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Inhibition of MEK and PI3K/mTOR Suppresses Tumor Growth but Does Not Cause Tumor Regression in Patient-Derived Xenografts of RAS-Mutant Colorectal Carcinomas

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ABSTRACT

Purpose: Gene mutations along the Ras pathway (*KRAS*, *NRAS*, *BRAF*, *PIK3CA*) occur in approximately 50% of colorectal cancers (CRC) and correlate with poor response to anti–EGF receptor (EGFR) therapies. We assessed the effects of mitogen-activated protein (MAP)/extracellular signal-regulated kinase (ERK) kinase (MEK) and phosphoinositide 3-kinase (PI3K)/mTOR inhibitors, which neutralize the major Ras effectors, in patient-derived xenografts from RAS/RAF/PIK3CA-mutant metastatic CRCs (mCRC).

Experimental Design: Forty mCRC specimens harboring *KRAS*, *NRAS*, *BRAF*, and/or*PIK3CA* mutations were implanted in nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice. Each xenograft was expanded into four treatment arms: placebo, the MEK inhibitor AZD6244, the PI3K/mTOR inhibitor, BEZ235, or AZD6244 + BEZ235. Cases initially treated with placebo crossed over to AZD6244, BEZ235, and the anti-EGFR monoclonal antibody cetuximab.

Results: At the 3-week evaluation time point, cotreatment of established tumors with AZD6244 + BEZ235 induced disease stabilization in the majority of cases (70%) but did not lead to overt tumor regression. Monotherapy was less effective, with BEZ235 displaying higher activity than AZD6244 (disease control rates, DCRs: AZD6244, 27.5%; BEZ235, 42.5%). Triple therapy with cetuximab provided further advantage (DCR, 88%). The extent of disease control declined at the 6-week evaluation time point (DCRs: AZD6244, 13.9%; BEZ235, 16.2%; AZD6244 + BEZ235, 34%). Cross-analysis of mice harboring xenografts from the same original tumor and treated with each of the different modalities revealed subgroups with preferential sensitivity to AZD6244 (12.5%), BEZ235 (35%), or AZD6244 + BEZ235 (42.5%); another subgroup (10%) showed equivalent response to any treatment.

Conclusions: The prevalent growth-suppressive effects produced by MEK and PI3K/mTOR inhibition suggest that this strategy may retard disease progression in patients. However, data offer cautionary evidence against the occurrence of durable responses.

INTRODUCTION

The introduction of the humanized monoclonal antibodies cetuximab and panitumumab, which target the EGF receptor (EGFR), has widened the therapeutic opportunities for patients with metastatic colorectal cancer (mCRC; refs. 1–3). Both agents achieve incremental gains greater than those of standard chemotherapy, yet a large fraction of patients do not receive clinical benefit from such therapies (4). Several studies have unequivocally showed that mutations of the *KRAS* gene, which occur in 35% to 40% of CRCs, are associated with extremely low response rates to anti-EGFR antibodies (5–13). As a consequence, the use of cetuximab and panitumumab is now limited to patients with KRAS wild-type tumors. Furthermore, several retrospective analyses have provided preliminary evidence that mutations of other components of the Ras pathway (*NRAS*,*BRAF* and, possibly, *PIK3CA*) seem to negatively affect sensitivity to EGFR inhibitors (14–17). When considering the cumulative distribution of these mutations in all mCRCs, more than 50% of patients can be predicted to be nonresponsive to EGFR-targeted antibodies (17, 18). Once these patients become refractory to standard chemotherapy, the remaining therapeutic armamentarium is substantially ineffective. Together, these observations indicate that treatment of mCRC tumors exhibiting *KRAS* mutations (and—most likely—also other oncogenic mutations along the Ras pathway) represents a largely unmet medical need.

We recently developed and characterized an experimental platform that allows the design of exploratory trials at the preclinical level and on a population-based scale (19). This platform consists of a series of human mCRC surgical specimens directly transplanted into mice, to produce a study population that can be concurrently subjected to molecular analysis and assessed for response to targeted agents (“xenopatients”). By combining the versatility of preclinical analysis with the informative merit of population-based approaches, xenopatients are deemed to represent a valuable source for reliable, biomarker-driven drug development (20, 21). Indeed, when we did a validation trial aimed to challenge the clinical predictability of the system using cetuximab as a reference treatment, we found that xenografted mCRCs responded to EGFR inhibition with rates and extents similar to those described in the clinic. Importantly, anti-EGFR therapy proved to be ineffective in all cases exhibiting activating mutations of *KRAS*, *NRAS*, and *BRAF* (19). To assess the efficacy of novel therapeutic regimens in these tumor settings, we devised a multiarm trial in which the activity and efficacy of investigational compounds that target the Ras-dependent effectors mitogen-activated protein (MAP)/extracellular signal-regulated kinase (ERK) kinase (MEK) and phosphoinositide 3-kinase (PI3K)/mTOR was evaluated.

PATIENTS AND METHODS

Specimen collection and annotation

A total of 150 consecutive tumor samples and matched normal samples were obtained from patients treated by liver metastasectomy at the Institute for Cancer Research and Treatment (Candiolo, Torino, Italy) and Mauriziano Umberto I Hospitals (Torino, Italy). All patients provided informed consent and samples were procured and the study was conducted under the approval of the Review Boards of the Institutions. Clinical and pathologic data were entered and maintained in our prospective database.

Molecular analyses

Analyte extraction and mutational profiling were done as described (14, 19, 22, 23). Briefly, fresh specimens from surgically resected CRC liver metastases and from matched normal liver tissue were incubated overnight in RNAlater (Ambion), quick-frozen at −80°C, and mechanically fragmented. Genomic DNA was isolated using the Blood & Cell Culture DNA Midi Kit (Qiagen).

DNA concentrations were quantified using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific). Exon-specific and sequencing primers were designed using Primer3 software and synthesized by Sigma. Purified PCR products were sequenced using BigDye Terminator version 3.1 Cycle Sequencing kit (Applied Biosystems) and analyzed with a 3730 ABI capillary electrophoresis system.

Explant xenograft models

Tumor material not required for histopathologic analysis was collected and placed in medium 199 supplemented with 200 U/mL penicillin, 200 μg/mL streptomycin, and 100 μg/mL levofloxacin. Each sample was cut into 25- to 30-mm^3 pieces in antibiotic-containing medium; some of the pieces were incubated overnight in RNAlater and then frozen at −80°C for molecular analyses; another piece was coated in Matrigel (BD Biosciences) and implanted in a subcutaneous pocket produced in the right flank of one female nonobese diabetic/severe combined immunodeficient (NOD/SCID) mouse. After mass formation, the tumor was passaged by single-step propagation into 4 mice (19, 23). Established tumors (average volume 400 mm^3) were treated twice weekly with 20 mg/kg cetuximab (Merck) or daily with 40 mg/kg BEZ235 (Selleck Chemicals) or 25 mg/kg AZD6244 (Sequoia Research Products). Tumor size was evaluated once weekly by caliper measurements and the approximate volume of the mass was calculated using the formula $4/3 \times \pi(d/2)^2 \times D/2$, where d is the minor tumor axis and D is the major tumor axis. All animal procedures were approved by the Ethical Commission of the Institute for Cancer Research and Treatment and by the Italian Ministry of Health.

Immunohistochemistry

Immunohistochemistry was done as described (24). Briefly, 4-μm paraffin tissue sections were dried in a 37°C oven overnight. Slides were deparaffinized in xylene and rehydrated through graded alcohol to water. Endogenous peroxidase was blocked in 3% hydrogen peroxide for 30 minutes. Microwave antigen retrieval was carried out using a microwave oven (750 W for 10 minutes) in 10 mmol/L citrate buffer, pH 6.0. Slides were incubated with individual primary antibodies overnight at 4°C inside a moist chamber. After washings in PBS, anti-rabbit secondary antibody (Dako Envision+System-horseradish peroxidase–labeled polymer, Dako) was added. Incubations were carried out for 1 hour at room temperature. Immunoreactivities were revealed by incubation in DAB chromogen (DakoCytomation Liquid DAB Substrate Chromogen System, Dako) for 10 minutes. Slides were counterstained in Mayer's hematoxylin, dehydrated in graded alcohol, cleared in xylene, and the coverslip was applied by using DPX. A negative control slide was processed with secondary antibody, omitting primary antibody incubation. The following antibodies were used: phospho-S6 ribosomal protein rabbit mAb (Ser235/236, clone D57.2.2E; Cell Signaling Technology); phospho-p44/42 MAPK (ERK1/2) rabbit mAb (Thr202/tyr204, clone D13.14.4E; Cell Signaling Technology); cleaved caspase-3 rabbit mAb (Asp175, clone D3E9; Cell Signaling Technology); rat mAb anti-mouse Ki67, clone TEC-3 (Dako). Images were captured with the Image-Pro Plus 6.2 software (Media Cybernetics) using a BX60 Olympus microscope.

Statistics

Statistical analyses were done by the 2-tailed Student t test. For all tests, the level of statistical significance was set at $P < 0.05$.

RESULTS

Patient population

A summary of the clinical and molecular characteristics for the study population can be found in Table 1 and detailed information is provided in Supplementary Tables S1 and S2. Independent fragments of the original mCRC specimens used for xenotransplantation procedures were sequenced for the presence of hotspot mutations in *KRAS* and *NRAS*(exon 2: codons 12 and 13), *BRAF* (exon 15: codon 600), and *PIK3CA* (exon 9: codons 542, 545, and 546; exon 20: codon 1047), as well as for the presence of rare mutations in*KRAS* and *NRAS* (exon 3: codon 61; exon 4: codon 146).

The trial involved 40 specimens extracted from our series of 150 mCRCs. Such liver metastases harbored mutations of *KRAS*, *NRAS*, *BRAF*, and/or *PIK3CA* and successfully engrafted in mice. Thirty-two samples (67.6%) harbored a *KRAS* mutation: 22 (55%) in codon 12; 5 (12.5%) in codon 13; 1 (2.5%) in codon 61; and 4 (10%) in codon 146. Seven tumors (17.5%) exhibited a *PIK3CA* mutation located in exon 9 [4 (10%)] or exon 20 [3 (7.5%)]. With the exception of one exon 20 mutation, all other PIK3CA mutations [6 (15%)] occurred concomitantly with KRAS mutations. Four cases (10%) exhibited *NRAS* mutations [1 in codon 12 (2.5%) and 3 in codon 61 (7.5%)], and 3 tumors (7.5%) displayed a *BRAF*mutation at codon 600 (Table 1; Supplementary Table S2). Among the 33 cases for which relevant information was available, 4 (12%) had undergone previous adjuvant chemotherapy, 8 (24%) had undergone neo-adjuvant chemotherapy, and 9 (27%) had undergone both treatments.

Trial design and treatment plan

The major signaling pathways controlled by Ras include the RAF/MEK/ERK cascade and the PI3K/AKT/mTOR axis. On the basis of preclinical studies, there is now general agreement that individual or concomitant blockade of these pathways might affect growth of tumors displaying constitutive Ras hyperactivation (24). However, Ras signals include a wealth of components whose net biochemical output is a result of multilayered feedback and crosstalk, implying that single transducer inhibition can be bypassed or dampened by compensatory signaling routes. For example, selective inhibition of BRAF in a KRAS-mutant context results in paradoxical stimulation of MEK/ERK signaling by favoring the formation of active BRAF–CRAF or CRAF–CRAF dimers (25, 26); similarly, inhibition of mTOR disrupts the negative feedback that mTOR normally operates on PI3K signaling, leading to increased activity of the PI3K downstream effector AKT (27, 28). At the therapeutic level, the critical issue is finding a rationale for identification of the Ras-dependent signals that, once neutralized, are likely to exert the highest collapsing activity on the system while avoiding the emergence of escape mechanisms.

We reasoned that a meaningful approach to intercept RAF signaling, while minimizing the deleterious consequences of potential CRAF activation, could be inhibition of MEK, which is the direct substrate of both BRAF and CRAF; along the same line, we decided to block PI3K signaling by simultaneous obstruction of both PI3K and mTOR. As therapeutic tools, we chose the MEK inhibitor AZD6244 (selumetinib) and the dual PI3K/mTOR inhibitor BEZ235. Selection of these compounds relied on the finding that combination of these 2 inhibitors induced regression of established tumors in a genetically defined mouse model of KRAS-driven lung tumorigenesis (24). *In situ* examination of representative tumors using phospho-specific antibodies directed against MEK and PI3K downstream transducers revealed that target inhibition was successful: administration of AZD6244 impaired phosphorylation of the MEK substrate ERK, with minor effects on the PI3K distal effector S6, whereas BEZ235 induced depletion of S6 phosphorylation without affecting phospho-ERK levels; as expected, combined treatment with AZD6244 and BEZ235 abrogated phosphorylation of both ERK and S6 (Fig. 1A).

Our recent validation trial with cetuximab was done using mCRC specimens that were propagated through serial *in vivo* passaging until production of treatment cohorts consisting of 6 to 12 mice (19). One strong element that emerged from this effort was the widespread consistency in response to cetuximab among tumors belonging to the same cohort (i.e., to the same patient), indicating that tumor behavior in one mouse was largely representative of the entire cohort and likely recapitulated the biologic characteristics of the original cancer lesion. This prompted us to design this study using a different strategy: by single-step *in vivo* propagation, we obtained 4 mice bearing tumors from one original sample/patient. These 4 mice represented independent arms that underwent treatment in parallel with 4 different regimens: (i) placebo, (ii) AZD6244 alone, (iii) BEZ235 alone, (iv) AZD6244 + BEZ235 ("double therapy"; Fig. 1B). In this format, in which each tumor in each mouse identifies a "case," results are to be interpreted mainly by looking at the distribution of response rates in the overall population. Evaluation time points were established at 3 weeks for mice receiving placebo (a time point when tumor burden in most animals neared the maximum volume allowed by Institutional and International Ethical Guidelines) and 3 and 6 weeks for mice receiving the targeted inhibitors. To assess whether inactivation of Ras signals could restore sensitivity to cetuximab, a fifth treatment arm was planned in which mice treated with placebo for the first 3 weeks crossed over to combinatorial treatment with AZD6244, BEZ235, and cetuximab ("triple therapy") for the subsequent 3 weeks (Fig. 1B).

For monitoring tumor response to therapy, we measured volumetric changes and used an arbitrary classification method partially based on clinical practice (19): (i) tumor regression (or shrinkage) was defined as a decrease of at least 50% in tumor volume with respect to the baseline tumor volume; (ii) disease progression was defined as an increase of at least 35% in the volume of target lesions; and (iii) finally, responses that were neither sufficient reduction to categorize regression nor sufficient increase to categorize progression were considered as disease stabilization.

Effects of MEK and PI3K/mTOR inhibitors

Figure 2 depicts a waterfall plot of the effect of the various therapeutic regimens on tumor growth after 3 weeks of compound administration. For each treatment arm, cases are independently ranked by tumor volume compared with values at baseline. Spontaneous tumor growth rates in placebo-treated animals are shown in Supplementary Fig. S1. We detected disease stabilization in 11 cases treated with AZD6244 alone (27.5%; Fig. 2A; Supplementary Table S2), 17 cases treated with BEZ235 alone (42.5%; Fig. 2B; Supplementary Table S2), and 28 cases treated with the AZD6244 + BEZ235 combination (70%; Fig. 2C; Supplementary Table S2). Mean tumor growth was +206% (range, +44% to +440%) in the arm treated with placebo, +107% (range, −18% to +327%) in the arm treated with AZD6244 ($P = 4 \times 10^{-5}$), +71% (range, −28% to +351%) in the arm treated with BEZ235 ($P = 4 \times 10^{-8}$ against placebo; $P = 0.066$ against AZD6244), and +17% (range, −49% to +150%) in the arm treated with AZD6244 + BEZ235 ($P = 6 \times 10^{-11}$ against placebo; $P = 4 \times 10^{-7}$ against AZD6244 alone; $P = 5 \times 10^{-5}$ against BEZ235 alone; Supplementary Table S2). Finally, concomitant inhibition of both MEK and PI3K/mTOR proved to be the most effective therapeutic modality, although the best response was limited to tumor growth arrest rather than overt tumor regression. In monotherapy, BEZ235 displayed superior activity compared with AZD6244.

We analyzed modulation of phospho-ERK and phospho-S6 levels in representative samples from mice that displayed the full spectrum of response to double therapy with AZD6244 and BEZ235: specifically, 5 cases with evident progression (43% to 150% increase of tumor growth at 3 weeks compared with baseline: M018, M019, M043, M050, M120); 5 cases that underwent "pure" disease stabilization (tumor growth at 3 weeks compared with baseline ranging from +10% to −13%: M021, M036, M048, M057, M097); and 5 cases that displayed a certain (albeit modest) degree of tumor shrinkage (tumor growth at 3 weeks compared with baseline ranging from −34% to −49%: M004,

M080, M087, M100, M115). In all cases, the AZD6244 + BEZ235 combination abolished phosphorylation of ERK and S6 (Supplementary Fig. S2A), indicating that target inhibition occurs independently of the extent of the response. The same cases were also scored for changes in the proliferation index (Ki67 positivity) and for the presence of apoptotic cells (as evaluated by active caspase-3 staining). Treatment with AZD235 + BEZ235 did not induce evident apoptosis (Supplementary Fig. S2B). However, it led to a reduction of the proliferation index that correlated with the degree of response (Supplementary Fig. S2C). These findings reinforce the notion that the effect of combined MEK and PI3K/mTOR inhibition seems to be cytostatic rather than cytotoxic.

We then assessed therapeutic efficacy at the single-case level by cross-evaluation of the 3 mice harboring xenografts from the same original tumor and treated with the 3 different modalities. We arbitrarily assumed treatments as equipotent when the difference in the $\log_2$ratio of growth rates among the xenografts obtained from the same patient and treated with the different therapeutic regimens was less than 0.5. Using this approach, we could identify xenopatient subsets that shared similar responses to the compounds (Fig. 3): (i) in the first subgroup, AZD6244 proved to be more effective than BEZ235, and double therapy was not superior to single AZD6244 ("MEK dominance," 5 tumors, 12.5%; Fig. 3A; Supplementary Table S2); (ii) a mirror subset proved to be more sensitive to BEZ235 than to AZD6244 and, again, response was not substantially improved by combinatorial treatment ("PI3K/mTOR dominance," 14 tumors, 35%; Fig. 3B; Supplementary Table S2); (iii) different from the former categories, another subset had more benefit from double therapy than from monotherapy with either compound ("codominance," 17 tumors, 42.5%; Fig. 3C; Supplementary Table S2); (iv) finally, a small subset responded to any of the treatment modalities with similar outcomes ("indifference," 4 tumors, 10%; Fig. 3D; Supplementary Table S2). When correlating such drug sensitivity subgroups to the distribution of mutant genotypes, we found that the 3 BRAF codon 600 mutant tumors seem to be poorly sensitive to MEK inhibition and all segregated in the subset that showed preferential responsiveness to BEZ235 (Fig. 3C; Supplementary Table S2). No evident correlations emerged for the other genotypes.

After the first evaluation time point at 3 weeks, placebo-treated animals were crossed over to triple therapy with AZD6244, BEZ235, and cetuximab for the subsequent 3 weeks. In this treatment arm, 6 mice (15%) became emaciated and eventually died, likely because of therapy-related toxicity. In the 34 mice that survived treatment, addition of cetuximab provided further significant advantage: when comparing the efficacy of double and triple therapy, we found that addition of the antibody improved disease control rate (30 of 34, 88%; Fig. 4; Supplementary Table S2). Mean tumor growth in mice treated with triple therapy was −2% (range, −51% to +100%; $P = 0.011$ against double therapy).

Mice treated with AZD6244, BEZ235, or double therapy for the first 3 weeks continued on the same therapy for additional 3 weeks. Along this second observation period, 10% of mice (12 of 120) died: 4 mice were undergoing treatment with AZD6244, 3 with BEZ235, and 5 with double therapy. Tumor assessment of survivors revealed that prolonged therapy maintained disease stabilization in 5 of 36 cases treated with AZD6244 alone (13.9% vs. 27.5% at 3 weeks; Fig. 5A), 6 of 37 cases treated with BEZ235 alone (16.2% vs. 42.5% at 3 weeks; Fig. 5B), and 12 of 35 cases treated with AZD6244 + BEZ235 (34% vs. 70% at 3 weeks; Fig. 5C; Supplementary Table S2). Mean tumor growth was +267% (range, +19% to +852%) in the arm treated with AZD6244, +222% (range, −10% to +1216%) in the arm treated with BEZ235 ($P = 0.37$ against AZD6244), and +77% (range, −33% to +401%) in the arm treated with AZD6244+BEZ235 ($P = 1 \times 10^{-6}$ against AZD6244 alone; $P = 0.0014$ against BEZ235 alone; Supplementary Fig. S3). The slopes of the curves describing mean tumor growth rates experienced by the different treatment groups in the second observation period were similar to those monitored in the first 3 weeks of treatment (Supplementary Fig. S3). This indicates that the biologic activity of compounds seems to be maintained over time in the majority of xenopatients; however, in several cases, this growth-

inhibitory effect was not sufficient to fully contrast residual tumor cell proliferation and failed to induce prolonged overall benefit.

DISCUSSION

Preclinical studies in KRAS-mutant colorectal cell lines have shown that individual blockade of MEK or combined inhibition of MEK and mTOR induce cell-cycle arrest and/or apoptosis (29, 30). Accordingly, we observed that pharmacologic neutralization of MEK and PI3K/mTOR suppressed growth in a high percentage of mCRC patient–derived xenografts, an effect that was magnified by further addition of cetuximab. Although these findings provide initial evidence of widespread disease control in a tumor setting for which no effective therapies are currently available, some caveats need to be stated. First, these regimens failed to induce overt tumor shrinkage. This occurrence cannot be attributed to technical limitations of the xenopatient platform, because we observed massive regression of a subset of KRAS wild-type tumors treated with cetuximab (19).

Although objective response is a surrogate endpoint that likely predicts clinical benefit more reliably than disease stabilization, there are situations in which tumor stabilization leads to a substantial improvement of life expectancy. For example, patients with KRAS-mutant CRCs normally do not show objective response to anti-EGFR antibodies (5–13), but cetuximab-treated subjects that specifically bear mutations in codon 13 display higher rates of disease stabilization than subjects with other KRAS-mutant genotypes, which results in significantly longer overall and progression-free survival (31). This notwithstanding, it remains difficult to anticipate whether the prevalence of disease stabilization in the absence of overt tumor regression is an encouragement to, or a warning against, implementing this therapy into the clinical setting. A corollary of this finding is that the rates of tumor stabilization tended to decrease after prolonged therapy. Because the growth rates of treated tumors did not become rampant over time, this effect should not be ascribed to evasive resistance of cancer cells as a consequence of sustained drug exposure; rather, we suspect that the extent of tumor growth inhibition produced by target blockade was not sufficient to contain mass expansion in the long period. Although the kinetics of tumor growth in mouse xenografts are strongly accelerated compared with those occurring in humans, this observation suggests that disease stabilization induced in patients by MEK and PI3K/mTOR inhibition often might not translate into a durable therapeutic effect. Given the higher efficacy of the triple therapy combination with cetuximab, periodic administration of anti-EGFR antibodies might prove useful to retard tumor progression more effectively.

Intriguingly, we could identify drug sensitivity subgroups in which single inhibition of either pathway was prevalent, with little advantage provided by additional blockade of the other. This suggests that KRAS-mutant tumors might be further stratified in cases that display higher dependency on the PI3K/mTOR axis and others that preferentially rely on the RAF/MEK/ERK cascade. The sample size of the present trial is too small to conduct statistically solid correlation analyses, but at least some preliminary clues merit discussion. First, when considering individual target inhibition at the population level, inactivation of PI3K/mTOR seems to be more effective than inactivation of MEK, suggesting more widespread tumor reliance on PI3K signals rather than on the RAF/MEK/ERK pathway. Second, the 3 cetuximab-resistant BRAF-mutant tumors included in our series all underwent growth suppression in response to PI3K/mTOR inhibition but proved to be insensitive to MEK inhibition. This piece of information is counterintuitive, given the hierarchical position of RAF at the apex of the MEK/ERK cascade, and requires future studies to confirm the observation and to explore its mechanistic underpinnings.

Another aspect that deserves discussion deals with the actual clinical predictability of patient-derived, direct-transfer xenografts. We are well aware that this experimental model remains a contentious subject: the subcutaneous milieu in which specimens are routinely implanted does not

recapitulate the orthotopic context of spontaneous tumorigenesis and the host immune system is heavily compromised. These limitations could, in principle, weaken the “human mimicry” of this approach and undermine its validity in anticipating clinical response to therapy. We analytically tackled these issues in a previous study, in which we challenged the predictive value of the xenotransplantation setting by investigating whether the effects of cetuximab in mice could recapitulate findings in humans. We observed that mCRC xenopatients responded to cetuximab with rates and extents analogous to those observed in the clinic and could be stratified as responders or nonresponders on the basis of several predictive biomarkers, including *KRAS* mutations (19). Although these findings confirm the robustness of the system in mimicking the human situation, there still remains the fact that human tumors grown as mouse xenografts contain a species mismatch between cancer cells and the host stroma. Overall, it is conceivable that xenograft platforms like ours will have strong predictive potency in drug development efforts aimed to interfere with tumor-autonomous features, including driver oncogenic alterations such as the *RAS*/*RAF*/*PIK3CA* mutations explored here. Conversely, patient-derived xenografts will likely have little—if any—value for studies that explore the contribution of microenvironmental parameters to the tumorigenic phenotype, including cancer-associated fibroblasts, inflammatory cells, and angiogenic cells. Finally, xenografts from patients with prior exposure to chemotherapy might react differently to targeted therapies compared with samples from chemonaive subjects. Also in this respect, our previous validation study with cetuximab showed that the therapeutic outcome was not influenced by previous treatment history (19).

In conclusion, concomitant blockade of MEK and PI3K/mTOR resulted in prevalent induction of disease stabilization in patient-derived mCRC xenografts exhibiting *KRAS*,*NRAS*, *BRAF*, and/or *PIK3CA* mutations. Although these observations suggest potential therapeutic opportunities to delay disease progression in patients with no alternative treatment options, they also offer cautionary evidence against the occurrence of durable responses.

Disclosure of Potential Conflicts of Interest

S. Marsoni is a consultant/advisory board member for BergenBIO and EOS. No potential conflicts of interest were disclosed by other authors.

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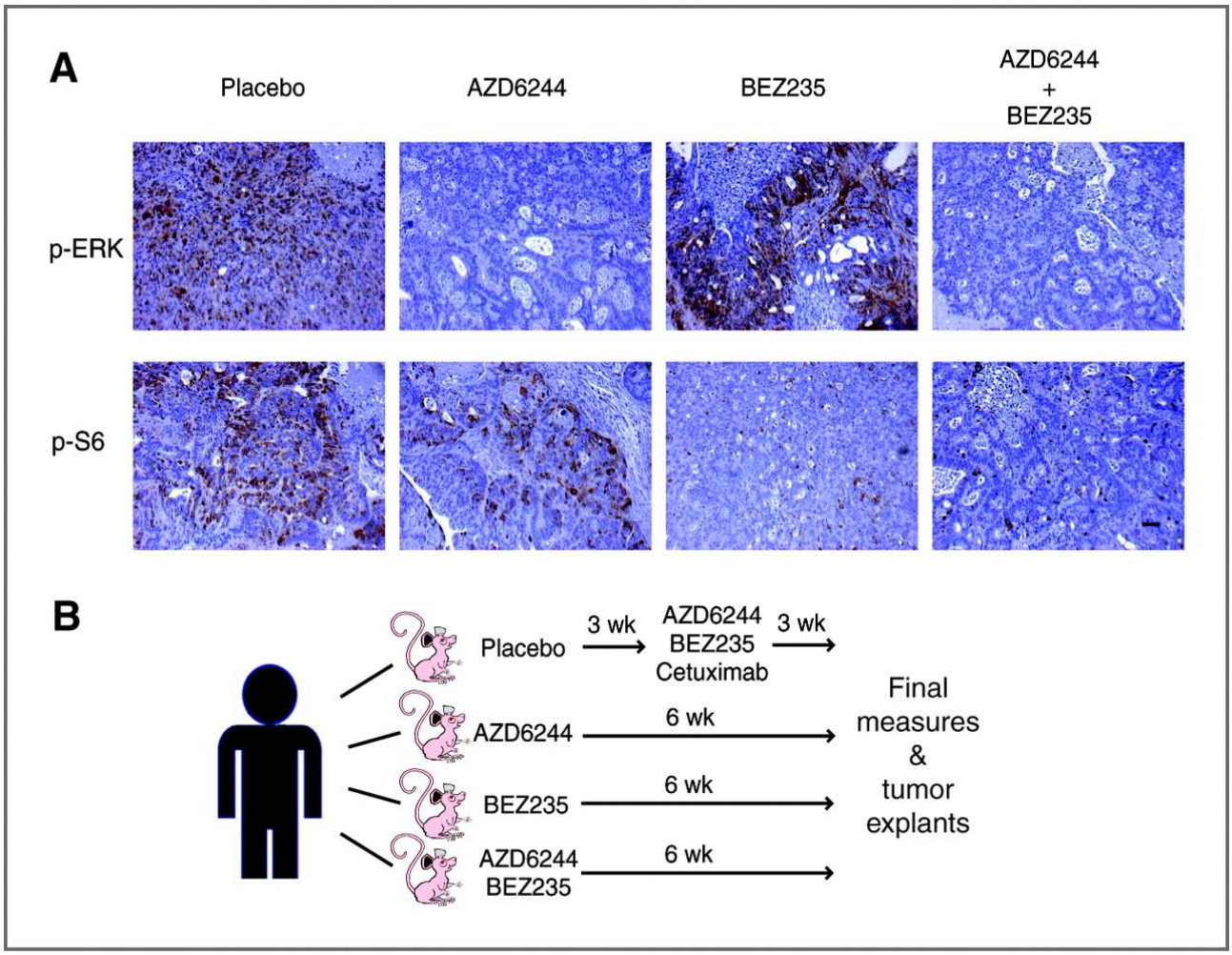

Figure 1.

Pharmacodynamic effects of AZD6244, BEZ235, and AZD6244 + BEZ235 and trial design. A, immunohistochemical assessment of the phosphorylation levels of MEK and PI3K/mTOR downstream effectors [phospho-ERK (p-ERK) and phospho-S6 (p-S6), respectively] in representative tumors derived from case M019 at the end of treatment. Scale bar, 100 μm. B, trial design. Each patient-derived sample was passaged by single-step propagation into 4 mice that underwent treatment in parallel with the indicated different regimens. Mice treated with placebo for the first 3 weeks crossed over to combinatorial treatment with AZD6244, BEZ235, and cetuximab for the subsequent 3 weeks.

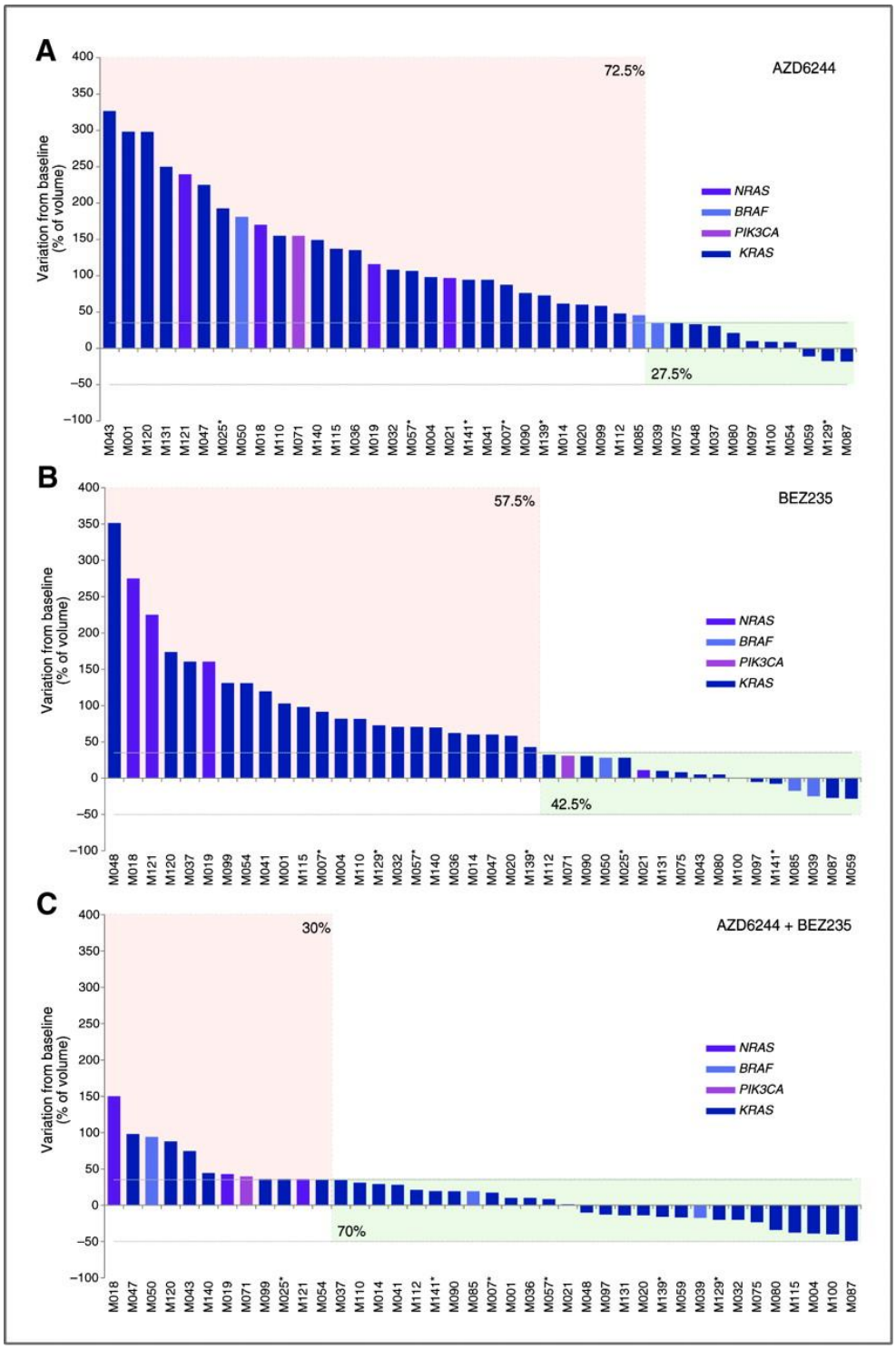

Figure 2.

Effects of monotherapy with AZD6244 or BEZ235 and double therapy with AZD6244 + BEZ235 in mCRC xenopatients after 3 weeks of treatment. A–C, waterfall plot of response to AZD6244 (A), BEZ235 (B), and double therapy with AZD6244 + BEZ235 (C), normalized against tumor volume at baseline. Dotted lines indicate the cutoff values for arbitrarily defined categories of therapy response: cases experiencing disease progression or stabilization are shaded in light brown and aquamarine, respectively. Mutational status is specified by the color of histogram bars. When*PIK3CA* mutations coexist with *RAS* mutations, the color refers to *RAS* mutations and*PIK3CA* mutations are denoted with an asterisk placed after case identity under each histogram bar.

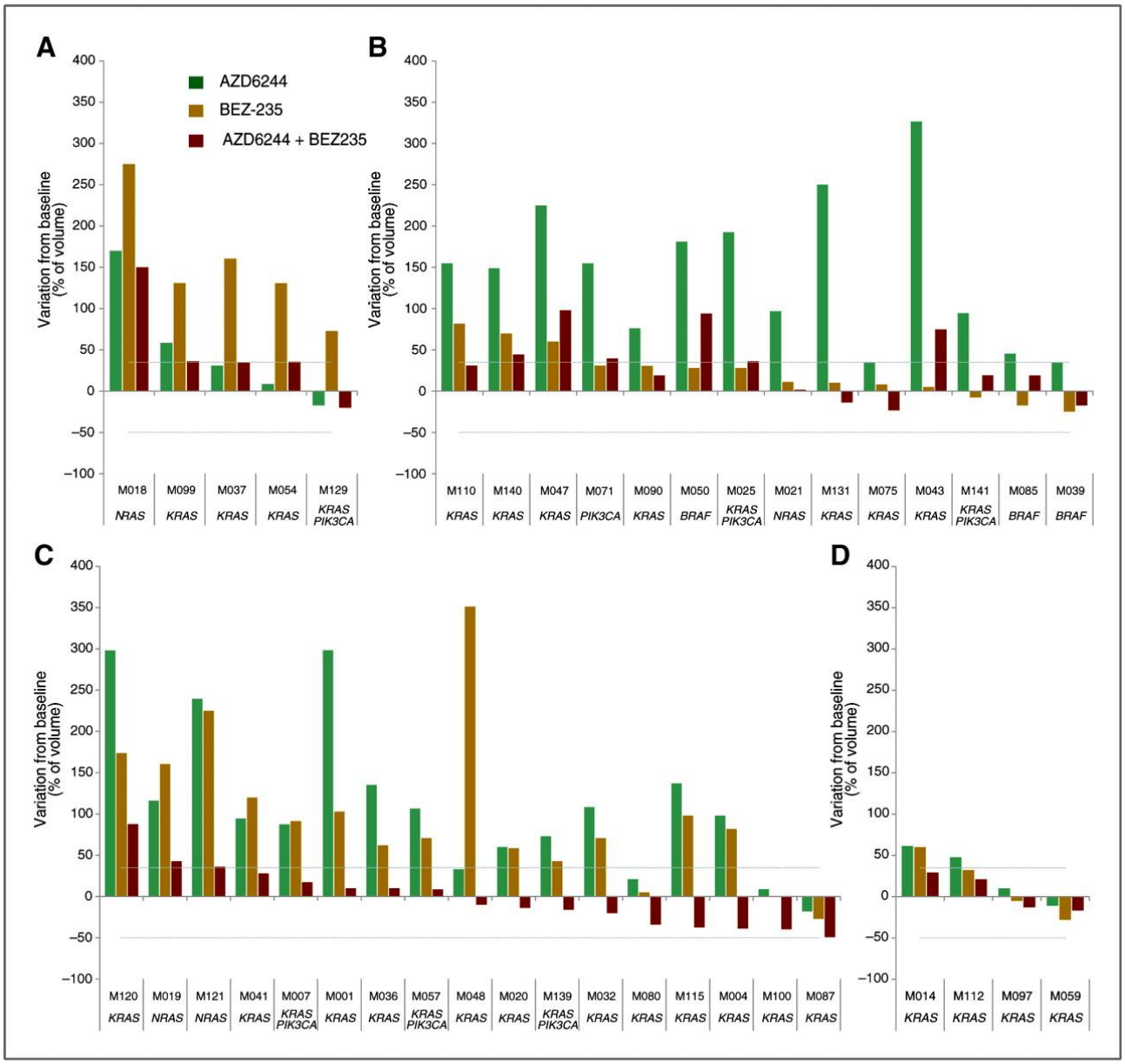

Figure 3.

Analysis of response to AZD6244, BEZ235, or AZD6244 + BEZ235 at single-case level and identification of response clusters to specific regimens. A, MEK dominance; best response is produced by AZD6244. B, PI3K/mTOR dominance; best response is produced by BEZ235. C, codominance; best response is produced by double therapy with AZD6244 + BEZ235. D, indifference; any of the treatments is equally effective. Colors of the histogram bars indicate type of treatment. Genetic mutations are specified for each sample/xenopatient under the case identities.

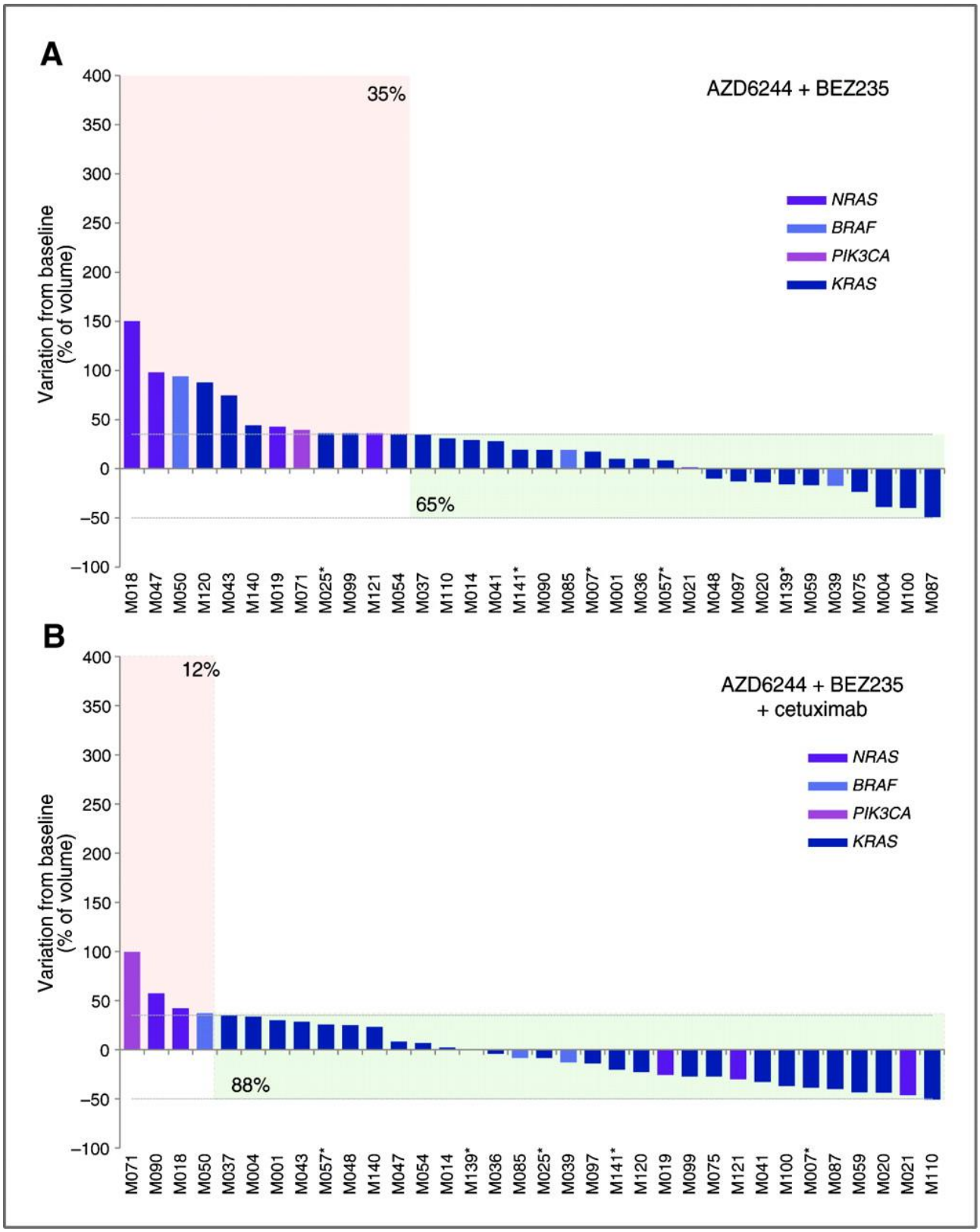

Figure 4.

Effects of double therapy with AZD6244 + BEZ235 and triple therapy with AZD6244, BEZ235, and cetuximab in mCRC xenopatients after 3 weeks of treatment. A, waterfall plot of response to double therapy for the same cases treated with triple therapy, extracted from data shown in Fig. 2. B, waterfall plot of response to triple therapy.

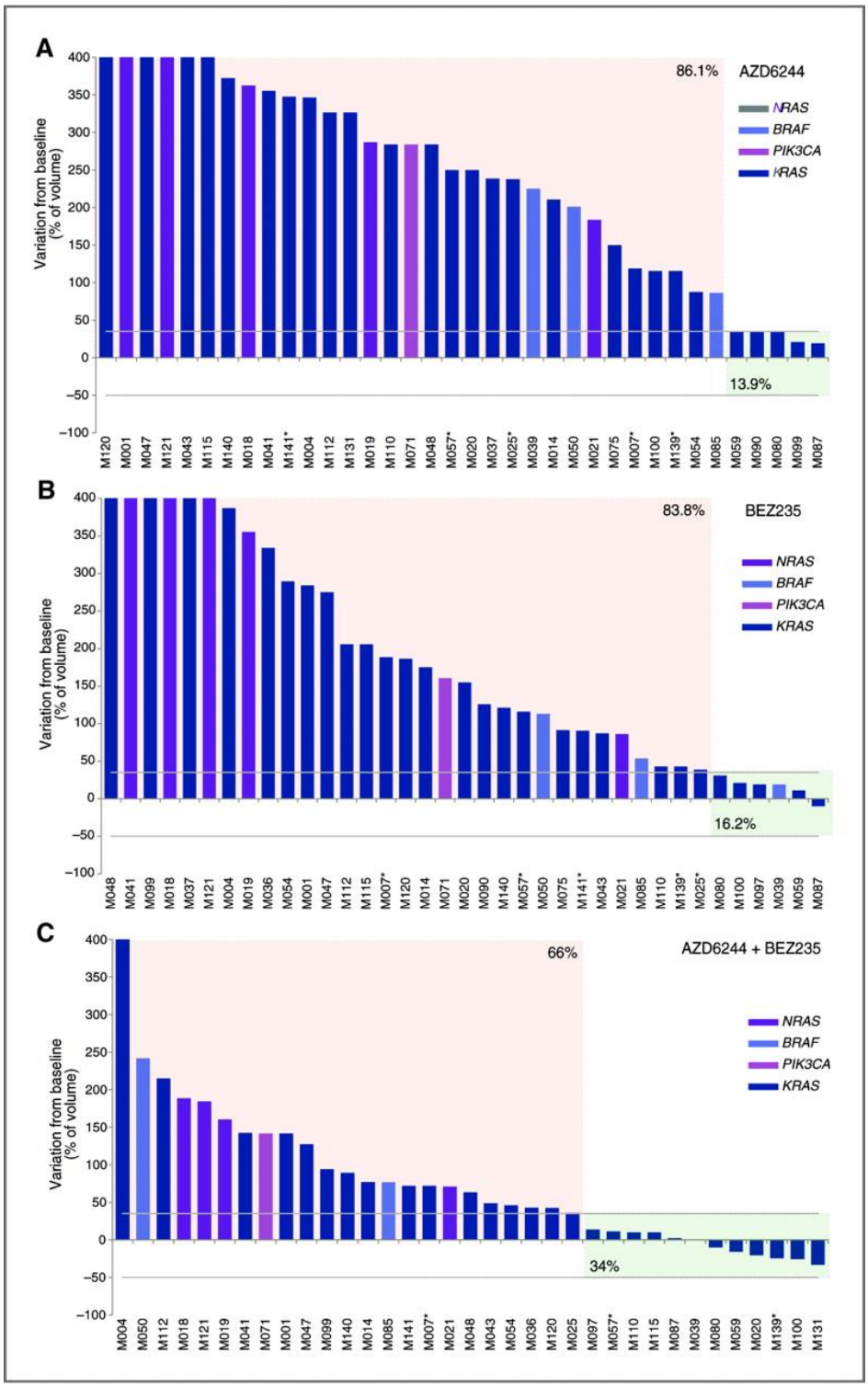

Figure 5.
Effects of monotherapy with AZD6244 or BEZ235 and double therapy with AZD6244 + BEZ235 in mCRC xenopatients after 6 weeks of treatment. A–C, waterfall plot of response to AZD6244 (A), BEZ235 (B), and double therapy with AZD6244 + BEZ235 (C). Tumor volume increases over 400% with respect to volume at baseline are not represented.