing and remained saturated throughout the dosing period. In addition, as seen in previous trials (11, 12), levels of cellular SEMA4D decrease on T cells upon dosing, due to internalization of the pepinemab/SEMA4D complex on the surface of these cells, and levels of total soluble SEMA4D (complex of drug bound to target) increase upon dosing due to the increased half-life of the pepinemab/SEMA4D complex in the periphery. Because of the limited sample collection interval of 2 weeks postdose, true terminal half-life could not be captured. However, based on the observed 2- to 3-fold accumulation ratio, and assuming time-independent linear pharmacokinetics, the effective half-life is calculated to be 15 to 20 days, which is in agreement with the half-life calculated in a previous single-agent pepinemab trial (12).

PD-L1 expression in patients with NSCLC is associated with increased response to anti-PD-1/L1 therapies including avelumab (13, 24). The population enrolled in this study was skewed towards PD-L1-negative or -low tumors, likely, because pembrolizumab was approved as first-line therapy for NSCLC not long before this study was initiated. The Dako PD-L1 IHC 73–10 pharmDx assay for avelumab is relatively sensitive, and a high PD-L1 status of 80% in the 73–10 assay corresponds to approximately 50% PD-L1 expression using the Dako 22C3 assay (17). Employing the 73–10 assay, only 2% (1/51) of the PD-L1 evaluable patients had high PD-L1 expression (≥80%) whereas 43% (22/51) and 37% (19/51) of patient tumors were respectively PD-L1 low (1–49%) or negative (<1%). Although we are mindful of the difficulty of comparing response rates in different trials, and with the caveat that this was a relatively small study, it may be instructive that the activity of the combination therapy reported here appears to exceed by a factor of 2- to 3-fold the objective response rate consistently reported for single-agent avelumab in similar PD-L1-negative and PD-L1-low NSCLC populations (13, 25).

The beneficial reprogramming of the TME by pepinemab, increasing antigen presentation and secretion of immune-activating cytokines like IFNγ and TNFα by activated APC, may contribute to lowering the threshold of PD-L1 required for avelumab efficacy. Additional investigation of activity and mechanism of action for pepinemab combinations with PD-1 blocking agents is warranted in patients with PD-L1-low disease.

Patients with immunotherapy-resistant tumors are an increasingly unmet need in NSCLC and many other cancers where response to ICI is low and of relatively short duration. The majority of the IOF patients enrolled in this trial had progressed on either pembrolizumab or nivolumab. The DCR in evaluable IOF patients was 59% (17/29), including 2 PRs and 17 SDs, with durable benefit lasting ≥1 year in 1 subject and ≥6 months in 5 subjects. Although the majority of the disease control after switching to pepinemab and avelumab was achieved in subjects that had at least documented disease stabilization on prior immunotherapy, 2 subjects that were primary refractory to prior immunotherapy achieved SD on this trial.

Biomarkers are useful to inform mechanism of action and design of future studies. Expression of SEMA4D was confirmed in the TME, predominantly expressed on immune cells; further investigation into expression of SEMA4D and its receptors as potential biomarkers of response is warranted. Immune cell composition is another potential biomarker for immunotherapies. Our data did not demonstrate significant correlations between baseline levels or treatment-related changes in circulating lymphocytes or NK cells, although others have recently reported an association of altered levels of circulating $CD8^+$ T lymphocytes (26) with treatment benefit of PD-1 inhibition. Low sample size in each study and/or differences in analysis technique, that is, gene expression CIBERSORTx analysis versus cytometric analysis presented here may explain this. In contrast and consistent with recent reports, we observed lower baseline levels of MDSC in blood of patients with SD and PR (**Fig. 3B**), and lower baseline AMC and ANC levels were significantly associated with prolonged PFS (Supplementary Fig. S4). High frequency of MDSCs correlated with disease progression and negative impact on overall survival rate has been reported by others (15, 27). Furthermore, within the TME, clinical benefit favored a shift in the balance of suppressive and inflammatory APC following treatment (Supplementary Fig. 4A). Notably, these results were consistent with preclinical findings following treatment with anti-SEMA4D antibodies in preclinical models (6). These biomarker data highlight the importance of myeloid cell composition in regulating and predicting response to treatment in this study.

Identifying mechanisms of resistance in IOF patients could help guide rational combination therapies. The immune response is dynamic and, as T cells are activated in response to ICI, resistance mechanisms are activated through negative feedback in the immune system and cues from the TME. Resistance may develop through exclusion of T cells from TME, downregulation of APC, either by mutations in antigen processing machinery or reprogramming of APC, and recruitment/amplification of other suppressive cell populations, such as MDSC (28). We had the opportunity to directly compare ION and IOF patients to investigate potential mechanisms of acquired resistance. We did not observe a significant difference in CD8 density within the tumor between ION and IOF, but we did observe a reduced number of CD8 and increased MDSC and other suppressive myeloid cells in circulation of IOF patients compared with ION, suggesting a smaller pool of available tumor reactive CD8 and a shift in the balance of immunity that favored immune suppression in IOF. Importantly, although this undesirable immune status is not predicted to respond well to ICI (22, 29), the addition of pepinemab to PD-1 blockade was able to overcome this suppressive immune balance in the IOF cohort.

A key feature of this study was the comparison of lesion-matched pre- and on-treatment biopsies to investigate changes within the TME and confirmation of proposed mechanism of action. This is the first report of increased $CD8^+$ T-cell infiltration and a shift in the APC balance in clinical samples of pepinemab- and avelumab-treated patients. Although other myeloid-targeted strategies, such as CSF1R inhibitors, have not translated to clinical successes (30), SEMA4D is a multifaceted target that regulates signaling on multiple myeloid

lineages, including DC, macrophage and, importantly, MDSC. It may be necessary to reprogram myeloid subpopulations to facilitate effective T-cell activity. Preclinical models (6, 7) showed a high concentration of SEMA4D expression at the invasive tumor margin, with the concomitant exclusion of activated DC, APC, and CTL from the tumor, suggesting that the SEMA4D gradient serves as a protective barrier against immune cell infiltration. In contrast, antibody neutralization of SEMA4D increased infiltration of DC and CD8 into the tumor, increased CTL activity, and reduced the frequency and spatial distribution of MDSC, M2 TAM, and Treg, while changing the overall cytokine milieu in the TME to favor immunoactivation and tumor destruction.

In conclusion, pepinemab has demonstrated a well-tolerated safety profile and trends toward enhanced efficacy and antitumor immune activity were observed when combined with avelumab in patients with NSCLC. These data serve to support additional combination trials with avelumab and other checkpoint inhibitors in patients and cancer indications for which checkpoint inhibitors cannot overcome PD-L1 dependent and independent mechanisms of immune suppression and resistance.

Authors' Disclosures

M.R. Shafique reports personal fees from Jazz Pharmaceuticals outside the submitted work. T.L. Fisher reports other from Vaccinex, Inc. outside the submitted work. T.L. Fisher also has a patent for anti-CD100 antibodies and methods for using the same issued, a patent for anti-SEMA4D antibodies and epitopes issued, and a patent for semaphorin-4D antagonists for use in cancer therapy pending. E.E. Evans reports other from Vaccinex outside the submitted work. E.E. Evans also has a patent for use of anti-semaphorin-4D antibodies in combination with an immune modulating therapy to inhibit tumor growth and metastases issued to Vaccinex and a patent for Use of antibodies or antigen-binding fragments thereof that specifically bind semaphorin-4D to increase tumor infiltrating leukocyte frequency issued to Vaccinex. D.E. Pastore reports personal fees from Vaccinex during the conduct of the study, as well as personal fees from Tarus Therapeutics outside the submitted work. C.L. 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Mishra also has a patent for anti-SEMA4D antibodies and epitopes issued to Vaccinex, Inc., a patent for use of antibodies or antigen-binding fragments thereof that specifically bind semaphorin-4D to increase tumor-infiltrating leukocyte frequency issued to Vaccinex, Inc., a patent for use of semaphorin-4D inhibitory molecules with an immune modulating therapy to inhibit tumor growth and metastases issued to Vaccinex, Inc., and a patent for humanized anti-CD100 antibodies issued to Vaccinex, Inc. A. Schröder reports personal fees and other from Merck KGaA during the conduct of the study, as well as personal fees and other from Merck KGaA outside the submitted work. K.M. Chin reports other from EMD Serono Research & Development Institute, Inc.; an affiliate of Merck KGaA during the conduct of the study. J.T. 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Zauderer also is a patent inventor on several patents related to this work issued to Vaccinex, a patent for anti-SEMA4D antibodies and epitopes issued to Vaccinex, a patent for use of antibodies or antigen-binding fragments thereof that specifically bind semaphorin-4D to increase tumor infiltrating leukocyte frequency issued to Vaccinex, a patent for use of semaphorin-4D inhibitory molecules with an immune modulating therapy to inhibit tumor growth and metastases issued to Vaccinex, and a patent for humanized anti-CD100 antibodies issued to Vaccinex. No disclosures were reported by the other authors.

Authors' Contributions

M.R. Shafique: Resources, supervision, investigation, writing–review and editing. **T.L. Fisher:** Conceptualization, data curation, formal analysis, supervision, visualization, methodology, writing–original draft, project administration, writing–review and editing. **E.E. Evans:** Conceptualization, data curation, formal analysis, validation, visualization, methodology, writing–original draft, writing–review and editing. **J.E. Leonard:** Conceptualization, supervision, project administration, writing–review and editing. **D.R.E. Pastore:** Conceptualization, data curation, formal analysis, visualization, methodology, writing–original draft, project administration, writing–review and editing. **C.L. Mallow:** Formal analysis, methodology, writing–review and editing. **E. Smith:** Conceptualization, supervision, writing–review and editing. **V. Mishra:** Formal analysis, methodology, writing–review and editing. **A. Schröder:** Conceptualization, funding acquisition, writing–review and editing. **K.M. Chin:** Conceptualization, funding acquisition, writing–review and editing. **J.T. Beck:** Resources, supervision, investigation, writing–review and editing. **M.A. Baumgart:** Resources, supervision, investigation, writing–review and editing. **R. Govindan:** Resources, supervision, investigation, writing–review and editing. **N.Y. Gabrail:** Resources, supervision, investigation, writing–review and editing. **A.I. Spira:** Resources, supervision, investigation, writing–review and editing. **N. Seetharamu:** Resources, supervision, investigation, writing–review and editing. **Y. Lou:** Resources, supervision, investigation, writing–review and editing. **A.S. Mansfield:** Resources, supervision, investigation, writing–review and editing. **R.E. Sanborn:** Resources, supervision, investigation, writing–review and editing. **J.W. Goldman:** Resources, supervision, investigation, writing–review and editing. **M. Zauderer:** Conceptualization, supervision, funding acquisition, visualization, writing–original draft, writing–review and editing.

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