MET-driven resistance to dual EGFR and BRAF blockade may be overcome by switching from EGFR to MET inhibition in *BRAF* mutated colorectal cancer

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Abstract

A patient with metastatic *BRAF*-mutated colorectal cancer initially responded to combined EGFR and BRAF inhibition with panitumumab plus vemurafenib. Pre-existing cells with increased MET gene copy number in the archival tumor tissue likely underwent clonal expansion during treatment, leading to the emergence of *MET* amplification in the re-biopsy taken at progression. In *BRAF*-mutated colorectal cancer cells, ectopic expression of MET conferred resistance to panitumumab and vemurafenib, which was overcome by combining BRAF and MET inhibition. Based on tumor genotyping and functional *in vitro* data, the patient was treated with the dual ALK-MET inhibitor crizotinib plus vemurafenib, thus switching to dual MET and BRAF blockade, with rapid and marked effectiveness of such strategy. Although acquired resistance is a major limitation to the clinical efficacy of anticancer agents, the identification of molecular targets emerging during the first treatment may afford the opportunity to design the next-line of targeted therapies, maximizing patient benefit.

Statement of significance

MET amplification is here identified – clinically and pre-clinically - as a new mechanism of resistance to EGFR and BRAF dual/triple block combinations in *BRAF*-mutated colorectal cancer. Switching from EGFR to MET inhibition, while maintaining BRAF inhibition, resulted in clinical benefit after the occurrence of MET-driven acquired resistance.

Introduction

Anticancer targeted therapies are hardly ever curative. Even in the setting of precision medicine, targeted agents fail to achieve prolonged responses in the vast majority of cases. Molecular characterization of tumor samples taken at relapse can inform about the mechanisms underlying acquired drug resistance and drive subsequent lines of therapy with the ultimate goal of prolonging disease control.

Combinations of anti-EGFR monoclonal antibodies and BRAF inhibitors (BRAFi), with or without other agents such as MEK or PI3K inhibitors, are emerging as an effective strategy to target *BRAF* V600E mutated metastatic colorectal cancer (CRC) (1-6). Therefore, *BRAF* mutations are evolving from a poor prognostic biomarker and a negative predictive factor for single-agents anti-EGFRs or BRAFi (7,8), to a positive selection marker for BRAFi-based dual or triple combinations. Nevertheless, the long-term efficacy of these targeted combinations is limited by the relatively rapid emergence of drug resistance (1, 2). Scant data are available about the molecular mechanisms underlying acquired resistance to targeted agents combinations in CRC. A previous study including three patients reported that acquired resistance to BRAFi in combination with either EGFR or MEK blockade in CRC cell lines and/or clinical samples is caused by the emergence of MAPK pathway molecular alterations including amplification of *KRAS* or mutated *BRAF* V600E, or mutations in *KRAS* or *MAP2K1* (9). However, the identification of the above mentioned molecular mechanisms has had no or very limited impact on the clinical management of individual *BRAF* mutant patients. In this work, we present how defining the spectrum of resistance mechanisms is critical to develop optimal subsequent treatment strategies aimed at prolonging survival. By genotyping a tissue biopsy, we identified the emergence of *MET* gene amplification as a mechanism of acquired drug resistance in a *BRAF* mutated metastatic CRC patient initially treated with the combination of panitumumab and vemurafenib. We describe how the combined data obtained by tumor molecular profiling and functional experiments in cell lines assisted therapeutic decision-making and offered the opportunity to deliver precision oncology.

Results

Emergence of *MET* gene amplification upon acquired resistance to panitumumab and vemurafenib

The patient clinical history is summarized in **Figure 1**. Approximately two years after multimodality treatment for *BRAF* V600E mutated, microsatellite stable mucinous rectal cancer, a 48-year-old man was diagnosed with pelvic relapse and liver metastases. First-line therapy with FOLFOXIRI obtained a disease stabilization lasting 9 months, indicating that intensive triplet treatment may achieve best outcomes in the poor-prognosis BRAF mutant mCRC patient subset. Following liver disease progression, the patient was given off-label treatment with panitumumab and vemurafenib according to a previously published phase II study (5). Within days of treatment start the patient symptoms improved and, after 2 months, a CT scan showed a partial response according to RECIST version 1.1 criteria **(Figure 2)**. Unfortunately, after 4 months, disease progression occurred (**Figure 2**). At this time, a liver tumor biopsy was obtained in order to investigate molecular mechanisms of acquired drug resistance. Molecular analyses were carried out in parallel both in pre-treatment primary tumor tissue and in post-treatment liver biopsy. Amplicon-based next-generation sequencing (NGS) of selected exons in 110 genes (Supplementary Table S1) did not identify nucleotide variants previously implicated in resistance to EGFR monoclonal antibodies or BRAF target therapies. In details, this analysis ruled out secondary mutations in likely candidate resistance genes including *EGFR*, *KRAS*, *NRAS* or *MAP2K1*.

Since amplicon-based NGS approaches do not allow accurate assessment of gene copy number changes, we performed immunohistochemical and *in situ* hybridization (ISH) analyses of HER2 and MET, as the activation of these genes had previously been associated with acquired resistance to EGFR monoclonal antibodies in CRC (10,11). No changes in HER2 expression or gene copy number status were observed (Supplementary Table S2). However, a marked difference was seen in MET expression (**Figure 2**). Analysis of the archival rectal sample showed heterogeneous MET immunostaining, with a strong membrane signal in about 50% neoplastic cells (H-score: 150), while the remaining cell population showed a weak to moderate membranous staining. Heterogeneity was also observed in *MET* gene copy number. By ISH (FISH and SISH) analyses, we estimated that the signals corresponding to the MET and CEP7 (chromosome seven α-centromeric) probes ranged from 1 to 8 (mean values of 3.52 and 2.89 for MET and CEP7, respectively), with a gene-to-chromosome ratio of 1.2, indicating chromosome 7 polysomy. However, in a small fraction of cells within the pre-treatment sample, the gene-to-chromosome ratio was ≥6, indicating subclonal *MET* gene

amplification **(Figure 2**, Supplementary Table S2). In the post-progression liver biopsy, MET was strongly expressed at the membrane level in about 90% neoplastic cells (H-score: 270). In this sample, *MET* copy number varied from 1 to 30 (mean 8.5), while CEP7 ranged from 1 to 16 (mean 3.24) with an overall gene-to-chromosome ratio of 2.62. At least 75% cells in the liver biopsy were found to carry *MET* gene amplification (**Figure 2**, Supplementary Table S2) (defined by a gene-to-chromosome ratio ≥6), indicating that dual EGFR-BRAF blockade had positively selected for *MET* amplified clones.

Since *MDM2* gene amplification has frequently been found to co-occur with *MET* genetic alteration in lung cancer (12), we assessed MDM2 gene copy number by ISH. This analysis suggested that dual BRAF and EGFR treatment had selected for *MDM2*-coamplified clones (**Supplementary Figure S1,** Panels **A-B**).

In order to investigate the prevalence of *MET* amplification in the context of BRAF mutant CRC, we performed *MET* ISH analysis on archival tumor tissue of 17 BRAF V600E advanced CRC samples. Three out of 17tumors (18%) from chemonaïve patients displayed *MET* amplified subclones (**Supplementary Figure S2,** Panels **A-D**).

Overexpression or amplification of MET confers resistance to targeted therapy combinations in BRAF mutant CRC cell lines

In order to assess whether MET overexpression alone is causally responsible for resistance to combinatorial vemurafenib and panitumumab treatment, we conducted *in vitro* forward genetic experiments. We took advantage of the *BRAF* mutant colorectal cancer cell line WiDr, which we had previously shown to be refractory to either single agent BRAF or EGFR inhibitors, but sensitive to their combination (13). Ectopic overexpression of MET in WiDr parental cells was able to confer resistance to combined vemurafenib and panitumumab by activating ERK signaling (**Figure 3A**) and sustaining proliferation (**Figure 3B**). Importantly, addition of the dual ALK-MET inhibitor crizotinib was able to restore sensitivity to combined vemurafenib and panitumumab in MET transduced cells (**Figure 3B**).

In vitro modeling of acquired resistance in cancer cell lines has proven effective in identifying resistance mechanisms that occur clinically (14,15). From a comprehensive effort aimed at defining the molecular landscape of resistance to targeted therapy combinations in *BRAF* mutant CRC cell lines (16), we detected the emergence of MET increased gene copy number and overexpression (**Figure 3C**) in a drug resistant WiDr subline (WiDr Res.) obtained by prolonged exposure of parental cells with cetuximab in association with the BRAF inhibitor encorafenib and the selective

PI3K-α inhibitor alpelisib (**Figure 3D**), a triplet regimen that is being tested in clinical trials (1). Functional experiments indicated that ectopic expression of MET in WiDr parental cells is able to confer resistance not only to combined BRAF and EGFR blockade, but also to the triplet regimen that includes PI3K inhibition (**Figure 3D**). WiDr resistant derivatives exhibited basal levels of activated RAS-GTP protein (**Supplementary Figure S3**) and constitutive phosphorylation of ERK despite combinatorial treatment with encorafenib+cetuximab+alpelisib (**Figure 3E**). WiDr resistant cells displaying *MET* amplification were clearly refractory to EGFR, BRAF and MEK targeted therapies, either as single agents or in combination (**Figure 3F**). Importantly, crizotinib alone showed no effects on the proliferation of WiDr resistant cells, whereas its combination with BRAF kinase inhibition by vemurafenib led to a marked decrease of cell viability over prolonged times (**Figure 3F**).

Altogether, these results directly underpin a causal relationship between *MET* amplification and resistance to targeted therapy combinations in *BRAF* mutant CRC and suggest a potential therapeutic strategy to overcome MET-mediated resistance.

Subsequent MET and BRAF targeted therapy overcomes clinical resistance to EGFR-BRAF dual block

The subsequent clinical history of the patient is again summarized in **Figure 1**. After 2 cycles of TAS-102, pelvic pain became severe and opioids were insufficient to achieve optimal symptom relief. Disease re-assessment with CT and FDG-PET/CT scans confirmed the rapid progression of the disease both loco-regionally (pelvic relapse with penis bulbs involvement and bilateral inguinal lymph nodes) and in the liver (**Figure 4A**). Prescription of regorafenib was contraindicated due to urinary fistula. In absence of further treatment options, based on tumor genotyping and preclinical data, and after ethical approval, combined MET and BRAF inhibition was considered as a rationale choice. Given the recent phase 1 data of crizotinib and vemurafenib combination (17), the patient started this combinatorial regimen in January 2016. After one week from treatment start, pelvic pain completely disappeared and the use of opioids was dramatically reduced. After 10-day treatment, a FDG-PET/CT scan showed an early and dramatic metabolic response (**Figure 4B**). Serum CA19-9 marker declined by >50% after only 2 weeks, and almost by 90% after 4 weeks (**Figure 1**). Treatment is still ongoing without significant toxicity.

Discussion

In this case study, we found for the first time that *MET* amplification and protein over-expression was the main molecular driver of clinical resistance to an anti-EGFR plus BRAFi combination. *MET*

amplification has been previously identified as a mechanism of resistance to targeted agents in other settings, including *BRAF* mutated melanoma treated with vemurafenib (10,18,19). *MET* amplification was also found in approximately 5%-20% of *EGFR*-mutated non-small cell lung cancers with acquired resistance to EGFR tyrosine kinase inhibitors (20) and in 10-20% *RAS/BRAF* wild-type CRC at progression upon anti-EGFR monoclonal antibodies (9). It was previously reported that *MET* amplification emerges in malignancies with pre-existing clones of *MET* amplified cells (21), which undergo positive selection during anti-EGFR based therapy in both NSCLC and CRC patients (10). The same seems to be true for the patient in our study, as molecular and histological analyses identified the presence of a subpopulation of *MET* amplified cells in the tumor sample before treatment with BRAF targeted therapy. The percentage of cells carrying *MET* gene copy amplification increased by at least five-fold in the post-treatment liver biopsy, indicating that dual EGFR-BRAF blockade had positively selected for *MET* amplified clones. We argue that the presence of a detectable fraction of pre-existing *MET* amplified cells has impacted negatively on the duration of response to dual EGFR and BRAF blockade, which was relatively short-lived. It is possible that in this case, combining upfront EGFR, MET and BRAF inhibition might have led to a more durable response and we suggest that future clinical studies should further investigate this aspect.

The proportion of *BRAF* mutant colorectal tumor samples co-harboring MET molecular alterations is unknown, but a large series of 1115 samples from any tumor types has previously reported that *MET* amplification was associated with a higher prevalence of *BRAF* mutation compared with non-amplified tumors (22). In our collection of 17 additional *BRAF* mutant CRC samples from chemonaive patients, we were able to find MET amplified subclones in three cases, Although none of the three patients actually received BRAF target agents, it is tempting to speculate that subclonal genetic diversity in these tumors could negatively influence the duration of response to BRAF combinatorial therapies due to likely clonal selection of MET amplification, On the other hand, it is possible that *MET* amplification could also be implicated in *de novo* resistance to target therapy combinations in BRAF mutant colorectal cancer. Pre-treatment tumor samples from non-responding patients enrolled in phase I-II studies with BRAFi combination therapies (2-6) will be essential to address this clinically relevant question.

Our preclinical experiments indicated that *MET* amplification emerged also as a driving mechanism of resistance in *BRAF* mutant CRC cells selected with cetuximab in combination with the BRAFi encorafenib and the PI3K inhibitor alpelisib. Ectopic overexpression of MET was able to confer resistance not only to panitumumab plus vemurafenib but also to the above clinically relevant triplet

which includes PI3K inhibition (1). This strongly suggests that MET activation is able to confer resistance to several BRAFi+EGFRi-based combinations, irrespective of the specific drugs used. Pharmacological data in CRC cells indicated that MET inhibition alone was ineffective to overcome resistance and that crizotinib should be combined with BRAF blockade to affect the proliferation of MET-overexpressing cells both in short-term and in long-term growth assays. This is consistent with what has been reported very recently in BRAF inhibitor resistant melanoma patient derived xenograft models, in which only combined vertical blockade of the pathway with MET and BRAF inhibitors was shown to induce durable regression in mice (23).

Based on preclinical modeling and functional characterization of MET aberrant signaling as a resistance mechanism to BRAF and EGFR dual blockade, the patient was treated with the combination of crizotinib and vemurafenib. The ALK inhibitor crizotinib is well known to bind also to MET tyrosine kinase domain and has shown clinical activity in MET amplified cancers (24,25). Moreover, recent phase 1 data showed that crizotinib may be safely combined with vemurafenib without occurrence of significant dose-limiting toxicities (17). Imaging revealed that switching from EGFR to MET blockade, while maintaining BRAF inhibition induced an extremely rapid and dramatic response following the initiation of treatment with crizotinib plus vemurafenib.

In this case, molecular analysis of a single-lesion biopsy did not identify any additional MAPK pathway molecular alterations beyond MET amplification that could eventually lead to treatment failure. Yet we admit that our strategy could have missed other resistance alterations occurring at subclonal level in the same lesion or as main drivers in other disease sites, since tumor heterogeneity may lead to the simultaneous selection of several inter- or intra-metastatic clones carrying different mechanisms of secondary resistance (26). However, metabolic response was observed across all disease sites, with *ex juvantibus* confirmation that MET might have been driving resistance in a relatively dominant and homogeneous fashion in this individual case of *BRAF*-mutated CRC. We acknowledge that early assessment with FDG-PET/CT may not be routinely accepted as an endpoint of treatment activity. However, the role of early metabolic response has gained robust evidence since the introduction of targeted therapies. In the era of precision medicine, the dramatic FDG-PET/CT response, necessarily coupled with the clinical and laboratory data, is indicative of immediate treatment benefit – even if longer follow up is needed to assess the duration of such benefit and potential emergence of further resistance mechanisms.

Extreme caution should be applied not to over-interpret the implications of this work. Indeed, we are well aware of the potential pitfalls of selecting a targeted therapy strategy based on the molecular profile of a single resistant lesion, as exemplified by recent case reports in CRC and breast cancer

patients (26, 27). Nevertheless, we believe that this case study may highly impact future research. In fact, specific targeting of MET-driven resistance to dual EGFR and BRAF block may lead to the design of biomarker-driven trials of second-line targeted therapy. Triple EGFR, MET and BRAF co-inhibition may also be investigated as additional strategy to prevent or overcome resistance in molecularly selected patients.

Methods

Patient Care and Specimen Collection

Tumor samples were collected in accordance with an Institutional Review Board–approved protocol, to which the patient provided written informed consent, and all studies were conducted in accordance with the Declaration of Helsinki. Targeted NGS on clinical specimens using was performed in the Department of Diagnostic Pathology and Laboratory Medicine at Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy. The patient's insurance company covered the cost of off-label combinatorial therapies (panitumumab+ vemurafenib; crizotinib+vemurafenib), to which the patient gave informed consent. CT scans were obtained as part of routine clinical care, while FDG-PET/CT scans were performed as part of an ancillary study protocol for patients receiving targeted treatments. The clinical course of the patient is summarized in **Figure 1**.

Next-generation sequencing

Next Generation Sequencing **(**NGS) analysis was performed as detailed in the Supplementary Methods and in Supplementary Table S2.

Immunohistochemistry

Immunohistochemistry (IHC) was performed on 3 μm formalin fixed paraffin-embedded (FFPE) tissue sections or on WiDr cytoclots. MET protein expression was detected by using a rabbit monoclonal anti-MET antibody (dilution 1:200; clone SP44, Spring Bioscience, Pleasanton, CA), directed against the synthetic peptide derived from C-terminus of human MET displaying membranous and/or cytoplasmic epitope. Other information is provided in the Supplementary Methods.

In situ hybridization (SISH and FISH)

MET gene status was assessed respectively by both SISH and FISH analyses scored blindly by two observers (A.G. and E.V.). Bright field dual-color SISH analysis was performed on 3 μm FFPE tissue sections by using the MET DNP Probe along with the Chromosome 7 DIG Probe (Ventana Medical

Systems) on a BenchMark Ultra Platform (Ventana Medical Systems) according to the manufacture's protocol. Dual color FISH analysis, both on metaphase chromosomes and interphase nuclei, obtained from WiDr cultured cells and on 3 μm FFPE tissue sections, was performed by using D7Z1 (7p11.1-q11.1) / c-MET (7q31.2) probes (Cytocell), respectively labelled with FITC and Texas Red. Procedure information is described in the Supplementary Methods.

For both SISH and FISH analyses the probes signals were counted in at least 100 non overlapping tumor cells nuclei from each case. Two independent molecular pathologists (A.G. and E.V.) scored the slides in a blinded fashion. *MET* gene amplification was defined as positive when: a) *MET*/CEP7 ratio was $\geq$ 2 or b) average number of *MET* signals per tumor cell nucleus was > 6.

SISH analyses for *MET* status were also performed on archival tumor tissue of 17 advanced colorectal tumors carrying mutant *BRAF* V600E diagnosed at Fondazione IRCCS Istituto Nazionale dei Tumori from 2012 to 2016.

Similarly, *HER2* gene amplification was defined as positive when: a) *HER2*/CEP17 ratio was $\geq$ 2 or b) average number of *HER2* signals per tumor cell nucleus was > 6.

Bright field dual-color SISH analysis was performed for *MDM2* by using the MDM2 DNP Probe along with Chromosome 12 DIG Probe (Ventana Medical Systems) on a BenchMark Ultra Platform (Ventana Medical Systems) according to the manufacture's protocol.

Cell lines and Treatments

WiDr parental cells were obtained from Dr René Bernards (Amsterdam, The Netherlands) in July 2011. The *KRAS* mutant SW480 cell line was purchased from ATCC (LGC Promochem) in January 2011. All cells were cultured at 37°C and 5% CO2 in RPMI 1640 (Invitrogen), supplemented with 10% fetal bovine serum, 2 mM L-glutamine and antibiotics (100 U/ml penicillin and 100 mg/ml streptomycin). The genetic identity of parental cell lines and their resistant derivatives was authenticated by short tandem repeat profiling (Cell ID System; Promega) at 10 different loci (D5S818, D13S317, D7S820, D16S539, D21S11, vWA, TH01, TPOX, CSF1PO and amelogenin) not fewer than 2 months before drug profiling or biochemical experiments. Cell lines were tested and resulted negative for Mycoplasma contamination with the VenorGeM Classic Kit (Minerva Biolabs). WiDr were made resistant by continuous drug exposure to the combination of cetuximab (5 μg/ml), encorafenib (1 μM) and alpelisib (0.5 μM). A detailed protocol of the drug proliferation assays is reported in the Supplementary Methods.

Western Blot analysis

Total cellular proteins was extracted by lysing cells in boiling Laemmli buffer (1% SDS, 50 mM Tris-HCl [pH 7.5], 150 mM NaCl). The following primary antibodies were used (all from Cell Signaling Technology, except where otherwise indicated): anti-MET (clone D1C2, 1:1000); anti-phospho MET (Tyr1234/1235, Cat.#3126; 1:1,000); anti-phospho p44/42 ERK (Thr202/Tyr204; 1:1,000); anti-p44/42 ERK (1:1,000); anti-phospho AKT (Ser473; 1:1,000); anti-AKT (1:1,000); HSP90 (Santa Cruz Biotechnology; 1:500).

Viral Infection

Lentiviral vector stock were produced by transient transfection of the pCCL-MET wild-type or lenti-control plasmid (a gift of Elisa Vigna, IRCCS, Candiolo, Turin) the packaging plasmid MDLg/pRRE and pRSV.REV, and the vesicular stomatitis virus (VSV) envelope plasmid pMD2.VSV-G (25, 2.5, 6.25 and 9 μg, respectively, for 15-cm dishes) in HEK-293T cells in presence of 1mM/L sodium butyrate (Sigma) and 10 ng/ml of Doxicicline. Cells were tranduced in six well plates (3 x 10^5 per well in 2 ml of medium) in the presence of polybrene (8 μg/ml) (Sigma).

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Figure Legends

Figure 1. Summary of patient clinical history.

The clinical course of the patient with colorectal cancer is summarized, with serum cancer antigen 19-9 tumor marker levels shown throughout treatment (green line). Shaded boxes indicate periods of administration of the indicated chemotherapeutic agents. Blue vertical lines indicate timing of tumor specimen acquisition from surgical procedures or biopsy, as well as dates of tumor assessment by either CT scan or FDG-PET/CT scan.

Figure 2. MET amplification is selected for in a BRAF mutant CRC biopsy from a patient who developed resistance to combined BRAF and EGFR targeted treatments

In panel A, baseline CT scan prior to start of panitumumab and vemurafenib, showing liver involvement; in panel B, partial response to treatment according to RECIST 1.1 at week 8; in panel C, progressive disease during treatment at week 16.

In panel A1-4 and C1-4: analyses performed on archival surgical specimen and on post-progression liver rebiopsy, respectively. A1-C1. Conventionally stained section using Hematoxylin & Eosin, showing poorly differentiated carcinoma of the rectum (A1) and liver metastasis (C1) (A1-C1, original magnification x20); A2-C2. Immunohistochemical detection of MET protein. The primary tumor displays heterogeneous immunostaining ranging from weak to moderate (at the center) intensity (A2). Metastatic tumor cells display homogeneous, strong immunostaining (C2) (A2-C2, original magnification X20); A3-C3. Dual color bright field in situ hybridization (ISH) for *MET* gene (black dots) and CEP7 (red dots). The primary tumor, though not amplified, display heterogeneous, *MET* gene copy number, ranging from 1 to 7 (A3). Metastatic tumors cells contain high *MET* gene copy number (ranging from 2 to 20) featuring *MET* gene amplification (C3) (A3-C3, original magnification X20). A4-C4. Dual color FISH analysis using D7Z1 (7p11.1-q11.1) / c-MET (7q31.2) probes (Cytocell), respectively labeled with FITC and Texas Red. The primary tumor (A4, original magnification x100) shows heterogeneous, MET gene copy number, ranging from 1 to 8, while metastatic tumors cells (C4, original magnification x100) contain high *MET* gene copy number (ranging from 2 to 30) featuring *MET* gene amplification.

Figure 3. MET overexpression or gene amplification confers resistance to targeted therapy combinations in BRAF mutant CRC cells. A, BRAF mutant WiDr cells were transduced with either control (empty) or MET–expressing lentiviral vectors. Cells were then treated with vemurafenib (2 μM), panitumumab (5 μg/ml) and their combination for 5 hours prior to protein

extraction and Western blotting with total MET, phosphorylated–MET (Tyr 1234-1235), total AKT, phosphorylated-AKT (Ser473), total ERK1/2 and phosphorylated-ERK1/2 (Thr 202 /Tyr 204) antibodies. HSP90 was included as a loading control. **B,** After 96-hour treatment with panitumumab (5 μg/ml), vemurafenib (1 μM), crizotinib (0.3 μM) or their combinations, the viability of empty or lenti-MET transduced cells was assessed by relative ATP content measurement. Results represent mean ± SD of at least 2 independent observations, each performed in triplicate. ** P<0.01; *** P<0.001 Bonferroni's adjusted ANOVA *p* values; **C**, FISH analysis and immunohistochemistry (IHC) showing *MET* gene amplification (top) and MET protein overexpression (bottom), respectively, in WiDr resistant derivatives (WiDr res) generated from WiDr parental (WiDr par) cells after several weeks' continuous treatment with the triple combination encorafenib+cetuximab+alpelisib; MET (7q31)–specific probe is labeled with Texas red, whereas chromosome 7 centromeric probe D7Z1 (7p11.1–q11.1) is marked in green. FISH, original magnification 100×; IHC, original magnification ×40; **D**, Cell viability by ATP assay of WiDr parental cells and resistant derivatives, as well as WiDr transduced with MET after treatment for 96 hours with the indicated molar concentrations of encorafenib in association with constant 5 μg/ml cetuximab and 0.5 μM alpelisib. Results represent mean ± SD of at least 2 independent observations, each performed in triplicate. **E**, WiDr parental cells and their *MET* amplified subline with acquired resistance to the triple combination encorafenib+cetuximab+alpelisib were treated with the combination of encorafenib (0.4 μM), cetuximab (5 μg/ml) and alpelisib (1 μM) for 5 hours prior to protein extraction and Western blotting with the indicated antibodies. **F**, Long-term colony forming assay of *MET* amplified encorafenib+cetuximab+alpelisib resistant WiDr cells treated for 12-14 days in the absence or presence of panitumumab (EGFRi, 5 μg/ml), trametinib (MEKi, 10 nM), crizotinib (0.3 μM), vemurafenib (BRAFi, 1.5 μM) or their combinations. Results are representative of at least 2 independent observations.

Figure 4. 18F-FDG PET/C T scans at baseline and 10 days after the start of combination treatment with crizotinib and vemurafenib. Panel **A** (coronal and axial fused images) shows liver metastases (red arrows) and pelvic/inguinal lymph node involvement (white arrows) at baseline. Panel **B** (coronal and axial fused images) shows marked metabolic response at all sites of disease.

Figure 1

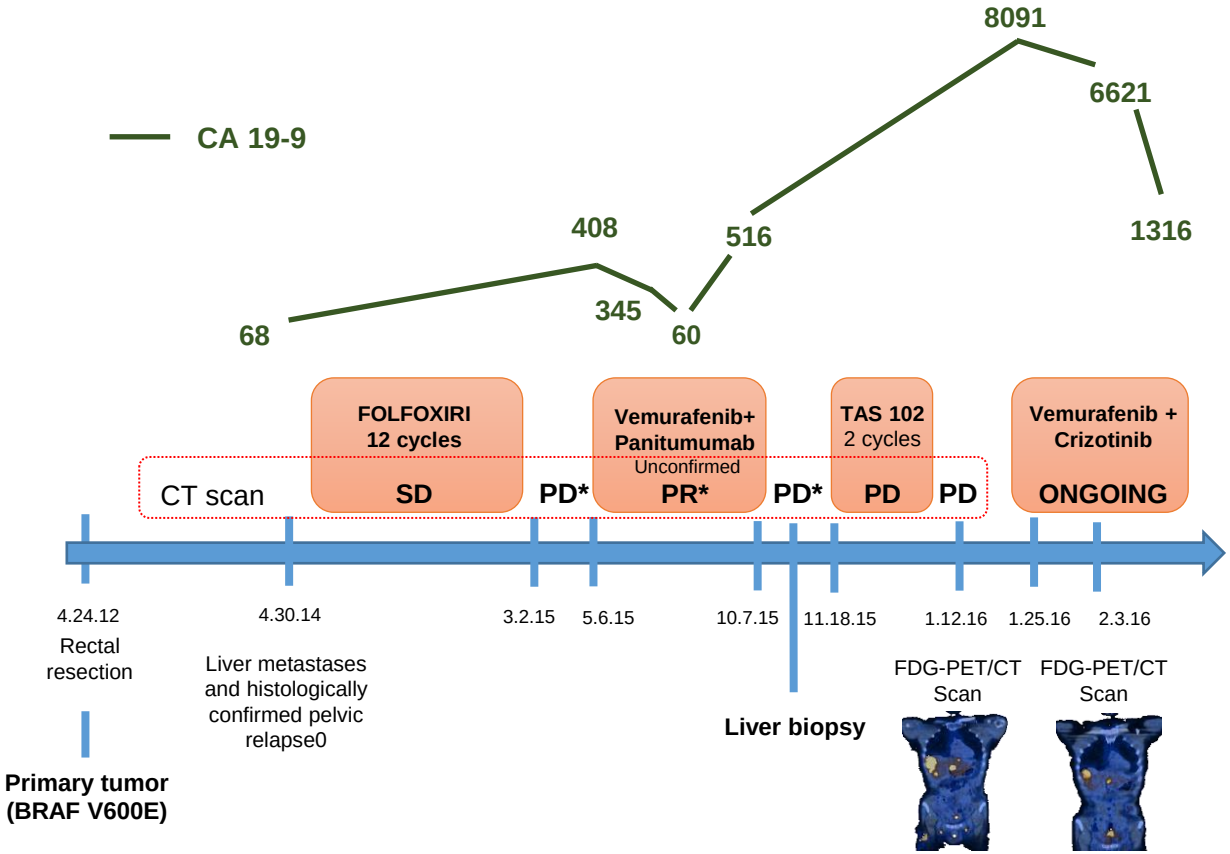
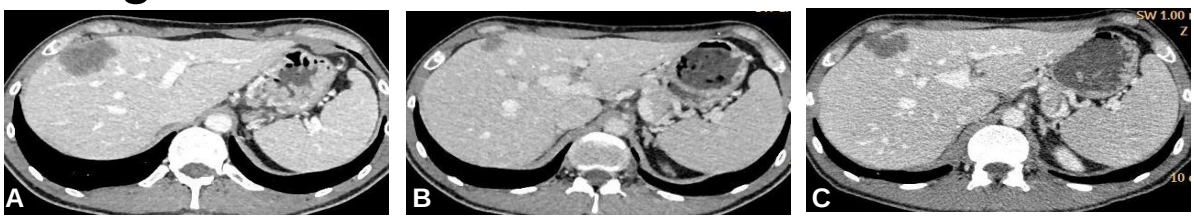
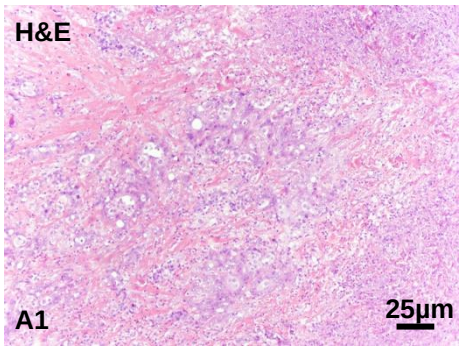
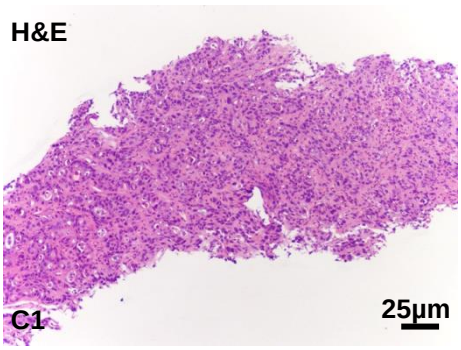
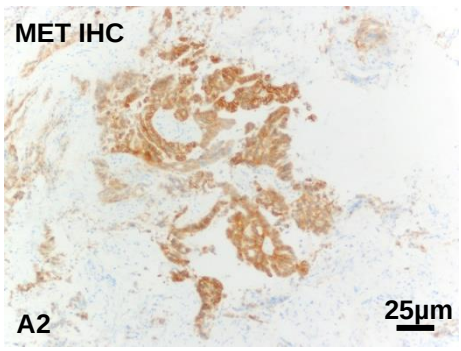


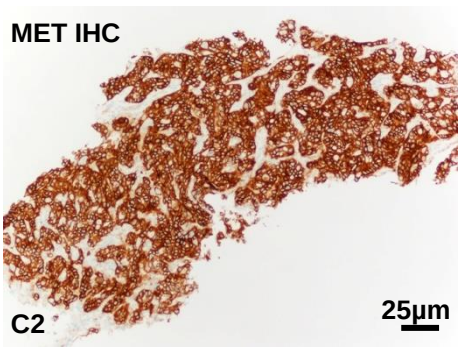
Figure 2

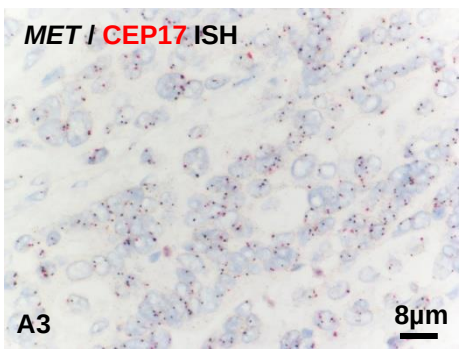


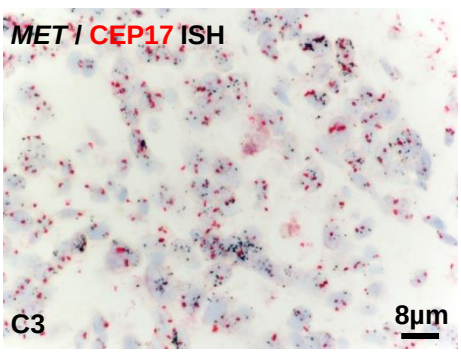


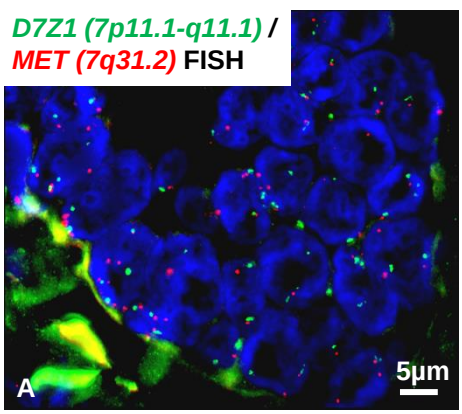


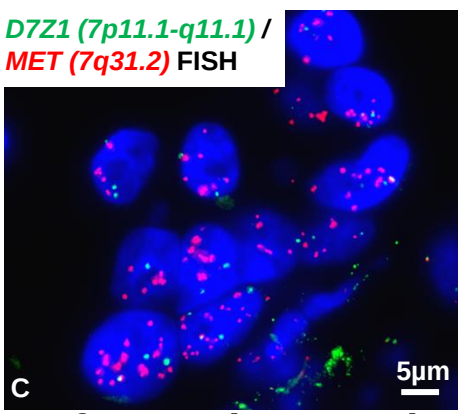












Pre-treatment

After panitumumab +vemurafenib

Figure 3

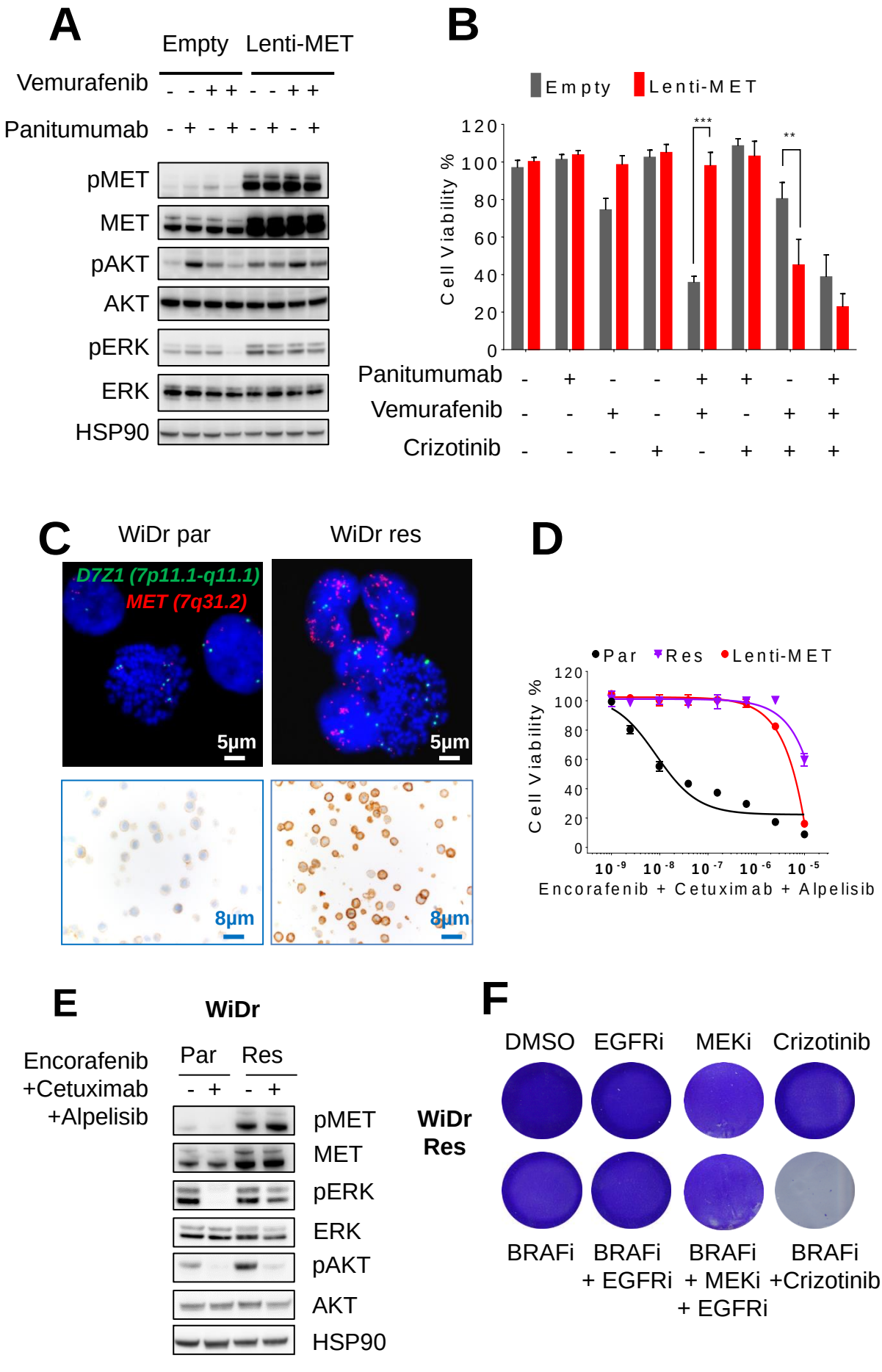
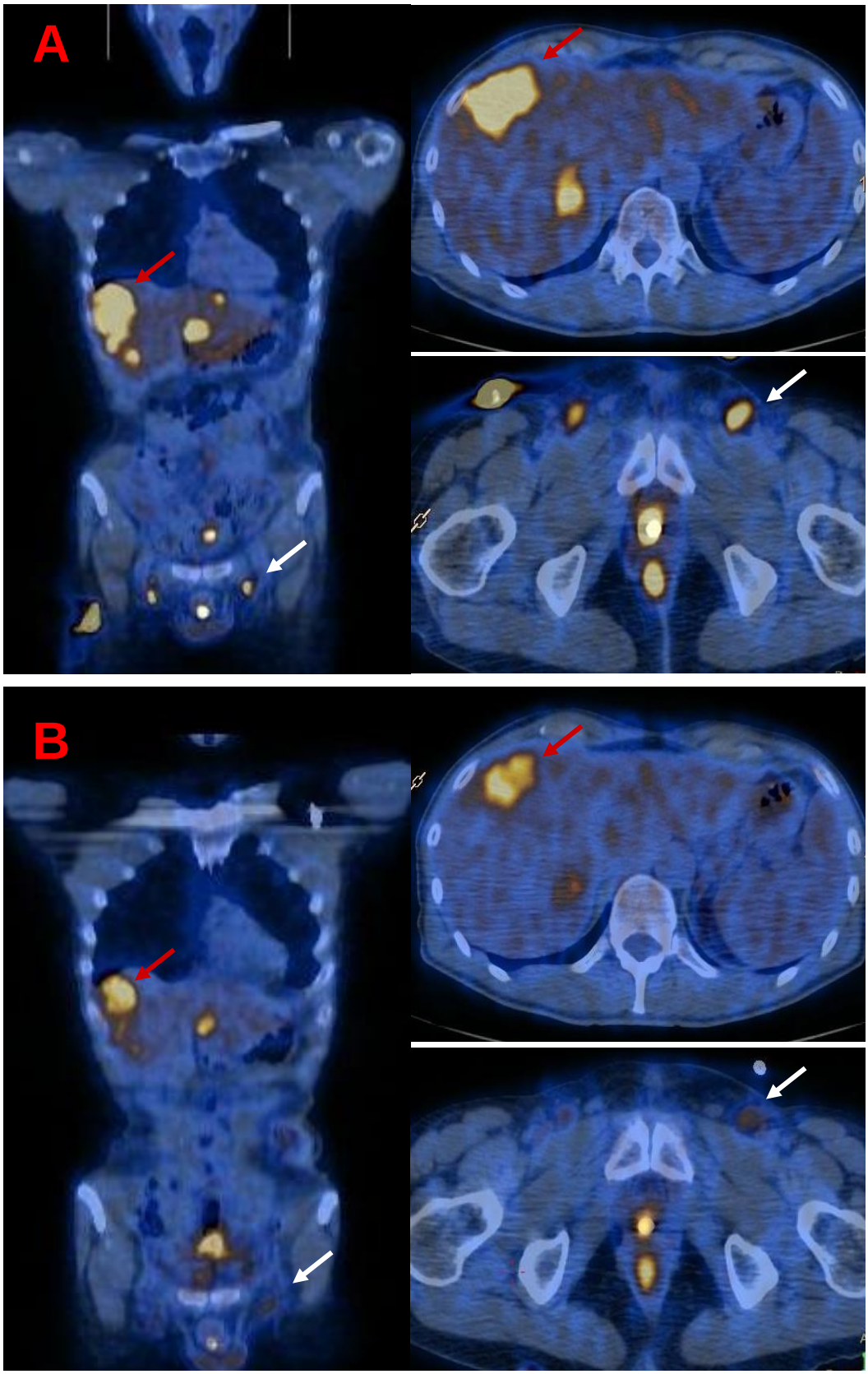


Figure 4



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CANCER DISCOVERY

MET-driven resistance to dual EGFR and BRAF blockade may be overcome by switching from EGFR to MET inhibition in BRAF mutated colorectal cancer

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