Journal for ImmunoTherapy of Cancer

Efficacy, safety, and biomarker analyses of bintrafusp alfa, a bifunctional fusion protein targeting TGF-β and PD-L1, in patients with advanced non-small cell lung cancer

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To cite: Rajan A, Abdul Sater H, Rahma O, *et al*. Efficacy, safety, and biomarker analyses of bintrafusp alfa, a bifunctional fusion protein targeting TGF-β and PD-L1, in patients with advanced non-small cell lung cancer. *Journal for ImmunoTherapy of Cancer* 2024;**12**:e008480. doi:10.1136/jitc-2023-008480

► Additional supplemental material is published online only. To view, please visit the journal online (https://doi.org/10.1136/jitc-2023-008480).

Accepted 06 February 2024

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ABSTRACT

Background Bintrafusp alfa, a first-in-class bifunctional fusion protein targeting transforming growth factor-β (TGF-β) and programmed cell death ligand 1, has demonstrated encouraging efficacy as second-line treatment in patients with non-small cell lung cancer (NSCLC) in a dose expansion cohort of the phase 1, open-label clinical trial (NCT02517398). Here, we report the safety, efficacy, and biomarker analysis of bintrafusp alfa in a second expansion cohort of the same trial (biomarker cohort).

Methods Patients with stage IIIb/IV NSCLC who were either immune checkpoint inhibitor (ICI)-naïve (n=18) or ICI-experienced (n=23) were enrolled. The primary endpoint was the best overall response. Paired biopsies (n=9/41) and peripheral blood (n=14/41) pretreatment and on-treatment were studied to determine the immunological effects of treatment and for associations with clinical activity.

Results Per independent review committee assessment, objective responses were observed in the ICI-naïve group (overall response rate, 27.8%). No new or unexpected safety signals were identified. Circulating TGF-β levels were reduced (>97%; p<0.001) 2 weeks after initiation of treatment with bintrafusp alfa and remained reduced up to 12 weeks. Increases in lymphocytes and tumor-associated macrophages (TAMs) were observed in on-treatment biospies, with an increase in the M2 (tumor trophic TAMs)/M1 (inflammatory TAMs) ratio associated with poor outcomes. Specific peripheral immune analytes at baseline and early changes after treatment were associated with clinical response.

Conclusions Bintrafusp alfa was observed to have modest clinical activity and manageable safety, and was associated with notable immunologic changes involving modulation of the tumor immune microenvironment in patients with advanced NSCLC.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Immune checkpoint inhibitor monotherapy or combination therapy improves the survival of patients with non-small cell lung cancer (NSCLC); however, clinical activity is limited by primary or acquired resistance, which ultimately results in disease progression. Bintrafusp alfa has been evaluated previously in patients with heavily pretreated advanced solid tumors, including NSCLC, and has demonstrated a manageable safety profile and encouraging clinical activity.

WHAT THIS STUDY ADDS

⇒ This is the first study of bintrafusp alfa in a heterogeneous population of patients with relapsed NSCLC that includes comprehensive immune analyses to evaluate the effects of concurrent transforming growth factor-β and programmed death ligand 1 inhibition in tissue and blood. Safety and efficacy signals of bintrafusp alfa were consistent with previous observations in patients with NSCLC. Changes in specific immune cell populations in the tumor microenvironment, and the peripheral immunome were associated with clinical outcomes.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our results showed that bintrafusp alfa treatment was associated with significant changes in immune cell profiles and modulation of the tumor immune microenvironment. This study may help support further evaluation of TGF-β-directed therapies in combination with other immunomodulatory agents.

BACKGROUND

Lung cancer accounts for 21% of all cancer-related deaths globally, with non-small cell lung cancer (NSCLC) being the most common type of lung cancer.[1] The availability of targeted therapies and immunotherapies has improved the survival rates of patients with NSCLC.[2 3] Patients with driver mutations receive targeted therapies (eg, epidermal

growth factor receptor (EGFR) or anaplastic lymphoma kinase (ALK) inhibitors)[2] and those with advanced and unresectable NSCLC without driver mutations receive programmed death-1 (PD-1)/programmed death ligand 1 (PD-L1)-directed immune checkpoint inhibitors (ICIs) as monotherapy or in combination with chemotherapy.[4–7]

However, primary and acquired resistance limits the clinical benefits of these therapies, ultimately resulting in disease progression.[3] Thus, treatment options for patients with recurrent NSCLC after treatment with ICIs are limited. Therefore, developing new treatments to improve the clinical outcomes of such patients is necessary. The tumor microenvironment (TME) of solid tumors is complex, with studies reporting that dysregulation within the TME contributes to tumor progression and poor treatment response.[8] In addition, the immunosuppressive functions of the PD-L1 pathway within the TME are well established.[9]

Transforming growth factor-beta (TGF-β) signaling is highly dysregulated in the TME of advanced tumors, including in NSCLC, and has been implicated in the development of resistance to drugs, including to PD-L1 inhibitors.[10] The aberrant upregulation of TGF-β expression has been correlated with tumor metastasis and cancer progression due to epithelial-mesenchymal transition induction, and the driving of several key features of tumorigenesis, such as cancer fibrosis, sustained angiogenesis, innate and adaptive immunity suppression, and immune surveillance evasion.[11] Within the TME, TGF-β and PD-L1 are non-redundant immunosuppressive pathways, with preclinical studies reporting that the bifunctional inhibition of TGF-β and PD-L1 by a single molecule elicited synergistic antitumor immunity and tumor regression.[8 12]

A recent preclinical radiolabeling study demonstrated that bintrafusp alfa sequesters plasma TGF-β and localizes to the TME *in vivo*, and that the PD-L1 binding portion is largely responsible for tumor uptake, which then results in simultaneous inhibition of PD-L1 and TGF-β.[13] The imaging results of this study demonstrated significant tumor uptake of bintrafusp alfa and a highly favorable tumor-to-blood ratio.[13] In a phase 1 dose-escalation study, treatment with bintrafusp alfa was associated with a manageable safety profile and encouraging clinical activity in patients with heavily pretreated advanced solid tumors.[14]

In an NSCLC expansion cohort of the phase 1 study, bintrafusp alfa showed clinical activity (overall response rate [ORR], 21.3% and median overall survival [OS], 13.6 months) as second-line treatment in patients who progressed after platinum therapy and did not previously receive immunotherapy.[15] Here, we report the safety and efficacy of bintrafusp alfa in a second expansion cohort (NSCLC biomarker expansion cohort) of the phase 1 study, including patients with stage IIIb/IV NSCLC with relapsed, refractory, or progressive disease (PD) on/after a single line of platinum-based chemotherapy, targeted therapy, or anti-PD-(L)-1 monotherapy (ICI-experienced), and in patients who had not previously received anti-PD-(L)-1 therapy (ICI-naïve), with histologically confirmed stage IV or recurrent NSCLC. We also report the results from biomarker analyses in this cohort.

MATERIALS AND METHODS

Study design and participants

In this NSCLC biomarker expansion cohort from the global, multicenter, phase 1, open-label NCT02517398 study of bintrafusp alfa, patients with advanced NSCLC who were either ICI-naïve or ICI-experienced but had relapsed, were refractory, or had PD were evaluated. Patients aged ≥18 years with a life expectancy of ≥12 weeks, those who had an Eastern Cooperative Oncology Group performance status of 0 or 1, and those who had adequate renal, hepatic, and hematological functions were eligible for the study. ICI-naïve (with/without a history of systemic therapies, but not having received PD-(L)-1 inhibitors) and ICI-experienced (received prior treatment with PD-(L)-1 inhibitors) patients with non-squamous NSCLC were required to undergo testing for *EGFR* mutation, ALK translocation, and ROS1 rearrangement and receive appropriate tyrosine kinase inhibitor therapy if these genomic alternations were present.

The disease had to be measurable by Response Evaluation Criteria in Solid Tumors V.1.1 (RECIST V.1.1). The study was conducted in accordance with all applicable regulatory requirements, and the protocol was approved by the institutional review board/ethics committee of the US National Cancer Institute (NCI) and participating institutions. All patients provided written informed consent before study enrollment. The study complied with international standards of Good Clinical Practice and the Declaration of Helsinki.

Procedures

Participants were treated with bintrafusp alfa 1,200 mg once every 2 weeks via intravenous infusion over 1 hour until confirmed PD, unacceptable toxicity, or withdrawal from the trial; treatment was continued after disease progression if clinically justified.[16] Extensive immune analyses, including interrogation of the tumor immune microenvironment (TIME) in tumor biopsies and peripheral blood, were performed in patients (n=14) treated at the NCI where available.

Paired (pretreatment and on-treatment) samples were used to evaluate the immunological effects of treatment in tumor biopsies (n=9) and peripheral blood (n=14, additional details in supplementary methods and online supplemental table 1). The pretreatment biopsy was performed not more than 28 days prior to the first dose of bintrafusp alfa. The post-treatment biopsy was performed within 7 days of the week 7 dose of bintrafusp alfa (days 44–50). After an amendment to the protocol, the post-treatment biopsy was collected within 7 days of the week 3 dose (days 16–22). This amendment affected only the last patient enrolled at the NCI and paired biopsy samples

from this patient were insufficient for biomarker analysis and not included in the results of tissue analyses.

Outcomes

The primary endpoint was the best overall response (BOR) assessed according to RECIST V.1.1 by an independent review committee (IRC). Secondary endpoints included the BOR per investigator; safety with adverse events (AEs) coded according to the Medical Dictionary for Regulatory Activities terms, V.21.0, and classified by grade according to the NCI Common Terminology Criteria for Adverse Events, V.4.03; and pharmacokinetics (PK). Exploratory endpoints included the duration of response (DOR), disease control rate (DCR), and progression-free survival (PFS) determined according to RECIST V.1.1 by the IRC and investigator and OS. Additional analyses included the evaluation of biomarker data.

Statistical analysis

The target number of patients for enrollment in the biomarker cohort was 30. With 30 eligible patients with NSCLC, an observed ORR of 30% (responders, n=9/30) will rule out a ≤15% ORR (null hypothesis). Efficacy and safety were analyzed in all patients who received ≥1 dose of bintrafusp alfa. Although initially the protocol intended to enroll 30 patients (ICI-naïve vs ICI-experienced, n=15 each), the mandatory requirement of paired biopsies resulted in slower-than-expected accrual. Therefore, otherwise eligible patients with at least one biopsy were permitted to enroll and the accrual ceiling was increased to 41 in order to have sufficient tissue for biomarker analyses. The ORR was determined as the proportion of patients with a confirmed BOR of complete response (CR) or partial response (PR). Additional details are available in online supplemental methods.

To determine whether immune cell populations in tumor biopsies or immune parameters in peripheral blood change on treatment with bintrafusp alfa, Wilcoxon matched-pairs signed-rank test was performed. Statistical differences between two groups for a given immune correlate were assessed using the Mann-Whitney test. All graphs and statistical analyses were performed using GraphPad Prism (GraphPad Software, La Jolla, California, USA) or RStudio (Boston, Massachusetts, USA).

RESULTS

Cohort characteristics

Between February 2017 and March 2020, 41 patients with NSCLC who were ICI-naïve (n=18) or ICI-experienced (n=23) were enrolled in the biomarker expansion cohort and were included in the full analysis and safety sets. The median age was 66 (range, 50–83) years and 65 (range, 52–74) years in the ICI-naïve and ICI-experienced groups, respectively, and only one patient in each group was treated with prior immunotherapy other than PD-(L)-1 inhibitor (table 1).

Table 1 Baseline patient and disease characteristics

Characteristics, n (%)	ICI-naïve (n=18)	ICI-experienced (n=23)
Sex		
Male	10 (55.6)	13 (56.5)
Female	8 (44.4)	10 (43.5)
Age		
Median (range), years	66 (50–83)	65 (52–74)
<65 years	8 (44.4)	9 (39.1)
≥65 years	10 (55.6)	14 (60.9)
ECOG performance status		
0	6 (33.3)	3 (13.0)
1	12 (66.7)	20 (87.0)
Tumor cell PD-L1 expression*		
≥1%	6 (33.0)	13 (56.6)
<1%	7 (39.0)	5 (21.7)
Not available	5 (28.0)	5 (21.7)
EGFR mutation status†		
Wild type	9 (69.2)	17 (94.4)
Mutated	3 (23.1)	1 (5.6)
Not available	1 (7.7)	0
Tumor histology		
Adenocarcinoma	10 (55.6)	17 (73.9)
Squamous cell carcinoma	5 (27.8)	5 (21.7)
Other	3 (16.7)	1 (4.3)
Number of prior anticancer regimens		
0	6 (33.3)	0
1	7 (38.9)	0
2	0	12 (52.2)
3	3 (16.7)	6 (26.1)
≥4	2 (11.1)	5 (21.7)
Type of prior anticancer therapy for metastatic or locally advanced disease		
Anti-PD-(L)1	0	23 (100.0)
Cytotoxic therapy	10 (55.6)	20 (87.0)
Endocrine therapy	0	0
Monoclonal antibody therapy	0	7 (30.4)
Small molecules	4 (22.2)	3 (13.0)
Immunotherapy other than anti-PD-(L)1	1 (5.6)	1 (4.3)
Others	0	0

Data are presented as n (%) or median (range).
*PD-L1 immunohistochemistry data were obtained using clones 73–10, E1L3N, 22C3, SP263, and SP142.
†The percentage is calculated based on the number of patients with non-squamous histology.
ECOG, Eastern Cooperative Oncology Group; EGFR, epidermal growth factor receptor ; ICI, immune checkpoint inhibitor; PD-L1, programmed death ligand 1.

Of the 41 patients, 14 were biomarker evaluable (ICI-naïve, n=6; ICI-experienced, n=8) (online supplemental table 2). The baseline PD-L1 expression data for all patients is summarized in online supplemental tables 2 and 3.

At the data cut-off (May 15, 2020), patients received bintrafusp alfa for a median duration of 16.8 (range, 2.0–61.9) weeks in the ICI-naïve group and 6.1 (range, 2.0–53.9) weeks in the ICI-experienced group. The median follow-up time since the first dose of bintrafusp alfa according to Kaplan-Meier analysis was 42.1 (range, 2.0–166.0) weeks and 47.3 (range, 2.0–162.0) weeks in the ICI-naïve and ICI-experienced groups, respectively. The most common reason for treatment termination was PD (ICI-naïve, 38.9%; ICI-experienced, 56.5%). Five patients (27.8%) in the ICI-naïve group and nine patients (39.1%) in the ICI-experienced group received at least one subsequent anticancer drug therapy, with most receiving cytotoxic therapy (ICI-naïve, 22.2%; ICI-experienced, 21.7%). Of the 7 (38.9%) and 16 (69.6%) patients in the ICI-naïve and ICI-experienced groups who discontinued the study, at data cut-off 7 (38.9%) and 13 (56.5%) patients had died, respectively.

Efficacy

Five (27.8%) patients in the ICI-naïve group and no patient in the ICI-experienced group had a confirmed PR as adjudicated by IRC (ORR, 27.8%; figure 1A and table 2).

Of note, one patient in the ICI-experienced group experienced radiological progression at the first restaging time point, but subsequently developed a durable PR, which is ongoing after more than 5 years of enrollment. The DCR per IRC was 38.9% versus 4.3% in the ICI-naïve versus ICI-experienced group, respectively; the median DOR per IRC was not estimable (NE) in both study groups (table 2). Overall, the median PFS by IRC

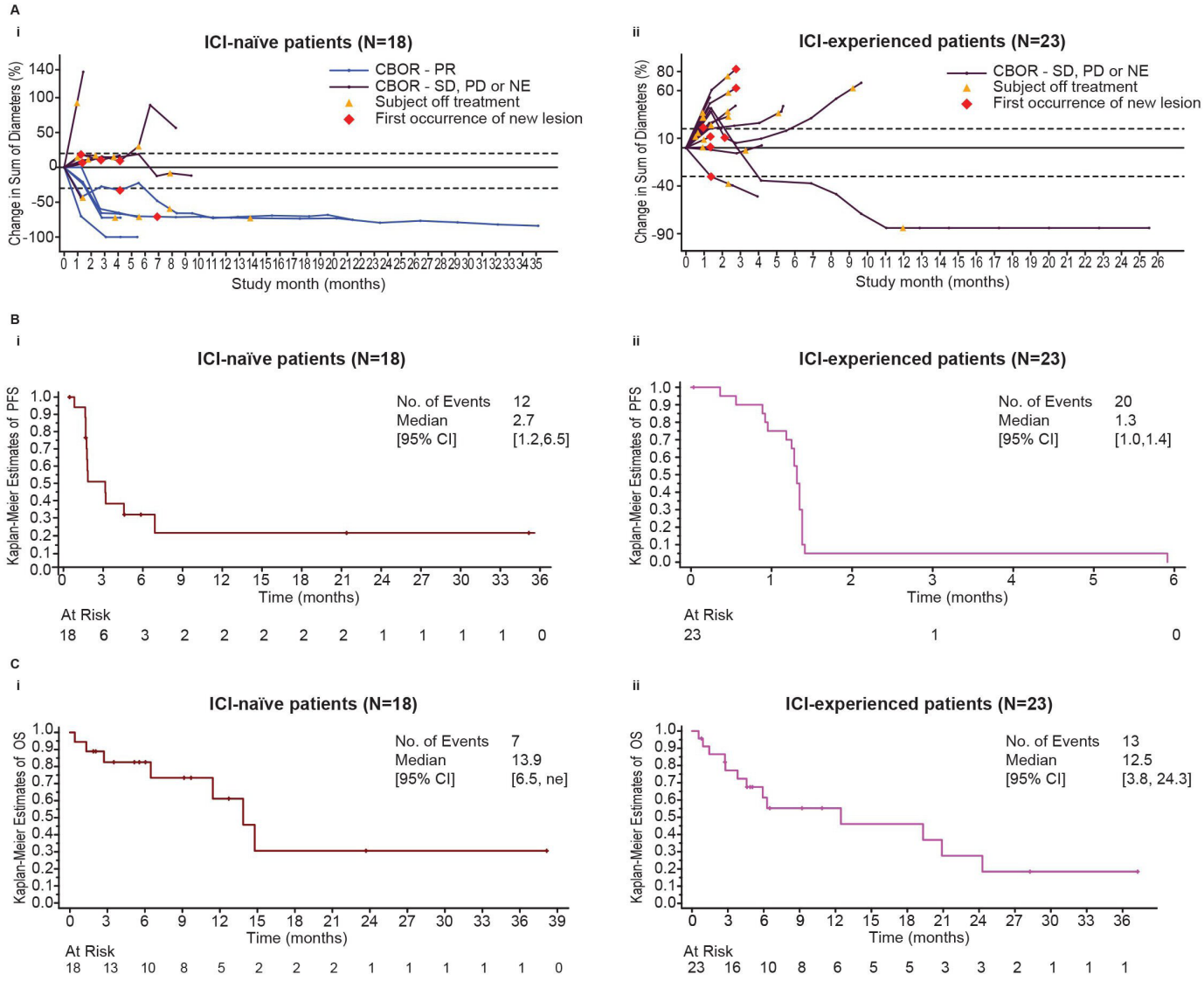

Figure 1 Clinical activity of bintrafusp alfa (A) Percent change from baseline in the sum of diameters according to Response Evaluation Criteria in Solid Tumors V.1.1 as adjudicated by the IRC for (i) ICI-naïve patients and (ii) ICI-experienced patients. (B) Kaplan-Meier curve for PFS per the IRC for (i) ICI-naïve patients and (ii) ICI-experienced patients. (C) Kaplan-Meier curve for OS for (i) ICI-naïve patients and (ii) ICI-experienced patients. CBOR, confirmed best overall response; ICI, immune checkpoint inhibitor; IRC, independent review committee; NE, not estimable; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease.

Table 2 Efficacy according to RECIST V.1.1 as adjudicated by the IRC

	ICI-naïve (n=18)	ICI-experienced (n=23)
Confirmed BOR		
CR	0	0
PR	5 (27.8)	0
Stable disease	2 (11.1)	1 (4.3)
Progressive disease	6 (33.3)	17 (73.9)
Not evaluable	5 (27.8)	5 (21.7)
Overall response rate (CR+PR), (95% CI)	5 (27.8) (9.7 to 53.5)	0 (0.0 to 14.8)
Disease control rate, (95% CI)	7 (38.9) (17.3 to 64.3)	1 (4.3) (0.1 to 21.9)
Median progression-free survival, months, (95% CI)	2.7 (1.2 to 6.5)	1.3 (1.0 to 1.4)
Median duration of response, months, (95% CI)	NE (3.8 to NE)	NE (NE to NE)

Data are presented as n (%) or n (%; 95% CI) unless specified otherwise.
BOR, best overall response; CR, complete response; ICI, immune checkpoint inhibitor; IRC, independent review committee; NE, not estimable; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors.

in the ICI-naïve group was 2.7 months (95% CI, 1.2 to 6.5 months) and 1.3 months (95% CI, 1.0 to 1.4 months) in the ICI-experienced group (figure 1B and table 2). The median OS by IRC was 13.9 months (95% CI, 6.5 months to NE) in the ICI-naïve group and 12.5 months (95% CI, 3.8 months to 24.3 months) in the ICI-experienced group (figure 1C). Efficacy outcomes by investigator assessment are shown in online supplemental table 4.

Safety

Any treatment-emergent AEs (TEAEs) were reported in 18 (100%) and 23 (100%) patients in the ICI-naïve and ICI-experienced groups. The rates of TEAEs leading to permanent discontinuation of any treatment occurred in 33.3% and 17.4% of patients in the ICI-naïve and ICI-experienced groups, respectively. The proportion of patients who experienced treatment-related AEs (TRAEs) of any grade in the ICI-naïve group was 77.8%, of which the most common (≥15%) were keratoacanthoma, maculopapular rash, and pruritus (16.7% each) (table 3).

In the ICI-experienced group, there were 17 patients (73.9%) with TRAEs of any grade of which the most common (≥10%) were keratoacanthoma and decreased appetite (13.0% each) (table 3). In the ICI-naïve group, TRAEs leading to permanent discontinuation were reported in 3 (16.7%) patients, including death (due to disease progression) in 1 patient (5.6%). Other TRAEs that required permanent discontinuation included colitis and infusion-related reaction, each observed in 1 (5.6%) patient. There were no TRAEs leading to permanent discontinuation or death in the ICI-experienced group.

The proportion of patients with immune-related adverse events of special interest (AESI) was 16.7% and 17.4% in the ICI-naïve and ICI-experienced groups, respectively (online supplemental table 5). Skin-related AESI rates were 22.2% and 21.7% in the ICI-naïve and ICI-experienced groups, respectively, of which the most common skin AESI was keratoacanthoma (ICI-naïve, 16.7% and the ICI-experienced, 13.0%) (online supplemental table 5). Immune-related pneumonitis was not reported in any patient in either of the groups.

Table 3 Treatment-related adverse events occurring at any grade or at grade ≥3 in ≥2 patients in either group

	ICI-naïve (n=18)		ICI-experienced (n=23)	
	Any grade, n (%)	Grade ≥3, n (%)	Any grade, n (%)	Grade ≥3, n (%)
Any TRAEs	14 (77.8)	6 (33.3)	17 (73.9)	1 (4.3)
Keratoacanthoma	3 (16.7)	0	3 (13.0)	0
Rash maculopapular	3 (16.7)	1 (5.6)	2 (8.7)	0
Pruritus	3 (16.7)	0	2 (8.7)	0
Dyspnea	2 (11.1)	1 (5.6)	0	0
Infusion-related reaction	2 (11.1)	1 (5.6)	0	0
Fatigue	2 (11.1)	0	2 (8.7)	0
Decreased appetite	2 (11.1)	0	3 (13.0)	0
Diarrhea	1 (5.6)	0	2 (8.7)	0
Nausea	1 (5.6)	0	2 (8.7)	0
Rash	1 (5.6)	0	2 (8.7)	0
Vomiting	1 (5.6)	0	2 (8.7)	0
Asthenia	0	0	2 (8.7)	0

ICI, immune checkpoint inhibitor; TRAEs, treatment-related adverse events.

Pharmacokinetics

PK analyses were performed in 39/41 patients with available samples. After the first dose, the geometric mean (geo coefficient variation percentage) for concentration after end of infusion was 454.0 µg/mL (56.3%), trough concentration (C_{trough}) was 73.3 µg/mL (62.1%), area under serum concentration-time curve was 66,500 µg×h/mL (29.0%), half-life was 140 hours (~5.8 days) (29.1%), and clearance was 0.207 (33%). The target C_{trough} (geometric mean C_{trough} of >100 µg/mL) was achieved by day 29 and was maintained throughout the treatment period following dosing at 1,200 mg once every 2 weeks.

Biomarker analyses

Extensive immune analyses were performed in a subset of biomarker-evaluable patients enrolled at the NCI (n=14). In analyses of changes in soluble analytes and peripheral immune cell subsets with bintrafusp alfa after 2 weeks of therapy, circulating levels of TGF-β1 were reduced by >97% (p<0.001, figure 2A), and remained reduced at both 6 and 12 weeks of therapy (online supplemental table 6A). In contrast, circulating levels of the ratio of sCD27:sCD40L (p=0.042), which is suggestive of immune activation, and the chemokine CCL17 (p=0.049) were increased after 2 weeks of therapy (figure 2A). Transient increases in total white blood count (p=0.006) and absolute neutrophil counts (p=0.002) were also observed after 2 weeks of therapy (figure 2B), with levels returning to baseline after 6 and 12 weeks of therapy (online supplemental table 6B). Evaluation of peripheral blood mononuclear cell (PBMC) subsets, which included 10 parental cell types ($CD4^+$ and $CD8^+$ T cells, regulatory T cells, natural killer [NK] cells, natural killer T cells [NKT] cells, conventional dendritic cells, plasmacytoid dendritic cells [pDC], B cells, myeloid-derived suppressor cells [MDSC], and monocytes) and 148 refined subsets related to their maturation/function, revealed reductions in total $CD8^+$ T cells (p=0.049), effector memory (EM, $CCR7^-$ $CD45RA^-$) $CD8^+$ T cells (p=0.030), $ki67^+$ $CD8^+$ T cells (p=0.020), and NKT cells (p=0.025), and increases in pDC (p=0.030) and MDSC (p=0.030) after 2 weeks of therapy (figure 2C, online supplemental table 6C), with changes in $CD8^+$ T-cell subsets, NKT cells, and pDC persisting after 6 weeks of therapy (online supplemental table 6C).

Differences in the peripheral immune profile of patients with ICI-naïve versus ICI-experienced disease, and in patients with a history of heavy smoking versus non-smokers showed notable differences in terms of soluble analytes, complete blood counts, and PBMC subsets prior to therapy with bintrafusp alfa. ICI-naïve patients had lower circulating levels of sCD27 (p=0.043), sPD-1 (p=0.008), sCD73 (p=0.029), CD8a (p=0.001) and IL-10 (p=0.013), and trends of lower levels of sPD-L1 (p=0.081) than ICI-experienced patients (online supplemental figure 1A). ICI-naïve patients also had trends of higher absolute lymphocyte counts (ALC, p=0.059) and a lower neutrophil-to-lymphocyte ratio (NLR, p=0.059) than ICI-experienced patients (online supplemental figure 1B), and higher frequencies of total B cells (p=0.020), $PD\text{-}1^+$ $CD4^+$ T cells (p=0.030) and terminally differentiated effector memory (EMRA; $CD45RA^+CCR7^-$) $CD4^+$ T cells (p=0.045) than ICI-experienced patients (online supplemental figure 1C). Different changes in the peripheral immune profile on receiving bintrafusp alfa were also observed between ICI-naïve and ICI-experienced patients. After 2 weeks of therapy, ICI-experienced patients had greater decreases in total $CD8^+$ T cells (p=0.005), EM $CD8^+$ T cells (p=0.043), EMRA $CD8^+$ T cells (p=0.043), $ki67^+$ $CD8^+$ T cells (p=0.008) and regulatory T cells (Tregs; p=0.030), and greater increases in total B cells (p=0.008) than ICI-naïve patients (online supplemental figure 1D). Notable differences in the immune status of patients with a history of heavy smoking versus non-smokers were also observed. Prior to therapy, smokers had a higher NLR (p=0.036), a well-known poor prognostic factor, and lower frequencies of activated $PD\text{-}1^+$ EM $CD4^+$ T cells (p=0.028) than non-smokers (figure 2D). After 2 weeks of bintrafusp alfa, smokers had greater reductions in total NK cells (p=0.036) and NK cells expressing the activating receptor NKp46 (p=0.034), and trends of greater decreases in proliferative $ki67^+$ $CD8^+$ T cells (p=0.054) and increases in total monocytes (p=0.076) compared with non-smokers (figure 2E).

Analysis on the association between the peripheral immune profile and clinical response showed that the immune status of patients prior to therapy was associated with PFS. For these and other biopsy-based immune analyses, a PFS cut-off of >3 months versus <3 months was used to group patients as responders versus non-responders, and two patients with radiologic pseudoprogression who showed PD at first restaging followed by SD or PR were classified as responders. Additionally, PFS was calculated based on efficacy assessment by investigators (online supplemental table 4). Patients with a PFS >3 months had lower baseline levels of circulating mucin-16 (p=0.029) and angiopoietin 2 (p=0.029) and trends of higher frequencies of NKT cells (p=0.059) and lower frequencies of MDSC (p=0.059) than patients with a PFS <3 months (figure 2F). Specific early immune changes induced by bintrafusp alfa were also associated with PFS. Compared with patients with a PFS <3 months, patients with PFS >3 months had less of an increase in interleukin (IL)-6 (p=0.081) after 2 weeks of therapy (figure 2G), and a greater increase in the ratio of sCD27:sCD40L (p=0.019) after 4 weeks of therapy (figure 2H). Although there was a limited number of patients in each group, evaluations with BOR also showed notable associations between the immune profile and clinical outcome. Prior to therapy, patients developing a BOR of CR/PR had higher levels of sCD40L (p=0.006), IL-7 (p=0.006), TNFSF14 (p=0.006), CCL17 (p=0.039), and CXCL5 (p=0.022), and trends of lower levels of sPD-L1 (p=0.088) and trends of higher ALC (p=0.088) than patients who developed SD or PD (online supplemental figure 2A). After 2 weeks of bintrafusp alfa, patients who subsequently developed a BOR of CR/PR had greater increases in CXCL10 (p=0.011),

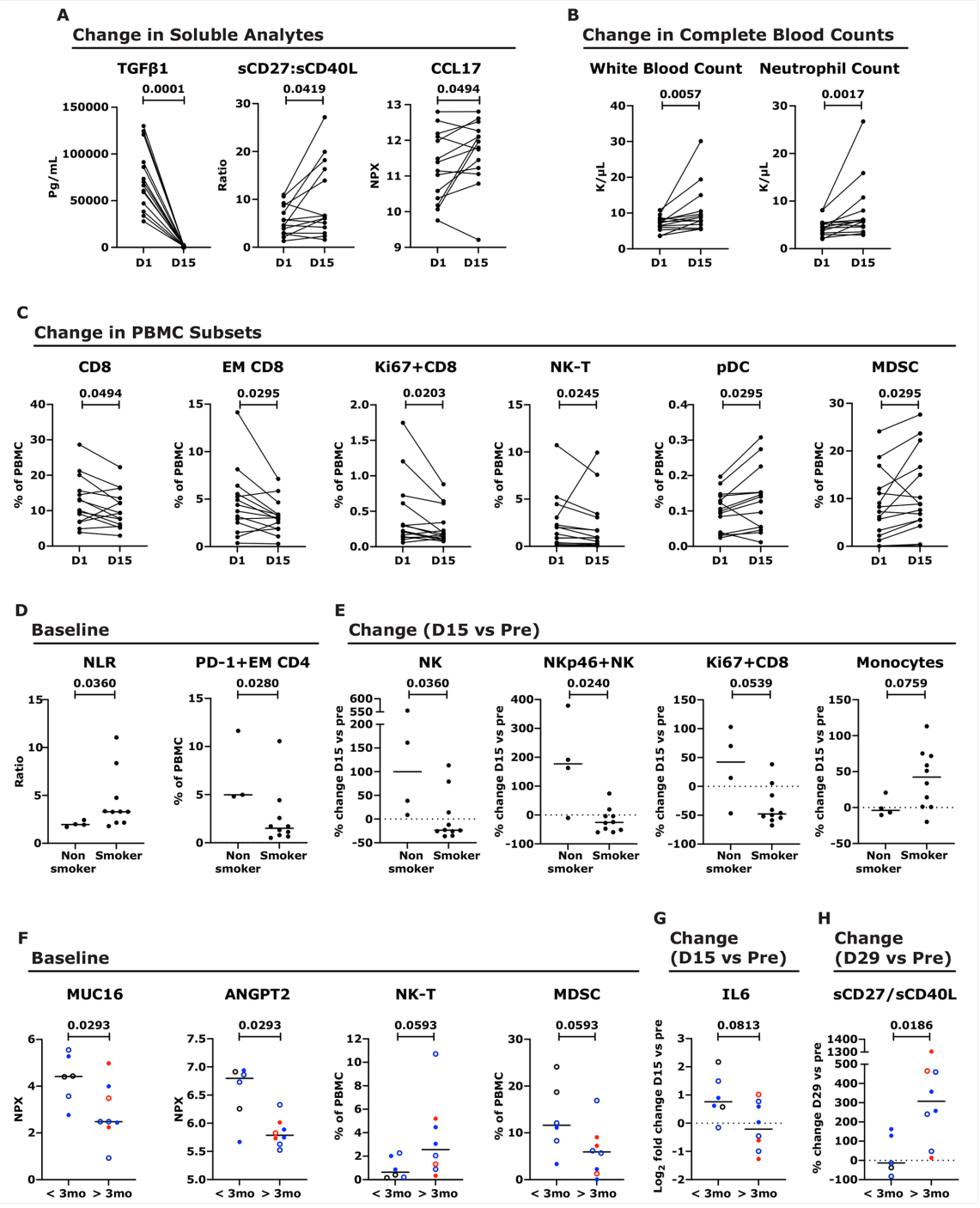

Figure 2 Immune changes in peripheral blood in pretreated and on-treated samples, and immune associations with smoking history and clinical response to bintrafusp alfa. Changes in (A) soluble analytes, (B) complete blood counts, and (C) PBMC immune subsets at pre (D1) and day 15 (D15) post-initiation of bintrafusp alfa in 14 patients in the biomarker cohort enrolled at the National Cancer Institute. Differences in the peripheral immune profile at (D) baseline and (E) after one cycle of therapy (D15 vs baseline) in patients with a prior history of heavy smoking (smoker, n=10) versus those without a history of smoking (non-smoker, n=4). The peripheral immune profile of patients at baseline (F) and changes after one cycle (G) and three cycles (H) of bintrafusp alfa associate with progression-free survival (PFS). (F–H) Patients with PFS <3 months (n=6) were compared with those with PFS >3 months (n=8). In (F–H) black indicates patients with a best overall response of progressive disease, blue indicates stable disease, red indicates partial/complete responses, an open circle identifies patients who are ICI-experienced, and a closed circle marks patients who are ICI-naïve. In (A–C) p values were calculated using the Wilcoxon signed-rank test. Graphs in (D–H) display median frequency of analytes and p values were calculated using the Mann-Whitney test. ANGPT2, angiopoietin-2; CD, cluster of differentiation; D, day; EM, effector memory; IL-6, interleukin-6; MDSC, myeloid-derived suppressor cells; Mo, months; MUC16, mucin 16; NK, natural killer cells; NKT, natural killer T cells; NLR, neutrophil-to-lymphocyte ratio; NPX, normalized protein expression; PBMC, peripheral blood mononuclear cells; PD-1, programmed cell death protein 1; pDC, plasmacytoid dendritic cells; s, soluble.

interferon-gamma (p=0.022), and trends of a greater increase in sCD27 (p=0.088) than patients who did not respond (online supplemental figure 2B). Patients who developed a CR/PR also had trends of greater decrease in IL-8 at both 2 (p=0.051) and 4 (p=0.051) weeks of therapy, and greater decreases in eosinophil count after 2 (p=0.039), 4 (p=0.014), and 6 (p=0.007) weeks of therapy (online supplemental figure 2B–D).

Routine histopathology examination of pretreatment and on-treatment tumor biopsies (online supplemental figure 3A and B) showed necrotic tissue with no definitive viable tumor and an inflammatory infiltrate in two subjects with objective response (including one subject who initially had radiological pseudoprogression) and two subjects with SD on-treatment. Additionally, three subjects with worsening pleural effusion, including an individual with a PR, had acute inflammatory cells in pleural fluid with no malignant cells. Another subject with SD subsequently experienced disease progression resulting in death. At autopsy, several sites of disease showed widespread inflammation and necrosis.

Results of multiplex immunofluorescence staining and multispectral imaging in a subset of patients with paired biopsies available (n=9) showed that bintrafusp alfa treatment increased tumor-infiltrating lymphocytes in NSCLC. The cut-off of PFS for 3 months was used to group patients as responders versus non-responders (figure 3A). Densities of lymphocytes ($CD4^+$ and $CD8^+$ T cells) were higher in bintrafusp alfa treated samples compared with baseline (mean from 3178.2 to 5156.7 cell/mm^2, p=0.0078). This increase in lymphocytes was slightly greater in patients with better outcomes (PFS>3 months). Densities of T helpers defined as $CD4^+$ and $FOXP3^-$, Tregs defined as $CD4^+$ $FOXP3^+$, and $CD8^+$ T cells showed an increase after treatment (mean 2276.2 vs 3199.5 cell/mm^2 p=0.027, mean 262.2 vs 517.1 cell/mm^2, p=0.05 and mean 637.7 vs 1440 cell/mm^2 p=0.027, respectively). The ratio of $CD8^+$/Tregs did not show any change after treatment in all samples and did not associate with outcomes.

Densities of inflammatory macrophages M1 ($CD68^+$/$CD163^-$) and tumor-trophic M2 macrophages ($CD68^+$/$CD163^+$) were also assessed (figure 3B and online supplemental figure 4A and B). Tumor-associated macrophage (TAM) densities (M1+M2) were slightly increased after treatment in all samples (mean 1902 vs 3005.3 cell/mm^2, p=0.49), and particularly in patients with PFS >3 months (p=0.06). M1 macrophage densities tended to decrease in non-responders (1148.75 vs 279.2 cell/mm^2, p=0.25) and increase in responders (102.4 vs 1298.4 cell/mm^2, p=0.18). M2 macrophage densities increased in treated samples for all patients. This increase was surprisingly more robust in responders changing from 1320.4 to 3394.4 cell/mm^2, p=0.06. M2/M1 ratios were found to be higher in treated samples; this increase was the highest among non-responders, rising from 1.47 to 51.86 cell/mm^2 (p=0.12). These findings highlight the role of M2 macrophages in tumor resistance to immune therapy and their potential to promote tumor growth.

T-cell receptor (TCR) sequencing of biopsy tissue in patients treated at the NCI with paired biopsies available (n=9) showed that TCR diversity, calculated by the number of clones comprising the top 25% of the repertoire, changed with the administration of bintrafusp alfa, and that these changes were associated with BOR (figure 3C). In the biopsy tissue of responders and subjects with SD ≥6 months, the number of clones comprising the top 25% of the repertoire generally decreased with treatment. In other words, the repertoire was composed of fewer, but more frequent TCR rearrangements, or a more clonal population. The opposite trend in TCR diversity was seen in 3/5 subjects with a BOR of PD or SD <6 months, where the top 25% of the repertoire was composed of more, but less frequent rearrangements after treatment, or a more diverse population. A subject with a response just below the 6-month cut-off (SD, 5.5 months) followed the pattern of the responders, with a decline in the number of highest frequency clones from 99 to 52. Another subject had a highly diverse selection of low-frequency clones at baseline that were maintained after treatment. No consistent changes were associated with the outcome in TCR sequencing of PBMCs corresponding to these biopsy time points.

DISCUSSION

This study evaluated the role of a TGF-β-inhibiting agent in a heterogeneous population of patients with NSCLC (different tumor histology, PD-L1 expression levels and different prior cancer therapies) who were previously treated with systemic therapies and were either ICI-naïve or ICI-experienced. Bintrafusp alfa showed modest clinical activity and had a manageable safety profile in this population. Treatment responses per IRC were only observed in the ICI-naïve group (ORR, 27.8%). Although there were no patients with objective responses by IRC in the ICI-experienced group, one patient developed a durable PR after initial radiological pseudoprogression at the first restaging time point. The response is ongoing (now a near-CR) after more than 4 years of discontinuation of bintrafusp alfa.

The efficacy results from this cohort are consistent with results from two other NSCLC cohorts of this phase 1 study. The ORR of 27.8% in the ICI-naïve group was comparable with the ORR of 25.0% observed in a cohort of ICI-naïve patients with advanced NSCLC that progressed after platinum doublet therapy, or after platinum-based adjuvant or neoadjuvant treatment (n=10/40).[15] The lack of response per IRC assessment in the ICI-experienced group was consistent with only modest clinical activity of bintrafusp alfa observed in a cohort of patients with NSCLC that progressed following ICI therapy (ORR: 4.8% [n=4/83]).[17] In addition to the phase 1 results, a recent phase 3 study of patients with advanced NSCLC and high PD-L1 expression failed to demonstrate superior efficacy of first-line bintrafusp alfa over the PD-1 inhibitor pembrolizumab.[18] However,

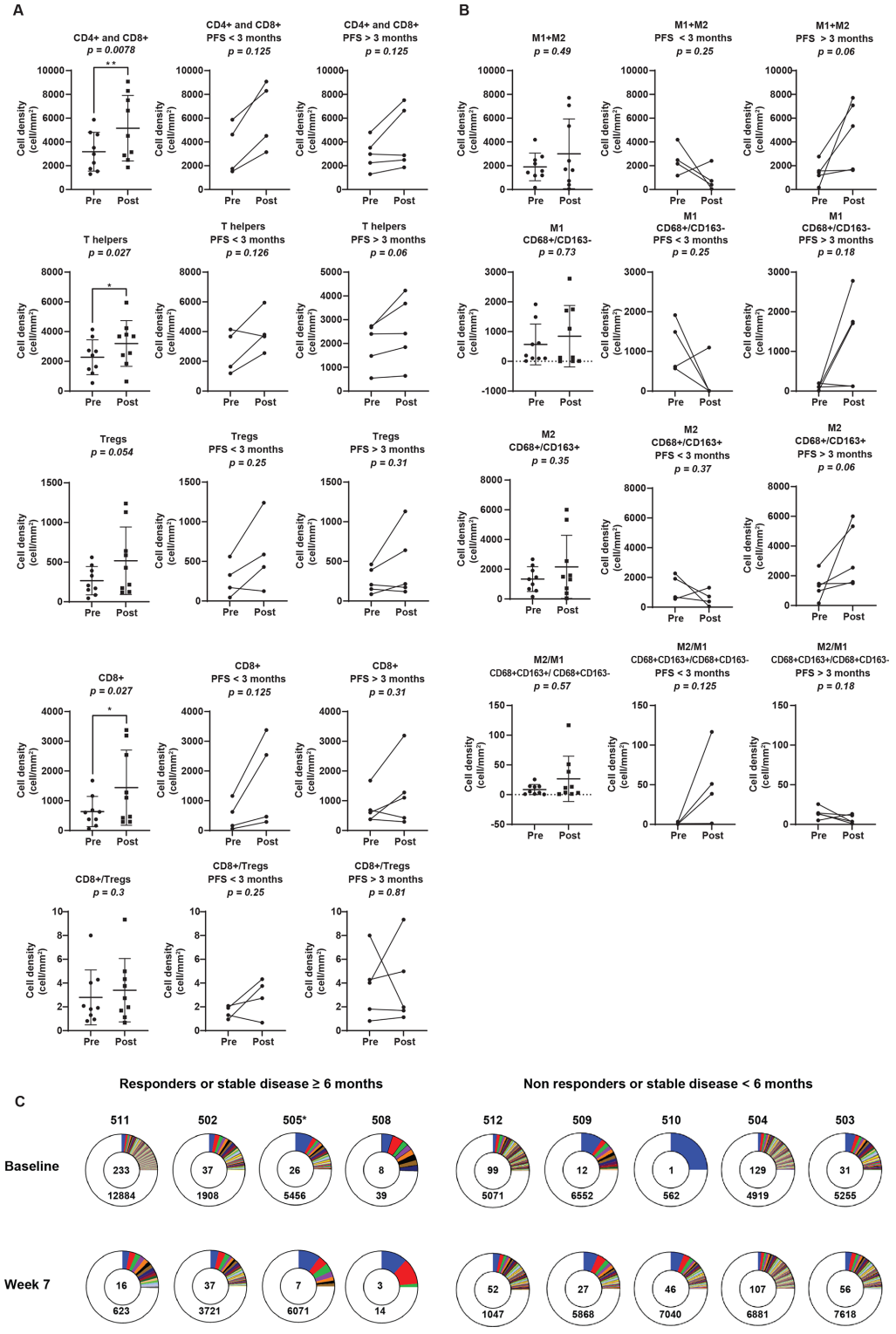

Figure 3 Immune changes in tumor tissue pretreatment and on-treatment with bintrafusp alfa. Densities of immune cells including (A) lymphocytes (T cells CD4$^+$ and CD8$^+$, T helpers, Tregs, CD8$^+$ T cells), and (B) TAMs (M1 and M2) were quantified in paired biopsies for nine patients. Graphs show immune cell densities in pretreatment and on-treatment biopsies and based on PFS less or more than 3 months. (C) Changes in TCR diveristy. Each doughnut plot indicates the number and frequency of productive TCR gene rearrangements in the baseline and week 7 biopsies by BOR. The total number of productive rearrangements is indicated on the bottom of each plot, while the number of clones comprising the top 25% of the repertoire is indicated in the center. Subject identifiers are indicated at the top of each plot. *Subject 505 is included with the responders due to a delayed durable PR, though RECIST was documented as PD for radiological pseudoprogression at first restaging. BOR, best overall response; CD cluster of differentiation; M1, inflammatory macrophages; M2, tumor-trophic macrophages; PD, progressive disease; PFS, progression-free survival; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; TAMs, tumor-associated macrophages; TCR, T-cell receptor; Tregs, regulatory T cells.

the response rate in the ICI-naïve group (27.8%) of the present study was higher than the response rates reported in the CheckMate 017 (nivolumab arm: 20%), CheckMate 057 (nivolumab arm: 19%), and OAK (atezolizumab arm: 18%) trials. Similarly, the PFS rate in the ICI-naïve group (2.7 months) of the present study was comparable with phase 3 trials of nivolumab and atezolizumab in patients with stage IIIb/IV NSCLC who had received prior treatment with platinum-based therapies (CheckMate 017 vs CheckMate 057 vs OAK: 3.5 months vs 2.3 months vs 2.8 months, respectively).[19–21]

Taken together, these results suggest that there are possible mechanisms of resistance independent of PD-(L)-1, and potentially related to TGF-β that cannot be overcome by targeting TGF-β in addition to PD-L1. TGF-β is a known pleiotropic cytokine that depending on the cellular/tissue context can have protumor or antitumor effects[22]; therefore, the lack of response observed in patients enrolled in this cohort could be independent of resistance to PD-(L)-1 inhibition and related to a net antitumor effect of TGF-β, the inhibition of which can cause tumor growth. Given the encouraging preclinical data across different tumor types and combination regimens, and the mixed clinical outcomes of bintrafusp alfa in clinical studies across different tumor types,[12 23–26] a better understanding of tumor biology, and identification of appropriate biomarkers is needed to identify patients who may benefit from bintrafusp alfa.[18 23] The C_{trough} reported in this study is indicative of a target occupancy being reached for all four targets of bintrafusp alfa (TGF-β1, TGF-β2, TGF-β3, and PD-L1),[27] similar to other studies with bintrafusp alfa in other cancer types.[14 28] Similarly, the first dose PK parameters are also in line with those reported previously for other cancer types.[29]

The biomarker analysis suggests that although there is an increase of both M1 and M2 TAM subtypes in NSCLC TIME after treatment with bintrafusp alfa, the interplay between the two TAM subtypes (M2/M1 ratio) showed a better correlation with patient outcome, and therefore could be used to better understand the effect of this treatment in modulating TAMs in the TIME. We were unable to perform any correlative studies between M1 and M2 TAM subtypes and TGF-β in biopsy samples due to the limited number of tissue sections available for analysis. However, in murine models it has been shown previously that TGF-β promotes the M2-like polarization of bone marrow-derived macrophages through the transcription factor SNAIL and PI3K/AKT and Smad2/3 pathways.[30] Moreover, in vitro silencing of SNAIL has been shown to result in M1-like macrophage polarization.[30]

While this study involved extensive interrogation of tissue and blood-based biomarkers in patients with NSCLC receiving dual inhibition of TGF-β and PD-L1, these analyses were performed in a small number of patients and were not directly compared with a similar patient population receiving PD-1/PD-L1 or TGF-β inhibition. It is therefore difficult to conclude whether the biomarkers identified in the current study are specific to the inhibition of the PD-1/PD-L1 or TGF-β signaling pathways targeted by bintrafusp alfa.

Taken together, this study shows that bintrafusp alfa treatment was associated with remodulation of the TIME, and induced a higher infiltration of various immune cells, including both lymphoid and myeloid cells into the TIME. Although the bintrafusp alfa program has been discontinued, we believe our data provide early insights into the biology of NSCLC, especially with respect to the impact of TGF-β inhibition (with or without concurrent PD-1/PD-L1 inhibition) on the NSCLC TIME, and these results are relevant for other TGF-β-directed therapies that are currently under evaluation in ongoing clinical trials.

Conclusions

In summary, bintrafusp alfa is clinically active in a subset of patients with advanced NSCLC and has an acceptable safety profile. Notable immunologic changes associated with treatment include modulation of the TIME and changes in peripheral immune cell subsets and soluble factors. These observations support further evaluation of TGF-β-directed therapies in combination with other immunomodulatory treatments.

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Acknowledgements The authors thank the patients and their families, investigators, coinvestigators, and study teams at each of the participating centers and at Merck. The authors also thank Susan Sansone, BA, for her many contributions to the care of patients enrolled in this study. Medical writing assistance was provided by Satrupa Das, PhD, CMPP (Merck Specialities Pvt. Ltd., Bangalore, India, an affiliate of Merck KGaA), and funded by Merck (CrossRef Funder ID: 10.13039/100009945). The authors thank Debra Weingarten for her editorial assistance in the preparation of this manuscript.

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Funding This study was supported in part by the Intramural Research Program of the Center for Cancer Research, National Cancer Institute (NCI), National Institutes of Health, and through a Cooperative Research and Development Agreement between the NCI and Merck (CrossRef Funder ID: 10.13039/100009945), which was previously part of an alliance between the Merck KGaA and GlaxoSmithKline.

Competing interests AR reports research funding to his institution from Promontory Therapeutics Inc. OR reports leadership roles as vice president and global clinical strategy head of gastrointestinal oncology at AstraZeneca and reports having stocks at AstraZeneca. RI reports employment with Merck Biopharma Co., Ltd., Tokyo, Japan, an affiliate of Merck KGaA and has attended the independent data monitoring committee/steering committee as part of employment responsibilities. YV reports employment with EMD Serono Research & Development Institute, Inc., Billerica, Massachusetts, USA, an affiliate of Merck KGaA and patent applications. MS reports employment with Merck Biopharma Co., Ltd., Tokyo, Japan, an affiliate of Merck KGaA. AM reports employment with EMD Serono Research & Development Institute, Inc., Billerica, Massachusetts, USA, an affiliate of Merck KGaA. JS reports patents. JLG reports patents and is vice president of the Society for Immunotherapy of Cancer. The remaining authors declare no conflicts of interests.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by Copernicus Group IRB (111) Comitato Etico IRCCS di Candiolo (520) Istituto Nazionale Tumori Fondazione G. Pascale (521) Greenslopes Research and Ethics Committee (601) Peter MacCallum Cancer Centre Ethics Committee (RGO) (609) Comité d'Ethique de l'Insitut Jules Bordet (551) Comitato Etico dell'Università Cattolica del Sacro Cuore e annesso Policlinico "A. Gemelli" (535) WIRB (118) Mary Crowley Medical Research Center Institutional Review Board (106) Port Macquarie Health Campus RGO (602) Copernicus Group IRB (117) WIRB (139) US Oncology, Inc. IRB (135) Medical University of South Carolina IRB (158) Comité d'Ethique Grand Hôpital de Charleroi (501) IRB of Severance Hospital, Yonsei University Health System (805) US Oncology, Inc. IRB (128) US Oncology, Inc. IRB (129) US Oncology, Inc. IRB (132) Comitato etico degli IRCCS Istituto Europeo di Oncologia e Centro Cardiologico Monzino (522) University of Illinois at Chicago Office of Research Services (110) Comitato Etico Indipendente Azienda Ospedaliera Gaetano Rummo di Benevento (523) Comitato Etico Indipendente della Fondazione IRCCS Istituto Nazionale dei Tumori di Milano (526) Commissie Medische Ethiek GZA (502) Dartmouth Commitee for the Protection of Human Subjects (CPHS) IRB (151) University Hospitals Case Medical Center IRB (153) US Oncology, Inc. IRB (134) City of Hope Institutional Review Board (141) Azienda Ospedaliero Universitaria Pisana (536) Copernicus Group IRB (123) Comité d'Ethique du CHA (503) Calvary Health Care (Newcastle) Limited (614) Comitato Etico IRCCS Ospedale S. Raffaele di Milano (527) National Cancer Institute IRB (101) Copernicus Group IRB (121) Emory IRB (142) IRB of Pusan National University Hospital (809) Bellberry Human Research Ethics Committees (Non Lead Sites) (616) Chris O'Brien Lifehouse RGO (603) Sydney Local Health District RPAH Zone – HREC (603) Michigan State University Office of Regulatory Affairs (126) Mackay Memorial Hospital, Institutional Review Board (901) National Cancer Center IRB (704) Commission d'ethique CHU Sart Tilman Liege 1 (504) UC Davis IRB (143) Arbogast Pharmaceuticals – PARENT (613) South Metropolitan Area Health Service Human Research Ethics Committee (613) IRB of Seoul National University Bundang Hospital (801) IRB of Seoul National University Hospital (802) Kindai University Hospital IRB (703) St George's Healthcare NHS Trust (611) IRB of Asan Medical Center (806) IRB of Chungbuk National University Hospital (807) University of North Carolina at Chapel Hill IRB (125) Copernicus Group IRB (116) IRB of Samsung Medical Center (803) National Taiwan University Hospital, Research Ethics Committee (902) Commission d'Ethique Hospitalo-Facultaire (544) Comitato Etico di Area Vasta Sud Est (552) Copernicus Group IRB (115) US Oncology, Inc. IRB (130) Bellberry Human Research Ethics Committees (607) University of Cincinnati (112) WIRB (112) MD Anderson Cancer Center IRB (103) Copernicus Group IRB (120) IntegReview IRB (105) Northern Sydney Local Health District – RGO (610) Northern Sydney Local Health District HREC (610) Comitato Etico Referente per l'area di Pavia (528) Copernicus Group IRB (114) Salus IRB (113) Dana Farber Cancer Institute (157) Ethisch Comité UZA (550) WIRB (119) US Oncology, Inc. IRB (131) Cabrini Human Research Ethics Committee (604) US Oncology, Inc. IRB (136) Central Adelaide Local Health Network Incorporated operating as The Queen Elizabeth Hospital (RGO) (605) The Queen Elizabeth Hospital Human Research Ethics Committee (605) Ethisch Comité Universitair Ziekenhuis Gent (506) IRB of Asan Medical Center (804) Rhode Island Hospital IRB (149) Comitato Etico Indipendente dell'Azienda Ospedaliero-Universitaria Policlinico S. Orsola-Malpighi BO (518) Health Research Ethics Board of Alberta - Cancer Committee (HREBA-CC) (200) Istituto Clinico Humanitas (529) WIRB (155) Taipei Medical University, Joint Institutional Review Board (903) Comitato Etico MILANO Area C (545) Fondazione IRCCS CA' Granda Ospedale Maggiore Policlinico (545) Copernicus Group IRB (154) US Oncology, Inc. IRB (137) Bellberry Human Research Ethics Committees (Non Lead Sites) (606) Washington University in St. Louis IRB (107) Chang Gung Medical Foundation, Institutional Review Board (906) Henry Ford Health System (108) Western University Office of Research Ethics Board (201) Copernicus Group IRB (104) US Oncology, Inc. IRB (138) University of Kansas IRB (147) Western Sydney Local Health District HREC – RGO (615) Penn State Milton S. Hershey Medical Center IRB (144) South Western Sydney Local Health District (RGO) (612). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Any requests for data by qualified scientific and medical researchers for legitimate research purposes will be subject to Merck's Data Sharing Policy. All requests should be submitted in writing to Merck's data sharing portal (portal https://www.merckgroup.com/en/research/our-approach-to-research-and-development/healthcare/clinical-trials/commitment-responsible-data-sharing.html). When Merck has a co-research, co-development, or co-marketing or co-promotion agreement, or when the product has been out-licensed, the responsibility for disclosure might be dependent on the agreement between parties. Under these circumstances, Merck will endeavour to gain agreement to share data in response to requests.

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