Supplementary Methods – Full eligibility criteria

Inclusion Criteria

Patients were eligible for the study if they were ≥18 years of age, had a histologically confirmed diagnosis of advanced NSCLC and:

a) Had not received prior systemic therapy (although completion of treatment with cytotoxic chemotherapy, biological therapy, and/or radiation as part of neoadjuvant/adjuvant therapy was allowed as long as therapy was completed at least 6 months prior to the diagnosis of metastatic disease).

b) Had measurable disease (RECIST 1.1).

c) Had a life expectancy of at least 3 months.

d) Available tumor tissue (<6 months old excluding bone biopsies) before the first dose, or a fresh biopsy if the patient received local therapy after the archival biopsy.

e) PD-L1 high status by central testing (≥80% PD-L1 positive tumor cells by the PD-L1 IHC 73-10 assay).

f) Did not have *EGFR ALK, ROS1*, or *BRAF*V600E mutations.

ECOG PS 0-1 and adequate organ function [hematological function defined by absolute neutrophil count (ANC) ≥1.5 × 10^9/L, platelet count ≥100 × 10^9/L, and hemoglobin ≥9 g/dL; hepatic function defined by a total bilirubin level ≤ upper limit of normal (ULN), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels ≤1.5 × ULN and alkaline phosphatase ≤2.5 × ULN; renal function defined by creatinine ≤1.5 × ULN or calculated creatinine clearance (CrCL) ≥30 mL/minute for patients with creatinine >1.5 × ULN); coagulation function defined as international normalized ratio or prothrombin time ≤1.5 × ULN unless the patient was receiving anticoagulant therapy, and activated partial thromboplastin time ≤1.5 × ULN unless the patient was receiving anticoagulant therapy).

Contraceptive use by males or females was consistent with local regulations; male patients were eligible to participate if they refrained from donating sperm and either: abstained from intercourse with a female or used a male condom when having sexual intercourse with a woman of childbearing potential (WOCBP), who was also using a highly effective contraceptive method with a failure rate of <1% per year (for at least 4 months after the last dose). Female participants were not pregnant (as determined by appropriate contraception, investigation of menstrual and sexual history, and pregnancy tests) or not breastfeeding and using a highly effective contraceptive method (with a failure rate of <1% per year), or not a WOCBP.

All patients were required to provide informed consent.

Exclusion Criteria

Patients with the following conditions were excluded:

a) Patients with nonsquamous NSCLC histologies whose tumor harbored molecular alterations that can be treated with targeted therapy, specifically, an *EGFR* sensitizing

mutation, *ALK* translocation(s) *ROS1* rearrangement(s) or the *BRAF* V600E mutation; testing was not required for patients known to have a tumor of predominantly squamous histology

b). Had received major surgery within 4 weeks prior to the first dose of study intervention or had received thoracic RT within 6 months prior to the first dose of study intervention.

c). Had a previous malignant disease within the last 3 years (except patients with a history of cervical carcinoma in situ, superficial or noninvasive bladder cancer, or basal cell or squamous cell carcinoma in situ).

d) Had active CNS metastases and/or carcinomatosis meningitis unless they had fully recovered from treatment demonstrated by 2 brain images.

e) Active autoimmune disease that required systemic treatment in the past year or receiving systemic steroid therapy or any other form of immunosuppressive medication (although hormone replacement with corticosteroids at low doses and c and corticosteroid use for IV contrast allergies/reactions was allowed).

f) Had a history or evidence of any condition, therapy, or laboratory abnormality that potentially confounded the results of the study or interfered with participation for the full duration of the study, including patients with clinically relevant bleeding events of hemoptysis Grade ≥ 2.

g) Was administered a prior/concomitant therapy with an anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, or anti-cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) antibody, systemic or localized antineoplastic therapy (including maintenance therapy with another agent for NSCLC, RT, and/or surgical resection), a prohibited concomitant drug, a live vaccine (within 30 days prior to the first dose though seasonal flu vaccines were permitted), systemic therapy or antibiotics due to an active infection, an investigational agent or investigational device within 4 weeks of the first dose of treatment.

Patients with a known active alcohol or drug abuse issue or any psychiatric condition that would interfere with providing informed consent or consistent participation in study procedures, as well as patients with legal incapacity or limited legal capacity were excluded.

Table S1. mPFS per RECIST 1.1 by IRC by PD-L1 status for different PD-L1 assays

		73-10		SP263		22C3	
		Number	mPFS, months (95% CI)	Number	mPFS, months (95% CI)	Number	mPFS, months (95% CI)
Treatment arm	**PD-L1 status**						
Pembrolizumab	High	141	14.9 (8.1-NR)	104	14.9 (6.9-NR)	98	11.1 (8.1-NR)
	Not-high	8	8.4 (5.6-NR)	13	5.7 (1.3-NR)	0	—
Bintrafusp alfa	High	138	7.9 (4.2-NR)	107	7.0 (4.1-NR)	104	7.9 (4.1-NR)
	Not-high	13	2.8 (1.2-NR)	15	6.9 (1.3-NR)	3	1.2 (0.8-NR)

CI, confidence interval; IRC, Independent Review Committee; m, median; NR, not reached; PFS, progression-free survival; RECIST 1.1, Response Evaluation Criteria in Solid Tumors Version 1.1.

Table S2. AEs of special interest

	Bintrafusp alfa, n (%) n=151		Pembrolizumab, n (%) n=152	
	Any grade	Grade ≥3	Any grade	Grade ≥3
TGF-β inhibition–mediated skin AEs, n (%)*	32 (21.2)	5 (3.3)	0	0
Keratoacanthoma	15 (9.9)	—	0	—
Squamous cell carcinoma of skin	9 (6.0)	—	0	—
Hyperkeratosis	8 (5.3)	—	0	—
Actinic keratosis	3 (2.0)	—	0	—
Immune-related AEs, n (%)	80 (53.0)	35 (23.2)	53 (34.9)	12 (7.9)
Immune-related rash	63 (41.7)	23 (15.2)	25 (16.4)	1 (0.7)
Immune-related endocrinopathies	24 (15.9)	1 (0.7)	22 (14.5)	1 (0.7)
Thyroid disorders	17 (11.3)	0	15 (9.9)	0
Adrenal Insufficiency	6 (4.0)	0	5 (3.3)	0
Type 1 diabetes mellitus	2 (1.3)	1 (0.7)	2 (1.3)	1 (0.7)
Pituitary dysfunction	1 (0.7)	0	1 (0.7)	0
Immune-related hepatitis	8 (5.3)	7 (4.6)	1 (0.7)	0
Immune-related colitis	7 (4.6)	2 (1.3)	2 (1.3)	0
Immune-related nephritis and renal dysfunction	2 (1.3)	1 (0.7)	3 (2.0)	3 (2.0)
Immune-related pneumonitis	4 (2.6)	2 (1.3)	3 (2.0)	3 (2.0)

Other immune-related AEs	5 (3.3)	3 (2.0)	4 (2.6)	4 (2.6)
Bleeding events, n (%)	55 (36.4)	8 (5.3)**	18 (11.8)	3 (2.0)**
Anemia, n (%)	47 (31.1)	17 (11.3)**	18 (11.8)	4 (2.6)**

AE, adverse event; TGF-β, transforming growth factor-β.

*Includes keratoacanthoma, squamous cell carcinoma of skin, hyperkeratosis, actinic keratosis, basal cell carcinoma, lip squamous cell carcinoma, and Bowen's disease.

**Represent Grade 3 events only.

Table S3. Overview of TRAEs by histology and any cause AESIs (squamous vs nonsquamous)

	Bintrafusp alfa n=151		Pembrolizumab n=152	
	Squamous (n=45)	Nonsquamous (n=106)	Squamous (n=44)	Nonsquamous (n=108)
TRAEs, n (%)	37 (82.2)	87 (82.1)	29 (65.9)	76 (70.4)
Any grade ≥3 TRAE	22 (48.9)	42 (39.6)	7 (15.9)	13 (12.0)
Any cause AESIs				
Any TGF-β inhibition–mediated skin AEs, n (%)*	10 (22.2)	22 (20.8)	0 (0.0)	0 (0.0)
Any immune-related AEs, n (%)	21 (46.7)	59 (55.7)	16 (36.4)	37 (34.3)
Any bleeding AEs, n (%)	18 (40)	37 (34.9)	5 (11.4)	13 (12.0)
Any anemia, n (%)	12 (26.7)	35 (33.0)	4 (9.1)	14 (13.0)

AE, adverse event; AESIs, AEs of special interest; TGF-β, transforming growth factor-β; TRAE, treatment-related adverse event.

*Includes keratoacanthoma, squamous cell carcinoma of skin, hyperkeratosis, actinic keratosis, basal cell carcinoma, lip squamous cell carcinoma, and Bowen's disease.

Table S4. Bintrafusp alfa serum C_{trough} and C_{eoi} over time

	Day 1	Day 15	Day 29	Day 43		Day 85		Day 127	Day 169	Day 211
Statistics	C_{eoi}	C_{trough}	C_{trough}	C_{trough}	C_{eoi}	C_{trough}	C_{eoi}	C_{trough}	C_{trough}	C_{trough}
n (N=146)	130	124	103	88	84	65	64	58	46	36
Mean	381	57.8	79.8	88.3	467	101	470	102	99.7	103
StD	107.3	22.45	33.90	33.63	140.1	40.27	141.2	36.32	40.62	29.36
CV%	28.2	38.8	42.5	38.1	30.0	39.9	30.0	35.5	40.7	28.6
GeoMean	365	53.0	71.2	79.9	436	91.7	446	95.7	91.7	99.0
logStD	0.3052	0.4556	0.5305	0.5335	0.4242	0.4839	0.3542	0.3887	0.4290	0.2761
GeoCV%	31.2	48.0	57.0	57.4	44.4	51.4	36.6	40.4	45.0	28.1

Concentration values BLQ were set to zero for parameter calculation and descriptive statistics.

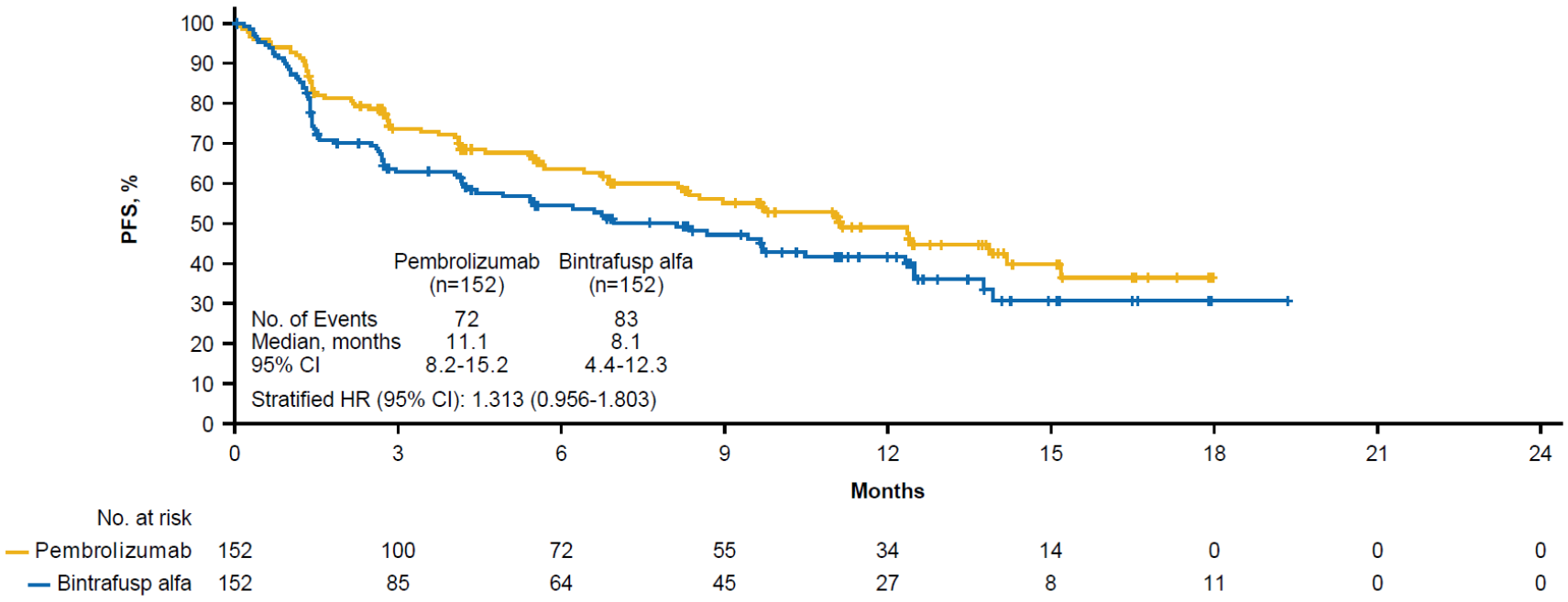
BLQ, below the lower limit of quantification (LLOQ); CV%, coefficient of variation; Geo, geometric; logStD, standard deviation of log-transformed data; n, number of non-missing observations; StD, standard deviation.

Table S5. Immunogenicity of bintrafusp alfa

	Bintrafusp alfa n=144
ADA Result	
Never positive, n (%)	77 (53.5)
Ever positive, n (%)	67 (46.5)
Preexisting n/N1 (%)	15/138 (10.9)
ADA Bintrafusp alfa boosted n/N2 (%)	5/129 (3.9)
ADA treatment-emergent n/N3 (%)	52/121 (43.0)
Transient positive n/N3 (%)	27/121 (22.3)
Persistent positive n/N3 (%)	25/121 (20.7)
ADA treatment-emergent ALL n/N4 (%)	57/135 (42.2)
ADA non-treatment-emergent ALL n/N4 (%)	78/135 (57.8)

N1, Number of patients with valid baseline result.
N2, Number of patients with valid baseline and at least one valid postbaseline result.
N3, Number of patients with at least one valid postbaseline result and without positive baseline results (including missing, NR).
N4, Number of patients with at least one valid postbaseline ADA result at any time point.
ADA, anti-drug antibody

Figure S1. PFS per RECIST 1.1 by investigator



CI, confidence interval; HR, hazard ratio; PFS, progression-free survival; RECIST 1.1, Response Evaluation Criteria in Solid Tumors Version 1.1.