

Anti-tumour activity in RAS-driven tumours by blocking AKT and MEK

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Running title: Targeting KRAS signalling through MEK and AKT.

Key Words: Phase I Trial, RAS, MEK, AKT, Combination

Preliminary data were presented in part as oral and poster presentations at the 47th and 48th American Society of Clinical Oncology Annual Meeting, Chicago, Illinois, USA (2011, 2012 respectively).

CONFLICTS OF INTEREST

ER, PH, KS, AM, ET, Y-MJ, and LY are current or former employees of Merck & Co., Inc., Whitehouse Station, NJ and may hold stock/stock options in the company. JMS, PS, and ML were employees of AstraZeneca, Alderley Park, Macclesfield, Cheshire, UK at the time this study was conducted and may own stock in the company.

Statement of Translational Relevance: RAS mutations that result in the unregulated activation of RAS signalling are common in cancer and a key unmet medical need. Therapeutic strategies that block RAS signalling could benefit many cancer patients suffering from KRAS mutant cancers.

Preclinical studies indicate that combined MEK and AKT blockade can abrogate KRAS signalling. We conducted a combination Phase I study of a MEK inhibitor (selumetinib) and an AKT inhibitor (MK2206).

To minimise the drug toxicities of this combination, while maximising antitumor activity, we evaluated several drug schedules. This trial demonstrates the complexity of drug combination trials and demonstrates that this combination strategy has antitumor activity against KRAS mutant cancers at tolerable doses.

ABSTRACT

Purpose: *KRAS* is the most commonly mutated oncogene in human tumours. *KRAS*-mutant cells may exhibit resistance to the allosteric MEK1/2 inhibitor selumetinib (AZD6244; ARRY-142886) and allosteric AKT inhibitors (such as MK-2206), the combination of which may overcome resistance to both monotherapies.

Experimental Design: We conducted a dose/schedule-finding study evaluating MK-2206 and selumetinib in patients with advanced treatment-refractory solid tumours. Recommended dosing schedules were defined as MK-2206 135 mg weekly and selumetinib 100 mg once-daily.

Results: Grade 3 rash was the most common dose-limiting toxicity (DLT); other DLTs included grade 4 lipase increase, grade 3 stomatitis, diarrhoea, and fatigue, and grade 3 and grade 2 retinal pigment epithelium detachment. There were no meaningful pharmacokinetic drug-drug interactions. Clinical anti-tumour activity included RECIST 1.0-confirmed partial responses in non-small cell lung cancer and low-grade ovarian carcinoma.

Conclusion: Responses in *KRAS*-mutant cancers were generally durable. Clinical co-targeting of MEK and AKT signalling may be an important therapeutic strategy in *KRAS*-driven human malignancies (Trial NCT number NCT01021748).

INTRODUCTION

Kirsten rat sarcoma viral oncogene homolog (*KRAS*) is frequently mutated. This results in deregulated signalling via the Ras/Raf/mitogen extracellular signal-regulated kinase (MEK)/extracellular-signal-regulated kinase (ERK) pathway in human cancers, which promotes neoplastic transformation and maintenance of a malignant phenotype. Ras signalling may be activated through direct interactions with numerous growth factor receptors, or independently stimulated by somatically-acquired mutations in approximately 20% of human cancers, making this protein an important therapeutic target (1, 2). Nevertheless, to date, direct targeting of Ras activation by guanosine triphosphate (GTP) interaction has not been clinically feasible despite substantial research efforts (3). Furthermore, single-agent inhibition of downstream effector pathways through the use of MEK or protein kinase B (AKT) inhibitors has not led to significant clinical anti-tumour activity in *KRAS*-mutant tumours (4, 5).

Numerous preclinical models have suggested that *KRAS*-mutant tumours require co-targeting of the Ras/Raf/MEK/ERK and phosphatidylinositol 3-kinase (PI3K)/AKT pathways due to multiple points of cross-talk, negative feedback, and redundancy (6, 7). Inhibition of MEK by selumetinib in *KRAS*-mutant cancers can result in reactive up-regulation of AKT phosphorylation (8), whilst co-targeting of PI3K and MEK ablates this compensatory effect and results in superior anti-tumour efficacy, in contrast to inhibition of either pathway alone (9). Mutations in the PI3K/AKT and Ras/Raf pathways frequently co-exist in advanced cancers (10), while coactivating mutations are often found in treatment-resistant *KRAS*-mutant tumour models (11). For example, in *KRAS*-mutant cell lines treated with MEK inhibitors, activating *PIK3CA* mutations or *PTEN* loss lead to MEK inhibition resistance, which can be reversed by

coinhibition of the PI3K/AKT pathway (12). *PIK3CA* mutations concurrent with *KRAS* mutations appear to drive AKT signalling, restoring cyclin D1 expression and allowing G1-S cell cycle progression by underlying mechanisms independent of *KRAS*-mediated MEK/ERK signalling (13). Indeed, in a *KRAS*-mutant lung cancer mouse model insensitive to MEK inhibition alone, combined MEK and PI3K/mammalian target of rapamycin (mTOR) inhibition resulted in synergistic induction of tumour regression (14). Combinatorial inhibition of key nodes within signal transduction networks to overcome both *de novo* and acquired resistance has wide clinical applications (15), and preclinical data supporting the present combination are a successful example of this treatment strategy.

We approached targeting *KRAS*-mutated tumours through the dual inhibition of two major downstream signalling pathways, RAF/MEK/ERK and PI3K/AKT. Two potent, allosteric, selective and orally administered inhibitors were combined: MK-2206, an allosteric inhibitor of AKT 1, 2, and 3 (IC_{50} = 8, 12, and 65 nmol/L, respectively), and selumetinib (AZD6244; ARRY-142886), a non-ATP competitive inhibitor of MEK (IC_{50} = 10-14 nmol/L) (16, 17).

METHODS

Preclinical studies

In vitro cell line models

Determining the combination index by the Chou-Talalay method for cell lines treated with selumetinib and MK-2206

Combinations of MK-2206 with AZD6244 were evaluated in 9 cancer cell lines with various mutational activations in the PI3K and/or MAPK pathways: 3 colon cancer cell lines (HCT116, HCT15, and HT29), 3 pancreatic cancer cell lines (AsPC-1, BxPC-3, and MIA-Pa-Ca2), 2 lung

cancer cell lines (Calu-6, NCI-H460), and 1 melanoma (A2058) cell line. Human cell lines were purchased from American Type Culture Collection (ATCC) which employs short tandem repeat (STR) profiling to ensure cell line authenticity. The fixed-ratio experimental design originally described by Chou-Talalay was used (18). The *in vitro* anti-proliferative potencies (IC_{50}) of selumetinib and MK-2206 as single agents were first determined separately to yield the $IC_{50\text{-selumetinib}}/IC_{50\text{-MK-2206}}$ ratio. A dilution series of selumetinib/MK-2206 combinations in which the ratio of selumetinib/MK-2206 was fixed and equal to the $IC_{50\text{-selumetinib}}/IC_{50\text{-MK-2206}}$ ratio was then prepared. Corresponding single-agent dilution series of selumetinib and MK-2206 were also prepared. The 3 dilution series were tested in the proliferation/viability assay (CellTiter-Glo Luminescent Cell Viability Assay; Promega). The data were analyzed using CalcuSyn software that calculates the combination index (CI) for each combination of selumetinib/MK-2206. CI <0.9 indicates synergism; CI = 0.9 to 1.1 indicates additivity; and CI >1.1 indicates antagonism. Enhanced apoptotic cell death was assessed by luminescence assay for caspase-3/7 activity after 24 hours.

In vivo studies

CD1-nude mice bearing HCT116 tumour xenografts were selected as a model for study (Supplementary Table S1). Selumetinib at 25 mg/kg was orally administered twice daily (BID) on days 0–4 and days 7–11. MK-2206 at 120 mg/kg was orally administered once-every-other-day (QOD) for 2 weeks.

CD1-nude mice bearing A2058 tumour xenografts were selected as a model for study. Selumetinib at 25 mg/kg was orally administered BID on days 0–4 and days 7–11. MK-2206 at 120 mg/kg was orally administered QOD for 2 weeks.

Clinical study

This was a 2-part, Phase I study (Merck Sharp & Dohme Corp., MK-2206 Study Number 010; ClinicalTrials.gov identifier: NCT01021748) organized as below to achieve the following:

- Dose finding: Identify the maximum-tolerated dose (MTD) of combination therapy with oral MK-2206 and oral selumetinib (capsule formulation) in patients with locally advanced or metastatic solid tumours
- MTD expansion: Confirm the MTD of combination therapy of oral MK-2206 and oral selumetinib (capsule formulation) in a select cohort of *KRAS*-mutant non-small cell lung cancer patients

In the dose-finding portion of the study, sequential cohorts of 3–6 patients were enrolled into panels representing different dose levels of combination therapy to determine a preliminary MTD. The dose-escalation schedule initially followed a QOD schedule for MK-2206 in combination with selumetinib. Subsequent escalation included evaluation of the once-weekly (QW) dosing schedule of MK-2206 in combination with selumetinib.

Patients evaluated in the determination of dose-escalation decisions must have received $\geq 80\%$ of planned study combination therapy during the first 28-day cycle (unless they experienced a dose-limiting toxicity [DLT] prior to completing cycle 1). Patients who failed to begin trial treatment, or who did not complete at least 80% of study therapy, were replaced for determination of the dose-escalation decision. For any initial cohort of 3 patients, up to 3 additional patients were enrolled (to make the total number of evaluable patients = 6) to further evaluate safety and tolerability. The dose level administered to each subsequent cohort of patients was to be determined based on the total number of DLT observed at the current dose

relative to the total number of patients treated and evaluable for DLT at the current dose. Dose escalation continued until the MTD or maximum planned dose was reached according to the modified toxicity probability interval (mTPI) approach (Supplementary Table S2).

The study was conducted in accordance with Good Clinical Practice guidelines and in compliance with local and/or national regulations and in accordance with the ethical principles that have their origin in the Declaration of Helsinki. All patients gave informed consent, and approval was obtained from the ethics committees at each participating institution.

Patients were evaluated for tumour response using CT or MRI at baseline and every 8 weeks during the study. Tumour response was assessed by RECIST (Response Evaluation Criteria in Solid Tumours) 1.0 (19). Where appropriate, different tumour markers were used to assess the effects of the combination treatment on the respective tumour types. Patients received MK-2206 and selumetinib until disease progression, intolerable adverse event, or consent withdrawal.

Sampling for pharmacokinetic determinations of MK-2206 and selumetinib (including the metabolite *N*-desmethyl selumetinib) was conducted during the first cycle of combination therapy. Pre-dose and serial post-dose specimens were taken on either days 1 and 22 (QW dosing schedules) or days 1 and 27 (QOD dosing schedules). Additional pre-dose collection time points included days 2, 3, 5, 8, 15, 23, 24, 26, and 29 for patients on the MK-2206 QW dosing schedule, and days 2, 3, 7, 15, 21, 28, and 29 for patients on the QOD dosing schedule.

Plasma (circulating nucleic acid) and archival tumour (formalin-fixed, paraffin-embedded tissue) specimens were collected at baseline. Analysis focused on PI3K or BRAF pathway activation events such as the mutation status of *KRAS*, *BRAF*, and *PIK3CA*.

RESULTS

Previous preclinical studies of MK-2206 and selumetinib monotherapy each identified dose-dependent growth inhibition in tumour-bearing mouse models and showed evidence of target inhibition (20). We evaluated synergism between MK-2206 and selumetinib by conducting a fixed-ratio experiment as described by the Chou-Talalay method in 8 cancer cell lines derived from colon, pancreatic, melanoma, and lung cancers (18). Table 1 shows combination indices <0.9 , the threshold for synergy, in all *KRAS*-mutant cell lines and cell lines driven by Raf/MEK/ERK and PI3K/AKT signalling.

We then examined the effects of combined treatment on cell survival of *KRAS*-mutant HCT116 cells (containing the *KRAS* G13D mutation). Induction of activated caspase 3 and 7 in HCT116 cells was significantly higher after 24 hours of co-treatment with MK-2206 and selumetinib compared with either agent alone, as determined by luminescence assay (Fig. 1A). Fluorescence-activated cell sorting (FACS) cell-cycle profiles of HCT116 cells exposed to MK-2206 and/or selumetinib for 72 hours showed that co-treatment resulted in the accumulation of a significantly greater proportion of cells in the sub-G1 phase than either MEK or AKT blockade alone (Fig. 1B). Inhibition of downstream signalling biomarkers was assessed by immunoblotting following incubation of cells with MK-2206 and/or selumetinib for 24 hours (Fig. 1C). Selumetinib alone blocked pT202/Y204 ERK completely, but incompletely suppressed pS235/236 S6 ribosomal protein (S6RP) and pT70 4E-BP1, and did not affect pS473 AKT. In contrast, monotherapy with MK-2206 completely abrogated pS473 AKT, but incompletely inhibited pT70 4E-BP1, and had no effect on pT202/Y204 ERK or pS235/236 S6RP. Combined treatment resulted in significant inhibition of pS473 AKT, pT202/Y204 ERK, pS235/236 S6RP, and pT70 4E-BP1.

Xenograft studies of *KRAS*-mutant HCT116 demonstrated inhibition of tumour growth by selumetinib, but little anti-tumour activity with MK-2206 alone (Fig. 1D; Supplementary Table S1). Combined treatment with MK-2206 and selumetinib resulted in moderate but significant increase in tumour regression over time compared with selumetinib alone. All treatments were well tolerated by the animals, with no mortalities or adverse clinical signs. A decrease in body weight was observed in tumour-bearing nude mice. The body weight reduction was very slightly enhanced by the combination (Supplementary Figure S1).

In A2058 melanoma xenograft mouse models harbouring a *BRAF* V600E mutation and *PTEN* loss, but not *KRAS* or *PIK3CA* mutations, only modest anti-tumour activity was observed with MK-2206 and selumetinib monotherapy, while combination treatment again demonstrated enhanced anti-tumour responses (Supplementary Fig. S2; Supplementary Table S3). Preclinical *in vivo* toxicity studies for each agent alone indicated little evidence of overlapping histomorphological changes, with the exception of alterations in haemo-lymphatic and gastrointestinal tract systems at poorly tolerated doses in non-rodents. A decrease in body weight was observed in tumour-bearing nude mice with no mortalities or adverse clinical signs. The body weight reduction was very slightly enhanced by the combination (Supplementary Fig. S1).

Clinical Studies

Based upon the strong scientific rationale for the co-inhibition of AKT and Ras/Raf pathways, and preclinical evidence of synergy and tolerability, we initiated a phase I clinical study combining MK-2206 and selumetinib. No formal drug-drug interaction studies were conducted to assess the potential for an interaction between selumetinib and MK-2206, as the risk of a drug-drug interaction was considered low based upon the metabolism of each drug.

Specifically, neither of these drugs is a potent inhibitor of CYP1A2, CYP2C19, or CYP3A4 and although both selumetinib and MK-2206 are substrates of P-glycoprotein, selumetinib is not an inhibitor and MK-2206 is only a weak inhibitor of this transporter. We had previously characterized the pharmacokinetic (PK) and pharmacodynamic profile of each drug in studies with paired tumour biopsies (16, 17, 21). The maximum-tolerated dose (MTD) for MK-2206 is 60 mg QOD, or 200 mg weekly. At 60 mg QOD, the terminal half-life ($t_{1/2}$) of MK-2206 was 71.3 hours and the median suppression of post-dose tumour pS473 AKT was 81%. For selumetinib, the hydrogen sulphate oral capsule formulation was used, with prior phase I trials having established the MTD as 75 mg BID and the terminal $t_{1/2}$ as 5.3 hours (16) with this formulation; previously a dose of 100 mg BID with freebase powder formulation had been established (21). Suppression of pERK at 6 hours was observed in both peripheral blood mononuclear cells and paired tumour biopsies. Clinical toxicities of MK-2206 were mainly erythematous maculopapular rash and stomatitis, whereas those for selumetinib included acneiform dermatitis, diarrhoea, fatigue, nausea, and peripheral oedema. Rash was a dose-limiting toxicity (DLT) for both drugs, likely reflecting on-target effects of AKT and MEK inhibition for MK-2206 and selumetinib, respectively.

In this phase I study, initial cohorts of 3–6 evaluable patients with advanced, treatment-refractory solid tumours were recruited and given combinations of MK-2206 and selumetinib. Additional patients were enrolled to evaluate tolerability according to the mTPI approach as reflected in Supplementary Table S2. Fifty-one patients received treatment during the dose-escalation portion of the study, with 46 evaluable for dose-escalation safety assessment (Table 2). Five patients were considered non-evaluable for the dose escalation safety assessment because they did not complete $\geq 80\%$ of the first cycle of treatment or due to either non-

compliance with study medication (n=2), or non-dose-limiting toxicity (n=3; 1 of the 3 patients experienced rapid disease progression and was discontinued after 1 week of therapy). Dose escalation of either or both drugs aimed to define the MTD as the highest dose at which <20% patients experienced a DLT (22). A dose-expansion cohort at the MTD recruited an additional 11 patients with *KRAS*-mutant non-small-cell lung cancer (NSCLC); this cohort was selected based on preclinical anti-tumour activity (20)—observed anti-tumour activity during dose escalation (Fig. 2)—and was further supported by data from a parallel randomised phase II trial showing activity of selumetinib in combination with docetaxel in advanced *KRAS*-mutant NSCLC (23).

The starting dose for combination therapy was MK-2206 45 mg QOD, which represented 75% of the QOD MTD (60 mg), administered with the monotherapy MTD of selumetinib 75 mg BID of selumetinib. However, combined treatment at this dose resulted in an unacceptably high rate of DLT, with 2 of 3 evaluable patients having National Cancer Institute Common Toxicity Criteria (NCI-CTCAE) version 3.0 grade 3 maculopapular rash (Supplementary Fig. S3). At 45 mg QOD of MK-2206 and selumetinib 75 mg once daily (QD), grade 1 rash and grade 3 diarrhoea were reported, but no DLT were observed. This dose was determined to be the MTD of QOD dosing of MK-2206 with selumetinib.

The MK-2206 schedule was then changed to QW dosing based on monotherapy studies demonstrating its long terminal elimination half-life, reduced drug accumulation, improved tolerability, and pharmacodynamic data suggesting ongoing target inhibition at day 5 post-dose (17). The initial combination schedule of MK-2206 90 mg QW with selumetinib at 75 mg BID was not tolerable, with DLT of grade 2 retinal pigment epithelium detachment and grade 3 stomatitis and dermatitis acneiform observed in 3 out of 7 evaluable patients. Subsequent dose levels required dose and schedule modifications from this starting dosing schedule. Doses of

MK-2206 at 135 mg QW with selumetinib 100 mg QD resulted in only 1 of 6 DLT, comprising grade 3 fatigue, and this was therefore defined as the recommended phase II dose. Further exploration of this dose level in an additional 11 patients (all evaluable) confirmed this dose to be well tolerated, with only 2 further patients experiencing DLT of grade 3 rash and stomatitis (Table 3).

The most common drug-related adverse events (occurring in >5 patients) are summarized in Supplementary Table S4. Rash was the most frequent adverse event and DLT; two distinct appearances were noted: a reversible maculopapular rash associated with pruritus consistent with our previous experience with MK-2206 (17); and an erythematous acneiform rash associated with selumetinib that improved with topical steroids or oral tetracycline therapy (24). Other DLT included diarrhoea and stomatitis, which appeared to be dose-related. No drug-related haematological toxicities were observed in this study. Infrequent asymptomatic grade 3 increases in circulating hepatic transaminases also were reported (n=5), but fully normalised on temporary discontinuation of the drug. Dose-limiting detachment of retinal pigment epithelium (RPED), which was observed in 2 patients, was reversible following discontinuation of treatment.

Pharmacokinetics suggested no meaningful drug-drug interaction between MK-2206, selumetinib, and the active metabolite *N*-desmethyl selumetinib. At the combination MTD, the terminal $t_{1/2}$ of MK-2206 was 61.7 ± 15.2 hours, within range of that seen in monotherapy (88.9 ± 26.9). Published monotherapy PK data for selumetinib 100 mg capsule is not available for direct comparison with the combination MTD. However, exposure (mean C_{max} and t_{max}) following 75 mg selumetinib plus MK-2206 was within the range previously reported for monotherapy at the same dose (16). Exposure to selumetinib increases proportionally with dose (16), and therefore was slightly higher in the combination MTD cohort treated with selumetinib

100 mg QD (mean C_{\max} 1140 ng/mL [range 554-2540] and AUC_{0-10h} 4500 ng.h/mL [range 2297-7875]) than cohorts given 75 mg selumetinib.

Inter-patient variability was moderate for MK-2206 (%CV of C_{\max} and AUC_{0-168h} ranged from 33% to 47%), and high for selumetinib (%CV of C_{\max} and AUC_{0-10h} ranged from 19% to 74%). Preclinical models associated anti-tumour activity with MK-2206 concentrations above 57 nmol/L, a concentration at which >70% inhibition of pS473 AKT was achieved; this target steady-state trough concentration of >57 nmol/L was achieved in 100% of patients in 48 hours at the 135 mg QW MTD level. In previous monotherapy studies, we have shown that pAKT and pERK were robustly suppressed at the exposures achieved at the combination MTD of MK-2206 (135 mg QW) and selumetinib (100 mg QD), respectively (16, 17, 25).

In this study, 29 patients with *KRAS*-mutant cancers were treated, for whom confirmed RECIST (Response Evaluation Criteria in Solid Tumours) 1.0 partial responses were observed in 3 of 13 (23%) patients with NSCLC, and 1 of 2 (50%) patients with ovarian cancer. The best anti-tumour responses were observed in a 59-year-old Caucasian female with chemotherapy-refractory *KRAS*-mutant lung adenocarcinoma. Overall, she had a 71% RECIST response and remained on treatment for 15 months. A 63-year-old patient of Asian ethnicity with chemotherapy-refractory *KRAS*-mutant lung adenocarcinoma also had a 45% RECIST response and remained on study for 20 weeks (Fig. 3). One additional patient with pancreatic cancer achieved a RECIST partial response, and although *KRAS* mutations are known to occur in approximately 85% in pancreatic ductal carcinoma (26), mutation status was not available for this patient. RECIST stable disease >6 months was observed in 1 patient with NSCLC and another with low-grade ovarian cancer. In contrast, none of the 33 patients with confirmed *KRAS* wild-type tumours achieved a confirmed RECIST partial response or stable disease >6 months.

Interestingly, no confirmed objective responses were observed in the colorectal cancers with *KRAS* mutations (n=11; Fig. 2).

The majority of *KRAS* mutations were amino acid substitutions to cysteine (21%), valine (34%), or aspartate (14%) in codon 12. The type of *KRAS* mutation, or presence of concurrent *PIK3CA* or *BRAF* mutations, did not appear to influence whether an objective response was achieved, though notably, the limited number of responders in this study prevented us from drawing a definitive conclusion. Among patients with NSCLC, 2 of 13 patients (15.4%) had concurrent *PIK3CA* mutations detected, while 0 of 9 patients with colorectal cancer (CRC) had concurrent *PIK3CA* mutations detected.

DISCUSSION

While single-agent activity for MEK inhibitors in clinical *KRAS*-mutant disease has been modest, Raf/MEK/ERK signalling is considered to be a major Ras effector pathway (4, 5). The activity we observed in *KRAS*-mutant models appeared largely driven by selumetinib, which is consistent with findings from other preclinical models combining MEK and mTOR inhibitors in this setting (8). Therefore, based on these data, we attempted to prioritize maintenance of MEK blockade, while also attempting to combine AKT inhibition. This trial highlights the multiple challenges of combining targeted agents (27). The numerous permutations of dose, schedule, and sequence result in significant complexities, and there are currently no standardized trial designs to assess optimal combination strategies. The particular challenge of combining MEK and AKT inhibition included known overlapping monotherapy toxicities, especially rash and diarrhoea that were observed at the MTD of both selumetinib and MK-2206 (17, 21). Rash and diarrhoea expectedly limited dosing and ultimately required de-escalation of both MK-2206 and

selumetinib to improve tolerability of the combination. Although de-escalation of both selumetinib and MK-2206 from recommended monotherapy doses was required to mitigate tolerability issues, both drug doses at the combination MTD were previously shown to be biologically active in their respective single-agent studies. In this study, we successfully changed schedules of administration of both drugs (compared with single-agent phase I schedules and doses) to improve tolerability and is an example of how flexible design of early clinical trials can help circumvent toxicity (27). The pharmacokinetic data in this study suggested no drug-drug interaction between selumetinib and MK-2206, which supported the pre-clinical assessment of the combination having a low potential for interaction. However, as no formal drug-drug interaction assessment studies were conducted, this result must be interpreted with caution. Clinical anti-tumour activity was observed with durable RECIST tumour shrinkage in *KRAS*-mutant NSCLC and low-grade ovarian carcinoma. However, no responses were observed in *KRAS*-mutant colorectal or small-bowel carcinoma, possibly suggesting distinct biological context differences in these diseases. As this trial did not include prospective screening of concurrent mutations for study entry, the number of subjects where both *KRAS* and *PIK3CA* mutations were detected was very limited and precluded our ability to draw firm conclusions around whether these concurrent mutations could be associated with response in the clinic. Colorectal cancer has previously shown limited success to treatment with MEK inhibitor monotherapy (28). The observed heterogeneity of response among patients with *KRAS*-mutant cancers likely reflects the complexities of tumour biology and possibly the presence of other aberrant driver mutations or disruption of signalling feedback loops (29), although we have not excluded poor drug penetration to tumour in these studies. Furthermore, there may be mechanisms independent of PI3K and MEK that lead to maintenance of cellular proliferation,

such as up-regulation of p21-activated kinase (30) or LKB1 mutations (31). Ultimately, the selection of molecularly targeted agents to combine on the basis of molecular profiling remains a challenging and imperfect strategy, requiring the ever-evolving application of an array of modern technologies, including DNA sequencing, genomics, bioinformatics, and computational approaches (15).

A combination of selumetinib and docetaxel was recently evaluated in a phase II study with *KRAS*-mutant NSCLC patients, demonstrating an improvement in response rate, progression free survival (5.3 months vs 2.1 months) and median overall survival (OS) in the selumetinib combination arm compared with docetaxel alone (9.4 vs. 5.3 months), although the combination resulted in more toxicities (23).

In conclusion, these are the first clinical data to demonstrate that different *KRAS*-mutant cancers may show differential sensitivity to the co-targeting of MEK and AKT and to present a novel and rational anti-tumour strategy against cancers driven by a common driver mutation. Multiple other drug combinations targeting different components of the Ras/Raf and PI3K/AKT signalling pathways have also entered clinical development based on robust preclinical biology. We envision that the complex and multifaceted clinical evaluation of these drug combinations will lead to a new therapeutic avenue for many *RAS*-mutant cancers, including *KRAS*-mutation driven NSCLC and low-grade ovarian cancer. Moreover, as with other rationally designed molecularly targeted strategies (e.g., poly[ADP-ribose] polymerase [PARP] inhibitors in *BRCA1/2*-mutant cancers), we have observed anti-tumour activity in patients whose tumours have the same molecular defect, but which arise from diverse geographical origins (lung, ovary, and pancreas) (32). Due consideration must continue to be given in oncological drug

development and registration studies to select patients based, not simply on disease origin, but also on the underlying cancer biology.

AUTHOR CONTRIBUTIONS

The following author contributions are noted: conceived, designed and/or planned study: JS, AWT, LY, JD, ER, PH, KS, Y-MJ, PS; collected/assembled the data: JMS, AWT, LY, KK, DG, VM, AP, BK, KPP, CRG, JD, MO, AM, DO, UB, KS, PS, ML; performed or supervised analyses: JMS, AWT, LY, KK, JD, MO, TAY, KS, Y-MJ, PS, ET, ML; interpreted results: JMS, AWT, LY, KK, DG, VM, JD, MO, ER, RDB, TAY, VP, KS, PS, ET, ML; wrote draft and/or critically reviewed subsequent drafts: all authors.

ACKNOWLEDGMENTS

This study was sponsored by Merck & Co., Inc., Whitehouse Station, NJ, in collaboration with AstraZeneca, Alderley Park, Macclesfield, Cheshire, UK. The authors wish to thank Michael Lam and Emmet Schmidt, both of Merck & Co., Inc., Whitehouse Station, NJ, for their contributions in interpreting the preclinical data presented in this manuscript. Editorial assistance was provided by Amy O. Johnson-Levonas, PhD, and Kristen Lewis, both of Merck & Co., Inc., Whitehouse Station, NJ, as well as Jennifer Granit, PhD, of Integrus Scientific, a division of Medicus International New York. This assistance was funded by Merck & Co., Inc., Whitehouse Station, NJ.

Funding: This study was sponsored by Merck & Co., Inc., Whitehouse Station, NJ, in collaboration with AstraZeneca, Alderley Park, Macclesfield, Cheshire, UK.

Grant information: The Drug Development Unit of the Royal Marsden NHS Foundation Trust and The Institute of Cancer Research is supported in part by a programme grant from Cancer Research UK (grant number: C347/A18077). Support was also provided by an Experimental Cancer Medicine Centre grant (no grant number) and the National Institute for Health Research Biomedical Research Centre (jointly to the Royal Marsden NHS Foundation Trust and The Institute of Cancer Research) (grant numbers: A46/CCR - CCR4057 & CCR4058).

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Table 1. Combination index (CI)[†] by Chou-Talalay method for selumetinib and MK-2206[‡]

Cell line	Cancer type	<i>KRAS</i>	<i>PIK3CA</i>	<i>BRAF</i>	<i>PTEN</i>	CI: ED ₅₀	CI: ED ₇₅	CI: ED ₉₀
HCT116	Colon	G13D	H1047R	<i>WT</i>	<i>WT</i>	0.20	0.14	0.11
HCT15	Colon	G13D	E545K	<i>WT</i>	<i>WT</i>	0.12	0.12	0.12
HT29	Colon	<i>WT</i>	P449T	V600E	<i>WT</i>	0.68	0.73	0.82
A2058	Melanoma	<i>WT</i>	<i>WT</i>	V600E	Del	0.35	0.19	0.12
AsPC-1	Pancreatic	G12D	<i>WT</i>	<i>WT</i>	<i>WT</i>	0.18	0.09	0.15
MIA-Pa-Ca2	Pancreatic	G12C	<i>WT</i>	<i>WT</i>	<i>WT</i>	0.42	0.25	0.58
Calu-6	Lung	Q61K	<i>WT</i>	<i>WT</i>	<i>WT</i>	0.25	0.20	0.18
HCI-H460	Lung	Q61H	E545K	<i>WT</i>	<i>WT</i>	0.04	0.03	0.02

[†]Cell Lines were studied across two-fold serial dilutions spanning the IC₅₀ for each cell line for each individual drug. Shown is the combination index (CI) which calculates the combination effects as a function of the slopes of the inhibition curves independent of any specific drug concentration

[‡]An additional analysis was performed on an expanded set of colorectal cancer cell lines harboring either wildtype or mutant K-RAS, B-RAF or PIK3CA were analyzed for sensitivity to MEKi or AKTi + MEKi. The results suggest that PIK3CA mutant status predicts resistance to single agent MEKi treatment while combination treatment of AKTi + MEKi reversed this effect. MEKi sensitivity was not altered in the presence of either wildtype or mutant K-RAS or B-RAF status in these lines.

Table 2. Patient demographics

Patient characteristics	No. of patients (%)
Number of patients	62
Age, years	
Median	58
Range	33–81
Performance status	
0	22 (35)
1	40 (65)
Male/females	26/36 (42/58)
Patients with prior chemotherapy	62
Median number of prior chemotherapy regimens	3
Range	1–10
Tumour types	
	Total
Non–small-cell lung	19 (31)
KRAS mutant	13 (21)
Colorectal	14 (23)
KRAS mutant	9 (15)
Ovarian	5 (8)
KRAS mutant	2 (3)
Pancreatic	5 (8)
KRAS mutant	4 (6)
Breast	3 (5)
Leiomyosarcoma	3 (5)
Ewing’s sarcoma	2 (3)
Prostate	2 (3)
Melanoma	2 (3)
Appendix, cervical (squamous), chondrosarcoma, esophageal, liver, Merkel cell, thyroid	7 total [1 each] (11 [2])
KRAS mutant	1 (2)

Table 3. Related grade 3/4 adverse events (AEs)

Dose level	MK-2206	Selumetinib	n (evaluable for safety)	DLT n (%)	Patients with C1-related grade (Gr) 3/4 AEs		Patients with C≥2-related Gr 3/4 AEs	
					Event	n	Event	n
QOD-1	45 mg QOD	75 mg BID	4 (3)	2 (66.7)	Gr 3 rash	2 ^a	Gr 3 ALT/AST elevation	1
QOD-1	45 mg QOD	75 mg QD	6 (6)	0 (0)	Gr 3 diarrhoea	1	None	0
QW-1	90 mg QW	75 mg BID	9 (7)	3 (42.8) ^b	Gr 3 stomatitis Gr 3 rash	1 ^a 1 ^a	Gr 3 anaemia Gr 3 fatigue Gr 3 retinal pigment epithelium detachment ^b	1 1 1
QW-1a	90 mg QW	75 mg QD	7 (6)	1 (16.7)	Gr 3 diarrhoea	1 ^a	Gr 3 ALT elevation	1
QW-1b	90 mg QW	50 mg BID	7 (6)	2 (33.3)	Gr 3 rash	2 ^a	Gr 3 CPK elevation Gr 3 diarrhoea	1 1
QW-1c	90 mg QW	100 mg QD	3 (3)	0 (0)	None	0	None	0
QW-1d	90 mg QW	150 mg QD	3 (3)	2 (66.7)	Gr 4 lipase elevation Gr 3 retinal pigment epithelium detachment	1 ^a 1 ^a	None	0
QW-1e ^c	135 mg QW	100 mg QD	17 (17)	3 (17.6)	Gr 3 fatigue G3 lymphocyte count decreased Gr 3 pruritus Gr 3 rash Gr 3 stomatitis	1 ^a 1 1 3 ^a (1 DLT) 1 ^a	Gr 3 ALT/AST elevation Gr 3 dry skin Gr 3 pruritus Gr 3 rash	1 1 1 3
QW-1f	100 mg QW	100 mg QD	6 (6)	0 (0%)	None		None	0

DLT = dose-limiting toxicity.

No grade 5 events considered by the investigator to be related to either drug were reported.

^aEvent counted as DLT.

^bOne grade 2 DLT of retinal pigment epithelium detachment was observed during cycle 1 in the same patient who experienced a grade 3 event after beginning cycle 2.

^cDose considered the maximum-tolerated dose (MTD) for the combination; 6 enrolled in dose escalation period, 11 *KRAS* mutant NSCLC enrolled in confirmation period.

Figure Legends

Fig. 1. Activated caspase induction, cell-cycle profiles, and pERK/pAKT profiles of HCT116 cell lines and HCT116 xenografts in rodents treated with MK-2206 and/or selumetinib. **(A)** Caspase-3/7 activity after 24 hours was determined by luminescence assay. Data was indicated as fold induction of caspase against the signals of DMSO treated cells. **(B)** Percent of sub-G1 population in cell cycle was determined with FACS when HCT116 cells were exposed with MK-2206 and/or selumetinib for 72 hours. **(C)** HCT116 colon cancer cells were treated with MK-2206 and/or selumetinib at the indicated concentration for 24 h. The cell lysates were analyzed by Western blot with the indicated antibodies. **(D)** Selumetinib alone showed potent anti-tumor efficacy in this animal model. However, MK-2206 had only marginal anti-tumour effect. The combination therapy produced a tumour regression with statistically significant increase in the anti-tumour response as compared to monotherapy ($P < 0.05$).

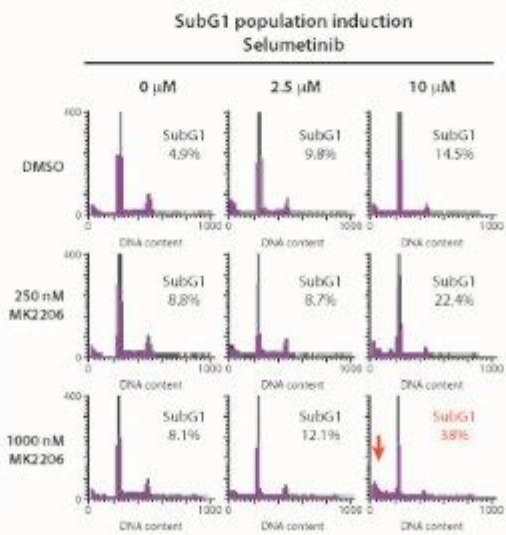
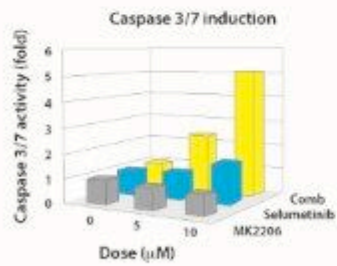


Fig. 2. Waterfall plot of RECIST responses in evaluable patients with *KRAS*-mutant cancers.

^aThese data only reflect subjects who were re-evaluated after the baseline scan; ^bRecommended phase II dose for the combination.

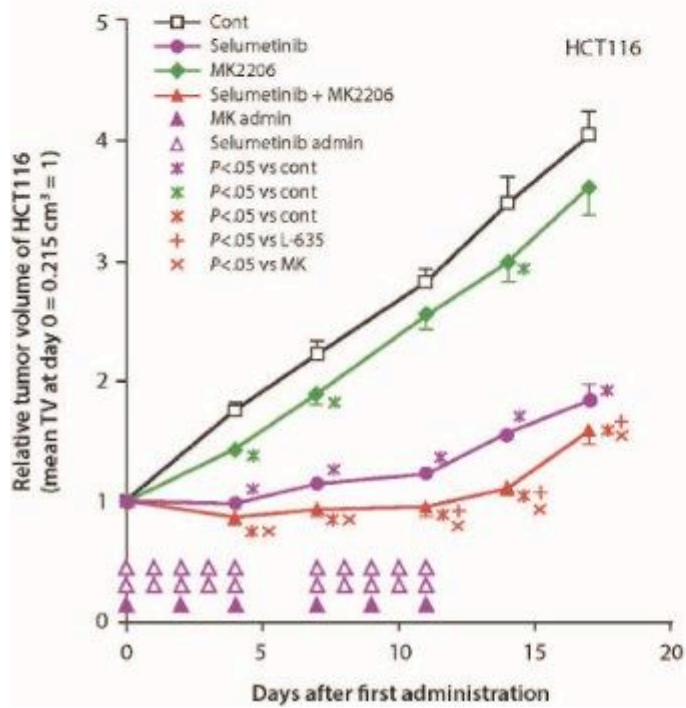
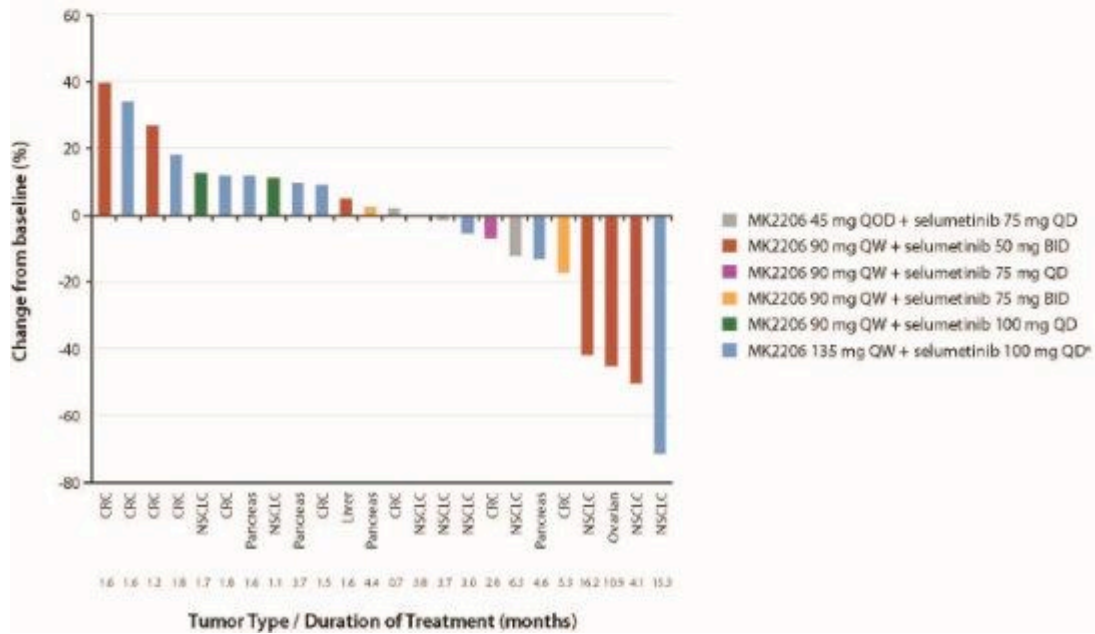


Fig. 3. Example responder: *KRAS*-mutant adenocarcinoma of lung. A 63-year-old female never-smoker of Asian ethnicity with metastatic adenocarcinoma of the lung, which was *KRAS* G12D mutant, but wildtype for epidermal growth factor receptor (*EGFR*), anaplastic lymphoma kinase (*ALK*), *PIK3CA*, and *BRAF* gene aberrations. Figures 3 A and B represent pretreatment scans, while the Figures 3 C and D represent the same slices of scans with best response to the treatment. This patient's prior treatments included (I) cisplatin and pemetrexed (2 cycles with disease progression); (II) carboplatin and paclitaxel (8 cycles with partial response); (III) docetaxel (2 cycles with disease progression); (IV) carboplatin and paclitaxel rechallenge (2 cycles with disease progression); (V) radiotherapy to spine metastases. She was treated with MK-2206 135 mg PO QW and selumetinib 100 mg PO OD without dose reduction, although she had a grade 2 skin rash that was tolerable. She had a RECIST partial response with 45% reduction in the sum of diameters of the target lesions. The patient eventually developed progressive disease after 6 cycles due to the development of symptomatic brain metastases.

Best RECIST Response - KRAS Mutant Cancers



Supplementary Table S1. CD1-nude mice bearing HCT116 tumour xenografts. * At day 10, % tumour volume compared with control.

Group	Compound(s)	Dose	Schedule	Route	n	% Tumour volume
1	Vehicle + vehicle	None	BID, M-F ×2 weeks	PO	5	Control
		None	QOD ×2 weeks	PO		
2	Selumetinib + vehicle	25 mg/kg	BID, M-F ×2 weeks	PO	5	13
		None	QOD ×2 weeks	PO		
3	Vehicle + MK-2206	None	BID, M-F ×2 weeks	PO	5	85
		120 mg/kg	QOD ×2 weeks	PO		
4	Selumetinib + MK-2206	25 mg/kg	BID, M-F ×2 weeks	PO	5	-5
		120 mg/kg	QOD ×2 weeks	PO		

Supplementary Table S3. CD1-nude mice bearing A2058 tumour xenografts. * At day 10, % tumour volume compared with control.

Group	Compound(s)	Dose	Schedule	Route	n	% Tumour volume
1	Vehicle + vehicle	None	BID, M-F ×2 weeks	PO	5	Control
		None	QOD ×2 weeks	PO		
2	Selumetinib + vehicle	25 mg/kg	BID, M-F ×2 weeks	PO	5	52
		None	QOD ×2 weeks	PO		
3	Vehicle + MK-2206	None	BID, M-F ×2 weeks	PO	5	52
		120 mg/kg	QOD ×2 weeks	PO		
4	Selumetinib + MK-2206	25 mg/kg	BID, M-F ×2 weeks	PO	5	30
		120 mg/kg	QOD ×2 weeks	PO		

Supplementary Table S4. Adverse events (worst grade, >5 patients overall) related to MK 2206 and selumetinib as a function of dose during the first course (and all courses). Values represent the number of drug-related adverse events in the first course (and in all courses) of treatment. Only the highest grade is counted for an individual subject. There were no grade 5 adverse events considered by the investigator to be related to either drug.

Preferred term	Dose Level								
	MK-2206 45 mg QOD selumetinib 75 mg QD N=6			MK-2206 45 mg QOD selumetinib 75 mg BID N=4			MK-2206 90 mg QW selumetinib 75 mg QD N=7		
	First course (all courses)			First course (all courses)			First course (all courses)		
	Grade 1	Grade 2	Grade 3	Grade 1	Grade 2	Grade 3	Grade 1	Grade 2	Grade 3
Skin and subcutaneous tissue disorders									
Rash NOS	3 (4)	0 (1)	0 (0)	1 (1)	0 (0)	2 (2)	1 (1)	0 (0)	0 (0)
Acneiform rash	1 (1)	0 (0)	0 (0)	1 (1)	1 (1)	0 (0)	2 (2)	2 (2)	0 (0)
Pruritus	0 (1)	0 (1)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)
Dry skin	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Gastrointestinal disorders									
Diarrhoea	1 (0)	0 (1)	1 (1)	3 (3)	0 (0)	0 (0)	1 (1)	1 (0)	0 (1)
Nausea	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (4)	0 (0)	0 (0)
Stomatitis	2 (2)	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)
Vomiting	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3)	0 (0)	0 (0)
General disorders and administrative site conditions									
Fatigue	1 (0)	0 (1)	0 (0)	0 (0)	1 (1)	0 (0)	0 (1)	1 (1)	0 (0)
Mucosal inflammation	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Metabolism and nutrition disorders									
Decreased appetite	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)
Investigations									
Liver transaminase increase	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	0 (1)	0 (0)
Preferred term	Dose Level								
	MK-2206 90 mg QW selumetinib 100 mg QD N=3			MK-2206 90 mg QW selumetinib 50 mg BID N=7			MK-2206 90 mg QW selumetinib 150 mg QD N=3		
	First course (all courses)			First course (all courses)			First course (all courses)		
	Grade 1	Grade 2	Grade 3	Grade 1	Grade 2	Grade 3	Grade 1	Grade 2	Grade 3
Skin and subcutaneous tissue disorders									
Rash NOS	2 (2)	0 (1)	0 (0)	2 (2)	0 (1)	0 (0)	0 (1)	0 (0)	0 (0)
Acneiform rash	1 (1)	0 (0)	0 (0)	2 (2)	2 (2)	1 (1)	1 (1)	0 (0)	0 (0)
Pruritus	1 (2)	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	1 (1)	0 (0)	0 (0)
Dry skin	2 (2)	0 (0)	0 (0)	2 (2)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)
Gastrointestinal disorders									
Diarrhoea	2 (2)	1 (1)	0 (0)	2 (2)	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)
Nausea	0 (1)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	2 (2)	0 (0)	0 (0)
Stomatitis	1 (1)	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)
Vomiting	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)
General disorders and administrative site conditions									
Fatigue	1 (2)	0 (0)	0 (0)	0 (0)	0 (3)	0 (0)	0 (1)	0 (0)	0 (0)
Mucosal inflammation	0 (0)	0 (0)	0 (0)	2 (2)	0 (1)	0 (0)	0 (1)	0 (0)	0 (0)
Metabolism and nutrition disorders									
Decreased appetite	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	0 (1)	0 (1)	0 (0)
Investigations									
Liver transaminase increase	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Preferred term	Dose Level								
	MK-2206 90 mg QW selumetinib 75 mg BID N=9			MK-2206 100 mg QW selumetinib 100 mg QD N=6			MK-2206 135 mg QW selumetinib 100 mg QD N=17		
	First course (all courses)			First course (all courses)			First course (all courses)		
	Grade 1	Grade 2	Grade 3	Grade 1	Grade 2	Grade 3	Grade 1	Grade 2	Grade 3
Skin and subcutaneous tissue disorders									
Rash NOS	1 (2)	2 (3)	0 (0)	2 (3)	1 (2)	0 (0)	4 (3)	2 (4)	0 (2)
Acneiform rash	1 (1)	1 (1)	0 (1)	2 (2)	0 (0)	0 (0)	3 (3)	2 (2)	0 (1)
Pruritus	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (5)	0 (0)	0 (2)
Dry skin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (5)	0 (0)	0 (1)
Gastrointestinal disorders									
Diarrhoea	2 (2)	1 (1)	0 (0)	0 (0)	1 (1)	0 (0)	7 (11)	0 (0)	0 (0)
Nausea	1 (1)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	6 (6)	0 (1)	0 (0)
Stomatitis	2 (2)	1 (2)	1 (1)	0 (0)	1 (1)	0 (0)	1 (2)	1 (1)	0 (1)
Vomiting	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)	0 (0)	0 (1)	0 (1)	0 (0)
General disorders and administrative site conditions									
Fatigue	0 (1)	1 (1)	0 (1)	0 (2)	0 (1)	0 (0)	5 (8)	0 (2)	1 (1)
Mucosal inflammation	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (5)	1 (2)	0 (0)
Metabolism and nutrition disorders									
Decreased appetite	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	0 (0)	1 (1)	0 (2)	0 (0)
Investigations									
Liver transaminase increase	0 (0)	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)	2 (2)	0 (1)	0 (1)

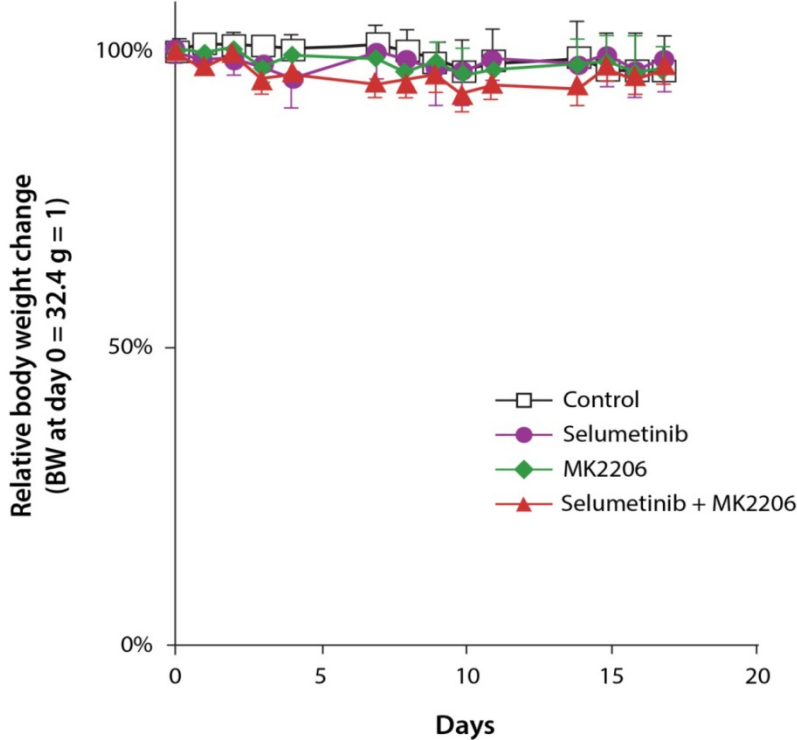
Figure Legends.

Supplementary Fig. S1. Effect of MK-2206 in combination with selumetinib on body weight. A decrease in body weight was observed in tumour-bearing nude mice. The body weight reduction was very slightly enhanced by the combination.

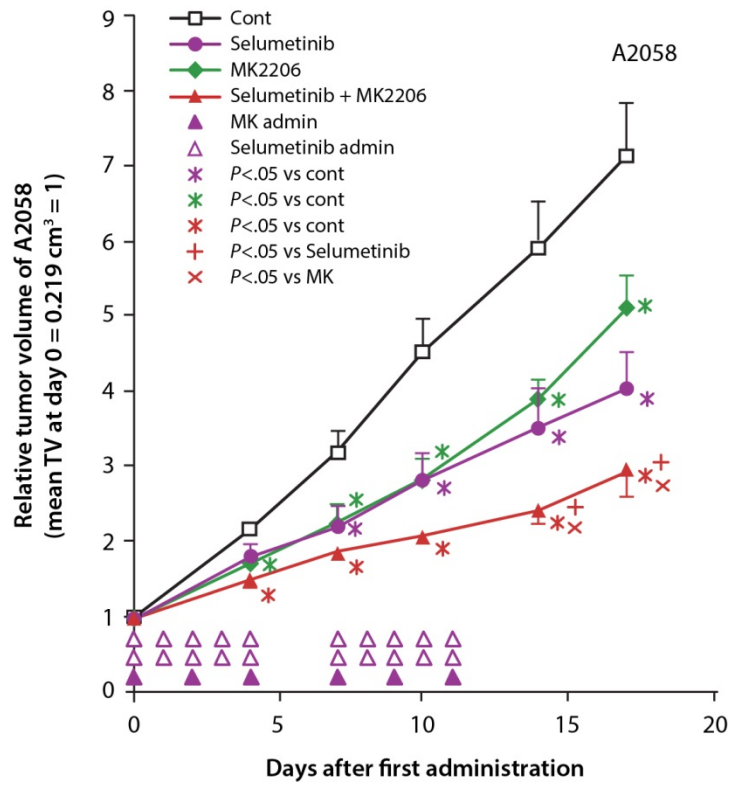
Supplementary Fig. S2. Treatment with MK-2206 or selumetinib alone had moderate anti-tumour effects. The combination therapy produced a statistically significant increase in the anti-tumour response as compared to monotherapy ($P<0.05$).

Supplementary Fig. S3. A 61-year-old male patient with metastatic adenocarcinoma of the jejunum at cycle (C) 1, day 21 with a grade III maculo-papular rash with pustules and desquamation. This patient received a dose of MK2206 90mg PO QW and selumetinib 50mg BD. He initially presented on C1D8 with a grade I maculo-papular rash; this became a grade II rash on C2D15, which was treated with a course of oral antibiotics and topical steroid cream. Continued drug treatment led to a grade III rash on C1D21 (pictured) when drug was stopped for a 1-week break along with an extended course of antibiotics. The rash resolved to grade I on day 28 after 1 week's break.

Supplementary Fig. S1.



Supplementary Fig. S2.



Supplementary Fig. S3.

