Ludwig & Du et al,

SUPPLEMENTARY METHODS

KPC-M09 cell line isolation

Isogenic cell lines were derived from *KPC* (*Kras*$^{LSL\text{-}G12D}$*; Trp53*$^{LSL\text{-}R172H}$*; Ptf1a*$^{Cre/+}$) mice. The pancreas from a *KPC* animal with large established disease was minced and digested with 1% collagenase type I, Dulbecco's Modified Eagle Medium (DMEM; Invitrogen), 10 mM HEPES, and 1% FBS at 37C to obtain a single-cell suspension. The resulting cells were centrifuged at low speed to pellet large debris, resuspended in wash buffer, and passed through a 70-µm cell strainer. The resulting cell suspension was plated at low density to isolate tumor cell populations using cloning rings. Cells were confirmed to be tumor cells by immunocytochemistry and PCR. These cell lines were expanded and stained for tumor cell markers. Cells were confirmed to be pathogen-free before use. Clone KPC-M09 was used in subsequent experiments. Cells were cultured in DMEM containing 10% FBS and maintained at 37C in a humidified incubator with 5% CO2 and 95% air.

Flow Cytometry

KPC-M09 tumors were established in Cre-negative mice from the *KPC* colony by injecting 100,000 cells subcutaneously. Animals with established tumors (150 mm^3) were treated with BGB324 (50 mg/kg, PO, BID, n=9) or vehicle (n=10). The treatment schedule was 5 days on 2 days off for a period of 2 weeks. BGB324 treatment did not affect tumor growth in this setting. Tumor tissue was harvested in the afternoon after therapy in the morning. Tumors were analyzed by flow cytometry by digesting with a cocktail containing collagenase I (45 u/ml; Worthington), collagenase II (15 u/ml; Worthington), collagenase III (45 u/ml; Worthington), collagenase IV (45 u/ml; Worthington), Elastase (0.075 u/ml; Worthington), hyaluronidase (30 u/ml; Sigma), and DNase type I (25 u/ml; Sigma) for 40 minutes at 37C and passed through a 70-µm cell strainer (Falcon). Cell suspensions were washed twice with PBS and stained with Fixable Viability Dye (Thermo Fisher) for an hour. The cell suspensions were then washed and stained with antibodies detecting CD11b (BD Bioscience, 557657), Ly-6C (BD Bioscience, 562728), Ly-6G (BD Bioscience, 740953), F4/80 (Biolegend, 123132), CD274 (BD Bioscience, 563369), CD11c (BD Bioscience, 564079), and I-A/I-E (BD Bioscience, 562009), for an hour at 4C. Surface-stained cells were fixed, permeabilized, and stained for intracellular cytokine Arginase 1 (R&D System, IC5868P). Cells were analyzed using FACS LSRFortessa SORP, and analysis was performed using FlowJo, with the help of Moody Foundation Flow Cytometry Facility at UT Southwestern.