Supplemental material

Inclusion criteria

Patients must meet all the following inclusion criteria to be eligible for participation in this study:

1. Voluntarily agree to participate by giving written informed consent
2. Age 18 years or greater
3. Have a histologically or cytologically confirmed diagnosis of a locally advanced or metastatic solid tumor for which no standard therapy is available (per local guidance) or standard therapy has failed
4. Have measurable disease on imaging based on Response Evaluation Criteria in Solid Tumors 1.1
5. Have a life expectancy of at least 3 months and an Eastern Cooperative Oncology Group performance status of 0–1
6. Have adequate organ function as defined in the protocol
7. Have available, sufficient, and adequate formalin-fixed tumor tissue sample (as specified in the Laboratory Manual) preferably from a biopsy of a tumor lesion obtained either at the time of or after the diagnosis of advanced disease has been made and from a site not previously irradiated. Alternatively, patients must agree to have a biopsy taken prior to entering the study to provide adequate tissue. Fine needle aspirates are not acceptable. Core needle or excisional biopsy or resected tissue is required
8. A negative serum pregnancy test is required for female patients (unless permanently sterile or greater than 2 years postmenopausal)

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9. Male and female patients of childbearing potential who engage in heterosexual intercourse must agree to use protocol-specified method(s) of contraception and refrain from egg or sperm donation
10. Lactating females must agree to discontinue nursing before the study drug is administered
11. Is willing and able to comply with the requirements of the protocol.

Exclusion criteria

Patients who meet any of the following exclusion criteria are not to be enrolled in this study:

1. Is currently participating and receiving study therapy or has participated in a study of an investigational agent and received study therapy or used an investigation device within 3 weeks of the first dose of treatment
2. Has received prior systemic cytotoxic chemotherapy, biological therapy, radiotherapy, or major surgery within 3 weeks; a 1-week washout is permitted for palliative radiation to non-central nervous system (CNS) disease with Gilead approval
3. Has persisting toxicity related to prior therapy of the national cancer institute CTCAE (NCI CTCAE) v5.0 of severity greater than Grade 1. **Note:** Alopecia and sensory neuropathy of Grade 2 or lower is acceptable
4. Is expected to require any other form of systemic or localized anticancer therapy while on study (including maintenance therapy with another agent, radiation therapy, and/or surgical resection)
5. Has known severe hypersensitivity reactions (NCI CTCAE Grade 3 or higher) to fully human monoclonal antibodies, dalutrafusp alfa formulation excipient, or severe reaction

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to Immuno-oncology agents, such as colitis or pneumonitis requiring treatment with corticosteroids, any history of anaphylaxis, or uncontrolled asthma

6. Is receiving systemic corticosteroid therapy 1 week prior to the first dose of study treatment or receiving any other form of systemic immunosuppressive medication. **Note:** The following corticosteroid uses are permitted: use as premedication for known hypersensitivity reactions (e.g., intravenous [IV] contrast, IV drug infusions); intraocular, intranasal, inhaled, and/or topical corticosteroids; and/or prednisone at doses of up to 10 mg per day or equivalent
7. Has concurrent active malignancy other than nonmelanoma skin cancer, carcinoma in situ of the cervix, or superficial bladder cancer and who has undergone potentially curative therapy with no evidence of disease. Patients with other previous malignancies are eligible if disease-free for more than 2 years
8. Has a known CNS metastasis(es), unless metastases are treated and stable and the patient does not require systemic corticosteroids for management of CNS symptoms at least 7 days prior to study treatment. Patients with history of carcinomatous meningitis are excluded regardless of clinical stability
9. Has active or history of autoimmune disease that has required systemic treatment within 2 years of the start of study treatment (i.e., with use of disease-modifying agents, corticosteroids, or immunosuppressive drugs). **Note:** Patients with diabetes type 1, vitiligo, psoriasis, hypothyroid disease, or hyperthyroid disease not requiring immunosuppressive treatment are eligible
10. Has had an allogeneic tissue/solid organ transplant
11. Has or had interstitial lung disease

12. Has a serious systemic fungal, bacterial, viral, or other infection that is not controlled or requires IV antibiotics
13. Has known history of human immunodeficiency virus
14. Has known active hepatitis B virus and/or hepatitis C virus
15. Patients with cardiovascular disease/abnormalities will be excluded per the following criteria:
    a. Has clinically significant (i.e., active) cardiovascular disease: cerebral vascular accident/stroke or myocardial infarction within 6 months of enrollment, unstable angina, congestive heart failure (New York Heart Association class ≥II), or serious uncontrolled cardiac arrhythmia requiring medication
    b. Has a mean QT interval corrected for heart rate (QTc) using the Fridericia formula (QTcF) of 470 ms or greater
    c. Has systolic dysfunction defined as ejection fraction less than 50% measured by echocardiogram (or multiple gated acquisition scan) at baseline
16. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the study, interfere with the patient's participation for the full duration of the study, or is not in the best interest of the patient to participate, in the opinion of the treating investigator
17. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the study
18. Is legally incapacitated or has limited legal capacity
19. Taking biotin supplements within 72 hours of screening
20. Has positive serum pregnancy test

21. Has had prior treatment with anti-cluster of differentiation (CD)73 or transforming growth factor beta (TGFβ) therapies
22. Breastfeeding female

Calculation of biomarker values

Biomarker values were measured at protocol specific timepoints

- Absolute values for free sCD73, sCD73 activity, TGFβ 1/2/3
- Derived values for CD73 TO on B-cells and CD8+ T-cells:
    - Target occupancy and CD73 expression on B-cells and T-cells data were derived from mean MFI using the following approach:
        - Per patient

$$\text{iMFI}_{Day\,1} \text{ of } 416 = MFI\ of\ 416_{Bcells>CD73+,Day\,1} * Percent\ of\ 416_{Bcells>CD73+,Day\,1}$$

$$\text{iMFI}_{Day\,1} \text{ of AD2} = MFI\ of\ AD2_{Bcells>CD73+,Day\,1} * Percent\ of AD2_{Bcells>CD73+,Day\,1}$$

        - CD73 expression

$$\text{CD73 expression Day10} = \left(\frac{iMFI\ of\ 416_{Day10}}{iMFI\ of\ 416_{predose}}\right) * 100$$

        - CD73 target occupancy

$$\text{CD73 Target Occupancy Day10} = \left(1 - \left(\frac{\text{iMFI of AD2}_{\text{Day 10}}}{\frac{\text{iMFI of 416}_{\text{Day 10}}}{\text{iMFI of 416}_{\text{predose}}} * \text{iMFI of AD2}_{\text{predose}}}\right)\right) * 100$$

Definition of dose-limiting toxicity

Toxicity will be graded according to NCI CTCAE v5.0. A dose-limiting toxicity (DLT) is a toxicity observed in the first 28 days as defined below and considered possibly related to dalutrafusp alfa. A DLT may lead to permanent withdrawal of dalutrafusp alfa for the patient after discussion between the investigator and sponsor.

Hematologic

- Grade 3 thrombocytopenia with clinically significant bleeding (i.e., requires hospitalization, transfusion of blood products, or other urgent medical intervention)
- Grade 3 or higher febrile neutropenia (absolute neutrophil count less than 1.0 × 109/L and fever greater than 101°F/38.3°C)
- Any Grade 4 hematologic laboratory abnormalities/adverse events (AEs) regardless of duration will be considered DLTs except for
    - Grade 4 lymphopenia
    - Grade 4 neutropenia lasting 7 days or fewer days that is not associated with fever (the use of growth factors is permitted)
    - Grade 4 anemia explained by underlying disease

Nonhematologic

- Grade 4 nonhematologic (laboratory and nonlaboratory) AEs will be considered DLT regardless of the duration

- Any Grade 2 or higher uveitis, blurred vision, eye pain, and/or reduction of visual acuity that does not respond to topical therapy and does not improve to Grade 1 severity within 2 weeks of the initiation of topical therapy OR requires systemic treatment will be considered as a DLT
- The following Grade 3 nonhematologic AEs **will be considered as DLTs:**
    - Any Grade 3 AE of unknown etiology consistent with an immune phenomenon that does not resolve to Grade 0–1 or to baseline with immunosuppressive therapy within 3 weeks of its onset
    - Any Grade 3 CNS related AE of unknown etiology consistent with an immune phenomenon regardless of duration or reversibility
    - Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) elevation that is 3 or more times greater than the upper limit of normal (ULN), also showing elevation of serum total bilirubin of greater than 2× ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase)
- Any other non–immune-related Grade 3 AE **except:**
    - Any Grade 3 endocrinopathy that is adequately controlled by hormonal replacement
    - Grade 3 AE of tumor flare (defined as local pain, irritation, or rash localized at sites of known or suspected tumor)
    - Transient (less than 3 days) Grade 3 fatigue, local reactions, headache, nausea, emesis, or diarrhea that are controlled with medical management and/or resolves to Grade 1 or less

- Transient (3 days or less) Grade 3 fatigue, local reactions, headache, nausea, emesis, or diarrhea that are controlled with medical management and/or resolves to Grade 0–1
- An event clearly associated with the underlying disease, progressive disease, a concomitant medication, or comorbidity.

Dosing/procedures-related toxicities

- Inability to receive the first 2 doses of dalutrafusp alfa because of related toxicity, even if the toxicity does not meet DLT criteria defined above (regardless of dosing schedule)
- Greater than a 2-week delay in starting the next cycle of therapy due to a treatment-related toxicity, even if the toxicity does not meet the DLT criteria determined above

Determination of maximum tolerated dose

The initial block of each dose in 3+3 escalation scheme consisted of 3 patients. Dose escalation occurred if no patients had a DLT during the first 28 days of study drug dosing. If 2 or more patients had DLTs within the first 28 days, dose de-escalation to a lower dose occurred. If 1 of the first 3 evaluable patients enrolled had a DLT within the first 28 days, then 3 additional patients were enrolled at the same dose level. If no DLTs were observed in the additional 3 patients, dose escalation occurred. If a DLT occurred in 2 or more patients in the total cohort of 6 patients, the maximum tolerated dose (MTD) was deemed to be exceeded, and the prior dose level was evaluated to determine the MTD by increasing enrollment to 6 patients. If the prior dose level was already deemed to be safe (i.e., 0 or 1 DLT) and enrolled 6 patients, then it was defined as the MTD.

SAEs related to the study drug

One patient receiving 30 mg/kg of dalutrafusp alfa had a serious AE of Grade 4 thrombocytopenia that was considered related to dalutrafusp alfa on study Day 191. The patient had dalutrafusp alfa withdrawn on study Day 193 and received medication and other treatment afterwards.

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Supplemental Table S1: Incidence of patients with Grade 3 or 4 laboratory abnormalities

	Dalutrafusp alfa doses (mg/kg)							
n (%)	**0.3 n=1**	**1 n=1**	**3 n=3**	**10 n=3**	**20 n=3**	**30 n=7**	**45 n=3**	**Total N=21**
Hemoglobin (anemia)	0	1	0	0	1	0	0	2 (9.5)
Platelets (decrease)	0	0	0	0	0	1	0	1 (4.8)
Alkaline phosphatase (increase)	0	0	0	1	0	0	0	1 (4.8)
GGT (increase)	0	0	0	1	0	1	0	2 (9.5)
Sodium (hyponatremia)	0	0	0	0	1	0	0	1 (4.8)

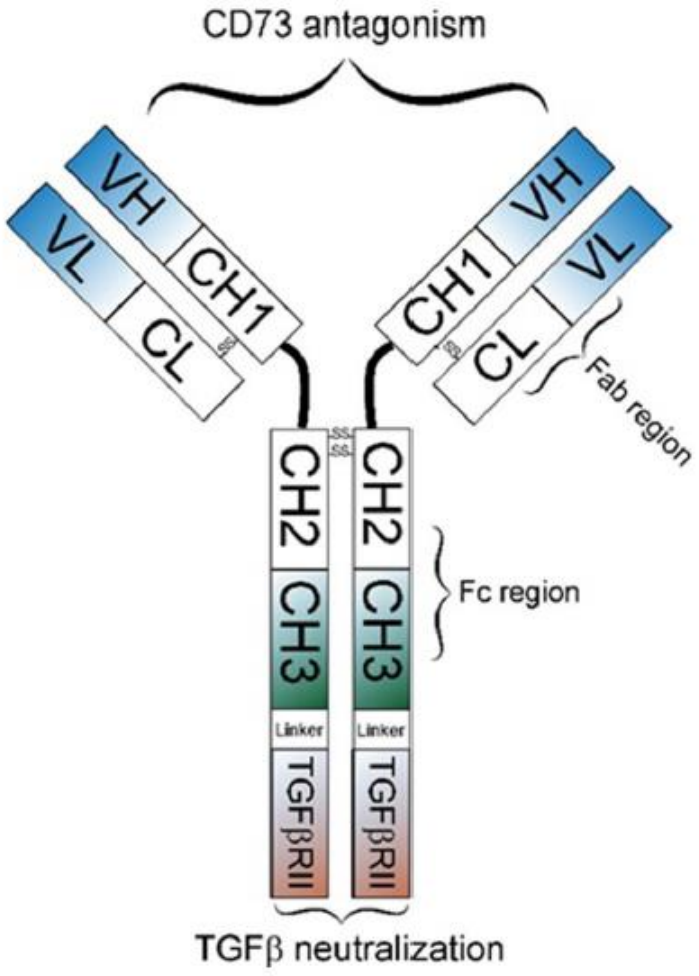
GGT, Gamma-Glutamyl Transferase.

Supplemental Table S2: Incidence of patients with positive anti–dalutrafusp alfa antibody

Visit	Dalutrafusp alfa doses (mg/kg)							
	0.3 n=1	**1 n=1**	**3 n=3**	**10 n=3**	**20 n=3**	**30 n=7**	**45 n=3**	**Total N=21**
Cycle 2 Day 1 (predose)	0	0	0	2	1	0	1	4 (19%)
Cycle 3 Day 1 (predose)	0	NA	1	1	2	0	0	4 (19%)
Cycle 4 Day 1 (predose)	NA	NA	1	NA	1	0	0	2 (9.5%)
Cycle 4 Day 15 (predose)	NA	NA	0	NA	1	0	NA	1 (4.8%)
Cycle 6 Day 1 (predose)	NA	NA	1	NA	NA	0	NA	1 (4.8%)
Day 30 follow-up	1	NA	0	NA	1	0	0	2 (9.5%)
Posttreatment follow-up	NA	NA	0	NA	1	0	NA	1 (4.8%)
Overall	1	0	2	2	2	0	1	8 (38.1%)

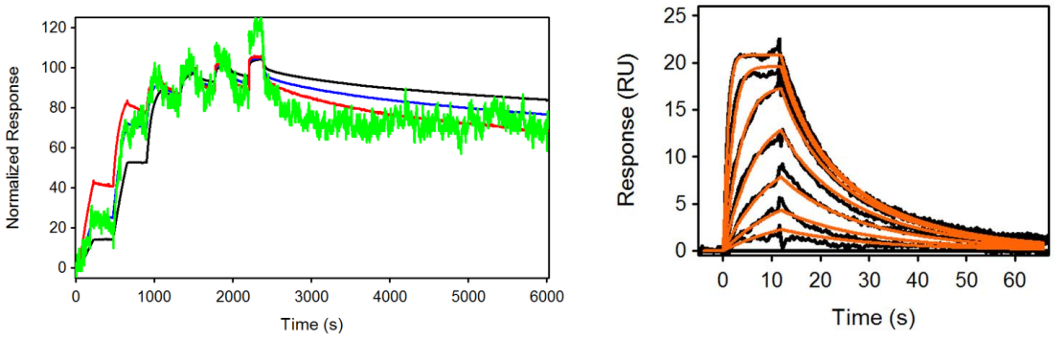
ADA, antidrug antibody; NA, not applicable.

Supplemental Figure S1: Dalutrafusp alfa design



CD, cluster of differentiation; CH1, constant domain, heavy chain 1; CH2, constant domain, heavy chain 2; CH3, constant domain, heavy chain 3; CL, constant domain, light chain; Fab, fragment antigen-binding; Fc, fragment, crystallizable; TGFβ, transforming growth factor beta; VH, variable domain, heavy chain; VL, variable domain, light chain.

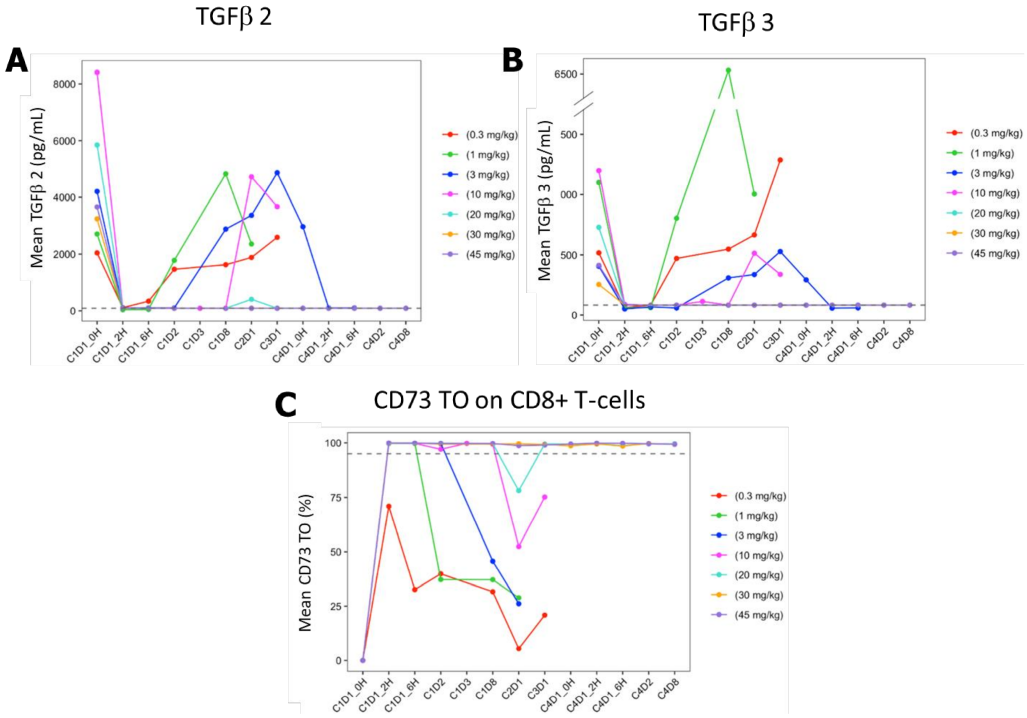
Supplemental Figure S2: Biosensor data of dalutrafusp alfa mAb and Fab data binding to immobilized CD73



Both the mAb (left panel) and a Fab of dalutrafusp alfa (right panel) were injected over surfaces of His-tagged CD73, captured on a Biacore nitrilotriacetic acid chip. MAb was injected at 6 concentrations for a contact time of 180 seconds, and a dissociation time of 300 seconds, except for the last injection, which was monitored for a 3600 second dissociation phase. MAb was injected over 4 surface densities (represented by the different colored traces; from lowest to highest density: green, red, blue, and black) and then normalized for response. As expected, evidence for both bivalent (slow dissociation) and monovalent binding (fast dissociation) can be observed. To avoid fitting complications due to the presence of both bivalent and monovalent binding, Fab was injected at various concentrations and fit to a simple bimolecular model. Fitting yielded a K_D value of 26 nM.

Fab, fragment antigen-binding; K_D, elimination rate constant; mAb, monoclonal antibody.

Supplemental Figure S3: Peripheral TGFβ and CD73 TO on CD8+ T-cells across all cohorts



A TGFβ2 in plasma on-treatment with dalutrafusp alfa. **B** TGFβ3 in plasma on-treatment with dalutrafusp alfa. **C** CD73 TO on CD8+ T-cells in whole blood on-treatment with dalutrafusp alfa. CD, cluster of differentiation; TGF, tumor growth factor; TO, target occupancy.