**Supplementary Materials**

**Supplementary Methods**

***Bioanalytical Sample Analysis***

Brain homogenate was prepared by homogenizing tissue with 4 volumes (w:v) of 15 mM PBS.

To evaluate protein precipitation, 30 µL aliquots of the following samples were added to a 96-well plate: unknown sample, calibration standard, quality control, dilute quality control, single blank, and double blank samples. The double blank sample was quenched with 600 µL acetonitrile (ACN) and all other samples were quenched with 600 µL IS1; the mixture was vortex-mixed for 10 min at 800 rpm and then centrifuged for 15 min at 3220 g (4000 rpm), 4°C. Next, 64 µL of supernatant was transferred to a clean 96-well plate and centrifuged for 5 min at 3220 g, 4°C. The supernatants produced were diluted 1:10 (i.e., 3 µL of sample was mixed with 27 µL of blank matrix) and subjected to liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis via direct injection.

For CSF analysis, each CSF sample was mixed with an equal volume of plasma (1:1 v:v). Samples underwent protein precipitation using 25 volumes of IS1 or ACN (double-blank only), and the mixture was vortex-mixed well (at least 15 s) followed by centrifugation for 15 min at 12000 g, 4°C. Supernatants were transferred to a 96-well plate and treated as described above.

***MDCK-MDR Cell Permeability Assay***

MDR1-MDCK cells (obtained from Piet Borst at the Netherlands Cancer Institute) were seeded onto polyethylene membranes in 96-well BD insert systems at 2.5 x 105 cells/mL for 4–7 days for confluent cell monolayer formation. Test compounds were diluted with the transport buffer (Hanks’ balanced salt solution [HBSS] with 10 mM Hepes, pH 7.4) from DMSO stock solution to a concentration of 2 µM (DMSO <1%) and applied to the apical or basolateral side of the cell monolayer. Permeation of the test compounds from A to B direction or B to A direction was determined in triplicate. Digoxin was tested at 10 μM from A to B direction or B to A direction as well, while nadolol and metoprolol were tested at 2 μM in A to B direction in duplicate. The plate was incubated for 2.5 hours in CO2 incubator at 37±1°C, with 5% CO2 at saturated humidity without shaking. In addition, the efflux ratio of each compound was also determined. Test and reference compounds were quantified by LC/MS/MS analysis based on the peak area ratio of analyte/IS.

Six wells per 96-well plate were selected for lucifer yellow rejection assay to determine the cell monolayer integrity. 75 µL of 100 µM lucifer yellow in transport buffer and 250 µL transport buffer were added to apical and basolateral chambers, respectively. The plate was incubated for 150 minutes at 37°C with 5% CO2 and 95% relative humidity without shaking. After 150 minutes incubation, 20 µL of lucifer yellow samples were taken from the apical sides, followed by the addition of 60 µL of transport buffer. Then 80 µL of lucifer yellow samples were taken from the basolateral sides. The relative fluorescence unit (RFU) of lucifer yellow was measured at 425/528 nm (excitation/emission) with a Envision plate reader.

***MDCK-MDR P-gp assay***

MRTX849 was synthesized at Array BioPharma, Inc., Boulder, CO, and a 100 mM DMSO stock was prepared and used as the dosing solution. The control LLC-PK1 cell line and the MDR1 LLC-PK1 cell line, stably transfected with human MDR1 cDNA, were obtained from BD Biosciences (San Jose, CA). Medium 199 was obtained from Sigma (St Louis, MO), phosphate buffer solution, trypan blue, trypsin/EDTA and versene were obtained from Invitrogen (Carlsbad, CA), millicell-96 cell culture insert plates were obtained from Sigma (St Louis, MO), hygromycin B was obtained from US Biological (Swampscott, MA), gentamycin was obtained from Calbiochem (San Diego, CA), methyl sulfoxide (DMSO; reagent grade) was purchased from EM Science (Gibbstown, NJ), and fetal bovine serum (FBS) was obtained from Fisher Scientific (Pittsburgh, PA). HBSS, digoxin, erythromycin, propranolol, quinidine, sulfasalazine, acebutolol, cefuroxime and lucifer yellow were obtained from Sigma (St Louis, MO). All other reagents and solvents were of the highest analytical grade supplied by Sigma (S Passage media was prepared using Medium 199, 2% FBS 50 µg/mL gentamycin and 100 µg/mL hygromycin B. Seeding media was prepared using Medium 199, 7% FBS and 50 µg/mL gentamycin. Assay buffer was prepared using HBSS, 1% DMSO and 10 mM HEPES. The pH of the assay buffer was adjusted to 7.4, using 1.0 M KOH.

Upon receipt of the frozen MDR1 transfected LLC-PK1 cells, they were quick-thawed in a 37°C water bath and seeded into tissue culture flasks (Corning, Acton, MA) with passage media. The cells were maintained in passage media, passing every three and a half days, when cells were 70–80 % confluent, until needed. Experiments were performed using MDR1 transfected LLC-PK1 cells below passage 30. The cells were plated into Millicell-96 cell culture insert plates at a density of 500,000 cells per well for both the MDR1 and parental LLC-PK1 cell lines. The plates were incubated at 37°C and in an atmosphere of 5% CO2 with saturating humidity. The media was replaced with new seeding media on day 2 of culture. The permeability experiments were conducted on culture day 6. Before conducting the assay, the integrity of the LLC-PK1 monolayers was monitored by measuring the trans-epithelial electrical resistance (TEER) using a Millipore Millicell®-ERS TEER device (Millipore, Billerica, MA). A reading greater than 300 ohms (Ω) from the TEER device indicated that the cells were acceptable for the permeability assay.

Propranolol, sulfasalazine, and cefuroxime (high, medium, and low permeability compounds, respectively) were used as negative controls for P-gp efflux. Erythromycin and acebutolol, digoxin, and quinidine (low, medium, and high permeability compounds, respectively) were used as positive controls for P-gp efflux. Stock solutions for assay controls and MRTX849 were prepared at 10 mM and 100 mM in DMSO, respectively. MRTX849 was assessed as a substrate of P-gp by preparing dosing solutions using the transport media, warmed to 37ºC, at 0.1, 0.3, 1, 3, 10, 30 and 100 µM for MRTX849 in both the MDR1 transfected LLC-PK1 cell line alone and in the LLC-PK1 vector-bearing control cell line. To assess MRTX849 as an inhibitor, dosing solutions were prepared using the transport media, warmed to 37ºC, at 0, 0.1, 0.3, 1, 3, 30 and 100 µM for MRTX849 along with 2.5 µM quinidine, and using the MDR1 transfected LLC-PK1 cell line. All dosing solutions contained 10 µM lucifer yellow to monitor the LLC PK1 cell monolayer integrity.

For the apical to basolateral determination (A to B), 75 µL of the test compound in transport buffer containing lucifer yellow were added to the apical side of the individual transwells and 250 µL of basolateral media without compound or lucifer yellow, were added to each well. For the basolateral to apical determination (B to A), 250 µL of test compound in transport buffer containing lucifer yellow were added to each well and 75 µL transport buffer without compound or lucifer yellow, were added to each transwell.

All tests were performed in triplicate, and each compound was tested for both apical to basolateral and basolateral to apical transport. The plates were incubated for 2 hours on a Lab-Line Instruments Titer Orbital Shaker (VWR, West Chester, PA) at 50 rpm and 37°C with 5% CO2. All culture plates were removed from the incubator and 50 µL of media were removed from the apical and basolateral portion of each well and added to 150 µL of 1 µM labetalol in 2:1 acetonitrile:water, v/v.

The plates were read using a Molecular Devices (Sunnyvale, CA) Gemini Fluorometer to evaluate the lucifer yellow concentrations at excitation/emission wavelengths of 425/535 nm. These values were accepted when found to be below 2% for apical to basolateral and 5% basolateral to apical flux across the MDR1 transfected LLC-PK1 cell monolayers. The plates were sealed and the contents of each well analyzed by LC MS/MS. The compound concentrations were determined from the ratio of the peak areas of the compound to the internal standard (labetalol) in comparison to the dosing solution.

The LC-MS/MS system was comprised of an HTS-PAL autosampler (Leap Technologies, Carrboro, NC), an HP1200 HPLC (Agilent, Palo Alto, CA), and a MDS Sciex 4000 Q Trap system (Applied Biosystems, Foster City, CA). Chromatographic separation of the analyte and internal standard was achieved at room temperature using a C18 column (Kinetex®, 30 x 3.0 mm, 2.6 µm particle size, Phenomenex, Torrance, CA) in conjunction with gradient conditions using mobile phases A (water containing 1% isopropyl alcohol and 0.1% formic acid) and B (0.1% formic acid in ACN). The total run time, including re-equilibration time, for a single injection was 1.2 minutes. Mass spectrometric detection of the analytes was accomplished using the ion spray positive mode. Analyte responses were measured by multiple reaction monitoring of transitions unique to each compound***.*** A 5-Parameter Logistic Model or Sigmoidal Dose-Response Model using IDBS software, XLfit (Bridgewater, NJ) was used to determine IC50 values for MRTX849.

***pERK Modulation Determination In Vivo***

For pERK modulation determination, tumors were flash frozen in homogenization tubes (#19-628-3; Omni International, Kennesaw, GA, USA), then lysed with ~500uL RIPA lysis buffer (#8990; Thermo Fisher Scientific, Waltham, MA, USA) with protease and phosphatase inhibitors added fresh before use. Lysis was performed on ice with 3 rounds of shaking, 20 seconds each using the FastPrep-24 system (MP Biomedicals, Santa Ana, CA, USA). Tumor lysates were then spun in a cold centrifuge at 14,000 g for 15 minutes. Cleared lysates were transferred to fresh Eppendorf tubes and stored at ‑80°C. For immunoblotting, 30 µg of protein was loaded into each well of a 12% Bis-Tris gel and run according to the manufacturer’s instructions (Bio-Rad [Hercules, CA, USA]). Phospho p44/42 MAPK (ERK1/2) (Thr202/Tyr204) (#9101; Cell Signaling Technology, Danvers, MA, USA), P44/42 MAPK (ERK1/2) (#9102; Cell Signaling Technology), and alpha-tubulin (#3873; Cell Signaling Technology) primary antibodies were added, with rocking, overnight. Next day, blots were washed 5 times with 1× PBS + 0.1% Tween-20, and incubated with IRDye secondary antibodies (#926-68070, 680RD goat anti-rabbit and #926-32211, 800CW goat anti-mouse; LI-COR Biosciences, Lincoln, NE, USA) for 2 hours at room temperature with rocking. Blots were then washed again and imaged using the Odyssey CLx Imaging system (LI-COR). The system was set to the AutoScan channel for both 700 and 800 nm wavelengths to measure the signal intensity from the anti-rabbit and anti-mouse antibodies, respectively. Images were imported into Image Studio software version 4.0 (LI-COR) and TIF files were exported to Windows Photo Viewer (Microsoft Corp., Redmond, WA, USA). To quantify pixel intensity, the Add Rectangle tool in the image viewer was used to identify a consistently sized area of interest for each band of a given target protein as well as a representative background region of the immunoblot. The corrected signal intensity was determined for both the phosphorylated protein and the total protein bands and data was exported to Excel (Microsoft). The level of ERK phosphorylation was determined by dividing the signal output of the phosphorylated protein by the signal output of the total protein. The level of phosphorylation was averaged within each vehicle or treatment group, and then vehicle was normalized to 1 by dividing all average values by the vehicle value; standard deviation was calculated from normalized values. Percent inhibition of ERK phosphorylation in adagrasib-treated tumors compared with vehicle-treated tumors was determined by subtracting average adagrasib-treated phosphorylation signal from average vehicle-treated signal, then dividing by the average vehicle-treated phosphorylation signal and multiplying by 100. GraphPad Prism 7 (GraphPad Software, LLC, San Diego, CA, USA) was used to display the data.

***KRYSTAL-1: Eligibility Criteria***

Additional key inclusion criteria were measurable or evaluable disease, according to Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1), adequate bone marrow and organ function, an Eastern Cooperative Oncology Group performance status (ECOG PS) score of ≤1, and discontinuation of most recent prior systemic therapy and radiation therapy >2 weeks prior to first adagrasib dose. Patients with a history of significant cardiovascular disease (unstable angina; prolonged corrected QT interval; and congestive heart failure, defined as New York Heart Association class 3 or higher) and those who underwent major surgery within 4 weeks of first dose date were excluded.

## Table S1. Clinicopathologic data for patients with KRAS mutant lung cancer treated at MGH between May 2015 and October 2019

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Feature** | **All patients**  | **KRAS G12C** | **KRAS non-G12C** | **p-value\*** |
| **Patients** | 374 (100) | 149 (40) | 225 (60) | - |
| **Median age at initial diagnosis, years (range)**  | 68(29–91) | 67 (44–91) | 68(29–91) | 0.51 |
| **Sex** |  |
|  Female  | 217 (58) | 90 (60) |  127 (56) | 0.46 |
|  Male  | 157 (42) | 59 (40) |  98 (44) |
| **Race/Ethnicity** |  |
|  White | 329 (88) | 131 (88) | 198 (88) | 0.96 |
|  Asian | 10 (3) | 3 (2) | 7 (3) |
|  Hispanic/Latinx | 6 (2) | 3 (2) | 3 (1) |
|  Black | 5 (1) | 2 (1) | 3 (1) |
|  Other/unknown | 24 (6) | 10 (7) | 14 (6) |
| **Smoking history** |  |
|  Never | 17 (5) | 3 (2) | 14 (6) | 0.16 |
|  Light (10 pack years or less) | 42 (11) | 14 (9) | 28 (12) |
|  Heavy (>10 pack years) | 306 (82) | 129 (87) | 177 (79) |
|  Unknown | 9 (2) | 3 (2) | 6 (3) |
| **Histology** |  |
|  Adenocarcinoma | 318 (85) | 122 (82) | 196 (87) | 0.30 |
|  Squamous cell carcinoma | 18 (5) | 10 (7) | 8 (4) |
|  NSCLC NOS | 21 (6) | 11 (7) | 10 (4) |
|  Other/unknown | 17 (5) | 6 (4) | 11 (5) |
| **ECOG performance status at initial diagnosis** |  |
|  0 or 1 | 246 (66) | 105 (71) | 141 (63) | 0.04\* |
|  2 or greater | 68 (18) | 18 (12) | 50 (22) |
|  Unknown | 60 (16) | 26 (17) | 34 (15) |
| **Stage IV disease at initial diagnosis** |  |
|  Yes | 249 (67) | 99 (66) | 150 (67) | 0.48 |
|  No | 122 (33) | 50 (34) | 72 (32) |
|  Unknown | 3 (0.8) | 0 (0) | 3 (1) |
| **Brain metastasis at any time** |  |
|  Yes | 150 (40) | 60 (40) | 90 (40) | 0.97 |
|  No | 218 (58) | 86 (58) | 132 (59) |
|  Unknown | 6 (2) | 3 (2) | 3 (1) |
| **PD-L1 status (%)** |  |
|  >49 | 86 (23) | 42 (28) | 44 (20) | 0.008\* (including unknown)0.02\* (omitting unknown) |
|  1–49 | 81 (22) | 30 (20) | 51 (23) |
|  <1 | 123 (33) | 36 (24) | 87 (39) |
|  Unknown | 84 (22) | 41 (28) | 43 (19) |

Data are shown as n (%) unless otherwise stated. Percentages may not add up to 100 due to rounding.

Categorical p-values, Fisher’s exact test; continuous p-value, Wilcoxon rank-sum test, \*denotes significance.

ECOG, Eastern Cooperative Oncology Group; NSCLC, non-small cell lung cancer; NOS, not otherwise specified; PD-L1, programmed death-ligand 1.