Clonal selection of RAS mutant metastatic colorectal cancer into RAS wild-type during first-line Therapy

Susanne Klein-Scory[1], Ingo Wahner I[1], Marina Maslova[2], Yosef Al-Sewaidi [2], Michael Pohl[3], Thomas Mika[3], Swetlana Ladigan[3], Roland Schroers[3], Alexander Baraniskin[1,3*]

[1] IMBL Medical Clinic, Ruhr University Bochum, University Hospital Knappschaftskrankenhaus Bochum GmbH, Germany
[2] Department of Radiology, Ruhr University Bochum, University Hospital Knappschaftskrankenhaus Bochum GmbH, Germany
[3] Department of Medicine, Ruhr University Bochum, University Hospital Knappschaftskrankenhaus Bochum GmbH, Germany

Introduction

Treatment options for patients with metastatic colorectal cancer (mCRC) are limited. This particularly affects the largest group of patients with RAS mutations, who are ineligible for therapy with EGFR antibodies. In this liquid biopsy-based study, we performed the first in-depth analysis of the course of mutational status in initially RAS-mutated patients during 1st-line therapy.

Methods

RAS mutation status of 12 pat. with initially RAS-mutated mCRC was monitored longitudinally in 69 samples using liquid biopsy. We focused on patients with stable disease (SD) or partial remission (PR) as response to first-line therapy (11 pat.). Detection of fragmented RAS-mutated circulating cell free tumor DNA (ctDNA) in plasma was performed by digital droplet PCR (ddPCR) and BEAMing.

Results

All patients with PR or SD at first follow-up revealed a consistent decrease of RAS mutation load. The ctDNA-based RAS mutation status of 10 patients (91%) even converted to wild type in ddPCR. Remarkably, the conversion was observed already after the first cycle of chemotherapy. Plasma load of ctDNA was controlled methylated WIF1- and NPY-promotor ctDNA burden, as a second tumor marker for mCRC. Persistent presence of methylated WIF1- and NPY-promotor fragments confirmed the ongoing release of ctDNA during treatment.

Conclusions

In patients with RAS-mutated mCRC, RAS mutations rapidly disappeared during first-line therapy in liquid biopsy, independent of type and intensity of chemo- and anti-VEGF therapy. This novel observation raises the important question whether these patients may benefit from treatment with anti-EGFR-AB analogous to RAS wt tumors following conversion of initial RAS-mutated status. Currently, our study group is initiating a randomized phase II trial to investigate whether patients with left sided RAS-mutant mCRC will have a PFS benefit from addition of cetuximab to first-line therapy after RAS-mutation status has changed to wild-type during 1st-line treatment as monitored by liquid biopsies (**MoLiMoR**-trial).

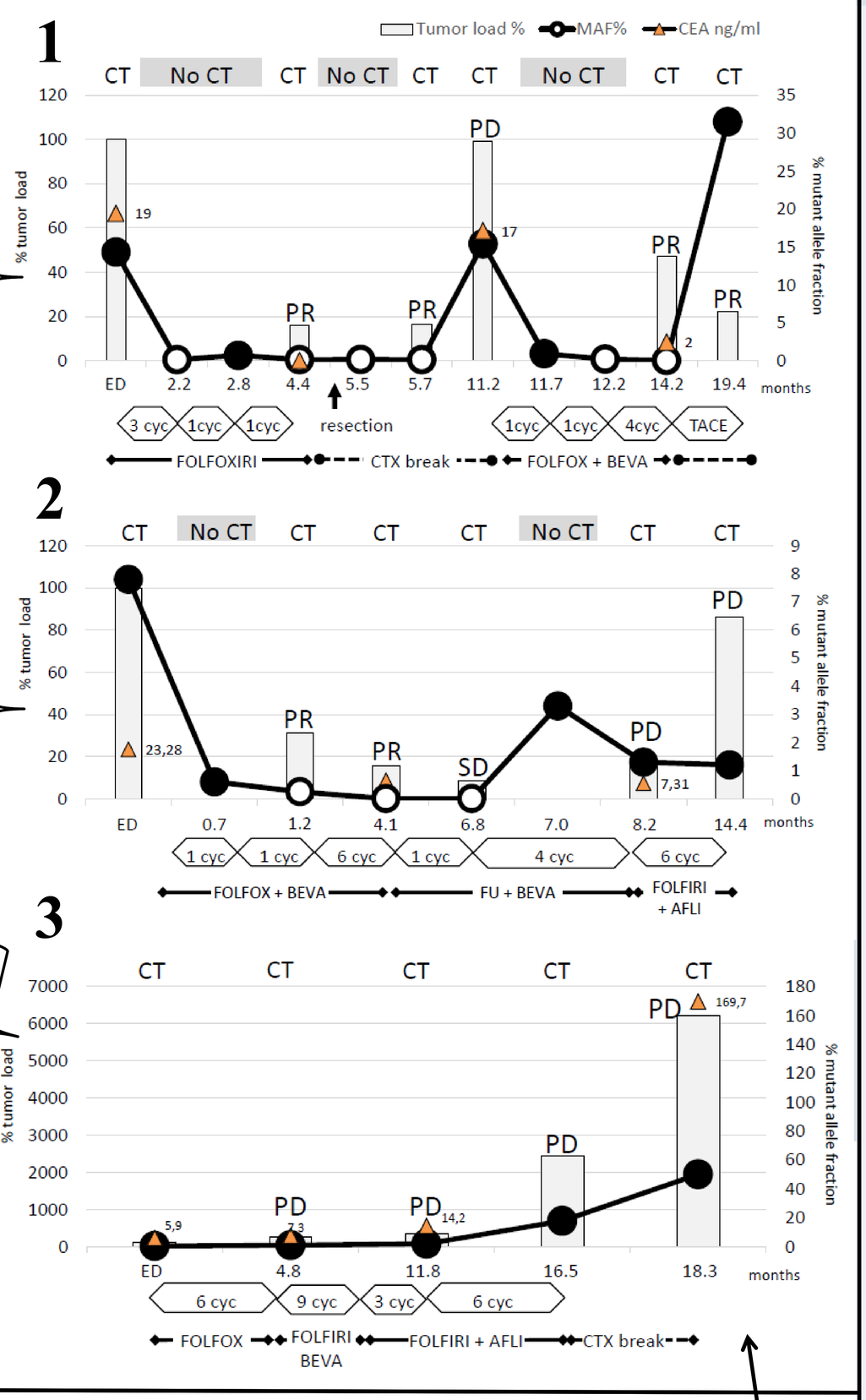
Grant support: This study was supported by a grant (PURE) from the Ministry of Science, North Rhine-Westphalia, Germany

Dynamics of KRAS mutant clones measured in plasma samples

Patient 1: RAS mutations disappeared already after 3xFOLFOXIRI and remained not detectable for 9 mo. PR after 6x CTX made the patient eligible for tumor resection (arrow). After the CTX break PD occurred at the same time with renewed rise of RAS mutation load. Already after 1xFOLFIRI+beva the RAS mutations disappeared again and rose after the next CTX break due to TACE. This case showed that even multiple conversions of RAS status are possible with appropriate CTX.

Patient 2: After 2xFOLFIRI+beva the RAS mutations disappeared and remained not detectable for 6 mo. Due to PR after 8 further cycles, the treatment was deescalated to 5-FU+beva. During this period, RAS mutation load increased again. Next, PD was diagnosed and the subsequent treatment change to FOLFIRI and aflibercept failed to achieve response. However, RAS mutation load did not increase. This case points out that RAS mutation load can decrease during PD indicating that PD is caused by a RAS wild-type clone.

Patient 3: Neither FOLFOX nor FOLFIRI or addition of anti-VEGF antibody therapy led to a decrease of RAS mutation load or tumor response.



Change of WIF1 and NPY promotor methylation proportion vs change of RAS MAF

Figure 1: Samples of patients at the diagnosis vs. samples of the time point of disappearance of RAS mutations were measured by methylation specific ddPCR. WIF1 promotor methylation proportion remained detectable in samples with massive RAS MAF% reduction. (solid line with circle, change of RAS mutant allele frequency; dashed line with cross, change of WIF1 promotor methylation proportion) .

Figure 2: Comparison WIF1 and NPY promotor methylation proportion vs. change of RAS MAF

Figure 3: Comparison WIF1 and NPY promotor methylation proportion vs. change of RAS MAF in case of ***pat. 3***

Figure 1

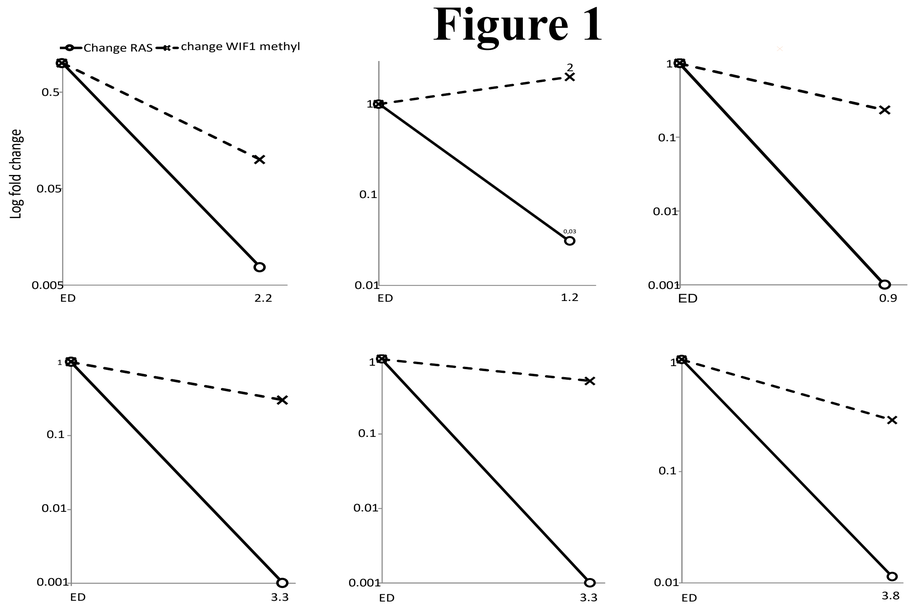


Figure 3

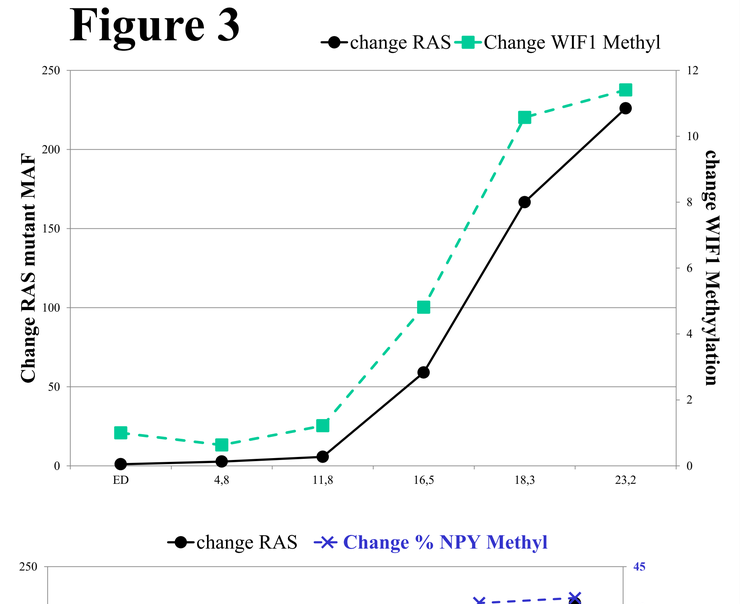


Figure 2