Supplementary Data

Supplementary Methods

Study procedures

The concept of the 'Actionable Targets in Cancer Metastasis' (MetAction) study (ClinicalTrials NCT02142036) was to undertake mutation profiling of the individual patient's progressing metastatic cancer in order to offer molecularly matched medication. The initial stage of the study established the required diagnostic infrastructure, implemented security-approved systems for handling of sensitive information, educated the study tumor boards, and estimated costs of the initiative within the public health services. The endeavor enabled expedite and safe mutation profiling of metastatic tumors (1). The aim of the principal study stage was to investigate the utility of the MetAction pipeline in clinical practice.

The study design and conduct have been detailed previously (1). An eligible patient had metastatic disease from a solid tumor of any origin, had failed documented systemic therapies that might provide meaningful benefit, and was eligible for repeat biopsy sampling of a metastatic lesion that was radiographically measurable. Specifically, the patient had been on the previous line of systemic therapy for 6 or more weeks with radiographic evaluation intervals of 6-12 weeks and disease progression according to the Response Evaluation Criteria in Solid Tumors v1.1. Importantly, the patient showed Eastern Cooperative Oncology Group performance status 0-1 and adequate organ function.

The study design considered an individual-based intervention by means of molecularly matched medication based on actionable target identification (ATI) in a biopsy from a metastatic tumor sampled at study enrolment. A study amendment approved by the designated authorities in February 2016, resulting from experiences in the initial study stage, provided the Molecular Tumor Board extended liberty to interpret the mutation data and the sequential Clinical Tumor Board the opportunity to conclude on a molecularly matched systemic agent alone or in combination with other tumor-directed medications, the latter conditional on established safety data. A study medication had to hold approval for clinical use by the Norwegian Medicines Agency. In the end-of-study incident when no ATI was found, the patient was further managed to the discretion of the referring oncologist. Following ATI, the workflow on commencement of therapy included repeat biopsy sampling after 2 weeks (solely for research purposes) followed by a clinical visit at every new treatment cycle. Radiographic work-up and sampling of plasma for analysis of circulating tumor DNA were performed at pre-defined intervals. The primary objective was to compare progression-free survival on study treatment with progression-free survival for the most recent therapy. The incidences of diagnostic adverse events and treatment-related grade 3-5 Common Terminology Criteria for Adverse Events v4.0 toxicities were secondary end points.

DNA/RNA isolation and sequencing

DNA/RNA was isolated from study biopsies and whole blood by the AllPrep DNA/RNA Universal Kit and QIAcube (Qiagen). The nucleic acid samples were quantified by NanoDrop (Thermo Fisher Scientific). The procedure of targeted DNA-sequencing accommodated to the applied Ion Oncomine™ Comprehensive Assay v1 (Thermo Fisher Scientific), designed to detect hotspot mutations, copy-number variants, and gene-fusion drivers in a total of 143

genes. In brief, 20 ng DNA and 10 ng RNA were required as input for the assay. The panel included two multiplexed primer pools that used 10 ng of DNA each (*i.e.,* a total of 20 ng) to amplify approximately 2,530 genomic areas of interest in the 143 genes. Library preparation for each sample was performed using the Ion AmpliSeq™ Library Kit 2.0 following the manufacturer's protocol. The libraries were barcoded using the Ion Xpress™ Barcode Adaptors 33-48 kit. A 40-pmol/L pool of tumor DNA and RNA libraries in a 5:1 ratio (DNA to RNA) was used for templating and loading of enriched Ion Sphere™ particles onto an Ion 318™ v2 BC chip using the Ion Chef™ Instrument and the Ion PGM™ Hi-Q™ View Chef Kit. The samples were sequenced on the Ion Torrent PGM™ Personal Genome Machine. The median sequencing depth for the amplicons was ~3000×, enabling calculation of the approximate mutant-allele fraction in the heterogeneous tissue samples.

For RNA sequencing, RNA quality was assessed by using the Agilent 2100 Bioanalyzer RNA 6000 Nano chip (Agilent). The two biopsy samples had RNA Integrity Number of 8.0 and 7.2. Libraries were prepared using the TruSeq® Stranded Total RNA kit (Illumina). Here, poly(A) RNA was purified from 200 ng total RNA, fragmented, and reverse-transcribed into cDNA using reverse transcriptase and random primers, followed by second-strand cDNA synthesis using RNase H and DNA Polymerase I. Double-stranded cDNAs were adenylated at the 3'-ends and ligated to sequencing adaptors, followed by purification and enrichment with PCR to create cDNA libraries. The final libraries were size-validated by the Agilent 2100 Bioanalyzer System and concentration was validated by quantitative PCR. The uniquely barcoded cDNA libraries were normalized to 10 nM and pooled together. 10 pM of pooled libraries were loaded onto an Illumina cBot for cluster generation. Paired-end sequencing was carried out on an Illumina HighSeq™ 2000 for 100 cycles to achieve a minimum of ~35 million reads per sample.

Analysis of sequence data

For analysis of the DNA sequence data, applying the Torrent Suite™ software (Thermo Fisher Scientific), gene variant calls were quality-controlled with the Integrative Genomics Viewer (2) and functionally annotated with ANNOVAR, using RefSeq as the underlying gene model (3) and also information from the 1000 Genomes Project (1000genomes.org) and the Catalogue of Somatic Mutations in Cancer (cancer.sanger.ac.uk/cosmic). Copy-number variants and gene fusions were detected using the copy-number and fusion detection module, respectively, within the Ion Reporter™ software (Thermo Fisher Scientific).

The RNA sequence data was processed by the Genomics Core Facility at Oslo University Hospital, using Illumina's RNA-Seq application v1.1.0 workflow (Illumina), which consist of several programs. STAR v2.5.0b was used to align the reads to the RefSeq UCSC human hg19 transcript reference. Transcript assembly and abundance estimation were done using Cufflinks v2.2.1. The individual RNA sequence counts are provided in Table S1.

Quantification of the relative abundance of each transcript was reported as fragments per kilobase of transcript per million reads (FPKM). In order to identify differentially expressed genes when $n = 2$, two criteria were applied: a minimum expression level of FPKM ≥0.2 in one sample (yielding 15,339 genes) and a $\log_2$ fold-change (FC) of ±2 in expression level. When a gene was not expressed in one sample, this approach yielded a FC of infinity. Altogether, these criteria yielded 4,168 genes, of which 2,583 were up-regulated and 1,585 were down-regulated in the 2-week *versus* baseline liver metastasis sample (Table S2). The total of 4,168 genes was used as input into the Ingenuity® Pathway Analysis software for the

analysis of significant biological functions and signaling pathways inherent in the tumor phenotype responses.

Supplementary Results

Figure S1.
Plasma levels of thyroid-stimulating hormone (TSH) and free-T_4 over the study treatment. The start of a new treatment cycle corresponds to the given week numbers.

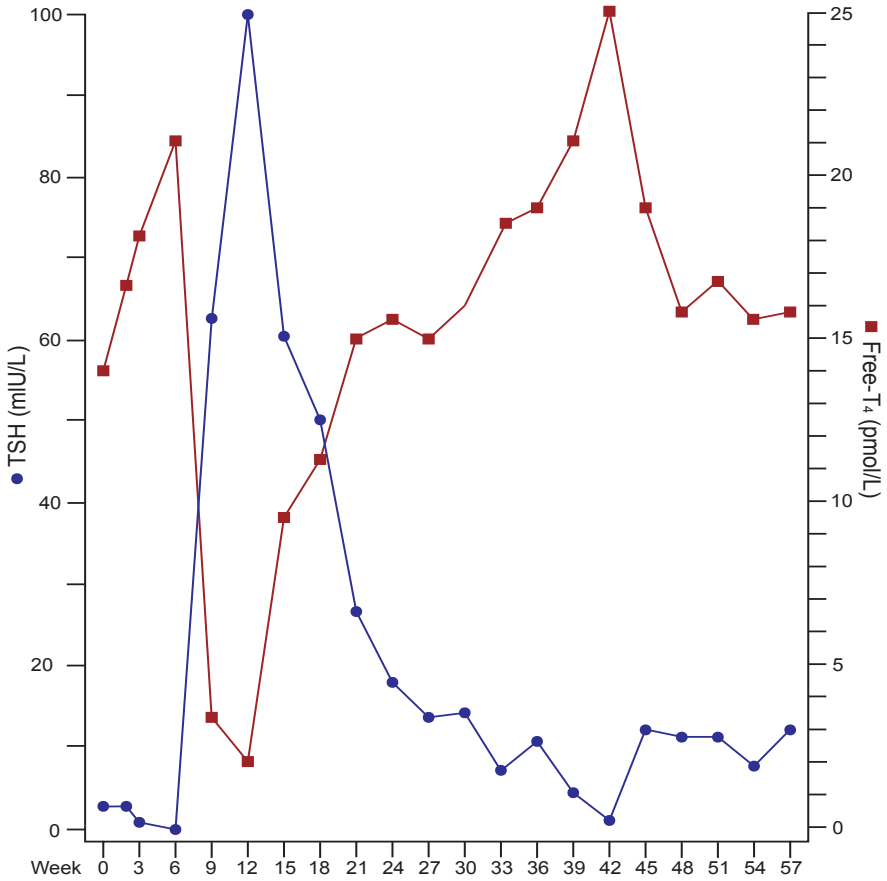

Table S1.
Individual RNA sequence counts in the baseline and 2-week liver metastasis biopsy specimens. The FPKM values are given for each RNA transcript, identified by its gene name.

The table is presented as a separate spreadsheet.

Table S2.
Differentially expressed genes (n = 4,168) between the baseline and 2-week liver metastasis biopsy specimens. FPKM values are given for transcripts with $\log_2$ FC of at least ±2 in expression level; inf, FC of infinity.

The table is presented as a separate spreadsheet.

Table S3.
Differentially expressed genes of the chromosome 9p24.1 locus between the baseline and 2-week liver metastasis biopsy specimens. FPKM values and $\log_2$ FC are given.

Gene	Locus	Baseline FPKM	2-week FPKM	$\log_2$ FC
CD274 (PD-L1)	chr9:5450503-5470567	10.1	3.82	–1.40
PDCD1LG2 (PD-L2)	chr9:5510438-5571282	2.11	8.36	1.99
JAK2	chr9:4985086-5128183	23.1	10.3	–1.16

Table S4.
Biological function annotation of input genes (n = 4,168) defined by the Ingenuity® Pathway Analysis software. Ranking is by Z-score; NaN, not a number. The p-value is given for each function. The molecules involved in each function are given by their gene names. Functions displayed by Fig. 3A of the main manuscript are marked by color.

The table is presented as a separate spreadsheet.

Table S5.
Signaling pathway annotation of input genes (n = 4,168) defined by the Ingenuity® Pathway Analysis software. Ranking is by p-value. Z-score is given for each pathway; NaN, not a number. The molecules involved in each pathway are given by their gene names. Pathways displayed by Fig. 3B of the main manuscript are marked by color.

The table is presented as a separate spreadsheet.

References

1. Ree AH, Russnes HG, Heinrich D, Dueland S, Boye K, Nygaard V, et al. Implementing precision cancer medicine in the public health services of Norway: the diagnostic infrastructure and a cost estimate. ESMO Open 2017;2:e000158.
2. Robinson JT, Thorvaldsdóttir H, Winckler W, Guttman M, Lander ES, Getz G, et al. Integrative Genomics Viewer. Nat Biotechnol 2011;29:24–6.
3. Yang H, Wang K. Genomic variant annotation and prioritization with ANNOVAR and wANNOVAR. Nat Protoc 2015;10:1556–66.