

Profiling of circulating tumor DNA and tumor tissue for treatment selection in patients with advanced and refractory carcinoma: a prospective, two-stage phase II Individualized Cancer Treatment trial

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Abstract

Background: Molecular profiling (MP) represents an opportunity to match patients to a targeted therapy and when tumor tissue is unavailable, circulating tumor deoxyribonucleic acid (ctDNA) can be harnessed as a non-invasive analyte for this purpose. We evaluated the success of a targeted therapy selected by profiling of ctDNA and tissue in patients with advanced and refractory carcinoma.

Patients and methods: A blood draw as well as an optional tissue biopsy were obtained for MP. Whole-genome sequencing and a cancer hotspot panel were performed, and publicly available databases were used to match the molecular profile to targeted treatments. The primary endpoint was the progression-free survival (PFS) ratio (PFS on MP-guided therapy/PFS on the last evidence-based therapy), whereas the success of the targeted therapy was defined as a PFS ratio ≥ 1.2 . To test the impact of molecular profile-treatment matching strategies, we retrospectively analyzed selected cases *via* the CureMatch PreciGENE™ decision support algorithm.

Results: Interim analysis of 24 patients yielded informative results from 20 patients (83%). A potential tumor-specific drug could be matched in 11 patients (46%) and eight (33%) received a matched treatment. Median PFS in the matched treatment group was 61.5 days [interquartile range (IQR) 49.8–71.0] compared with 81.5 days (IQR 68.5–117.8) for the last evidence-based treatment, resulting in a median PFS ratio of 0.7 (IQR 0.6–0.9). Hence, as no patient experienced a PFS ratio ≥ 1.2 , the study was terminated. Except for one case, the CureMatch analysis identified either a two-drug or three-drug combination option.

Conclusions: Our study employed a histotype-agnostic approach to harness molecular profiling data from both ctDNA and metastatic tumor tissue. The outcome results indicate that more innovative approaches to study design and matching algorithms are necessary to achieve improved patient outcomes.

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Introduction

The development of next-generation molecular profiling (MP) methods has enhanced our ability to interrogate multiple cancer-associated genomic changes within an individual patient's tumor. As a result of such advancements, an increasing number of molecular aberrations have now been rendered actionable targets. Cancer therapy, comprised previously by an arsenal of cytotoxic agents, is now being transformed by genome-targeted drugs, which has led to improved outcomes for several malignancies.¹ As of date, the US Food and Drug Administration (FDA) has approved over 80 targeted agents for solid and hematologic malignancies (<https://www.mycancergenome.org/content/page/overview-of-targeted-therapies-for-cancer/>), driving the possibility of individualized cancer treatment, that is, precision oncology.

Precision oncology clinical trials have typically been based on MP of tissue biopsy-derived deoxyribonucleic acid (DNA). In a pioneering pilot study investigating the efficacy of MP-based treatment in a cohort of refractory metastatic cancer patients, Von Hoff *et al.* reported a clinical progression-free survival (PFS) benefit in 27% of the patients.² These promising findings have encouraged the enrollment of patients onto trials matching cancer treatment to genetic profiles obtained from biopsy material. To date, however, results have been mostly disappointing, with overall response rates hardly exceeding 10%, thus leading some to question the promise of precision oncology approaches for improving patient benefit.^{3,4} Tumor tissue genotyping alone may not be sufficient enough to capture the complexity and heterogeneity of tumors,⁵⁻⁷ and recently, circulating cell-free DNA (cfDNA), which contains circulating-tumor DNA (ctDNA) in patients with cancer, has been shown to provide the most accurate snapshot of a patient's tumor, enabling the detection of tumor subclones from metastatic lesions.⁸⁻¹² Although studies have begun to prospectively use ctDNA to funnel patients into phase I clinical trials,¹³ such trials have only recently been initiated and only preliminary outcomes have been reported so far.

We hypothesized that MP of ctDNA in plasma or metastasis biopsy could enable the matching of the most current driver alterations to targeted therapies and improve outcomes in patients with advanced and refractory cancer. Furthermore, this approach reflects a true clinical scenario in which both plasma and/or tissue analytes inform treatment decisions.

As an initial test of this concept, we implemented the Individualized Cancer Treatment (ICT) trial, a single-center prospective interventional study, to investigate the efficacy of molecularly targeted cancer treatment based on the results of genomic profiling of plasma DNA and/or metastatic tissue. To the best of our knowledge, this is the first interventional study reporting survival outcomes from ctDNA-guided treatment.

Methods

Study design

The ICT trial, a prospective non-randomized open-label, clinical phase II study, was conducted at the Medical University of Graz. The primary endpoint of this trial was the PFS ratio (PFS on targeted therapy/PFS on the last evidence-based therapy), regardless of whether the treatment decision was based on ctDNA or tumor tissue results. We defined the success of the targeted therapy by a PFS ratio ≥ 1.2 . Secondary outcome measures were as follows: the number of patients for whom an anti-tumor drug could be defined based on the molecular profile; the overall survival (OS) measured from the date of enrollment to the date of death or censored at the date of last follow up; the objective radiographic response rate (ORR) and treatment safety.

This protocol was approved by the Ethics Committee of the Medical University of Graz (approval number 27-169 ex 14/15) and written informed consent was obtained from all patients. The study was performed in accordance with the Declaration of Helsinki and is registered with the EU Clinical Trials Register [EudraCT: 2014-005341-44].

Patient eligibility

Eligible patients were between the ages of 18 and 85 years and had unresectable locally advanced and/or metastasized carcinoma who had exhausted all standard evidence-based therapies. Patients were required to have measurable disease by RECIST version 1.1 and an Eastern Cooperative Oncology Group (ECOG) performance status between 0 and 2, with a life expectancy of at least 12 weeks. Key exclusion criteria included patients with severe comorbidities, pregnant or breast-feeding patients, as well as patients with untreated central nervous system metastases or a history of previously diagnosed malignancies, except for

adequately treated non-melanoma skin cancer, *in situ* cancer, or other cancer from which the subject had been disease free for at least 5 years.

Molecular profiling of plasma DNA and tumor tissue through whole-genome and targeted sequencing

Our approach represents a histotype-agnostic workflow [Figure 1(a) and (b)]. A mandatory blood draw for ctDNA analysis and collection of a buccal swab for the detection of germline DNA variants were performed prior to treatment start. An additional tissue biopsy or resection of a metastasis for molecular tumor profiling was optional, depending on the patient's consent. If a tissue biopsy had been performed in routine clinical practice within 3 months prior to study entry, this tissue was considered suitable for MP. DNA isolated from tissue biopsies of metastatic lesions was subjected to library preparation using the Ion AmpliSeq Cancer Hotspot Panel v2 (Thermo Fisher Scientific, Vienna, Austria) and libraries were sequenced on the Ion Torrent platform. Plasma DNA was isolated using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany) and shallow whole-genome sequencing (sWGS) libraries were prepared and sequenced using the plasma-Seq method described previously.¹⁴ Somatic copy number alteration (SCNA) data analysis and identification of significant tumor-specific focal events was performed as described previously.^{15,16} Estimation of tumor fraction (TF) from sWGS data was performed using the ichorCNA algorithm, a probabilistic Hidden Markov Model (HMM) model for the estimation of tumor fraction, roughly equivalent to tumor purity from bulk tumor analyses.¹⁷ As calling absolute copy number (ABCN) depends on TF estimation from cfDNA, we employed a previously published approach¹⁸ to determine ABCNs using log₂ ratios from sWGS data and TF estimated by ichorCNA. For mutation analysis, library preparation was performed with plasma DNA in duplicates using either the TruSeq Cancer Amplicon Panel (Illumina) or the NEBnext Direct Cancer Hotspot Panel (New England Biolabs). In addition, genomic DNA isolated from a buccal swab was analyzed to specifically call for somatic mutations. Bioinformatics analyses were performed using open-source software. Briefly, FASTQ files were preprocessed to remove adapters and filtered for quality and were aligned with Burrows–Wheeler Aligner (BWA).¹⁹ Unique molecular identifier (UMI) sequences were added to the resulting binary alignment map (BAM) files with the

AnnotateBamWithUmis function from the fgbio package (<https://fulcrumgenomics.github.io/fgbio/>). Duplicate reads were marked with the MarkDuplicates function from Picard (GATK) and variant calling was performed using the MuTect2 (GATK) program in tumor-only mode and variant call format (VCF) files were annotated with ANNOVAR.²⁰ Resulting VCFs were filtered to only keep variants present in both duplicates but absent in genomic DNA with variant allele frequencies (VAF) $\geq 5\%$. Molecular profiles including single nucleotide variants (SNVs), indels, and SCNAs were subsequently summarized in a patient report. Publicly available genomic databases (My Cancer Genome, DGidb, Mining the Druggable Genome, CIViC) were mined, and a comprehensive literature search was performed in order to identify targeted treatments associated with the genomic alterations detected in the tumor genome.

Study treatment administration

Molecular tumor board (MTB) assessment was based on MP results from plasma DNA, and, if available, from tumor tissue. With this strategy, we ensured that patients could still potentially qualify for targeted treatment in the case that our plasma DNA analyses were uninformative. Additional predictive biomarkers consisted of previously established parameters already in routine clinical use, such as hormone receptor status, human epidermal growth-factor-receptor 2 (HER-2)/neu-status, RAS mutation status, epidermal growth-factor receptor (EGFR) mutation status, ALK rearrangement and programmed cell-death 1 (PD-1) expression. The MTB was attended by a medical oncologist, a pathologist, and a clinical geneticist. Results were evaluated with respect to the previously performed tumor treatment, the comorbidities of the patient and the availability of suitable drugs. All potentially applicable tumor-specific drugs were prespecified and are listed in Supplemental Table S1. The drug with the strongest evidence for a gene target indication was selected for further therapy. If two or more promising biomarkers were identified which allowed for a promising combination treatment, then we preferred to administer a combination therapy over a single agent. According to the study protocol, treatment start was scheduled within 28 days after the first study visit. If a treatment was started after this 28-day time frame, a note to file was generated. All patients who received treatment with a matched molecular-targeted agent had biweekly treatment

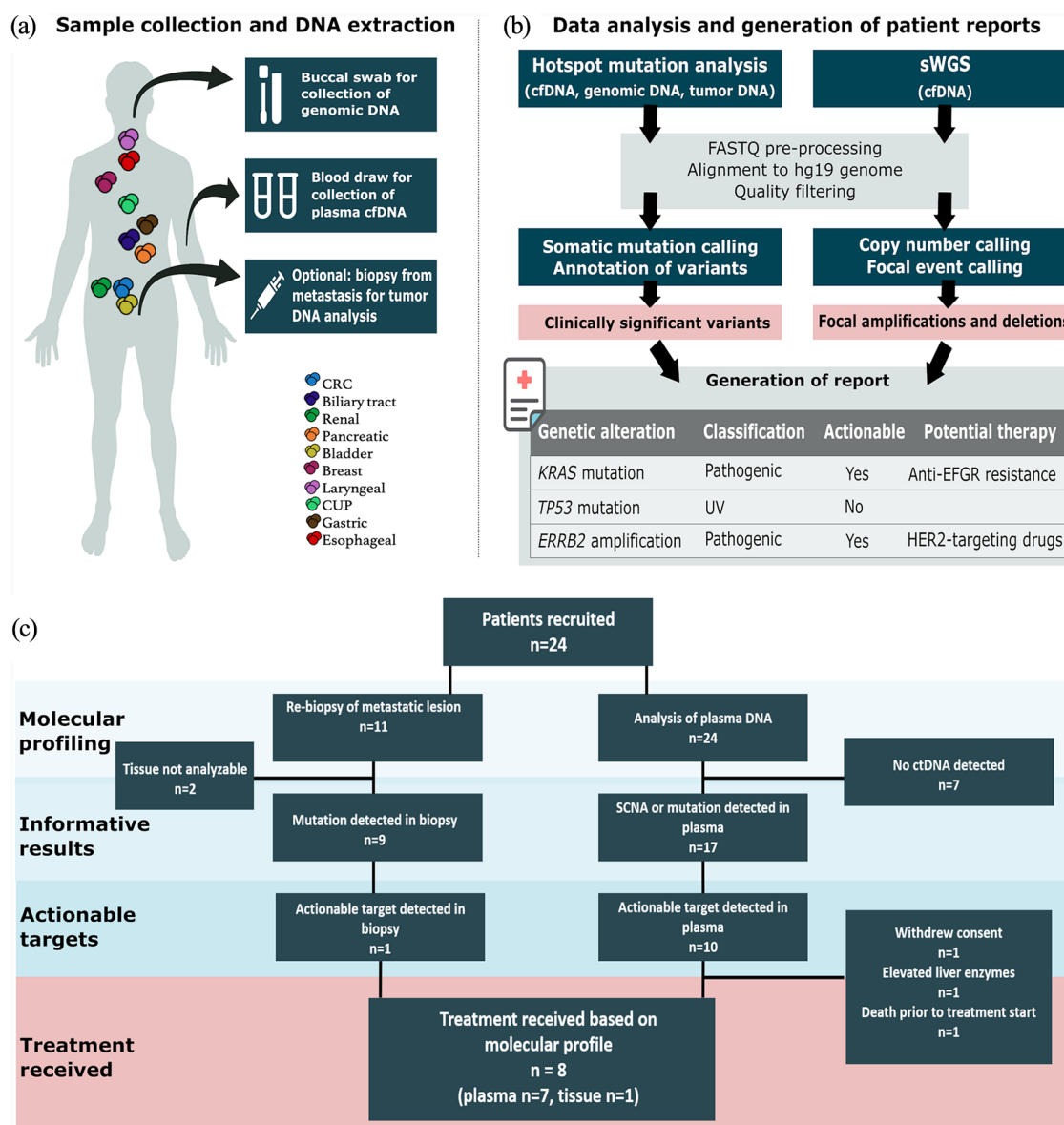


Figure 1. Study workflow.

(a) From each patient in this pan-cancer cohort, a mandatory buccal swab for the analysis of genomic DNA and blood draw for analysis of plasma DNA were collected. A re-biopsy of a metastatic lesion was optional and DNA from tissue was isolated and sequenced with a cancer hotspot panel. Plasma DNA was subjected to both targeted sequencing alongside germline DNA via a separate mutation hotspot panel and whole-genome sequencing via plasma-Seq for the detection of somatic copy number alterations (SCNAs). (b) After quality filtering of raw sequencing data, variant calling was performed with an open-source algorithm (MuTect2, GATK) and all novel variants not detected in germline DNA were annotated for clinical significance (ANNOVAR). For whole-genome sequencing data, copy number calling was performed to identify somatic focal events, that is, amplifications or deletions. These findings were summarized in a patient-specific report and further annotated with potential treatment options using publicly available databases. Results were discussed at an MTB and if a suitable targeted treatment could be aligned to the patient, the treatment was administered. (c) Flowchart of the study cohort.

CRC, colorectal cancer; CUP, cancer of unknown primary; DNA, deoxyribonucleic acid; cfDNA, circulating cell-free DNA; ctDNA, circulating tumor DNA; EFGR, epidermal growth-factor receptor; FASTQ, ; HER-2, human epidermal growth-factor-receptor 2; MTB, molecular tumor board; SCNA, somatic copy number alteration; sWGS, shallow whole-genome sequencing; UV, variant of uncertain significance.

visits according to a prespecified follow-up plan from the start of treatment until disease progression or death.

Study assessments

Radiological imaging, including CT scans of the chest, abdomen, and pelvis, optional magnetic

resonance imaging scans of the abdomen, as well as bone scans, were performed every 8 weeks and were assessed according to revised RECIST criteria version 1.1 or immune-related Response Criteria (irRC). Adverse effects were graded according to the National Cancer Institute Common Terminology Criteria of Adverse Events (version 3.0). PFS was measured from the date of enrollment to the date of documented disease progression (per revised RECIST criteria V1.1 or immune-related response criteria) or death. Alternatively, PFS was censored at the last radiologic imaging assessment date on which the patient was reported alive without progression. ORR was determined based on RECIST criteria 1.1 or irRC.

Samples size calculation and statistical analyses

Sample size was calculated based on the optimal two-stage design of Chen and Ng.²¹ Assuming a success (PFS of the targeted therapy $\geq 1.2 \times$ PFS of the last evidence-based drug therapy) rate of $p_1 = 0.25$ and $p_0 = 0.1$ at a significance level of $\alpha = 5\%$ and a power of $1 - \beta = 90\%$, 24^{21–28} patients should be included in the first stage of the trial. If the number of successes were > 2 with 21–24 or > 3 with 25–28 patients, then a total of 57–64 patients would have to be included in the study. Data were descriptively summarized using mean and standard deviation, median and interquartile range (IQR; first and third quartile) or range (minimum and maximum), or absolute and relative frequencies. Survival outcomes were presented with the Kaplan–Meier curve. R version 3.6.1. was used for all analyses.

Results

Patients

An interim analysis was performed after the recruitment of 24 patients and the study was terminated prematurely due to slow patient accrual and lack of treatment response [Figure 1(b)]. Median age of the study population was 59.5 years (IQR 53.8–67.0) and 11 patients were female (46%). All patients had an ECOG performance status of 0 or 1 and were heavily pretreated with a median number of 2.0 (IQR 2.0–3.2) previous palliative treatment lines. Additional baseline characteristics of the screening and treatment population are summarized in Table 1 and Supplemental Table S2. As outlined in the

following, the study was terminated and no further patients were recruited.

Informative molecular profiles and concordance of variants from tumor tissue and plasma

Plasma DNA was obtained from all patients for MP. Eleven patients (46%) also consented to a tissue biopsy. In one case, the biopsy did not yield malignant tissue (ICT11) and in another patient, no mutation could be identified (ICT20), thus achieving informative results from 9 (38%) biopsies. We did not detect ctDNA from plasma in 7 patients (29%) but were able to obtain informative results in the remaining 17 patients [71%; Figure 1(c)]. In the 17 cases in whom we were able to detect either at least 1 somatic mutation, focal SCNA or both, the median TF calculated from sWGS with ichorCNA in plasma was 22.7% (IQR 5.2–40.3) and was highest in colon cancer patients [Figure 2(b)]. Overall, a molecular profile obtained from plasma, tumor tissue or a combination of both could be obtained from 20/24 patients (83%). Median time from blood collection and tissue biopsy to report generation was 12 days (IQR 9–14) and 24 days (IQR 18.5–29.0), respectively.

For evaluating concordance of molecular profiles from tumor and plasma, we only considered mutations which were covered by both panels used for tissue and plasma analysis (Supplemental Table S3). Of the nine patients with informative molecular profiles from the tumor tissue, ctDNA with TF $> 5\%$ could be detected in six (67%) of the respective plasma samples, whereas in the other three patients (ICT08, ICT13, and ICT15), no tumor-specific mutation could be identified in plasma due to a low TF. In two other patients (ICT09 and ICT13), the same mutations detected in tissue were only observed in one duplicate and therefore not called by our algorithm. Instead, in one of these patients (ICT09), an *NRAS* mutation was identified in plasma, which was not found in the corresponding tissue. Likewise, in patient ICT19, a *CDKN2A* mutation was identified in plasma in addition to a *TP53* and a *PIK3CA* mutation known from the biopsy. In patients ICT18 and ICT16, the same *TP53* and *SMAD4* mutations were identified in plasma and metastatic tissue, respectively. However, in patient ICT18, plasma DNA analysis revealed a *KRAS* mutation that was originally identified in the primary tumor but missed in the metastatic biopsy. Vice versa, in patient ICT05, three out of four mutations identified in the biopsy (*APC*,

Table 1. Baseline characteristics of the study cohort.

Variables	Overall cohort (n = 24)	Treatment cohort (n = 8)*
Female sex	11 (46%)	5 (62.5%)
Age at baseline, years (IQR)	59.5 (53.8–67.0)	56.0 (51.5–63.5)
ECOG performance score at baseline		
0	11 (46%)	2 (25%)
1	13 (54%)	6 (75%)
Tumor location		
	/	/
Colon cancer	5 (25%)	4 (50%)
Pancreatic cancer	4 (16.7%)	2 (25%)
Gastric cancer	3 (12.5%)	0 (0%)
Biliary tract cancer	3 (12.5%)	1 (12.5%)
Rectal cancer	2 (8.4%)	0 (0%)
CUP	2 (8.4%)	0 (0%)
Esophageal cancer	1 (4.2%)	0 (0%)
Breast cancer	1 (4.2%)	0 (0%)
Bladder cancer	1 (4.2%)	0 (0%)
Kidney cancer	1 (4.2%)	1 (12.5%)
Laryngeal cancer	1 (4.2%)	0 (0%)
Previous lines of palliative chemotherapy	2.0 (2.0–3.2)	2.5 (2.0–5.0)
Metastasis present	24 (100%)	8 (100%)
Number of metastatic organs		
1	5 (20.8%)	1 (12.5%)
2	11 (45.8%)	5 (62.5%)
3	8 (33.3%)	2 (25%)
Liver metastasis present	13 (54.2%)	5 (62.5%)
Lung metastasis present	13 (54.2%)	4 (50%)
Time from date of tumor diagnosis to study enrollment (days)	674 (499, 1253)	715 (655, 1019)
Time from progression under last palliative chemotherapy until study enrollment (days)	10.0 (5.5, 19.5)	16.0 (7.8, 20.2)
Data represent medians (first–third quartile) for continuous data and absolute frequencies (%) for categorical data.* One patient was incorrectly treated with a molecular-targeted agent, as a tissue biopsy prior to 3 months of the treatment start was used for molecular profiling. This patient was consequently excluded from the treatment cohort. CUP, Cancer of unknown primary; ECOG, Eastern Cooperation Oncology Group performance status; IQR, interquartile range.		



Figure 2. Study cohort overview, molecular profiling and actionability summary. (a) Cohort composition by primary tumor entity ($n=24$); (b) bar plot representing total percent tumor content in plasma estimated from ichorCNA (right y axis) as well as the number (left y axis) of alterations detected combined from plasma and tissue, SCNAs detected in plasma and mutations per patient detected combined from plasma and tissue results; (c) oncoplot of most frequently detected mutations across the entire cohort from plasma DNA analyses (left) and from tissue DNA analyses (right); (d) overall percent actionability calculated for the 20 informative cases per profile combined from plasma and tissue results. CUP, cancer of unknown primary; DNA, deoxyribonucleic acid; ICT, Individualized Cancer Treatment; SCNA, somatic copy number alteration.

KRAS, and *TP53*) were also detected in plasma, but the *SMAD4* mutation could not be detected. A particularly interesting case was ICT06, in which the *TP53* mutation detected in the biopsy material could not be identified in plasma, but five completely different mutations were called (*APC*, *KDR*, *PTEN*, *RET*, *SMO*). Overall, the concordance between mutations from tissue detected in plasma and from plasma detected in tissue was rather low at 77% and 53%, respectively.

Molecular profiling confirms genomic diversity between patients

From both plasma and tissue analyses, missense mutations comprised the majority of somatic mutations in the cohort, affecting most commonly *TP53*, *KRAS*, *PIK3CA* and *APC* [Figure 2(c)]. In plasma, higher average VAFs generally correlated with the presence of higher overall tumor fractions established with ichorCNA, although there were several outliers, indicating a difference in

mutation-derived *versus* SCNA-derived VAFs (Supplemental Figure S1; Lin's concordance correlation coefficient = 0.6). Of the total number of alterations detected in plasma DNA, the majority were composed of either SCNAs or mutations, varying from patient to patient [Figure 2(b)]. For example, in 10 patients, mutations comprised $\geq 80\%$ of the alterations detected, whereas in 7 cases, SCNAs were more frequently detected than mutations [Figure 2(b)]. Notably, no two patient profiles were identical (Supplemental Table S3), demonstrating the concept of 'molecular snowflakes' when profiling tumors from individual patients.²²

Actionability of detected targets

Of the 20 informative patient profiles, 17 derived from plasma and 3 from tissue biopsies. Druggable alterations could be identified in 11 patients (55% of the patients with informative results, 46% of entire recruited cohort; Table 2). The majority were detected from plasma, as at least 1 actionable target could be identified in 10 patients (91%) according to analysis of plasma DNA and in only 1 patient according to tissue biopsy [Figure 1(c), Table 2]. The majority of druggable alterations were focal SCNAs and, in two cases, mutations in *PIK3CA* or *PTEN* were considered to be an actionable target (Table 2). We calculated the percent overall actionability from the MP results by dividing the number of actionable alterations by the total number of detected alterations and observed an overall percent actionability of 14.6% across the cohort [Figure 2(d), Supplemental Table S4].

Treatment matching

Our treatment matching algorithm (ICT algorithm) involved the further interpretation and discussion of the annotated MP results at an MTB consisting of oncologists, a pathologist, and a geneticist.

Results from plasma DNA analyses and tissue DNA analyses were combined prior to discussion. Based on the annotated sequencing results, a potential tumor-specific drug could be matched in 11 (46%) patients. In two colorectal cancer (CRC) cases (ICT01 and ICT05), a focal amplification of chromosome 13q12 harboring the *FLT3* gene was detected for which the targeted drug sorafenib was matched. Other matching examples include mammalian target of rapamycin inhibitors for *PTEN* and *PIK3CA* alterations (ICT04,

ICT07, and ICT19), the combination of trastuzumab and lapatinib for *ERBB2* amplifications (ICT13, ICT14), and nintedanib for receptor tyrosine kinase (RTK) targets such as *PDGFR* and *FGFR* (ICT22, ICT24). In one patient, a *RET* amplification was targeted with sunitinib (ICT15) and another patient had a targetable *MYC* amplification, but the patient died prior to the MTB. Together with two other patients who were not eligible for treatment due to withdrawal of consent (ICT14) or inadequate liver function (ICT22), this results in 27% of patients with targetable alterations who subsequently did not receive a therapy [Figure 1(c), Table 2]. Thus, eight patients (33% of total cohort) were treated with a matched drug in accordance with the MP results, of which only one received treatment within the predefined period of 28 days after study enrollment (Table 2).

Median time from study enrollment and written informed consent until initiation of treatment was 34 days (IQR 28.5–37.3).

Survival outcomes

Two patients (ICT07 and ICT15) reached a longer PFS with the MP-based treatment compared with the last evidence-based treatment [Figure 3(a)]. However, regarding the primary endpoint analysis, none of the eight treated patients experienced a PFS ratio ≥ 1.2 for the MP-guided therapy compared with the last evidence-based treatment line [Figure 3(a)]. Median PFS with the MP-based treatment was 61.5 days (IQR 49.8–71.0) compared with 81.5 days (IQR 68.5–117.8) with the last evidence-based treatment line, resulting in a median PFS ratio of 0.7 [IQR 0.6–0.9; Figure 3(b)].

Furthermore, radiologic response assessment according to RECIST criteria was performed in six of eight patients. The overall response rate was 0%, with five patients experiencing primary disease progression and with one patient achieving stable disease. In two patients, radiologic imaging was not performed due to prior clinical progression. The median OS from date of study enrollment up to date of death or censoring was 207 days (IQR 126–292) and 112 days (IQR 60–164) in patients who did and did not receive genome-guided treatment, respectively (Supplemental Figure S2). No treatment-related deaths or treatment-related serious adverse events were observed. (Supplemental Table S2).

Table 2. Patients successfully matched to treatment based on molecular target identified *via* molecular profiling.

Patient ID	Druggable target(s) identified	Absolute copy number	Profiling source (i.e. plasma or tissue) used to make decision	Matched treatment	Best treatment response
ICT01	FLT1, FLT3 amplification	FLT1: 17.25 FLT3: 17.25	Plasma	Sorafenib	PD
ICT04	PTEN mutation, PIK3CA mutation	NA	Plasma	Everolimus	SD
ICT05	FLT3 amplification	FLT3: 7.94	Plasma	Sorafenib	PD
ICT07	PTEN deletion	PTEN: -1.87	Plasma	Temsirolimus	PD
ICT13	ERBB2 amplification	ERBB2: 3.79	Plasma	Trastuzumab + lapatinib	PD
ICT14	ERBB2 amplification	ERBB2: 4.41	Plasma	Trastuzumab + lapatinib	Not given (withdrew consent)
ICT15	RET amplification	RET: NA	Tissue	Sunitinib	PD
ICT16	MYC amplification	MYC: 8.23	Plasma	Death prior to MTB	Not given (death)
ICT19	PIK3CA mutation	NA	Plasma	Everolimus	PD
ICT22	FGFR4 and CDK6 amplification	FGFR4: 7.89 CDK6: 5.85	Plasma	Nintedanib or palbociclib	Not given (elevated liver transaminases)
ICT24	KDR, KIT, PDGFRA amplification	PDGFRA: 3.09	Plasma	Nintedanib (in combination with nab-paclitaxel)	PD

ICT, Individualized Cancer Treatment; MTB, molecular tumor board; NA, not available; PD, progressive disease; SD, stable disease.

Retrospective analysis using CureMatch clinical decision support yields alternative combination therapy recommendations

The matching of molecular targets to the most suitable treatments is at present not standardized. Notably, our ICT algorithm, which consisted of mining annotation for SNVs, indels and SCNAs from publicly available genomic databases (My Cancer Genome, DGidb, Mining the Druggable Genome, CIViC) alongside comprehensive literature searches and discussions at the MTB, resulted in treatment matches which were based on actionable SCNAs in nine patients, while only two treatment decisions were based on mutations. Therefore, we sought to identify potential alternative treatment options and, to this end, we retrospectively analyzed seven patient profiles with the CureMatch decision support algorithm, a platform which assesses the entire molecular profile for matching combination therapies rather than evaluating individual targets for their actionability.²³ The results of these analyses were outside of the scope of the treatment algorithm in this study and were only evaluated as

retrospective information. Only the results from plasma DNA analyses were considered. The CureMatch algorithm detected 10 additional actionable mutations and 6 additional actionable focal SCNAs in comparison with our manual ICT matching algorithm [Figure 4(a)]. In all the retrospectively analyzed cases, with the exception of ICT13, the CureMatch platform yielded a higher overall percent actionability compared with our matching workflow [Figure 4(b), Supplemental Table S5]. We were unable to match a treatment to patient ICT18, whereas CureMatch analysis matched several three-drug and two-drug combinations to the molecular profile (Supplemental Table S6). Interestingly, for patient ICT13, our ICT algorithm matched the combination of trastuzumab and lapatinib to the presence of a focal *ERBB2* amplification, a result which aligned completely with CureMatch analysis and had a matching score of 98% [Figure 4(c), Supplemental Tables S6–7]. This was the highest obtained matching score compared with the remaining distribution of scores obtained for the other patient profiles [Figure 4(c)]. Four patients

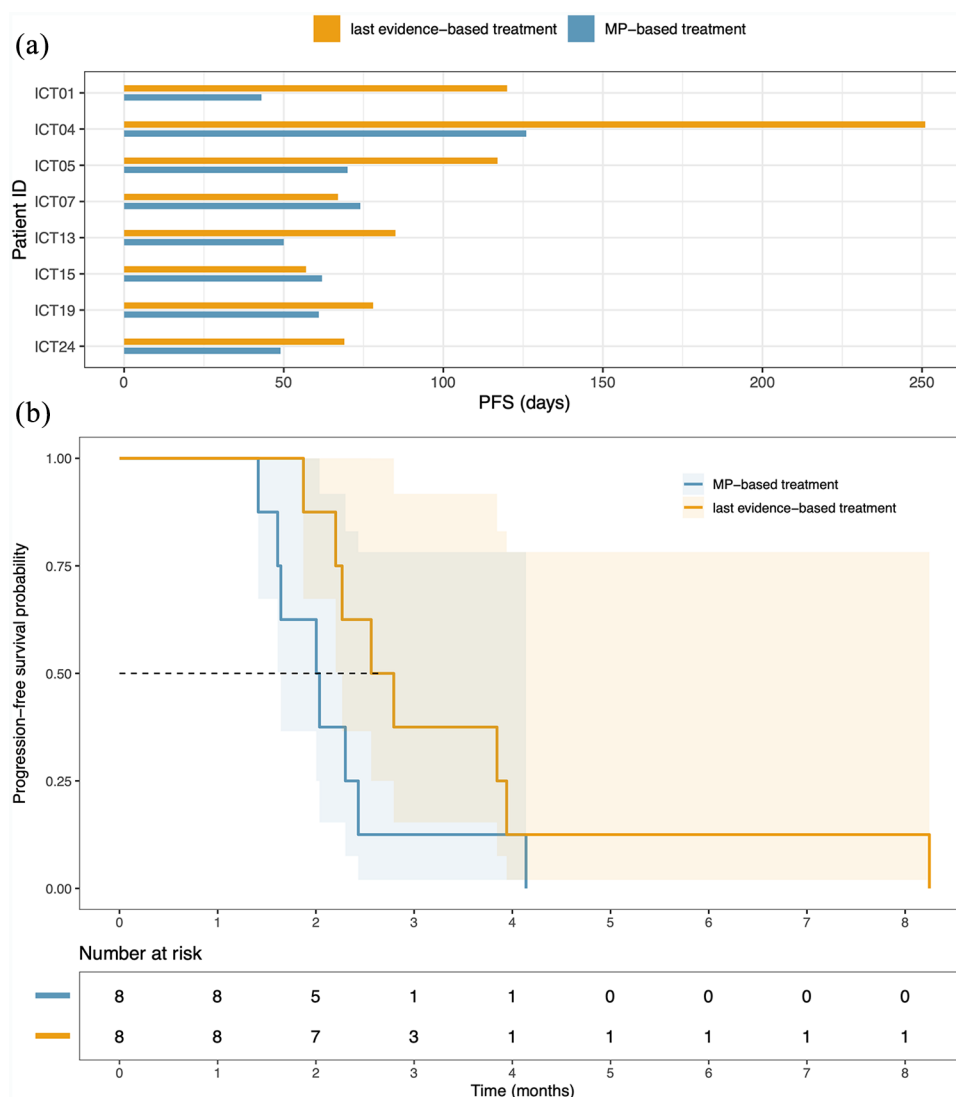


Figure 3. Progression-free survival of patients receiving MP-based treatment. (a) Swimmer's plot of the overall screening cohort presenting individual PFS data of MP-based treatment and the last evidence-based treatment line for each patient; (b) Kaplan-Meier curve comparing PFS from the MP-based treatment with the last evidence-based treatment line. MP, molecular profiling; PFS, progression-free survival.

had at least one treatment regimen corresponding to a matching score >50% [Figure 4(c)].

Discussion

The ICT trial represents the first prospective study reporting outcome results on the efficacy of ctDNA and metastatic tissue DNA profiling-based treatment in heavily pretreated metastatic cancer patients. Overall, a conclusive molecular profile could be achieved in 20/24 (83%) of patients screened and in almost 50% (11/24 patients), a potentially actionable molecular alteration could be identified. Eight patients (33%)

were treated with a molecularly-targeted agent, but based on our predefined criterium of a PFS ratio ≥ 1.2 , none of these derived a clinical benefit therefrom. As patient accrual was slower than expected and no clinical benefit could be demonstrated in the patients treated according to their molecular profile, the study was terminated after an interim analysis was performed.

Precision oncology hypothesizes that anticancer therapy should be matched to each patient in accordance with the molecular profile of the tumor. In contrast to MP of primary tumors, ctDNA represents an innovative and non-invasive option for

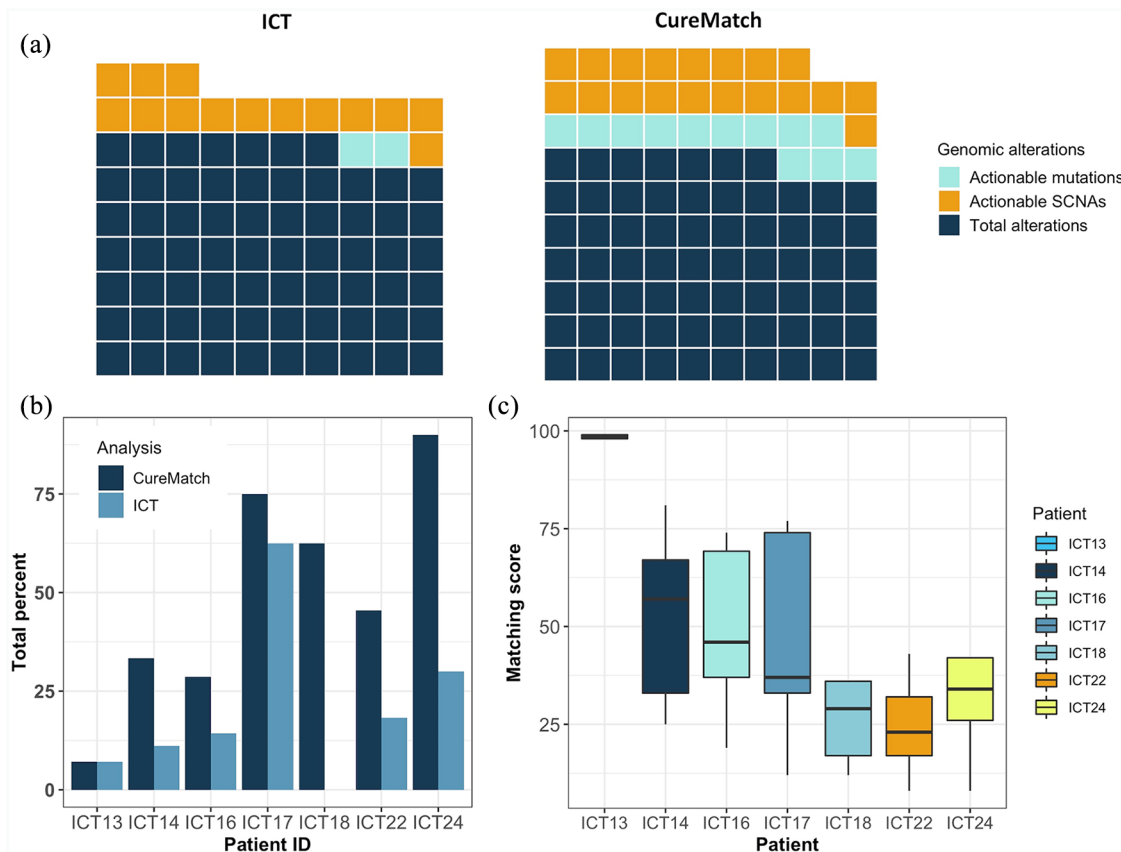


Figure 4. Comparison of actionability between ICT and CureMatch matching algorithms using plasma DNA analyses.

(a) Proportion of total alterations and actionable alterations detected according to the ICT algorithm (left) or according to the CureMatch algorithm (right); (b) comparison of overall percent actionability per patient determined by either the ICT algorithm (light blue) or CureMatch algorithm (dark blue); (c) distribution of matching scores across seven patient profiles analyzed retrospectively *via* CureMatch algorithm.

ICT, Individualized Cancer Treatment; SCNA, somatic copy number alteration.

assessing the current status of the molecular landscape of tumor genomes from patients with metastatic cancer.^{24–28} Only 11 patients volunteered for a re-biopsy of metastatic lesions, whereas all patients were willing to donate blood samples. This reflects a typical clinical setting in which tissue is not always available to inform treatment decisions. Therefore, the majority of treatment decisions described here were based on ctDNA profiling results. The negative ctDNA results in seven patients can be attributed to the limit of detection of plasma-Seq, which requires a minimum of 5% tumor-derived DNA fragments to yield informative copy number results. Furthermore, in order to avoid false positives, which could result in the over-treatment of patients with an already poor performance status, we applied very stringent filter criteria for the detection of ctDNA using the hotspot panels (limit of detection 5%).

For the limited cases in which we had metastatic biopsy samples, we used the tissue results to assess the concordance between plasma DNA and tissue. The observed concordance of tissue-derived variants detected in plasma was somewhat lower than rates described in other recent works,^{13,17,29,30} which may be attributed to the different panels and sequencing platforms which were used to analyze tissue and plasma³¹ and to the very stringent criteria for our hotspot panel analyses from ctDNA. The use of next-generation-sequencing panels employing UMIs with a much broader gene coverage and well-established bioinformatic workflows that have come to market since the completion of this study, now enable ctDNA detection down to 0.1% VAF and may lead to the detection of a higher number of (potentially actionable) genomic alterations in future studies, even at low VAFs.³²

Although our study had to be terminated after the interim evaluation, this report provides important considerations for future study designs. First, the timing of MP-based treatment trials may be decisive. As it is common for such studies, we selected patients with very advanced disease. However, advanced tumors are extensively heterogeneous both spatially across distinct regions and temporally in response to treatment. Furthermore, the general condition of patients with advanced cancer may have complicated treatment success, which was demonstrated by two patients in particular who were unable to receive the matched treatment as a result of clinical deterioration (ICT22) or death (ICT16). Thus, it can be hypothesized that personalized cancer treatment might be more effective in earlier treatment lines.

Second, as our primary endpoint, we assessed the PFS ratio using the patient as his or her own control. However, PFS in cancer patients generally becomes shorter with each subsequent line of therapy.² While two patients achieved a longer PFS with the MP-based treatment than with the previous evidence-based treatment, overall, each of the eight patients treated according to their molecular profile failed to achieve a PFS ratio ≥ 1.2 . This raises the possibility that meaningful PFS ratios as endpoints for such trials may have to be defined more precisely depending on the clinical status of the patients.

Third, due to a rapid tumor evolution, patients with advanced cancer may no longer be suitable for the selected treatment if the turn-around time from sampling to the treatment decision takes several weeks. Due to the limited clinical support for off-label use of targeted therapies, we faced reimbursement challenges and not all the matched targeted agents were immediately available. In fact, a median time frame from study enrollment until start of treatment was 34 days in our study, which was too long, considering the advanced disease stage of most of our patients.

Fourth, a major cause for treatment failure is founded in the complexity of cancer pathogenesis and its heterogeneity. For example, common driver alterations in one tumor type do not necessarily drive the tumor progression in another entity, which is why the effectiveness of targeted therapies depends on the tumor type. A prime example is the effective inhibition of *BRAF* V600E in melanoma and non-small cell lung cancer, whereas *BRAF* inhibitors alone have limited

activity in *BRAF* V600E-mutated CRC.^{33,34} This phenomenon was exemplified in the two CRC patients ICT01 and ICT05 harboring *FLT3* amplifications. *FLT3* is a well-known driver gene in hematological malignancies that can be targeted by the drug sorafenib.³⁵ A case study of a patient with metastatic colon cancer who was found to have *FLT3* amplification reported partial response to sorafenib.³⁶ Therefore, we categorized this alteration as druggable and both patients received sorafenib. Based on our knowledge today, it was not surprising that we did not observe a response in these patients. We recently characterized this chromosome 13q12 amplification to elucidate the candidate driver gene.¹⁶ We found that *FLT3* does not demonstrate an oncogenic role in CRC, but rather that an amplification of *POLR1D* is potentially the driving event in these cases. This is just one example of the evolving understanding of genomic actionable targets achieved through the systematic sequencing of tumor genomes, and such novel findings most likely would have influenced the decision reached at the respective MTBs. Another example for the ever-growing knowledge base leading to the identification of more molecular targets and the subsequent development of new targeted agents is patient ICT15, who presented with a *RET* amplification and was treated with the multi-kinase inhibitor sunitinib, since *RET* inhibitors were not yet available at the time the patient in question was treated.³⁷

The final call as to which treatment should be administered remains challenging. To this end, a recent comparison of clinical decision support platforms conducted by our group revealed a significant variation of treatment recommendations.³⁸ To further explore this in a retrospective setting, we analyzed selected molecular profiles using the CureMatch PreciGENE treatment matching algorithm. The CureMatch approach represents a multi-pathway molecular analysis for the identification of personalized treatment options, which are ranked using a predictive 'matching score' that reflects the degree to which therapies align to a patient's molecular profile.²³ This retrospective analysis demonstrates the complexity and diversity of existing matching algorithms that would ultimately lead to variable MTB decisions and highlights a potential shift in the current clinical trial paradigm. Hence, administering customized multi-drug treatments may represent an effective alternative to the one-aberration-one-drug model.³⁹

Taken together, our results align well with previous studies reporting a low molecular screening efficiency, with only a small fraction of cancer patients profiting from molecular genomic profiling. In 2018, still only 8% of all metastatic cancer patients were potential candidates for FDA-approved molecular-targeted treatment and only 5% were estimated to derive a clinical benefit therefrom.⁴⁰ In studies evaluating the feasibility and efficacy of molecular-targeted treatment outside their clinical indication, treatment benefit rates were reported to be even lower.⁴¹ The SHIVA trial,⁴² which still remains the only conducted randomized trial comparing the efficacy of targeted agents selected on the basis of tumor molecular profiling, did not meet its primary endpoint of improved PFS compared with treatment of the investigator's choice. Rather unpromising results were further demonstrated from the ProfILER trial, in which only 6% of patients in the large cohort ($n=2579$) received a matched treatment according to their profiling results, with only 13% achieving partial response, that is, 0.9% of the total study population.⁴³ These results are in line with previous studies such as the MOSCATO trial, which achieved an ORR of 2% in all patients screened.⁴⁴ The preliminary results from the largescale trials MATCH and MPACT from the United States National Cancer Institute are along this line,⁴⁵ and a number of reasons have been implicated for these rather disappointing experiences.⁴¹ Yet, preliminary results of the TARGET study, which used a ctDNA-guided therapy selection approach, achieved an ORR of 36% in matched patients.¹³ Another similar large-scale study employing ctDNA specifically geared towards matching advanced breast cancer patients to four predefined treatment cohorts was recently completed, demonstrating clinically relevant activity of targeted therapies against rare *HER2* and *AKT1* mutations.⁴⁶

In summary, despite the premature study termination and the small samples size, which led to risk of a potential underestimation of the treatment effect, studies like ours are critical to advancing the implementation of precision medicine and highlight the need to re-evaluate the current clinical trial design and adopt other approaches to matching molecular alterations to targeted therapies.

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Authors' contributions

Conceptualization: AG, MRS, GH, HS; resources: JMR, GP, FP, MP; data analysis: JMR, SOH, EH, KK, SWJ; data interpretation: JMR, SOH, EH, AG, MRS, GH; writing (original draft preparation): JMR, SOH; writing (review and editing): JMR, SOH, EH, MRS, AG; visualization: SOH; supervision: AG, EH, MRS, GH; project administration: KG; statistical analysis: AB; funding acquisition: MRS, EH, AG.

Conflict of interest statement

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Ethics approval and consent to participate

This protocol was approved by the Ethics Committee of the Medical University of Graz (approval number 27-169 ex 14/15) and written informed consent was obtained from all patients. The study was performed in accordance with the Declaration of Helsinki and is registered with the EU Clinical Trials Register [EudraCT: 2014-005341-44]. Trial registration: ISRCTN, ISRCTN12345678. Registered 28 September 2014, <http://www.isrctn.com/ISRCTN12345678>.

Consent for publication

All authors have read and agreed to the published version of the manuscript.

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Availability of data and material

The datasets generated during and/or analyzed during the current study are not publicly available due for data protection reasons but are available from the corresponding author on reasonable request.

Supplemental material

Supplemental material for this article is available online.

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