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ORIGINAL ARTICLE

Molecularly matched therapy in the context of sensitivity, resistance, and safety; patient outcomes in end-stage cancer – the MetAction study

Anne Hansen Ree[a,b], Vigdis Nygaard[c], Kjetil Boye[d,c], Daniel Heinrich[a], Svein Dueland[d], Inger Riise Bergheim[e], Christin Johansen[a], Klaus Beiske[f,b], Anne Negård[g,b], Marius Lund-Iversen[f], Vegard Nygaard[h], Eivind Hovig[c,i,j], Sigve Nakken[j,c,k], Salah Nasser[g], Lars Julsrud[l], Claudius H. Reisse[l], Espen A. Ruud[g], Vessela N. Kristensen[b,e], Vivi A. Flørenes[f], Gry A. Geitvik[e], Ole Christian Lingjærde[i,e], Anne-Lise Børresen-Dale[e,b], Hege G. Russnes[f,e], Gunhild M. Mælandsmo[c,m] and Kjersti Flatmark[c,n,b]

[a]Department of Oncology, Akershus University Hospital, Lørenskog, Norway; [b]Institute of Clinical Medicine, University of Oslo, Oslo, Norway; [c]Department of Tumor Biology, Oslo University Hospital, Oslo, Norway; [d]Department of Oncology, Oslo University Hospital, Oslo, Norway; [e]Department of Cancer Genetics, Oslo University Hospital, Oslo, Norway; [f]Department of Pathology, Oslo University Hospital, Oslo, Norway; [g]Department of Radiology, Akershus University Hospital, Lørenskog, Norway; [h]Department of Core Facilities, Oslo University Hospital, Oslo, Norway; [i]Centre for Bioinformatics, University of Oslo, Oslo, Norway; [j]Norwegian Cancer Genomics Consortium, Oslo, Norway; [k]Centre for Cancer Cell Reprogramming, University of Oslo, Oslo, Norway; [l]Department of Radiology, Oslo University Hospital, Oslo, Norway; [m]Institute for Medical Biology, University of Tromsø – The Arctic University of Norway, Tromsø, Norway; [n]Department of Gastroenterological Surgery, Oslo University Hospital, Oslo, Norway

ABSTRACT

Background: In precision cancer medicine, the challenge is to prioritize DNA driver events, account for resistance markers, and procure sufficient information for treatment that maintains patient safety. The MetAction project, exploring how tumor molecular vulnerabilities predict therapy response, first established the required workflow for DNA sequencing and data interpretation (2014–2015). Here, we employed it to identify molecularly matched therapy and recorded outcome in end-stage cancer (2016–2019).

Material and methods: Metastatic tissue from 26 patients (16 colorectal cancer cases) was sequenced by the Oncomine assay. The study tumor boards interpreted called variants with respect to sensitivity or resistance to matched therapy and recommended single-agent or combination treatment if considered tolerable. The primary endpoint was the rate of progression-free survival 1.3-fold longer than for the most recent systemic therapy. The objective response rate and overall survival were secondary endpoints.

Results: Both common and rare actionable alterations were identified. Thirteen patients were found eligible for therapy following review of tumor sensitivity and resistance variants and patient tolerability. The interventions were inhibitors of ALK/ROS1-, BRAF-, EGFR-, FGFR-, mTOR-, PARP-, or PD-1-mediated signaling for 2–3 cases each. Among 10 patients who received treatment until radiologic evaluation, 6 (46% of the eligible cases) met the primary endpoint. Four colorectal cancer patients (15% of the total study cohort) had objective response. The only serious adverse event was a transient colitis, which appeared in 1 of the 2 patients given PD-1 inhibitor with complete response. Apart from those two, overall survival was similar for patients who did and did not receive study treatment.

Conclusions: The systematic MetAction approach may point forward to a refined framework for how to interpret the complexity of sensitivity *versus* resistance and patient safety that resides in tumor sequence data, for the possibly improved outcome of precision cancer medicine in future studies.

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Introduction

Clinical evidence of efficacy and safety of therapies, demonstrated in prospective studies, provides the framework for oncology practice. This is also a premise for the prudent introduction of precision cancer medicine (PCM), commonly defined as using information encoded by the tumor genome as the dominant factor in prediction of therapy response. Over the past decade, a five-digit number of patients with advanced cancer have had their tumor analyzed by large-scale DNA sequencing in order to identify a molecular driver vulnerability for the possible off-label use of targeted medication within a study setting [1]. However, the early results have been disappointing, with estimations that PCM will benefit 1–3% of patients with relapsed or refractory solid

CONTACT Anne Hansen Ree a.h.ree@medisin.uio.no Department of Oncology, Akershus University Hospital, Lørenskog, Norway
A. H. Ree and Vigdis Nygaard share first authorship of this article. H. G. Russnes, G. M. Mælandsmo, and K. Flatmark share last authorship of this article.
Supplemental data for this article can be accessed here.

tumors [1,2]. In comparison, considering all patients with an advanced malignant disease, 5–6% respond to approved genome-based therapies with median duration of almost 30 months [3].

Recent examples from the refractory solid tumor setting include the Danish CoPPO study, which reported radiologically confirmed therapy response in 3% of all tested patients, with median progression-free survival (PFS) of 12 weeks [4]. Initial reports from the ongoing NCI-MATCH trial in the United States, featuring nearly 40 predefined treatment arms for patients with apparently actionable tumor mutations [5], have indicated objective response rate (ORR) of 0–5% [6–8]. The later much debated French SHIVA trial, which is the only one reported so far that has randomized patients with actionable tumor mutations to a matched molecular targeted agent or treatment at physician's choice, had PFS as primary endpoint and showed no improvement for the targeted therapy group [9].

Other PCM studies have adopted methods complementary to tumor DNA sequencing or single-agent therapy. The French MOSCATO trial employed array comparative genomic hybridization in addition to DNA as well as RNA sequencing and reported objective response in 11% of treated patients, corresponding to 2% of the enrolled population [10]. The TARGET study in the United Kingdom analyzed patients' circulating tumor DNA as template for molecularly matched therapy and resulted in ORR of 36% for treated patients and 4% for the total cohort [11]. The I-PREDICT study in the United States exploited several genetic alterations in the tumor and circulation to propose combinations of therapies, resulting in ORR of 20% for treated and 11% for all tested patients, and improved PFS when the theoretical concordance between actionable mutations and the chosen therapies (the matching score) was high [12]. The multinational WINTHER trial applied either large-scale DNA sequencing or RNA expression analysis of fresh metastasis specimens, resulting in ORR of 11% for treated patients and 4% for the total cohort, with median PFS of 2 months but again significantly longer for patients with high matching score [13]. Moreover, the DRUP initiative, providing therapy to 215 cases within DNA variant categories that were enabled to continuously manifest over the study conduct, reported a study-defined overall clinical benefit rate of 34% but 15% ORR [14]. A pooled analysis of 8 basket trials that administered PCM therapy to almost 1200 patients, of whom colorectal cancer (CRC) and sarcoma cases were common entities, showed 25% ORR [15].

Our Actionable Targets in Cancer Metastasis (MetAction) PCM study was set up to undertake DNA sequencing of fresh metastasis specimens from end-stage cancer in order to find molecularly matched therapy. The initial study stage established the workflow for the required diagnostic procedures, implemented security-approved systems for handling of sensitive information, educated the entire project staff within the context of tumor boards, and estimated costs within the national public health services [16]. The aim of the present study stage was to investigate the utility of the MetAction pipeline for routine oncology practice with emphasis on tumor DNA sensitivity and resistance variants and patient safety, response, and survival.

Materials and methods

Approvals and participants

The study was approved by the Institutional Review Boards, the Regional Committee for Medical and Health Research Ethics of South-East Norway, and the Norwegian Medicines Agency. Written informed consent was required for participation. An eligible patient had treatment-refractory end-stage cancer but life expectancy of more than 3 months, and metastatic tissue that was radiographically measurable and suited for biopsy sampling. Specifically, the patient had been on the previous line of systemic therapy for 6 or more weeks and had radiologic evaluation intervals of 6–12 weeks on this therapy with disease progression according to the Response Evaluation Criteria in Solid Tumors (RECIST) v1.1. The patient showed Eastern Cooperative Oncology Group performance status 0–1 and adequate organ function.

Conduct and endpoints

The design considered an individual-based intervention by means of molecularly matched medication based on actionable target identification (ATI) gene variants that indicated drug sensitivity and excluded therapy resistance in a biopsy from a metastatic tumor sampled at enrollment. When no variants of therapeutic implication or a drug-resistance variant that precluded targeted therapy was found (ATI-negative case), the patient was further managed at the discretion of the referring oncologist. When a drug-sensitivity ATI variant was found (ATI-positive case), the workflow on commencement of therapy included a clinical visit at every new treatment cycle. Radiologic evaluation was performed every 8–9 weeks according to RECIST or the guidelines for response assessment of cancer immune therapies (iRECIST) [17]. Study treatment continued until confirmed disease progression or its absence at 24 months, intolerable treatment toxicity, deterioration of the patient's condition corresponding to performance status $\geq$3, or death, whichever occurred first. In the first two instances, if the study participation criteria were still met, a patient could be offered a second enrollment with analysis of a new metastatic lesion biopsy for the purpose of detecting and prioritizing an alternative ATI.

The primary objective was to compare PFS on study treatment, termed Period-B, with PFS for the most recent systemic therapy, termed Period-A [18]; the rate of Period-B/Period-A $\geq$ 1.3 was the study's primary endpoint. Secondary endpoints were the ORR, defined as the fraction of ATI-positive cases obtaining complete or partial response based on the RECIST data, and overall survival (for both the ATI-negative and ATI-positive populations). Exploratory endpoints were the rate of ATI-positive cases along with the incidence of diagnostic adverse events (for the combined ATI-negative and ATI-positive population) and treatment-related grade 3–5 Common Terminology Criteria for Adverse Events (CTCAE) v4.0 toxicities. The durations of hospital admissions were recorded. The various endpoints were included to allow a broad analysis of the utility of this PCM approach in the public health services. Overall survival was measured from the date of enrollment (between 14 March 2016 and 8

March 2017) to death or final censoring (on 11 December 2019) and visualized by the Kaplan-Meier method.

The ATI procedure

The establishment of the MetAction diagnostic pipeline has been described previously [16]. It comprised sampling of metastatic tissue, mutation analysis, and data interpretation at the Molecular Tumor Board (MTB) before integration with clinical data at the Clinical Tumor Board (CTB). The procedure for DNA sequence analysis has been reported in detail [19]. The targeted sequencing was accommodated to the Ion Oncomine Comprehensive Assay v1 (Thermo Fisher Scientific), which is designed to detect hotspot mutations, indels, copy number variants, and gene fusion drivers in a total of 143 genes, followed by sequence variant calling and functional annotation. The called gene variants were classified in a tiered structure, essentially in accordance with the 2017 Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists [20]. Here, Tier 1 (level A or B) is variants with strong therapeutic significance, Tier 2 (level C or D) is variants of potential therapeutic significance, Tier 3 is variants of unknown significance, and Tier 4 is variants without therapeutic implication. If Tier 3 variants were predicted to have functional effects associated with molecularly matched therapy, they were retained for discussion of actionability based on biological rationale at the MTB. Some metastasis samples were also analyzed for copy number alterations or gene fusions of interest within designated fluorescence *in situ* hybridization protocols. The MTB interpreted the findings with regards to the likelihood of benefit from molecularly matched therapy in the context of variants predicting sensitivity or resistance of involved tumor-signaling activities. The succeeding CTB employed the conclusions to recommend use, or unsuitability, of tumor-directed medication as single agent or in combination with other systemic therapies, the latter conditional on established safety data.

Results

Cases and procedures

Twenty-six patients were screened (Table 1). Median age was 65 (range, 23–75) years. The most frequent tumor entity was CRC (4 right-sided, 8 left-sided, and 4 rectal cases). As the only case, a 67-year-old man with metastatic disease from a left-sided colon cancer was enrolled twice for the purpose of determining an alternative ATI when he experienced failure on the first ATI-based therapy. Moreover, 2 patients each had head-and-neck, upper gastrointestinal, or urinary tract primaries, and 1 woman had metastatic breast cancer. Biopsy procedures were guided by computed tomography or ultrasound at lung or pleural sites (6 cases), liver or peritoneal sites (19 cases), and an inguinal lymph node (1 case), and did not cause adverse events. The first study phase recorded a single procedure-specific adverse event among 22 patients [16]. Only 1 case in a total of 48 patients highlights the high safety of the diagnostic procedures. In the current study phase, histologic entities were 18 adenocarcinomas, 2 undifferentiated carcinomas, 1 case each of cholangiocarcinoma and squamous cell carcinoma, and 4 different sarcoma entities. The diagnostic procedures from written informed consent to CTB conclusion were completed in median 17.5 (range, 9–57) days, which compared with median 18 days in the initial study phase [16] highlights that the protocol amendments, making the diagnostic course more complex, were compensated for.

The ATI findings

Figure 1 summarizes the nature and frequencies of the detected gene aberrations across all cases. Table 1 lists the specific aberrations that were concluded to constitute the ATI findings. Details about each identified variant and type of data support for defining ATIs are given in Supplementary Table S1.

Among the 26 patients, genomic variants of therapeutic implication were identified in 22 cases. In 8 of them, molecularly matched medication could not be recommended due to the presence of known resistance variants, either as the only ATI (*KRAS* missense; $n = 4$) or in the context of resistance markers ($n = 4$), and patients were thus scored as ATI-negative. The latter category affected CRC cases, where the patients had a tumor mutation associated with oncogenic phosphatidylinositol 3-kinase (PI3K) signaling activity together with a *KRAS* hotspot mutation, when PI3K targeting is futile [21,22]. In a particularly complex case, a 69-year-old man with metastatic disease from a right-sided colon cancer had gain-of-function mutations in *ERBB2*, *KRAS*, and *PIK3CA*, collectively concluded as non-actionable, since single-agent targeting would likely be inefficacious because of compensatory signaling pathway activities and safety data from possible dual-pathway targeting regimens were lacking. The patient with metastases from a left-sided colon cancer who was enrolled twice, displayed identical mutations in his peritoneal biopsy sampled at the second enrollment as the ATI finding in the liver biopsy sampled the first time; thus, no alternative ATI-based therapy could be offered. The metastatic kidney epithelioid angiomyolipoma was devoid of detectable mutations. The gene variants detected in the cases of metastatic synovial sarcoma, undifferentiated pleomorphic sarcoma, and undifferentiated carcinoma originating in a paranasal sinus were without therapeutic implication. Altogether, 13 patients were scored as ATI-negative cases.

In addition, 2 patients with ATI-positive disease did not commence molecularly matched therapy. The metastatic retroperitoneal leiomyosarcoma showed *PTEN* loss, but the patient could not start mTOR-inhibiting medication at the CTB conclusion (after 17 days) because her performance status rapidly had become too poor. The metastatic pancreatic adenocarcinoma had a gene fusion variant (*CCDC6-BICC1*) that might be consistent with DNA repair deficiency [23], and the MTB decided to have this indication from the targeted DNA sequencing underpinned by exome sequencing. The entire diagnostic procedure took 57 days, and the patient's performance status was not consistent with commencement of PARP-inhibiting medication at its conclusion.

Table 1. The study cases.

Age (years)	Sex	Primary tumor site	Histology	Metastasis sampling site	ATI	Classification tier (a)	Study therapy	Period-B/ Period-A	BOR	DOR (days)	Post-study therapy (b)
35	F	Paranasal sinus	UC	Pleura	None						Not known
68	F	Oral cavity	SCC	Lung	*PIK3CA* missense (p.H1047R)	2C	Everolimus	<1.3	PD		
23	M	Thoracic wall	SS	Pleura	None						Regorafenib
52	F	Breast	AC	Liver	*FGFR1* gain	2C	Ponatinib	<1.3	PD		
60	M	Pancreas	AC	Liver	*CCDC6* fusion	2C (c)	Olaparib (d)				
58	F	Liver	CAC	Liver	*ALK* fusion	2C	Crizotinib	1.41	SD		
51	F	Right colon	AC	Liver	9p24.1 gain	2C	Pembrolizumab	∞	PR/CR	∞	
75	F	Right colon	AC	Liver	*BRAF* missense (p.V600E) and *KRAS* wild-type	2C	Vemurafenib + panitumumab	2.65	PR	119	
69	M	Right colon	AC	Liver	Missense of *ERBB2*, *KRAS*, and *PIK3CA*						None
73	M	Right colon	AC	Liver	*KRAS* missense						None
67	F	Left colon	AC	Peritoneum	High TMB	2C	Pembrolizumab	∞	CR	∞	
48	F	Left colon	AC	Liver	*KRAS* wild-type	1A	Panitumumab + FLIRI	2.40	PR	73	
67	M	Left colon	AC	Liver	*ATM* missense (p.P2353L)	3	Olaparib	<1.3	PD		
67	M	Left colon	AC	Peritoneum	*ATM* missense (e)						None
66	M	Left colon	AC	Lung	Missense of *KRAS* and *PIK3CA*						None
74	F	Left colon	AC	Liver	*KRAS* missense						None
75	F	Left colon	AC	Liver	*KRAS* missense						Not known
63	M	Left colon	AC	Liver	*KRAS* missense						Not known
68	M	Rectum	AC	Lung	*ROS1* fusion	2C	Crizotinib	1.34	SD		
46	M	Rectum	AC	Lung	*ALK* fusion	2C	Crizotinib	<1.3	PD		
67	F	Rectum	AC	Liver	*KRAS* missense and *PIK3R1* nonsense						Not known
63	M	Rectum	AC	Liver	Missense of *KRAS* and *PIK3CA*						Not known
49	F	Kidney	EAML	Liver	None						None
64	M	Urinary bladder	UC	Inguinal lymph node	High TMB	2C	Pembrolizumab (f)				
67	F	Retroperitoneum	LMS	Liver	*PTEN* loss	2C	Everolimus (d)				
42	F	Bone	UPS	Liver	None						Not known

AC: adenocarcinoma; BOR: best overall response; CAC: cholangiocarcinoma; CR: complete response; DOR: duration of response; EAML: epithelioid angiomyxolipoma; F: female; FLIRI: irinotecan 180 mg/m^2 on day 1 and bolus fluorouracil 500 mg/m^2 and folinic acid 100 mg on days 1 and 2 every second week; LMS: leiomyosarcoma; M: male; PD: progressive disease; PR: partial response; SCC: squamous cell carcinoma; SD: stable disease; SS: synovial sarcoma; UC: undifferentiated carcinoma; UPS: undifferentiated pleomorphic sarcoma; ∞: not reached at the final censoring.

(a) For ATI-positive cases, as concluded at the MTB between 30 March 2016 and 15 March 2017.

(b) The status of not known refers to cases with follow-up at referring hospitals.

(c) Evidence linking the *CCDC6* fusion partner to DNA repair deficiency led to confirmation by exome sequencing and detection of a *BRCA1*-like signature.

(d) Not commenced.

(e) The ATI in the patient's second study enrollment was identical to that of his first (the row directly above).

(f) Discontinued after the first cycle.

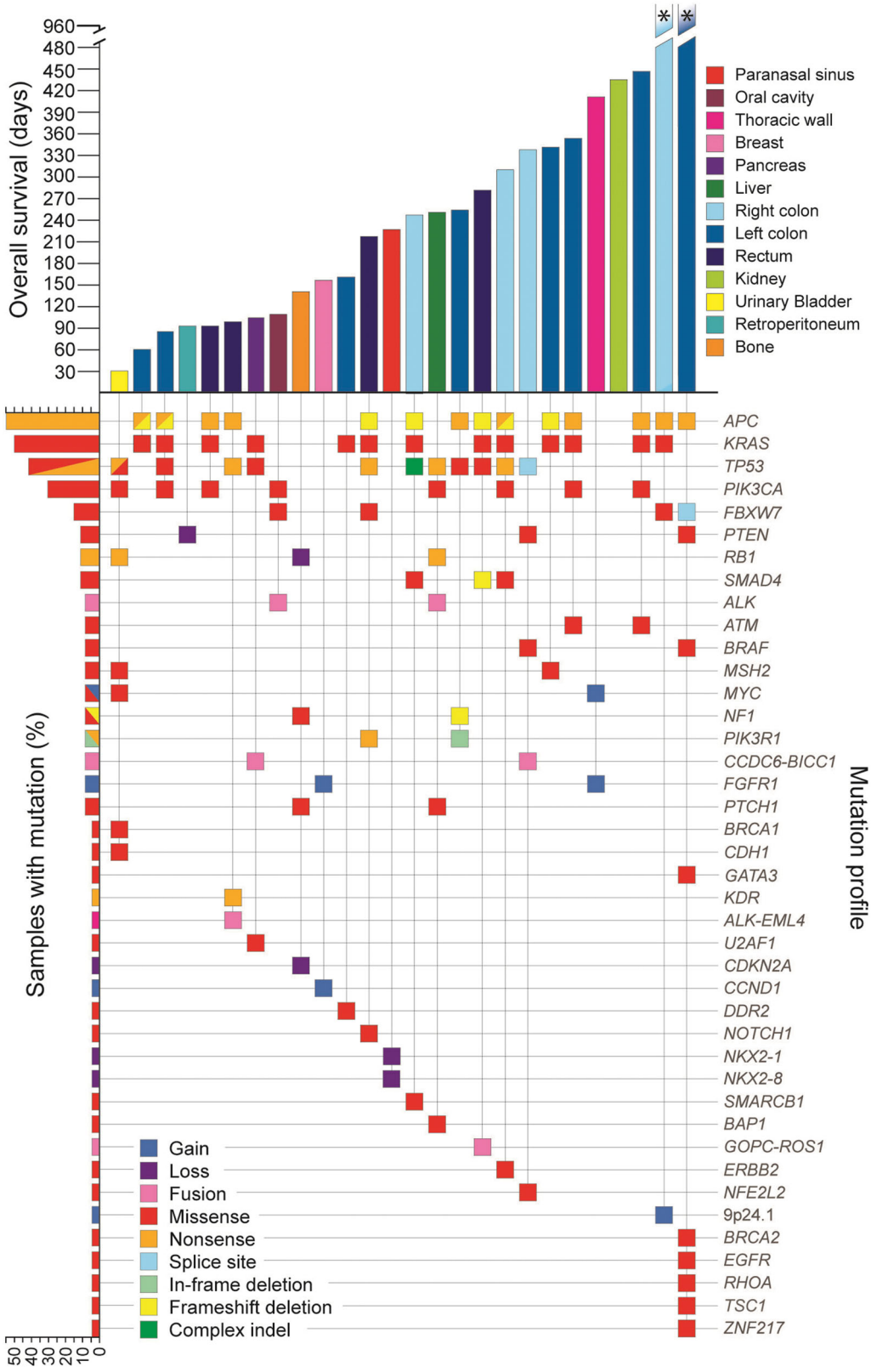

Figure 1. Overall survival for each study case (shown by the primary tumor site); *, alive at censoring (upper panel). The nature and frequencies of the detected gene aberrations for each case (lower panel).

Treatments and outcomes

In total, 13 patients were found eligible for molecularly matched therapy (Table 1); hence, the rate of ATI-positive cases was 50%. Eleven of the ATI-positive individuals commenced the treatment, but 1 (given PD-1 inhibitor for metastatic undifferentiated carcinoma of the urinary bladder) discontinued therapy after the first cycle because his general condition rapidly deteriorated.

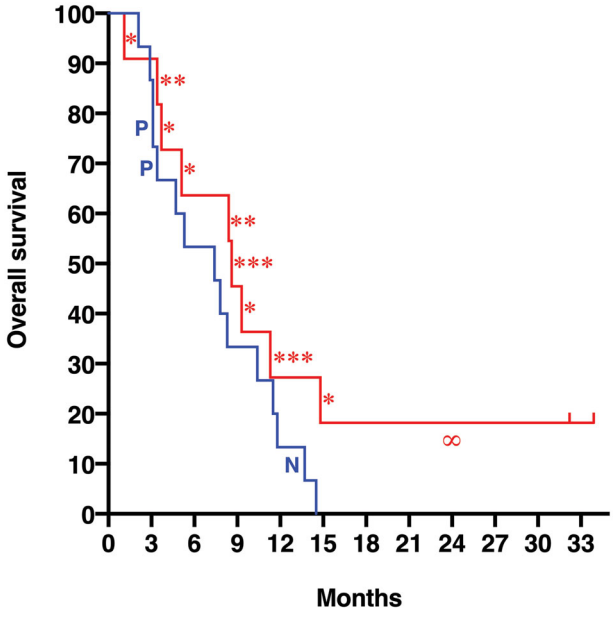

Figure 2. Overall survival for the patients who received MetAction therapy (red curve) and those who did not (blue curve). For each case in the former group, the best overall treatment response is indicated (*, progressive disease; **, stable disease; ***, partial response; ∞, radiologically or molecularly complete response). In the latter group, the labeled cases were concluded as ATI-positive but did not commence molecularly matched therapy (P) or ATI-negative but received alternative tumor-directed medication (N).

Among the 10 individuals who received study treatment until radiographically assessable, 6 met the primary endpoint; thus, the rate of Period-B/Period-A $\geq$ 1.3 was 46% (6 of 13 ATI-positive cases). Two patients given crizotinib (1 each with cholangiocarcinoma and a rectal primary) obtained Period-B/Period-A outcome slightly better than 1.3, with stable disease as the best overall response and progressive disease scored after 117 and 110 days, respectively.

Moreover, 2 patients with colon primaries, given a combination of panitumumab with either irinotecan-based chemotherapy or vemurafenib, had primary endpoint measures of 2.40 and 2.65 after partial response as the best overall response and with duration of response (the time from documented tumor response to progression) of 73 and 119 days, respectively. The patient who received the combination of vemurafenib and panitumumab, based on the conclusion that the mutant *BRAF* and wild-type *KRAS* genes constituted ATI positivity (which recently was evidenced [24]), completed the treatment period without hospital admission. The patient who received the combination of irinotecan-based chemotherapy and panitumumab, based on the absence of *KRAS* mutation in the liver metastasis biopsy, was admitted for a total of 7 days during the study treatment. Of note, she had not been given panitumumab (but had received irinotecan-based chemotherapy) within the standard-of-care therapies because the primary tumor had been scored with mutant *KRAS* status, which was reconfirmed within the MetAction study investigations. The study treatment caused a significant decline in the circulating level of the carcinoembryonic antigen tumor marker along with the temporary radiographic regression of the liver metastases and associated symptom alleviation. Simultaneously, the patient's asymptomatic mediastinal lymph nodes, regarded as non-target lesions within the RECIST assessment, increased modestly in size. It is tempting to interpret the radiologic findings to reflect that a heterogeneous primary tumor had consisted of a wild-type *KRAS* clone that had metastasized to the liver and a mutant *KRAS* clone that had metastasized to the mediastinal lymph nodes.

Finally, 2 patients with resected colon primaries and end-stage disease in the peritoneum or liver, respectively, were treated with PD-1 inhibitor based on the detection of high tumor mutational burden (TMB) or gene locus 9p24.1 copy number gain, the latter previously communicated as a case report [19]. Both patients had the immune checkpoint blockade (ICB) for 24 months before it was discontinued and were followed thereafter. The case with the copy number aberration resulted in partial response as the best overall radiographic response despite being negative for circulating mutant *KRAS* (interpreted as molecularly complete response) from the first RECIST assessment at 9 weeks [19]. The case with high TMB was scored as immune-unconfirmed progressive disease (according to iRECIST) at the first evaluations followed by partial response at 23 weeks and finally complete response from 59 weeks onwards. This was the only study patient who reported a serious adverse event – a single CTCAE grade 3 colitis event that immediately resolved on high-dose prednisolone. At final censoring, the 2 cases of end-stage colon cancer had ongoing responses (at 32–34 months).

Overall, the 1 complete and 3 partial radiographic responses resulted in ORR of 31% for the ATI-positive cases (or 15% for the total study population). Only 3 patients (11.5%) did not reach the inclusion-specified study criterion of life expectancy of at least 90 days (Figure 1). Median (range) overall survival was similar for ATI-negative and ATI-positive cases – 234 (62–436) days for the former and 251 (33 to not reached) days for the latter, and for patients who did not have or received study treatment – 222 (62–436) days *versus* 257 (33 to not reached) days (Figure 2). One ATI-negative patient received post-study tumor-directed medication (Table 1 and Figure 2); however, for 6 of the ATI-negative patients, who had follow-up at referring hospitals, the study approvals did not permit the collection of such information.

Of note, 2 of the 4 cases with objective treatment response would have been identified with the 50-gene Ion AmpliSeq Cancer Hotspot Panel used in the first MetAction project phase [16] – the colon cancer patients given combination therapy regimens (Supplementary Table S1). This panel would not have revealed the colon cancer patients offered ICB, since those decisions were based on high TMB or a gene locus copy number gain.

Discussion

The initial MetAction study phase (2014–2015) established the required diagnostic infrastructure for PCM in the Norwegian public health services; however, none of the 22 end-stage cancer cases analyzed with the initial 50-gene panel had an ATI within the conservative approach of a single medication strictly matched to a single driver mutation [16]. Hence, three principal protocol amendments were undertaken and approved by the designated authorities for the utility study phase reported here. First, we changed to a 143-gene panel that detected copy number variants and

gene fusion drivers, which are more likely to be driver genetic events [25], in addition to hotspot mutations. Next, the MTB had extended liberty to interpret the sequence data, specifically with regards to signaling activity in the tumor. Finally, the CTB had the opportunity to recommend combination therapy regimens if safety data were known.

As a result, 50% of the 26 enrolled patients were determined as ATI-positive. Of the 13 positive cases, 46% had PFS that was sufficiently long to meet the primary endpoint and 31% experienced objective treatment response (15% of the total study population). Other recent PCM studies in the refractory solid tumor setting have reported ORR of 0–11% for the total patient populations [4,6–8,10–13], underpinning that the revised strategy of the MetAction study was advantageous. Overall survival was essentially similar for patients who did and did not receive study treatment. Furthermore, the objective treatment responses came with negligible side-effects and hospital admission caused by the advanced disease (and not adverse treatment effects), altogether proving an indisputable benefit for the responding patients.

The 143-gene panel identified only 2 more cases with objective treatment response than the initial 50-gene panel would have done. Both were given ICB with molecularly or radiologically complete response. One was the only study patient that reported a serious adverse event, which was easily treatable; thus, the MetAction study demonstrated high treatment safety. Moreover, the other 2 cases with objective response were the only patients who were given combination therapy regimens, and both could have been identified with the 50-gene panel. Nevertheless, the larger gene panel provided a compelling additional value by the estimation of high TMB or detection of a copy number variant as the driver genetic event and the probable cure of end-stage malignancies by the resulting ICB. Two facets are of note in this regard. First, ICB is not approved by Norwegian health authorities for treatment of advanced high-TMB CRC within the public health services; thus, the relevant patient was ineligible in routine clinical practice and could only receive this treatment in a pertinent study setting. Second, the patient with the gene locus 9p24.1 copy number gain had a TMB rate of 5 [19], which is regarded as low [26]; thus, she would not have been identified as a typical CRC case for ICB [27] unless this particular driver alteration was found.

The study population consisted of 3 cases with *ALK* or *ROS1* gene fusion (16.7% of the 18 patients with gastrointestinal cancer), all given crizotinib and 2 of whom had disease stabilization of short duration. Both *ALK* and *ROS1* fusions have been described as rare genetic variants in CRC [28,29] and likewise, *ALK* fusion in cholangiocarcinoma [30]. Chromosomal rearrangements with *ALK* and *ROS1* have become strong biomarkers for crizotinib efficacy in advanced lung adenocarcinoma [31,32]. However, the outcome data reported here are only weakly supportive of these variants as sole driver genetic events in gastrointestinal cancer entities.

Limited success of PCM may lie in the existing knowledge gap linking the tumor genome with clinical intervention. In the presence of multiple gene alterations, the challenge is to prioritize driver events, account for resistance markers, and procure sufficient information for treatment that maintains patient safety. The MetAction study undertook variant classification within a tiered structure that is widely accepted [20]. However, published tier-based variant classification systems do not systematically take into account the impact of co-existing variants that may confer resistance or reduced sensitivity to molecularly targeted therapy, and ATI resistance markers are not clearly reported. For some patients in our study, the MTB relinquished a potential drug-sensitivity ATI due to concurrent resistance markers, e.g. in the case of co-existing hotspot mutations in *PIK3CA* and *KRAS*. The MTB also identified potential resistance variants or alternative drivers for the 4 ATI-positive patients who met the primary endpoint but had transient stable disease or partial response. These variants (Figure 1 and Supplementary Table S1) included concurrent mutations in DNA damage repair genes (in the presence of the *ALK* fusion), co-alterations within the PI3K signaling pathway, and *KRAS* mutation (in the presence of the *ROS1* fusion).

To summarize, PCM futility may reside in the small gene panels usable for clinical practice, the selective consideration of treatment sensitivity markers at the expense of resistance variants, and the effectiveness of targeted therapy being dependent of the tumor entity. In addition, opinion leaders have pointed to end-stage cancer and tumor heterogeneity, with the resulting incomplete pathway inhibition and biochemical plasticity to the chosen drug as well as undetectable co-existing drivers, as causes of failure of the PCM concept as it is employed today [1].

Acknowledging the limited case number, an evident weakness of MetAction, we still conclude that the study's strength when compared to large PCM initiatives [4,6–8,10–14] lies in the interpretation of somatic variants that provided insights into the complexity of tumor sensitivity *versus* resistance and patient safety for therapy decisions. Specifically, the study cohort consisted of many CRC and some sarcoma cases, which are entities with few lines of systemic therapies in the advanced setting and not uncommonly comprise patients in good performance status even at end-stage, amenable to off-label use of targeted medication. The study also emphasized the value and importance of MTB and CTB discussions for enhancing the utility of tumor genomic data in routine clinical practice.

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ORCID

Anne Hansen Ree http://orcid.org/0000-0002-8264-3223
Kjetil Boye http://orcid.org/0000-0002-5552-6283
Svein Dueland http://orcid.org/0000-0002-6125-6689
Marius Lund-Iversen http://orcid.org/0000-0002-2025-4062
Kjersti Flatmark http://orcid.org/0000-0001-7409-0780

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