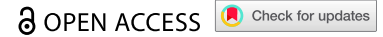



BRIEF REPORT



Complete response of metastatic microsatellite-stable *BRAF* V600E colorectal cancer to first-line oxaliplatin-based chemotherapy and immune checkpoint blockade

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ABSTRACT

The randomized METIMMOX trial (NCT03388190) examined if patients with previously untreated, unresectable abdominal metastases from microsatellite-stable (MSS) colorectal cancer (CRC) might benefit from potentially immunogenic, short-course oxaliplatin-based chemotherapy alternating with immune checkpoint blockade (ICB). Three of 38 patients assigned to this experimental treatment had metastases from *BRAF*-mutant MSS-CRC, in general a poor-prognostic subgroup explored here. The ≥ 70 -year-old females presented with ascending colon adenocarcinomas with intermediate tumor mutational burden (6.2–11.8 mutations per megabase). All experienced early disappearance of the primary tumor followed by complete response of all overt metastatic disease, resulting in progression-free survival as long as 20–35 months. However, they encountered recurrence at previously unaffected sites and ultimately sanctuary organs, or as intrahepatic tumor evolution reflected in the terminal loss of initially induced T-cell clonality in liver metastases. Yet, the remarkable first-line responses to short-course oxaliplatin-based chemotherapy alternating with ICB may offer a novel therapeutic option to a particularly hard-to-treat MSS-CRC subgroup.

ARTICLE HISTORY

Received 28 March 2024
Revised 7 June 2024
Accepted 22 June 2024

KEYWORDS

BRAF mutation; colorectal cancer; immune checkpoint blockade; metastasis; microsatellite-stable; oxaliplatin; T-cell receptor

Introduction


Immune checkpoint blockade (ICB) is efficacious in patients with advanced colorectal cancer (CRC) that is microsatellite-*instable*/mismatch repair (MMR)-deficient.^{1,2} Also, a rare patient subgroup with pathogenic mutations in the exonuclease domain of polymerase ϵ (*POLE*) or $\delta 1$ (*POLD1*), associated with a hypermutated phenotype and mostly observed in microsatellite-stable (MSS)/MMR-proficient tumors,³ shows ICB responsiveness.⁴ The majority of patients, however, present MSS/MMR-proficient CRC with low response rates to ICB alone.⁵ In patients with metastatic MSS-CRC given ICB, the presence of liver metastases was shown to be the most significant variable associated with rapid disease progression,⁶ which may have reflected the *de novo* ICB resistance manifested by the targeted elimination of cytotoxic T cells in preclinical liver metastasis models and the generally low fraction of this specific immune-cell population in liver metastasis specimens from MSS-CRC patients.^{7,8}

Moreover, among patients with MSS/MMR-proficient CRC, those with metastatic disease from *BRAF*-mutant tumors, comprising 14% of a population-based cohort,⁹ have particularly poor prognosis with median overall survival of less than a year found in pooled data analysis.¹⁰ While the median overall survival of first-line high-intensity chemotherapy containing both oxaliplatin and irinotecan was 13.6 months,¹¹ it was 18.3 months following a first-line three-drug combination of inhibitors directly targeting the intrinsically active tumor signaling pathways.¹² Median progression-free survival (PFS) was reported to 5 months or shorter for tumor signaling pathway inhibitors.^{12,13}

We conducted the METIMMOX randomized phase 2 trial, in which the experimental-group patients received short-course oxaliplatin-based chemotherapy (the Nordic FLOX regimen) alternating with ICB (nivolumab) as a first-line treatment of unresectable infradiaphragmatic (liver, peritoneal, and/or nodal) metastases from MSS-CRC. The trial was negative with regard to the primary endpoint PFS, which for the

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 Supplemental data for this article can be accessed online at <https://doi.org/10.1080/2162402X.2024.2372886>

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intention-to-treat experimental-group patients ($n = 38$) was identical (9.2 months) with that for the control-group patients ($n = 38$) receiving FLOX alone.¹⁴ Nevertheless, three experimental-group subjects had *BRAF* V600E disease, of whom all achieved complete response (CR) with PFS 20–35 months. Albeit a small number of cases, we present in-depth clinical and molecular characteristics that may elucidate the remarkable trajectories.

Materials and methods

Ethics approvals and consent to participate

Required approvals were given by the Regional Committee for Medical and Health Research Ethics of South-East Norway (2017/1850), the Norwegian Medicines Agency (17/12752), and the institutional review boards. The trial was conducted in accordance with Good Clinical Practice and the Helsinki Declaration. All patients provided written informed consent. The trial is registered with ClinicalTrials.gov, NCT03388190; by 2 January 2018.

Privacy protection

The data generated in this study are subject to patient confidentiality in accordance with the General Data Protection Regulation of the European Union. Hence, each subject that is individually presented by their detailed disease course is de-identified by partitioning the clinical characteristics into thematic sections with random order of patient presentation. This procedure is pursuant to the information in the informed consent form.

Study design, procedures, and endpoints

Details are provided in Supplementary Methods. In brief, MSS-CRC patients with unresectable infradiaphragmatic metastases were randomly assigned to first-line treatment with the FLOX regimen (oxaliplatin 85 mg/m² day 1 and bolus 5-fluorouracil 500 mg/m² and folinic acid 100 mg days 1–2) Q2W (control group) or alternating 2 cycles each of FLOX Q2W and nivolumab (240 mg flat dose) Q2W (experimental group), with pre-specified break periods (Supplementary Figure S1). Radiologic response assessment was done every 8 weeks with PFS as the primary endpoint. The intention-to-treat population consisted of patients who started the first therapy cycle. Because the first two therapy cycles were identical in the control and experimental groups (halfway toward the first radiographic reassessment), the per-protocol population included all subjects who adhered to treatment until this reassessment to enable objective comparison of the regimens.

Molecular procedures

Two of the molecular procedures reported here, comprising tumor DNA/RNA sequencing to determine somatic mutations and the tumor mutational burden (TMB), and the assessment of the dynamic plasma *BRAF* V600E variant-allele frequency (VAF), are both detailed in Supplementary Methods.

T-cell receptor (TCR) sequencing

For one study subject, the hsTCRB v4 immunoSEQ library preparation kit (Adaptive Biotechnologies) was used to generate TCR repertoire libraries from liver metastases at baseline (a biopsy from one of multiple metastases), from the first liver resection specimen (three samples from the two resected metastases), and from the second liver resection specimen (two samples from one of multiple resected metastases). The protocol used multiplex PCR with primers for all TCR- β gene V and J fragments, corrected for variability in primer efficiency. The protocol also included synthetic TCR templates and reference gene primers to facilitate accurate quantification of T cells in each sample. Pooled libraries were sequenced on the NextSeq 500/550 High Output (Illumina). TCR clonality was assessed using Hill diversity and evenness profiles, as previously described,¹⁵ by which the clonality index was defined as 10 minus the area under the curve of each evenness profile. The Hill equation integrates classic clonality variables – species richness ($\alpha = 0$), Shannon entropy ($\alpha = 1$; from which Pielou's evenness is derived), and Simpson's index ($\alpha = 2$) – into a unified framework.¹⁶ The tracking of clones across repertoires was done using the Adaptive Biotechnologies analyzer 3.0 software (Adaptive Biotechnologies). Only repertoires with sequencing coverage $>5\times$ were included in the analysis. T-cell fractions were determined by dividing the number of detected, productive, rearranged sequences (as a proxy for the number of T cells) with the total number of genomes in the sample which was calculated by total amount of DNA/6.6 pg (mean DNA content/cell). The full procedure was recently described in detail.¹⁵

Results

The patients with *BRAF* mutation

Thirteen individuals with *BRAF*-mutant MSS-CRC were enrolled between 31 August 2018 and 5 July 2021. The CONSORT diagram (Supplementary Figure S2), alongside details in Supplementary Results, accounts for the subject allocations and the intention-to-treat and per-protocol populations. For the present analysis, data cutoff was 15 August 2023 when the last patient with *BRAF*-mutant disease who completed the allocated treatment (11 of 13 intention-to-treat subjects; Supplementary Table S1) reached a prespecified endpoint. The eight control-group subjects had a median PFS of 4.0 months (95% confidence interval, 1.9–6.1) with partial response (PR) as best objective response occurring for only two patients. In contrast, all of the three experimental-group patients achieved CR with a median PFS of 33.0 months (minimum, 20.7; maximum, 35.0).

Figure 1(a) illustrates the course of study participation for each of the nine per-protocol subjects. Molecular tumor characteristics for the patients with PFS longer than 20 months are shown in Supplementary Tables S2–3. The six per-protocol control-group subjects are described in Supplementary Results.

Circulating *BRAF* V600E dynamics

Five patients had plasma samples with measurable *BRAF* V600E VAF at baseline and could be followed for circulating

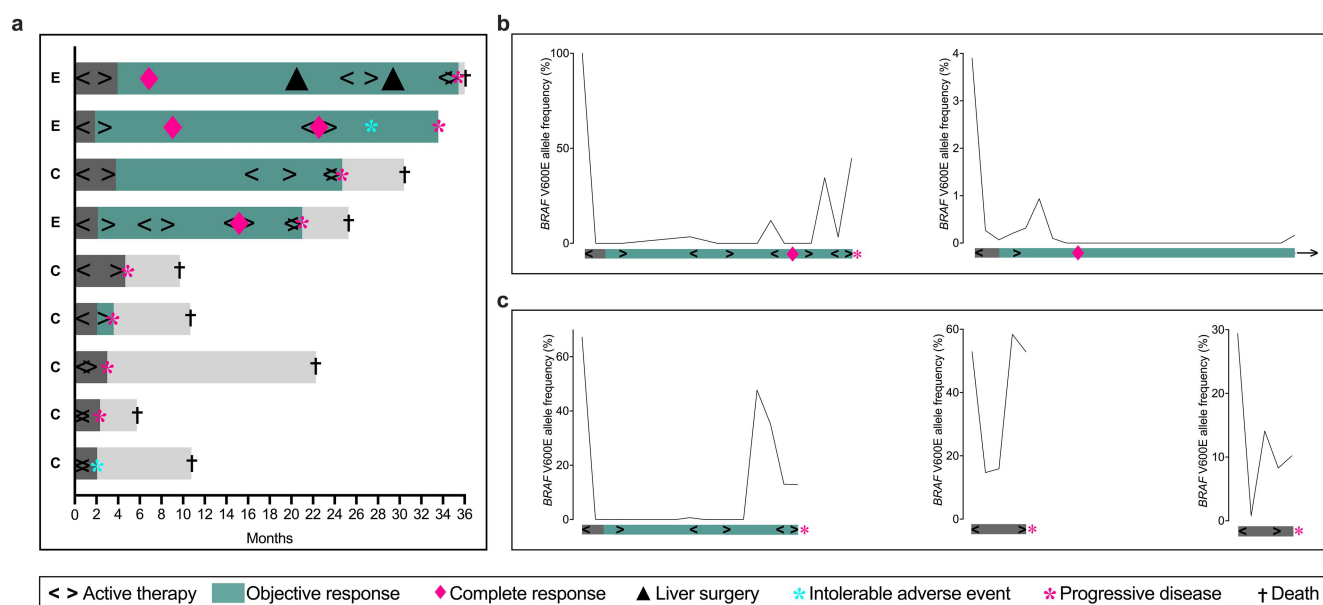


Figure 1. (a) Duration of study participation and efficacy assessment for the per-protocol *BRAF*-mutant population (experimental group (E), $n = 3$; control group (C), $n = 6$). Dynamics of plasma *BRAF* V600E DNA over the study treatment in (b) two experimental-group subjects and (c) three control-group subjects.

tumor DNA dynamics. The experimental-group patient with shortest PFS (20.7 months) presented with 100% of the circulating *BRAF* as the V600E variant but experienced rapid and complete clearance of this allele. The same dynamics occurred again in the second and third treatment sequences, albeit from lower starting VAF measures. In the fourth sequence, the mutant-allele *BRAF* decreased after the initial FLOX cycles but then increased following the sequential nivolumab cycles, portending the ICB failure (Figure 1(b), left panel). In contrast, the experimental-group subject with the longest PFS (35.0 months) had circulating *BRAF* V600E below the level of detection. The experimental-group patient with slightly shorter PFS (33.0 months) had low (3.9%) baseline *BRAF* V600E VAF, with durable clearance coinciding with the radiologic CR (Figure 1(b), right panel). Interestingly, the single control-group long-term responder (male aged >70; PFS 24.4 months) also showed rapid and complete clearance (at radiographic PR) of the high (67.2%) baseline *BRAF* V600E VAF before a sharp increase when nearing treatment failure (Figure 1(c), left panel). The other two control-group subjects with measurable plasma VAF for baseline *BRAF* V600E (none with objective response radiographically) had brief and transient responses (Figure 1(c), middle and right panels).

The three experimental-group patients

The patients were ≥ 70 -year-old females presenting with ascending colon adenocarcinomas (Supplementary Figures S3–4) with TMB 6.2–11.8 mutations per megabase and no *POLD1/POLE* mutations (Supplementary Tables S2–3). One patient had organ-infiltrating primary tumor (T4b), while the others had primaries confined to the bowel wall (T2).

All patients experienced CR of the primary tumor in the first treatment break. The T4b subject had the CR status confirmed by endoscopy showing slight mucosal edema and scarring, from where biopsies proved restored mucosa with crypt

branching and slight inflammation without residual tumor (Figure 2, left panel). Despite the early CR, one T2 subject proceeded to subsequent right hemicolectomy. The surgical specimen had sporadic residual tumor glands embedded in fibrous scar tissue in the submucosa and bowel wall in addition to a restored mucosa (Supplementary Figure S4). The primary tumor CR status was durable for all subjects.

One patient (Figure 1(a), second from the top; baseline plasma *BRAF* V600E VAF 3.9%), presenting with multiple peritoneal and para-aortal lymph node metastases, responded with CR in the first treatment break, which lasted almost a year before a new treatment sequence caused an immediate second CR. However, intolerable immune-mediated hepatotoxicity developed in the second break, making the patient ineligible for further study therapy when several liver metastases subsequently appeared (without radiologic evidence of the previous lymph node or peritoneal metastases), resulting in PFS 33.0 months. The patient proceeded to the FLOX regimen alone.

One patient (Figure 1(a), fourth from the top; baseline plasma *BRAF* V600E VAF 100%) presented with metastases in most liver segments and several lymph node stations below and above the diaphragm (Figure 2, right panel). She experienced early >90% shrinkage of the liver target lesions, followed by increasing lymph nodes and a second treatment sequence. In the second break, the liver target lesions were 97% reduced from baseline but lymph nodes at a previously unaffected site increased. Halfway into the third treatment sequence, CR occurred at all of the involved sites. A fourth treatment sequence, starting when previously unaffected lymph nodes increased, was immediately followed by adrenal gland and skeletal metastases (Supplementary Figure S5), resulting in PFS 20.7 months. The patient proceeded to second-line encorafenib and cetuximab¹³ with rapid clinical deterioration.

One patient (Figure 1(a), on the top; baseline plasma *BRAF* V600E VAF below the level of detection) presented with metastases affecting most liver segments (Supplementary Figure S6).

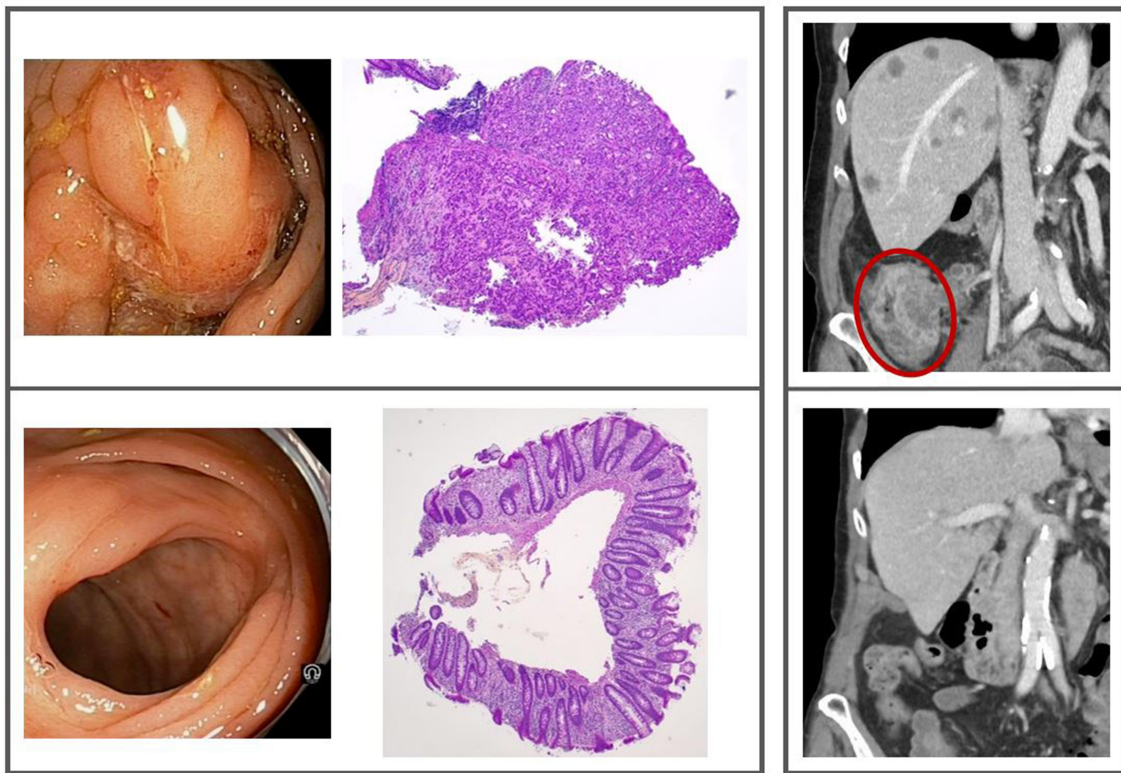


Figure 2. Left framed panel: The primary tumor site; baseline endoscopy and biopsy (top), endoscopy and biopsy at radiologic complete response (bottom). Right framed panel: The metastatic disease; the primary tumor (encircled) and multiple liver metastases at baseline (top), the ascending colon and liver at radiologic complete response (bottom). Further details are displayed in Supplementary Figures S3 and S5.

She experienced early CR, lasting a year until a few liver metastases relapsed. These were resected, which were also subsequently relapsing multiple liver metastases that appeared as end-stage cancer. The patient reached PFS 35.0 months, of which active therapy had been given only 10 months. TCR sequencing of liver metastases, detailed below, illustrated the metastatic evolution.

Changes in T-cell clonal diversity and evenness

TCR sequencing was done on a baseline liver metastasis biopsy, three metastasis samples from the first resection, and two samples from the resected end-stage metastases. The mean T-cell fraction of 3.2% at baseline had increased to 8.2% at the first resection but fallen to 0.07% at the second (Figure 3(a)). The corresponding mean clonality indices (higher values implying more monoclonal repertoires) were 2.9, 5.5, and 2.9 (Figure 3(b)). In addition to the increased T-cell fraction, the primarily relapsed liver metastases exhibited a repertoire with notable skew toward highly abundant clones, implying an influx of clonally expanded tumor-specific T cells. Specifically, the Hill diversity profiles (Figure 3(c)) revealed an absolute clonal diversity, averaging 27,423 clones, peaking in the primarily relapsed metastases, compared to 5,768 clones at baseline and 2,340 clones at end-stage ($\alpha = 0$). When weighing clonal diversity by more abundant clones, however, the disparity over the disease course diminished ($\alpha = 10$) with 3,029 clones at the first relapse, compared to 2,118 clones at baseline and 1,001 clones at end-stage. As illustrated by the Hill evenness profiles (Figure 3(d)), which are diversity profiles

normalized against the number of unique clones, the clonal frequency distribution was more uneven (the most rapid decline at increasing α values) for the primarily relapsed liver metastases than at baseline and end-stage. This indicates that the intratumoral T cells in liver metastases responded with a transient increase of more monoclonal repertoires to the experimental METIMMOX treatment.

Discussion

For *BRAF*-mutant metastatic CRC, several ongoing clinical trials that also include MSS/MMR-proficient disease evaluate combinations of RAF inhibitors with other molecularly targeted agents, some with the addition of oxaliplatin- or irinotecan-based chemotherapy or ICB.¹⁷ Of note for the first-line METIMMOX trial, eligible patients had unresectable abdominal metastases from MSS-CRC, commonly considered unresponsive to ICB.¹⁸ Moreover, the experimental-group patients received short-course oxaliplatin-based chemotherapy intended to invoke responsiveness to the sequential short-course ICB within a total sequence of only 4 months before treatment break, notably different from regimens given in other studies for this patient population. To our knowledge, the CR and PFS outcomes are unprecedented for *BRAF* V600E MSS-CRC. We have identified only one other reported case of this specific CRC entity responding with CR (to tumor signaling pathway inhibitors used at end-stage disease).¹⁹

All three patients had right-sided primary tumors that disappeared and never reappeared. The tumors, devoid of *POLD1/POLE* mutations, were characterized by intermediate

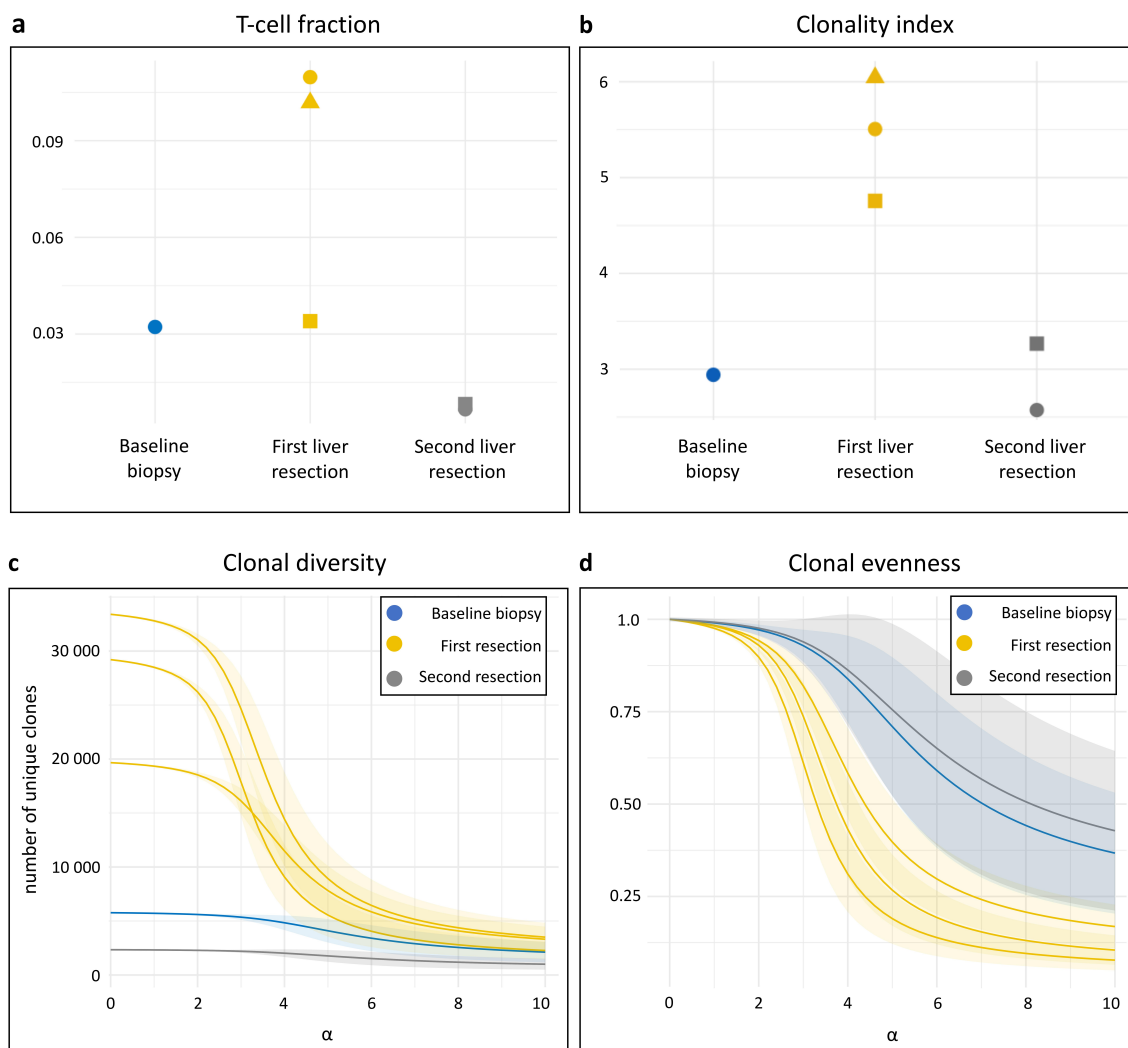


Figure 3. T-cell receptor (TCR) repertoire in sequential liver metastases. TCR sequencing was done on a baseline biopsy sample from one of multiple liver metastases, three samples from the two resected liver metastases that relapsed after the radiologic complete response, and two samples from the resected end-stage liver metastases. (a) T-cell fractions in the repertoire of each sample, stratified by sampling time. The mean number of TCR sequences detected was 22,185 at baseline, 82,263 at the first liver resection, and 7,252 at the second. (b) Repertoire clonality of each sample, stratified by sampling time. The clonality index is defined as 10 minus the area under the curve of the evenness profile, with higher values signifying more monoclonal repertoires. (c) Hill diversity profile and (d) Hill evenness profile for each repertoire, presented by mean (solid line) with 95% confidence interval (shaded area). The left part of the diversity curve ($\alpha = 0$) represents the number of unique clones present in the repertoire. The further and more rapidly each curve declines at increasing α values, the more uneven is the clonal frequency distribution (more monoclonal repertoires). The evenness profiles represent the diversity profiles normalized by clonal richness in the repertoire.

TMB, as such considered unresponsive to single-agent ICB.²⁰ One patient consistently experienced new metastatic progression at previously unaffected sites, also following CR. For her and one of the others, the disease when refractory to the METIMMOX regimen involved sanctuary organs solely. The third patient never experienced extrahepatic metastases. Instead, an intrahepatic tumor evolution was likely reflected in the subsequent loss by the end-stage metastases of the clonal T-cell expansion in the first metastases relapsing after long-lasting CR. We used the Hill equation¹⁶ to create diversity and evenness profiles for the TCR data. Using single-point values like Shannon entropy and Pielou's evenness for clonality would be challenging as they weight rare and common T-cell clones differently. The choice of indices might therefore have led to qualitatively different results from the same clonal frequency data.²¹

It was recently shown that the abundance and spatial distribution of different immune-cell subsets within MSS-CRC

metastases exposed to neoadjuvant chemotherapy (treatment duration not reported) varied in an organ-specific manner; in liver metastases, activated T cells were enriched in the outer invasive margin, but not in the tumor core as seen in lung metastases and primary tumors.²² Yet, we previously showed that neoadjuvant chemotherapy caused a transient increase in intratumoral T-cell density of MSS-CRC liver metastases,²³ a response retrieved in the case reported here by the expanded clonal TCR repertoires of the primarily relapsed liver metastases, lost shortly thereafter by the end-stage metastases.

We analyzed the dynamics of circulating *BRAF* V600E in an attempt to monitor the metastatic evolution, including emerging therapy resistance, in a noninvasive manner.^{24,25} Our observations echoed the recent findings for common CRC mutations, that both low baseline VAF and a rapid clearance from plasma are associated with improved clinical outcome in patients given standard chemotherapy regimens.²⁴ For patients treated with tumor signaling pathway inhibitors, a baseline

plasma *BRAF* V600E VAF exceeding only 2% was a surrogate of high tumor burden, liver involvement, and consequently poor outcome.²⁶ With this in mind, it is noteworthy that the METIMMOX regimen redirected the disease course even for the patient with baseline plasma *BRAF* V600E 100% VAF and liver metastases at the initial presentation.

The consensus molecular subtype classification of primary CRC tumors provided a gene expression-based framework for the role of the immune system by defining tumors enriched in immune response features as subtype-1.²⁷ Approximately half of *BRAF*-mutant MSS-CRC cases categorize to this particular subtype.^{28,29} *In situ* evaluation of *BRAF*-mutant primary tumors has shown high infiltration of cytotoxic T cells, even for MSS-CRC.³⁰ Of note, an impressive 45% response rate was observed in patients with treatment-refractory metastatic *BRAF* V600E MSS-CRC given nivolumab in combination with the signaling pathway inhibitors encorafenib and cetuximab.³¹ The METIMMOX schedule with de-intensified chemotherapy might have augmented subtype-1 biological features also in abdominal metastases. However, the serial relapses suggest a metastatic evolution process that might have been averted, for example, by maintenance ICB during treatment breaks.

In summary, previously untreated patients with unresectable abdominal metastases from right-sided *BRAF*-mutant MSS-CRC, in general a poor-prognostic subgroup, achieved CR (including the primary tumor) and remarkably extended PFS to alternating short-course oxaliplatin-based chemotherapy and ICB. If confirmed in a larger patient cohort, the METIMMOX schedule may offer a novel treatment option for a poor-prognostic MSS-CRC subgroup.

Acknowledgments

The authors are indebted to the Akershus University Hospital National Unit for Precision Medicine for the sequencing of tumor DNA/RNA.

Disclosure statement

AHR has received research support from Bristol-Myers Squibb (on behalf of Akershus University Hospital) and served on advisory board of Takeda. HMH has received personal honoraria from Bayer and Roche and served on advisory boards of AstraZeneca, Eisai, and InCyte. CK has served on advisory boards of AstraZeneca and Roche. HS has received personal honoraria from Ipsen, Pierre Fabre, Daiichi Sankyo, and SAM Nordic and served on advisory board of AAA Pharma. SM has served on advisory board of GSK. The other authors report there are no competing interests to declare.

Funding

This work was supported by the Norwegian Cancer Society under grant 182496; the South-Eastern Norway Regional Health Authority under Grants 2018054, 276940, and 2018014; and Bristol-Myers Squibb (by providing nivolumab free of charge and an associated research grant). This was an investigator-initiated trial and, hence, the funders had no role in the design and conduct of the study, the collection, management, analysis, and interpretation of the data, the preparation, review, or approval of the manuscript, or the decision to submit the manuscript for publication. Bristol-Myers Squibb reviewed the manuscript for possible intellectual property content before submission for publication.

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Abbreviations

CR	complete response
CRC	colorectal cancer
ICB	immune checkpoint blockade
MMR	mismatch repair
MSS	microsatellite-stable
PFS	progression-free survival
PR	partial response
TCR	T-cell receptor
TMB	tumor mutational burden
VAF	variant-allele frequency

Data availability statement

Requests for data can be made to the corresponding author. The data generated in this study are subject to patient confidentiality in accordance with the General Data Protection Regulation of the European Union, and the transfer of data or materials will require approval from the Data Privacy Officer at Akershus University Hospital and on some occasions from the Regional Committee for Medical and Health Research Ethics of South-East Norway. Any shared data will be de-identified.

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