Supplementary Methods

Formulation manufacturing processes

A 2-day process was used to manufacture GSK1795091 solution for injection, 0.001 mg/mL. The components and quantities used to generate the original formulation are listed in **Table S1**. On Day 1, a 1 mg/mL stock solution was formulated by adding a portion of water for injection to the manufacturing vessel followed by dextrose monohydrate (Roquette Freres, France) and then GSK1795091D. The vessel was placed in a sonication bath filled with chilled water to maintain a solution temperature of less than 15˚C. The pH was adjusted if necessary, and then the remaining portion of water for injection was added to make up the final stock solution batch volume. The solution was filtered through a sterile filter into a clean vessel. An assay measurement was taken, and the solution was stored overnight at 2–8˚C. On Day 2, a measured quantity (based on the assay result) of the 1 mg/mL stock solution prepared on Day 1 was added to a portion of water for injection in a vessel. Next, dextrose monohydrate and poloxamer P188 (BASF, New Jersey, USA) were added to the vessel and mixed. The pH was measured and adjusted if required. The remaining portion of water for injection was added to achieve the final solution volume. The contents of the vessel were passed through 2 sterile filters (0.22 um) (Millipore, New Hampshire, USA) and then filled into presterilized 5 mL type 1 glass vials. The vials were closed with presterilized rubber stoppers, sealed with presterilized flip-top overseals, and crimped.

A vehicle solution was formulated by adding a portion of water for injection to the compounding vessel, followed by poloxamer P188, dextrose monohydrate, and then mixing until full dissolution. The components and quantities used to generate the modified formulation are listed in **Table S2**. The remaining portion of water for injection was added, and the pH was adjusted if necessary, to make up the final vehicle solution batch volume. Next, a 1 mg/mL stock solution was formulated by adding GSK1795091D into dehydrated alcohol (Greenfield Global USA, Brookfield, Connecticut, USA) and

mixing until complete dissolution. The 1 mg/mL stock solution was added to the vehicle solution and mixed. The pH was measured and adjusted, if required. The solution was sterile-filtered through 2 sterile-grade 0.22 micron filters and aseptically filled into type I clear glass vials, which had been washed, sterilized/depyrogenated at the manufacturing facility. The filled vials were then sealed with prewashed and sterilized gray FluroTec stoppers (West Pharmaceutical Services, Pennsylvania, USA). The stopper was secured by a purple flip-top overseal.

Physicochemical characterization of the formulations

Formulation stability:

The original GSK1795091 formulation was found to be stable for up to 25 months at the long-term storage condition (5°C and ambient relative humidity) with no significant change in the total impurity levels. For the modified GSK1795091 formulation, a gradual increase in the total impurity level across the 9 months tested was observed.

Cryo-transmission electron microscopy:

The test sample was prepared on a copper grid with carbon-coated Quantifoil® (R1.2/2, 200 Mesh, copper) holey film (Electron Microscopy Sciences [EMS], Pennsylvania, USA) mounted on tweezers above a cryobox preparation chamber (Leica, Germany). After a glow discharge, a small drop (3 µL) of the sample was spread on the grid and excess liquid was blotted out. The grid was then immediately plunged in a container of liquid ethane maintained between 102°K and 105°K to achieve near instantaneous vitrification of the sample. The grid was transferred onto the sample holder, which was then mounted on the microscope. The sample temperature was allowed to stabilize at about 107°K before proceeding with observations under the transmission electron microscope (Libra120) (Zeiss, Germany) at 80 kV.

Dynamic light scattering (DLS) particle size measurements:

Dynamic light scattering (DLS) is a nondestructive method and effective in measuring particles in the range of approximately 1 nm to 10 µm. Particle size measurements were determined using a DynaPro Nanostar from Wyatt Technology Corporation (California, USA). The DynaPro Nanostar utilizes an avalanche photodiode (APD) to perform DLS analysis, which measures time-dependent fluctuations of light intensity by a fast photon counter. These fluctuations are directly related to the rate of diffusion of the molecule through the solvent via Brownian motion. Due to this relationship, particle size can be determined. Before taking measurements, the DynaPro Nanostar, the sample chamber, was equilibrated at 25°C. The sample was filled into a Nanostar disposable microcuvette (Wyatt Technology, California, USA) of the appropriate volume of sample as per cuvette manufacturer recommendations. Air bubble formation was avoided by placing the pipette tip in the corner of the well at the base and slowly ejecting the solution. The cuvette was placed into the chamber and properly aligned. To ensure proper system operation, samples were calibrated with a Nanosphere polystyrene standard (Thermo Fischer Scientific, Massachusetts, USA) having a similar hydrodynamic radius. The Dynamics software (version 7.1.8) (Wyatt Technology, California, USA) was used to determine the particle size.

Trial inclusion and exclusion criteria

- Inclusion criteria
    - Patients aged ≥18 years who provided informed consent and had histological confirmation of advanced solid tumor
    - Archival tumor tissue obtained at any time from the initial diagnosis to study entry or fresh biopsy at screening
        - Patients in the pharmacokinetics/pharmacodynamics (PK/PD) analysis population must have provided a fresh biopsy of a tumor lesion not previously irradiated

during the screening period and must have agreed to provide at least 1 additional on-treatment biopsy

    - Measurable disease, ie, presenting with at least 1 measurable lesion per Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 that has progressed after standard therapies or for which standard therapy is otherwise unsuitable (eg, intolerance)
    - Eastern Cooperative Oncology Group (ECOG) performance status 0–1
    - Life expectancy of at least 12 weeks
    - Adequate organ function
    - Female who is not pregnant, not breastfeeding, and meets either criteria:
        - Is not of childbearing potential *OR*
        - Agrees to follow the contraceptive guidance during the treatment period and for at least 120 days after the last dose of study treatment
- Exclusion criteria
    - Medical conditions:
        - Malignancy other than disease under study (those who have been disease-free for >2 years were excepted)
        - Symptomatic or asymptomatic central nervous system metastases requiring steroids within 2 weeks before first dose of study treatment
        - Active autoimmune disease requiring systemic disease-modifying or immunosuppressive treatment in the last 2 years
        - Concurrent condition requiring immunosuppressive treatment within 28 days before first dose of study treatment
        - Known human immunodeficiency virus infection
        - Current unstable liver or biliary disease
        - Presence of hepatitis B or C at screening or within 3 months before receiving first dose of treatment

- QT duration corrected for heart rate by Fridericia's formula (QTcF) >450 ms or QTcF >480 ms for participants with bundle branch block
- History of acute diverticulitis, inflammatory bowel disease, intra-abdominal abscess, or gastrointestinal obstruction within the past 6 months
- Recent history of allergen desensitization therapy within 4 weeks of starting study treatment
- History of severe hypersensitivity to monoclonal antibodies
- History or evidence of cardiovascular risk
- History of idiopathic pulmonary fibrosis, pneumonitis, interstitial lung disease, or organizing pneumonia, or evidence of active, noninfectious pneumonitis
- History of uncontrolled symptomatic ascites or pleural effusions in the past 6 months
- Any serious and/or unstable pre-existing medical, psychiatric disorder, or other condition that could interfere with the participant's safety, obtaining informed consent, or compliance with the study procedures

- Prior treatments:
  - OX40 or inducible T-cell costimulatory (ICOS) agonists at any time, or prior systemic or intra-tumoral therapy with a Toll-like receptor 4 (TLR4) agonist
  - Anti-cancer therapy or investigational therapy within 30 days or 5 half-lives of the drug (whichever is shorter)
  - Radiation therapy within 14 days if to extremities or 28 days to the chest, brain, or visceral organs
  - Allogeneic or autologous bone marrow transplantation or other solid organ transplantation
  - Toxicity Grade ≥3 related to prior immunotherapy and that lead to study treatment discontinuation

- Toxicity related to prior treatment has not resolved to Grade ≤1 (except alopecia, or endocrinopathy managed with replacement therapy)
- Transfusion of blood products ≤2 weeks before the first dose of study treatment
- Major surgery ≤4 weeks prior to first dose of study treatment
- Any live vaccine ≤4 weeks

Timing of blood sampling for pharmacokinetic and pharmacodynamic analysis

Blood samples for PK analysis of GSK1795091 were collected within 1 hour before start of infusion at Cycles 1–4 and at additional time points from the end of the infusion (Cycle 1: 10 minutes, 2, 4, 6, and 24 hours; Cycle 2: 10 minutes, 4 hours [latter not mandatory and if clinically feasible within the protocol window]; Cycle 3: 10 minutes, 4 and 24 hours).

Blood samples for PD analysis were collected within 1 hour before start of infusion at Cycles 1–3, 6, and 12, and at additional time points from the end of the infusion (Cycle 1: 2, 4, 6, and 24 hours; Cycle 2: 2 and 4 hours; Cycle 3: 2, 4, 6, and 24 hours; Cycle 6: 2, 4, and 6 hours; Cycle 9: 24 hours; Cycle 12: 2 and 4 hours).