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BRIEF REPORT

Anti-MEK and Anti-EGFR mAbs in RAS-Mutant Metastatic Colorectal Cancer: Case Series and Rationale

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ABSTRACT

KRAS (Kirsten rat sarcoma viral oncogene) or BRAF (v-raf murine sarcoma viral oncogene homolog B1) constitutive activation leads to anti-EGFR (epidermal growth factor receptor) therapy resistance of metastatic colorectal cancer patients. In this article we investigate the effects of anti-MEK (mitogen-activated protein kinase) antibody (trametinib) combined with anti-EGFR (cetuximab) on colon cancer cell lines with different RAS statuses. Even though cetuximab has no effect on RAS cell viability and ERK (extracellular-signal-regulated kinase) phosphorylation (one of the last kinases of the EGFR pathway), trametinib can induce cell death and inhibit the activation of ERK alone or in combination with cetuximab. In a more pathologic context, we observed that KRAS colon cancer patient biopsies treated ex vivo with trametinib and cetuximab also present less ERK phosphorylation. Finally, nine ovarian, endometrial and colon cancer patients with different KRAS statuses were treated with anti-EGFR/anti-MEK combination off label after molecular tumor board decision. KRAS exon 2 patients have significantly longer PFS (progression-free survival) than with previous lines of treatments. We believe that such observations provide a rationale for designing a clinical trial to test this association in RAS exon 2 mutated cancers.

Cédric Rébé and François Ghiringhelli share senior authorship of this article.

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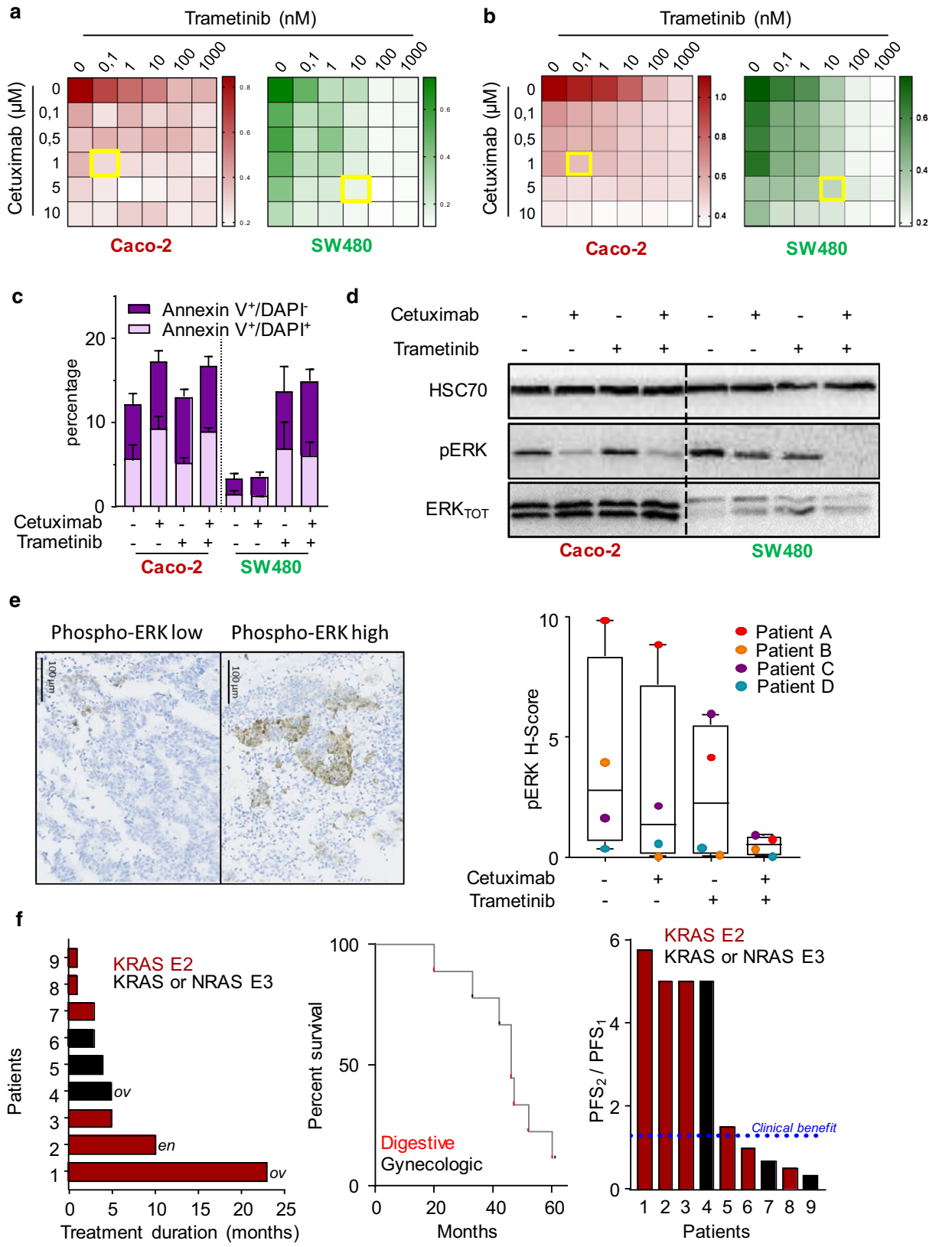
Metastatic colorectal cancer can be treated using several approaches, including targeted therapy or surgical procedures. Strategy choice depends not only on patients' general condition but also on EGFR (epidermal growth factor receptor) downstream pathways' mutational status. For instance, KRAS (Kirsten rat sarcoma viral oncogene) or BRAF (v-raf murine sarcoma viral oncogene homolog B1) constitutive activation leads to anti-EGFR therapy resistance limiting the therapeutic arsenal in patients presenting such mutations. Targeting downstream RAS effectors might be an alternative for treating these patients. However, the results with MEK (mitogen-activated protein kinase) inhibitors are modest and the number of adverse events important [1, 2]. ERK (extracellular-signal-regulated kinase) phosphorylation is a biomarker of MAPK (mitogen-activated protein kinase) signaling pathway activation [3], and targeting MEK protein or EGFR only does not completely inhibit the ERK signal [4]. However, the association of MEK inhibitors and anti-EGFR has shown a benefit on NRAS-mutant metastatic colorectal cancer cell viability in vitro [5]. This cell death induction might result from the multiple targeting proteins in the MAPK signaling pathway. Synergistic drugs can be used to treat resistant tumors with lower concentrations and decreased adverse events. In this way, we decided to investigate whether the association of an anti-MEK with an anti-EGFR can bypass resistance of KRAS mutant cells and patients. In this study, we used two human colon cancer cell lines: RAS wild-type Caco-2 cells and KRAS mutant SW480 cells. All procedures performed in this study involving human participants and material were in accordance with the ethical standards of the molecular tumor board research committee of the Centre GF Leclerc. and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Multiple concentrations of trametinib and cetuximab were tested to evaluate cellular viability by crystal violet coloration and cell proliferation with MTT assay

Fig. 1 **a**, **b** Human Caco-2 and SW480 colon cancer cell lines were treated or not with indicated concentrations of cetuximab and/or trametinib for 3 days. **a** Cell viability was analyzed using crystal violet staining, and **b** cell proliferation was assessed with MTT. Representative of the mean of three independent experiments. **c** Caco-2 and SW480 cell lines were treated or not with chosen concentrations of cetuximab and/or trametinib (see yellow squares in **a**) for 3 days, and cell death was assessed by annexin V/DAPI staining. **d** Caco-2 and SW480 cell lines were treated or not with chosen concentrations of cetuximab and/or trametinib (see yellow squares in **a**) for 2 h, and the expression of ERK and pERK was analyzed by Western blotting. HSC70 was used as a loading control. One representative of three independent experiments. **e** Colon cancer biopsies from four patients were treated or not ex vivo with cetuximab (1 μM) and/or trametinib (1 nM) for 5 h and fixed, embedded in paraffin and stained with anti-pERK. Representative images of low and high pERK expression are shown (left), and the H-score of pERK was calculated for each sample (right). **f** Six colon, one endometrial (en) and two ovarian (ov) cancer patients received trametinib (from the third to sixth line of treatment). Representation of treatment duration (left), overall survival (middle) or PFS_2 (treatment with anti-MEK)/PFS_1 (last treatment without anti-MEK) ratio evaluation (right). For more information see supplementary methods

(Fig. 1a, b and see supplementary methods). As expected, SW480 cells were resistant to cetuximab, whereas Caco-2 cells were sensitive. Moreover, trametinib inhibited cell viability in all cell lines. Starting from these results, we selected low concentrations of combined drugs (surrounded in yellow in Fig. 1a) that were able to synergistically decrease cell viability. As assessed by annexin V/DAPI staining, the effects of treatments can partly be attributed to cell death induction (Fig. 1c). The individual and combined drug effect on ERK phosphorylation was evaluated by Western blot (Fig. 1d). Results showed that cetuximab (Ctx) could not decrease the pERK signal on KRAS mutant cell lines. Trametinib was able to modestly reduce pERK, while the association of MEK inhibitor with anti-EGFR drastically inhibited ERK phosphorylation. On RAS wild-type Caco-2 cell lines, cetuximab decreased the pERK signal and trametinib did not have any further effect. To validate these observations, four KRAS colon


a
Trametinib (nM)
0 0,1 1 10 100 1000
Cetuximab (μM)
0 0,1 0,5 1 5 10
Caco-2
SW480
b
Trametinib (nM)
0 0,1 1 10 100 1000
Cetuximab (μM)
0 0,1 0,5 1 5 10
Caco-2
SW480
c
Annexin V+/DAPI-
Annexin V+/DAPI+
percentage
0 10 20
Cetuximab - + - + - + - +
Trametinib - - + + - - + +
Caco-2
SW480
d
Cetuximab - + - + - + - +
Trametinib - - + + - - + +
HSC70
pERK
ERKTOT
Caco-2
SW480
e
Phospho-ERK low
Phospho-ERK high
100 μm
100 μm
pERK H-Score
0 5 10
Patient A
Patient B
Patient C
Patient D
Cetuximab - + - +
Trametinib - - + +
f
KRAS E2
KRAS or NRAS E3
Patients
1 2 3 4 5 6 7 8 9
ov
en
ov
0 5 10 15 20 25
Treatment duration (months)
Percent survival
0 50 100
Digestive
Gynecologic
0 20 40 60
Months
KRAS E2
KRAS or NRAS E3
PFS2 / PFS1
0 2 4 6
Clinical benefit
1 2 3 4 5 6 7 8 9
Patients

patient tumor samples were collected, treated or not ex vivo with cetuximab, trametinib or both, fixed and paraffin embedded for immunohistologic analysis of ERK phosphorylation (Fig. 1e). As shown in the representative images, high pERK staining was discriminated from low pERK staining (Fig. 1e left panel) and a pERK H-score was calculated (Fig. 1e right panel). Results show the association of a MEK inhibitor with an anti-EGFR decreased the pERK signal in KRAS patients, similar to what has been previously observed in cell lines.

Finally, nine KRAS chemo-resistant patients (six colon, one endometrial and two ovarian cancers) were treated with an anti-EGFR/anti-MEK association off label, after molecular tumor board decision. Patient response was heterogeneous (Fig. 1f, left and middle panels). However, when the ratio between this combined treatment progression-free survival (PFS_2) and the previous treatment PFS (PFS_1) was evaluated, we observed that KRAS exon 2 patients were more prone ($PFS_2/PFS_1 > 1.3$) to respond to anti-EGFR/anti-MEK treatments than KRAS/NRAS exon 3 patients (Fig. 1f, right panel). Informed consent was obtained from all individual participants included in the study.

To conclude, our in vitro and ex vivo results show that the association of cetuximab and trametinib can inhibit pERK and induce KRAS mutant cell death. These observations were corroborated by patient data. Combination of BRAF inhibitor and cetuximab is in development in BRAF-mutant colorectal cancer [6]. However, to our knowledge, there is currently no available treatment targeting the MEK pathway of RAS-mutant patients. These results might be of importance as we provide here a rationale for using anti-MEK antibodies such as trametinib or cobimetinib to bypass the resistance to anti-EGFR in KRAS exon 2 patients. This work presents preliminary data, and our observations on patients will require further confirmation with a clinical trial on a larger cohort.

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Compliance with Ethics Guidelines. All procedures performed in this study involving human participants and material were in accordance with the ethical standards of the molecular tumor board research committee of the Centre GF Leclerc and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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