Supplementary Material and Methods

Dose Selection

LY3022855 was evaluated for safety and to establish a maximum tolerated dose in an all solid tumor dose escalation trial (NCT01346358). Doses for patients in Cohorts A, B, C, and D were selected based on the pharmacokinetics, pharmacodynamics, and safety data in this study Dose for patients in Cohort A was the lowest dose that showed a significant increase in the 2 ligands of CSF-1R (CSF-1, IL-34) and this dose had an acceptable safety profile. Dose for patients in Cohort B was expected to result in a cumulative dose of 4 mg/kg based on the JSCA study. It was a reasonable dose and schedule in terms of safety and was expected to enhance inhibition of CSF-1R, based on the preliminary pharmacodynamics data. Dose for patients in Cohort C was expected to result in enhanced target engagement, and dose for patients in Cohort D was selected because all patients who showed tumor shrinkage or stability in the JSCA study were treated on a Q2W schedule.

T Cell Activation/Exhaustion Flow Assay

Human peripheral blood mononuclear cell (PBMC) samples were thawed and stained with a fixable Aqua viability dye (Invitrogen) and a cocktail of antibodies to the following surface markers: CD8-Qdot605 (Invitrogen, 3B5), CD4-Qdot 655 (Invitrogen, S3.5), PD-1-PE (BD, MIH4), LAG-3-FITC (Enzo, 17B4), ICOS-PE-Cy7 (eBioscience, ISA-3), TIM-3-APC (R&D Systems, 344823). Next, cells were fixed and permeabilized with the FoxP3/Ki-67 Fixation/Permeabilization Concentrate and Diluent (eBioscience), and subsequently stained intracellularly with CD3-BV570 (Biolegend, UCHT1), Ki-67-AlexaFluor700 (BD, B56), FoxP3-eFluor450 (eBioscience, PCH101), and CTLA-4-PerCP-eFluor710 (eBioscience, 14D3). Stained cells were acquired on a BD Biosciences LSRFortessa and analyzed using FlowJo software (FlowJo, LLC).

Multiplex Cytokine Measurements

V-PLEX validated Human Proinflammatory Panel 10-plex kits (for IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IFNγ, TNFα, IL-1β, and IL-13) were purchased from Meso Scale Diagnostics (MSD, Cat #K15049D-1). All reagents were provided with the kit. The standards were reconstituted in the assay diluent provided. Frozen plasma samples were thawed, clarified by brief centrifugation to remove any particulate materials, and diluted two-fold in assay diluent. Diluted samples, controls, and standards were added at 50 µl per well into the MSD assay plates pre-coated with

10 capture antibodies per well against the cytokines of interest. The plate was sealed and incubated for 2 h at room temperature on an orbital shaker (600 rpm). At the end of the incubation the wells were washed three times using 150 µl wash buffer (PBS + 0.05% Tween 20). Detection antibodies conjugated to electrochemiluminescence labels were added at 25 µl per well, and the plate sealed and incubated for 2 h at room temperature on an orbital shaker (600 rpm). At the end of incubation the plate was washed three times as before. 150 µl of the MSD Read Buffer was added to each well and the plates were read on the MSD QuickPlex SQ 120 imager. The raw data was measured as light intensity detected by instrument photodetectors upon application of electricity to the plate electrodes. Data was analyzed using the MSD Discovery Workbench® software. A 4-parameter logistic fit calibration curve was generated for each analyte using the standards to calculate the concentration of each sample.