Utility of ctDNA to support patient selection for early phase clinical trials: The TARGET Study

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Introductory Paragraph

Next generation sequencing (NGS) of circulating tumour DNA (ctDNA) supports blood-based genomic profiling but is not yet routinely implemented in the setting of a phase I trials clinic. TARGET is a molecular profiling programme with the primary aim to match patients with a broad range of advanced cancers to early phase clinical trials based on analysis of both somatic mutations and copy number alterations (CNA) across a 641 cancer-associated gene panel in a single ctDNA assay. For the first 100 TARGET patients, ctDNA data showed good concordance with matched tumour and results were turned round within a clinically acceptable timeframe for Molecular Tumour Board (MTB) review. When applying a 2.5% Variant Allele Frequency (VAF) threshold, actionable mutations were identified in 41/100 patients and 11 of these patients received a matched therapy. These data support the application of ctDNA in this early phase trial setting where broad genomic profiling of contemporaneous tumour material enhances patient stratification to novel therapies and provides a practical template for bringing routinely applied blood-based analyses to the clinic.

Results and Discussion

The selection of patients to early phase clinical trials and clinical outcomes can be enhanced by molecular stratification (1-6) and most precision medicine strategies to date are based on DNA sequencing of archival or fresh tumour biopsies (7-9). However, genomic profiling of archival specimens can be limited by sample age, quality, low tumour content and tumour heterogeneity. Also, archival samples by their very nature, do not take into account on-going tumour evolution, particularly if patients have received therapies which may confer acquired resistance. Acquisition of fresh tissue is often challenging and not without patient risk, yet there is increasing demand for tumour material in the context of clinical trials and molecular profiling. ctDNA is extractable from a peripheral blood sample and provides a contemporaneous profile of the tumour genomic landscape. NGS technology has evolved for reliable sequencing of ctDNA (10,11), but clinical validation is needed to drive forward routine use of ctDNA in the clinic (12). The TARGET (Tumour chARacterisation to Guide Experimental Targeted therapy) study was designed to determine the feasibility of using ctDNA, relative to tissue-based testing to identify clinically actionable mutations in early phase clinical trial patients with a range of advanced stage cancers (Figure 1a). Our study was divided into Part A (100 patients) to establish an analytical workflow and assess feasibility of data turnaround in a timeframe of 2-4 weeks to support clinical decision-making, and Part B (450 patients) to test clinical utility following selection of patients in real-time to molecularly matched trials based on their ctDNA and/or tumour genomic profile. Here we present data from Part A of the TARGET trial demonstrating the 'real world' feasibility for routine

implementation of ctDNA profiling to increase the chance of matching patients with advanced cancers to a Phase I trial of an appropriate targeted therapy.

The first 20 patients' blood samples were used to optimise the ctDNA workflow with automated ctDNA purification demonstrating comparable yields to manual isolations (Extend Data Figure 1a). Hybridization and enrichment of a 2.1Mb Agilent SureSelect panel targeting 641 genes recurrently mutated in cancers (Supplementary Table ST1) to the ctDNA library and germline control for each patient resulted in an average 1322-fold enrichment (range 359-5804) of targeted genes (Extend Data Figure 1b). Sensitivity and reproducibility of the NGS assay was tested on a reference panel of five samples with highly characterized genotypes from the European Molecular Genetics Quality Network (EMQN). All 14 reference mutations in the five EMQN samples were detected with 100% specificity and sensitivity and >90% correlation of expected allele frequency across all mutations detected (Extend Data Figure 1c).

Having demonstrated the reliability of the ctDNA workflow, we expanded the cohort to 100 patients referred to the Experimental Cancer Medicine Team (ECMT) at The Christie NHS Foundation Trust for consideration of early phase trials. The patient cohort consisted of 22 different tumour types, with a median age of 56 years and patients had received a median of two prior lines of therapy (Extend Data Figure 2, Supplementary Table ST2). ctDNA NGS data was generated successfully for 99% of patients, compared to tumour tissue DNA analysis in 95% (Figure 1b). The average de-duplicated read depth across all ctDNA samples was 699 (range 108-1760) (Supplementary Table ST3). In this cohort of patients, 67% of tumour biopsies were >1 year

97 old and 36% >3 years old (range 0-5635 days pre-blood collection) (Figure
98 1b) highlighting the benefit of ctDNA sampling.

99 Critical to any molecular profiling program is turnaround of results within a
100 meaningful timeframe to facilitate clinical decision-making for an individual
101 patient and to minimise the risk of dropout from clinical trial participation due
102 to declining health. Our data show comparable report times for FFPE tumour
103 tissue analysis and ctDNA; with a mean report time from blood draw of 33
104 calendar days (range 20-80) for patients 21-100, comparable to a mean
105 tumour DNA report time of 30 calendar days (range 17-140) from date of
106 consent to receipt of result (Figure 1c).

107 All tumour samples were analysed in a National Health Service (NHS),
108 ISO15189 accredited clinical laboratory, initially using a 19-gene MassArray
109 assay (Sequenom OncoCarta™ v1.0; 57% patients) and more recently a 24-
110 gene GeneRead PCR amplicon assay (Qiagen Clinically Relevant Tumour
111 Targeted Panel V2; 43% patients), which represent cancer panel assays
112 clinically accredited in the UK NHS at the time of the study. A total of 69 non-
113 synonymous mutations were identified in tumours across 54 patients, with no
114 mutations reported for the remainder. Analysis of the corresponding mutations
115 in the ctDNA NGS data revealed good concordance, with 54/69 mutations
116 (78.6%) also detected (Figure 1d, Extend Data Figure 3). This level of
117 concordance, even accounting for differences between gene panels and
118 levels of sensitivity between the tumour and ctDNA assays compares
119 favourably with other recently described studies (10,13,14). The ctDNA assay
120 was also compared to the FoundationOne® panel in a subset of 39 patients
121 where the matched tumour also underwent Foundation Medicine testing

(Supplementary Table ST4). This enabled analysis across a broader panel of 230 genes present in both the 641-gene and FoundationOne® panels. In this patient subset 74 mutations were reported in the ctDNA, of which 52 were also reported in the tumour (70% concordance). A larger number of mutations were reported in the FoundationOne® tumour analysis for these patients, which most likely reflects a combination of a high tumour fraction in the input DNA and the ability to identify mutations belonging to minor tumour subclones that could not be picked up in ctDNA (Extend Data Figure 4).

For reporting mutations to the MTB, we applied a 2.5% VAF threshold to ensure reliability and robustness. Though more sensitive approaches are available (13), our rationale for TARGET was to evaluate whether a 2.5% VAF cut-off was suitable for clinical application and treatment decision making for phase I patients with advanced disease often having exhausted other treatment options. It has been shown that ctDNA yield is linked to tumour cell proliferation and death rates (15, 16) and therefore all ctDNA-based assays may have some bias towards higher tumour burden that should be taken into consideration when interpreting associated results. With this in mind, we asked whether the higher VAF threshold used here would result in bias towards patients with higher ctDNA yield or higher tumour burden. We did not find a significant correlation between VAF and cfDNA yield (Extend Data Figure 5a and 5b), which may be due to our cohort being phase I clinical trial patients, who will tend to have a large tumour burden and ctDNA yield. However, a significant correlation was observed between average VAF and number of metastatic sites ($p = 0.0118$), which was used here as a surrogate of tumour burden (Extend Data Figure 5c and 5d). Whilst our 2.5% VAF

147 threshold might result in ‘false negatives’ and inherently bias towards patients
148 with higher disease burden it will reduce ‘false positives’ and the assay
149 facilitates broad panel testing for a diverse range of alterations required in the
150 phase I trial setting, compared with smaller panel or single gene assays
151 where the sensitivity may be higher.
152 Using the 2.5% VAF threshold 70/94 patients with both tumour and ctDNA
153 analysed showed concordance of reported mutations (74.5%)(Figure 1e).
154 Discordance occurred in 24 patient samples: 20/24 had tumour mutations
155 undetected in ctDNA (9 of these mutations were detectable in ctDNA, but
156 below the 2.5% VAF threshold) and 4/24 had mutations in ctDNA, but not
157 corresponding tumour. No correlation between tumour biopsy age and
158 mutation discordance with ctDNA was evident (Extend Data Figure 6). Where
159 discordance was seen, this could often be ascribed to either a biological or
160 clinical consequence: for example, TAR-039, a colorectal cancer patient
161 exhibited a *KRAS* c.34G>T p.(Gly12Cys) mutation in their ctDNA (VAF 3.4%),
162 which was not detected in the archival tumour specimen collected 26 months
163 previously. This is likely linked to the administration of anti-EGFR therapy
164 (panitumumab) in the intervening period to which *KRAS* mutation is a well-
165 described mechanism of resistance (17).
166 A 641-gene panel was designed for application in the early phase ‘all cancer
167 types’ trial setting because of its potential to provide a broader coverage of
168 alterations/co-mutations, mechanisms of resistance and facilitate the selection
169 of novel targeted agents. The ctDNA assay provided a broad view of the
170 mutational landscape across the various cancer types, with ≥1 mutation
171 detected in 70% of patients (Extend Data Figure 7, Supplementary Table

ST5). Clear differences were seen in the number and allele frequencies of mutations across tumour types (Figure 1f), though patient numbers were too small to assign significance. We propose that this ctDNA assay will be most useful for certain patient populations/histological sub-types since in our study no mutations were detected in certain tumour types (e.g. adrenal cancer), whereas in others, for example breast cancer, SCLC and CUP >80% patients had detectable ctDNA mutations. These data are based on limited patient numbers and could be confounded by differences in tumour volume and as such require validation in larger patient cohorts.

Another advantage of the broad panel targeted enrichment approach is that it enables evaluation of CNA, as well as mutation profiling within the same assay. The ability to accurately call CNA is important as many clinically actionable alterations in cancer are structural alterations (18) as evidenced by the GENIE cohort (19) of 13,641 patients where structural variants accounted for 43% of 17,069 actionable mutations (personal communication, Dr Philip Beer). ctDNA CNA was compared to tissue-based CNA in a subset of 8 patients who had standard low-pass, whole genome sequencing (WGS) of their ctDNA (20), and in 23 patients where the matched tumour had CNA reported following FoundationOne® analysis (Figure 2a, Supplementary Table ST4). High concordance was seen between genome-wide CNA analysis of the 641-gene pull-down ctDNA and low-pass WGS profiles (Extend Data Figure 8). Concordant gene-level alterations were detected in 11/23 (48%) patients with both tumour FoundationOne® and ctDNA analysis available (Extend Data Figure 9, Supplementary Table ST6). As previously reported (21, 22) accurate CNA calling from ctDNA requires a higher fraction of ctDNA

in the sample and when we applied an average VAF ≥5% threshold (15/23 patients) for CNA analysis, concordance with tumour increased to 11/15 (73%, Extend Data Figure 9).

An important aim for Part A of TARGET was to establish a routine MTB for the formal reporting and discussion of tumour and ctDNA mutational profiles of the 100 Part A patients. A challenge identified at the MTB was efficient and effective integration of clinical and genomic data. This prompted the development of eTARGET, an in-house digital solution integrating a single overview of patients' clinical and genomic characteristics. eTARGET includes a storage account for data upload, a database for storing and integrating data and a web-application for data visualisation (Extend Data Figure 10). eTARGET enables the MTB to review summary patient data via a single portal (and remotely if required), capture meeting outcomes in real-time and upload information to electronic patient records.

A potential reason why large molecular screening programs have traditionally allocated only 10-15% of patients to studies may be in the interpretation of variants of unknown significance (VUS)(7,8,9). It is challenging for any MTB to have knowledge of all possible variants and databases are in development for pooling relevance of VUS (23,24). We addressed this issue by accessing software packages to aid interpretation of the relevance of specific variants and identify appropriate trials in different regions of the UK or in Europe. The Qiagen Clinical Interface (QCI) software package was considered valuable in differentiating actionable mutations (and recommended matched therapies) from those of unlikely clinical relevance and provided tiering following ACMG/AMP/CAP guidelines.

Following MTB review, 41 of the first 100 TARGET patients had an alteration considered to be actionable of whom 11 received a matched therapy, 17 received a non-matched therapy (largely due to trial availability at site) and 13 either had no trial available, did not meet study specific eligibility, deteriorated clinically or went on to a chemotherapy option (Figures 2b and 2c). For the 11 patients that received a matched therapy, partial response (PR) was achieved in 4/11 and stable disease (SD) (minimum of 3 months) was observed in 7/11 patients. Median duration on therapy was 6 months (range 1.5-20 months) (Figure 2d). Of the 17 patients that received non-matched therapy 0/17 showed response to therapy and 4/17 achieved SD (Figure 2c). An example of a patient matched to a clinical trial based on ctDNA analysis following discussion at the MTB is patient TAR-012; a 57-year-old female with lung adenocarcinoma who progressed through first-line cisplatin-pemetrexed chemotherapy. ctDNA profiling revealed an *NRAS* c.181C>A p.(Gln61Lys) mutation, also confirmed in her archival tumour. The patient was matched to a Phase I trial of a first-in-human MEK inhibitor and demonstrated PR with 60% reduction in marker lesions (RECIST 1.1) and symptomatic benefit (Figure 2e). Her disease remained controlled for 12 months. This is the first NRAS positive NSCLC patient reported, as far as we aware, to demonstrate radiological and clinical response to single agent MEK inhibition in keeping with pre-clinical data that strongly support this approach (25).

The overall intent of TARGET was to develop a robust workflow supporting clinical decision-making that can be delivered on a routine basis, with data turnaround time compatible with clinical practice, at an affordable cost (approximately £1600 per patient) that leads to benefit in a proportion of

phase I trial patients. With the feasibility of the workflow demonstrated in Part A, Part B of TARGET was initiated in Feb 2017 with the intention to recruit a further 450 patients over 3 years. In Part B, our primary aim is to improve matching of patients to clinical trials according to the molecular profile of their cancer and data will be prospectively collected for overall response rates and clinical outcomes for all patients to compare between matched and non-matched therapies. The turnaround time of results will also be shortened to 15-20 calendar days.

Our experience on the TARGET study encourages routine implementation of ctDNA testing as an adjunct to tumour testing. We suggest that with increased experience and on-going development of more sensitive ctDNA assays, such as incorporation of Unique Molecular Identifiers or other emergent methodologies, it may be possible to assign certain cancer patients to blood based testing. Tumour analysis would be applied only in cases with lower tumour burden or low ctDNA yields where blood analysis maybe unsuccessful, thereby reducing invasive procedures for patients and the associated healthcare system costs.

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Authors' contributions

D.G.R., A.M.H., G.B., C.D. and M.G.K. developed the clinical study, performed data analysis and wrote the manuscript. M.A., A.C., D.W., K.N., S.M. and N.S. performed ctDNA analysis. S.F., B.K., S.G. and C.M. provided bioinformatics support for the study. N.C., F.T., L.C., E.D., J.D., H.F., M.H., A.G., D.G., C.K., S.A., R.M., N.T., A.J.V., S.V., C.O., J.C. and R.K. recruited patients and provided clinical support for the study. J.S., S.S. and D.L. developed eTARGET and undertook software evaluations for the M.T.B. N.H., H.E. and A.W. performed tumour tissue analysis. A.J., K.F. and R.M. supported the MTB. All authors read and approved the final manuscript.

Competing Interests Statement

I declare that all authors have no competing financial or non-financial interests as defined by Nature Research

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Figure Legends

Figure 1. Overview of analysis of the first 100 patients recruited to the TARGET study. a) Outline of the approaches used for ctDNA and tumour analysis in the TARGET study. **b)** Average de-duplicated read depth for first 100 TARGET patients. A threshold of ≥100 average de-duplicated reads was set as a QC for reporting of data to the MTB (blue line). Reporting rate for tumour is indicated below the graph with failed samples indicated in red boxes, successful samples green boxes. The age of tumour biopsies at the time of analysis is indicated below the graph with biopsies <1 year old, 1 to 3 years and >3 years old indicated. **c)** Reporting times from the time of blood collection to generation of variant report for submission to the MTB in calendar days is shown for patients TAR-081 to TAR-100. The average time taken for patients 21-100 for ctDNA (mean=33 days, SD=+/-9 days SD, n=80) and tumour (mean=30 days, SD=+/-15 days, n=75) is indicated at the bottom of the graph. Calendar days taken to complete ctDNA isolation (red box), NGS generation (grey box) and bioinformatic analysis (blue box) are indicated. **d)** Bar graph showing concordance of mutations detected across 19 and 24-gene clinical panels in tumour and ctDNA for first 100 TARGET patients. Graph shows number of high confidence concordant mutations (dark green), mutations found below the 2.5% VAF Level of Detection (light green) and discordant mutations (red). **e)** Bar graph showing concordance of 94 TARGET patients for which combined tumour and ctDNA data was available. Concordant patients are indicated in blue (dark blue no mutations detected, light blue concordant mutations detected) and discordant patients in grey (mutation present only in tumour: light grey, mutation present only in ctDNA:

dark grey). **f)** Table showing number and VAF of mutations detected in extended 641-gene panel in ctDNA from first 100 TARGET patients according to disease type.

Figure 2. Analysis of CNA, actionable mutations and clinical response for the first 100 TARGET patients. a) Heat map showing CNA derived from ctDNA of 23 patients with corresponding Foundation Medicine CNA data. Regions of gain (red) and loss (blue) are indicated with chromosome number shown above. The average VAF and tumour type for each patient is indicated on the right of the heat map. Specific genes called amplified (red) or deleted (blue) within the tumour and ctDNA from three exemplar patients is shown on the far right. **b)** Schematic showing number of actionable mutations identified in the first 100 TARGET patients and efficiency of recruiting to a matched therapy (11%) using tumour and ctDNA mutation profiling. **c)** Consort diagram to show treatment decisions for the 41 patients with actionable alterations. The overall response rate (ORR) was 4/11 for patients on a matched therapy compared with 0/17 for those patients on an unmatched therapy. Stable disease rates were also higher in the matched trial cohort. **d)** Table showing details of the 11 patients recruited to matched therapies from TARGET Part A. All patients had partial response or stable disease with a median duration of response of 6 months. Actionability shown according to ACMG/AMP/CAP guidelines. ND = mutation not detected in ctDNA of patient. PR = partial response, SD = stable disease. **e)** Summary of ctDNA analysis for patient TAR-012 with non-synonymous mutation identified in ctDNA shown in the first box with mutations overlapping with the clinical tumour panel highlighted in

purple and clinical actionability according to ACMG/AMP/CAP guidelines indicated. CNA profile and genes amplified (red) or deleted (blue) are shown below mutation results. CT scans of patient showing clinical response pre and post 2-months of targeted therapy is also shown with yellow arrows identifying sites of disease.

Online Methods

Ethics approval

This study was undertaken in accordance with the ethical principles originating from the Declaration of Helsinki and in accordance with Good Clinical Practice. The study was approved by the North-West (Preston) National Research Ethics Service in Feb 2015, reference 15/NW/0078 and was registered on the NIHR Central Portfolio Management System, reference CPMS ID 39172. All patients were recruited within the Experimental Cancer Medicine Team at The Christie NHS Foundation Trust and provided fully informed written consent for provision of tumour and blood samples for genetic analyses. The University of Michigan Flexible Default Model was used for consent (26) that considers cancer related genetics from hereditary-related alterations. Whilst the study is focused predominantly on somatic alterations, the default is to inform patients of all genomic alterations, including those that could impact on family or risk of other diseases unless patients opt out. Specific optional consent was acquired for use of samples for cell culture or animal experiments.

Clinical workflow

TARGET is a two part study divided into Part A, feasibility of the workflow, ctDNA and tumour sequencing validation, formal reporting and setting up the MTB; and Part B, expansion to match patients to clinical trials and therapies in real-time (Figure 1a). Here we report results from Part A (N=100). The study recruited patients referred to the Experimental Cancer Medicine Team at The

Christie NHS Foundation Trust for consideration of early phase trials. Most patients had exhausted standard-of-care treatment options. Patients had to be ECOG PS0-1 and suitable clinical trial candidates, thus no or controlled co-morbidities and acceptable biochemical and haematology parameters in keeping with phase I trial inclusion criteria. The study excluded patients who were declining rapidly, poor performance status (PS) or high-risk blood sample donors. Following fully informed written consent blood and tissue samples were acquired and processed as detailed. Once results were available, data were discussed within a monthly MTB consisting of clinicians, clinical and translational scientists, bioinformaticians, basic scientists and biologists to interpret significance of variants and recommended trials or therapies. Software packages were also used to assist in determination of pathogenicity of VUS and a bespoke software package, eTARGET was developed as a digital solution to integrating clinical and genomic data digitally to facilitate MTB discussion, meeting outcome capture and to serve as a searchable database for data interrogation. The allocation of patients to treatment did not follow a specific algorithm as the process was dynamic and the treatment decision reached by the MTB was based on the specific mutations identified, VAF, associated pathogenicity (based on QCI tiering and evaluation), context in presence of co-mutations, patient treatment history, co-morbidities, fitness and available clinical trial options.

Blood Processing and Circulating Cell-Free DNA Extraction

Blood was collected in 10 ml BD Vacutainer K2E (EDTA) tubes (Becton-Dickinson) and 4 x 10 ml Streck Cell-Free DNA BCT blood collection tubes

(Streck) during routine phlebotomy. Germline DNA (gDNA) was isolated from EDTA whole blood using the QIAmp Blood Mini Kit (Qiagen, Hilden, Germany) as per manufacturer's instructions, and sheared to 200-300 bp on the Bioruptor Pico (Diagenode). Double-spun plasma was isolated from all Streck ctDNA BCT blood samples within 96 hours of blood collection and stored at −80 °C prior to ctDNA analysis. ctDNA was isolated using the QIAmp Circulating Nucleic Acid Kit (Qiagen) according to the manufacturer's instructions and/or the QIAsymphony with the Circulating DNA Kit (Qiagen). ctDNA and sheared gDNA yields were quantified using the TaqMan RNase P Detection Reagents Kit (Life Technologies).

Targeted sequencing of ctDNA and analysis

Sequencing libraries were generated from 0.5 to 25 ng ctDNA, or 25 ng sheared germline DNA in Accel-NGS 2S DNA Library Kits for the Illumina Platform (Swift Biosciences, Ann Arbor, MI) by the manufacturer's instructions with the following modifications. Library amplification and indexing was carried out with KAPA HiFi HotStart PCR Kits (Kapa Biosystems, Wilmington, MA) and NEBNext Index Primers for Illumina (New England Biolabs). 1 µg of each indexed library were pooled (up to 6 µg) as input for custom capture (641 gene panel) on SureSelectXT Reagent Kits (Agilent, Santa Clara, CA) by the manufacturer's instructions. Captured libraries were amplified using KAPA HiFi HotStart PCR Kits and quantified using the KAPA library quantification qPCR kit (Roche). Libraries were paired-end sequenced on an Illumina NextSeq 500, 2x 150bp High Output V2 kit (Illumina).

NGS Analysis of ctDNA sequencing data

FASTQ files were generated from the sequencer's output using Illumina bcl2fastq2 software (v.2.17.1.14, Illumina) with the default chastity filter to select sequence reads for subsequent analysis. All sequencing reads were aligned to the human genome reference sequence (GRCh37) using the BWA (v. 0.7.12) MEM algorithm. Picard tools (v.2.1.0) were used to mark/remove PCR duplicates and to calculate sequencing metrics. Somatic point mutations were called using both MuTect (v1) and also using the commercial software, Biomedical Genomics Workbench (BGW) v5.0 (Qiagen) by comparing plasma ctDNA to germline control DNA. Somatic InDels were called using both VarScan and Biomedical Genomics Workbench. Mutations called by two independent pipelines (MuTect+BGW or VarScan+BGW) were classed as high confidence and kept. Mutations within the 19 or 24-gene tumour panel were reported as low confidence if only called in a single pipeline. To ensure confidence in reported mutations a minimum of 10 variant reads at the reported loci and a 2.5% VAF threshold was applied to all ctDNA analysis.

Functional annotation of somatic variants was performed using ANNOVAR, the resultant VCF was analysed through the Qiagen Clinical Insight (QCI) for Somatic Cancer platform (Qiagen) and reports were generated for discussion in the TARGET Molecular Tumour Board. ‘Actionable’ was defined as a target of known pathogenic significance for which either a licensed or experimental agent or relevant clinical trial was available at the time of discussion.

CNA analysis of ctDNA

Standard low-pass WGS CNA analysis was performed on 8 patient samples as previously described (21) and analysed using HMM copy. CNA analysis of ctDNA hybridisation NGS data was performed using CNVkit software as previously described (27) and gene-level amplifications and deletions reported for the 641 cancer associated genes within the Agilent panel. For comparison to tumour CNA the gene list was restricted to the 315 genes reported by FoundationOne®.

Analysis of Tumour DNA

Between 1-3 5 µM thick sections from FFPET specimens were processed to extract genomic DNA using the Roche cobas® DNA Sample Preparation Kit. Tumour DNA was analysed using Sequenome OncoCarta panel v1.0 following the manufacturer's protocol or using the Qiagen Human Clinically Relevant Tumour GeneRead DNAseq Targeted Panel V2 as described. The OncoCarta™ v1.0 and Qiagen Clinically Relevant Tumour Targeted Panel V2 assays were validated to detect mutations to a VAF of 10% and 4% respectively. Following PCR based target enrichment; GeneRead libraries were prepared using the Illumina TruSeq PCR Free indexes and reagents. All NGS libraries were pair-end sequenced on an Illumina MiSeq using v2 sequencing chemistry (2x150cycles). Reads were aligned with BWA-MEM (version 0.6.2) hybrid to the human genome build GRCh37(hg19) followed by local realignment with ABRA (v0.96). Variant calling used a custom bioinformatics analysis pipeline which was validated to detect low level mosaic calls down to 4% allele fraction and uses a software consensus

between VarScan v2.3.9 and DREEP v0.7. Large indel events are assessed using Pindel (v0.2.4.t).

Variants identified bioinformatically were assessed for trueness and clinical relevance by two independent clinical scientists blinded to each other's interpretation. ACMG/ACGS & AMP guidelines on variant interpretation were followed in the assessment of pathogenicity and clinical relevance of variants.

Statistics and Reproducibility

The statistical methods used for each analysis are described within the figure legends and on the Life Science Reporting Summary associated with the manuscript.

Development of eTARGET

End-user and data requirements were defined based on the existing TARGET reports, exploration of data sources and interviews with the principal investigator and data controllers. After completion of a successful prototype, a beta version of eTARGET was developed in Microsoft Azure, a secure cloud-computing platform. Components included a storage account for data upload, a database for storing and integrating the data and a web-application to view the data. The web application, database and process server are backed up. Network traffic to resources is enforced and controlled by Network Security Group that contains a list of security rules. The data are stored within the European Economic Area (EEA) and all storage is encrypted.

Access to eTARGET is restricted to members of the MTB who have an account defined in the Azure Active Directory (AAD) and within the application

667 itself. Access to Azure File Upload Storage is restricted to users with an
668 account in the AAD, which has been defined as a contributor to the storage
669 account.

670

671 **Foundation Medicine FoundationOne® testing of tumour**

672 A subset of 51/100 TARGET patients had sufficient biopsy material for
673 FoundationOne® testing to be performed on FFPE biopsies of tumour tissue.
674 Of the 51 patients sent for testing 39 were successfully analysed with all 39
675 having at least 1 variant reported and 23 having CNA events reported
676 (Supplementary Table ST5). This data was used for comparison of variant
677 and CNA calling from the ctDNA of the corresponding patients.

Data availability statement

All the data generated or analysed during this study are included in this published article or are available from the corresponding author upon reasonable request. Genome data has been deposited at the European Genome-phenome Archive (EGA), which is hosted at the EBI and the CRG, under accession number EGAS00001003407.

Methods-only references

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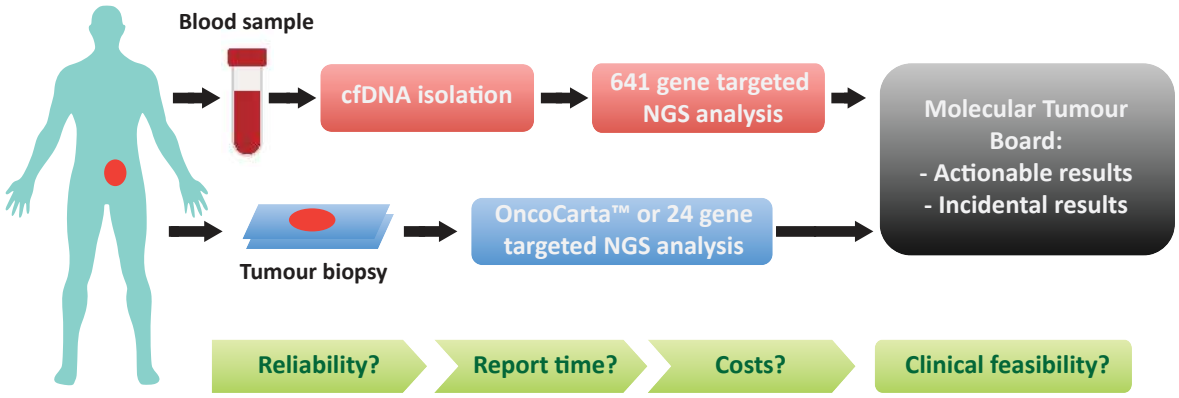

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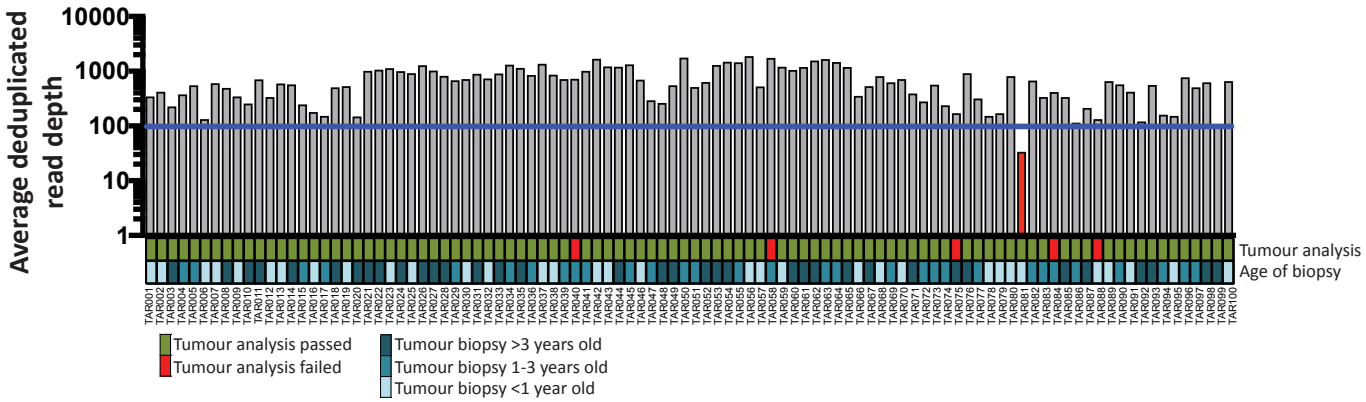

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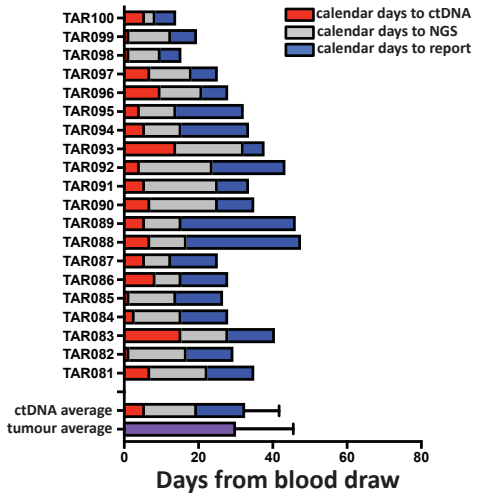

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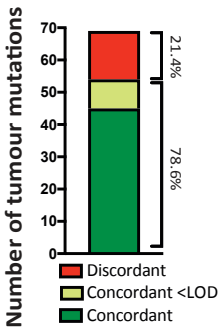

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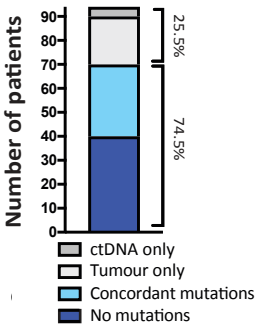

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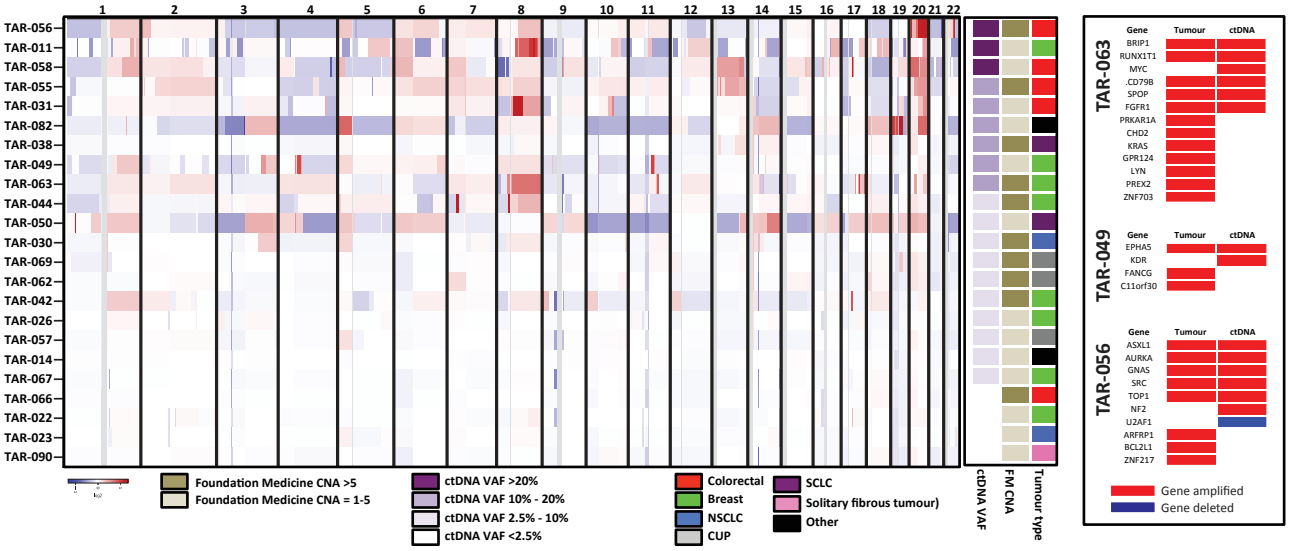
Disease type	Number patients	Patients ≥1 mutation	Mutation positive (%)	Average no. mutations (range)	Average VAF (%)	VAF range (%)
Colorectal	23	17	74	5.6 (1 - 16)	15.4	3.4 - 65.0
Breast	20	16	80	3.1 (1 - 6)	12.9	2.5 - 46.5
NSCLC	13	9	69	5.3 (1 - 10)	12.8	5.0 - 34.0
CUP	11	10	91	4.5 (2 - 16)	11.0	3.3 - 26.4
Sarcoma	5	2	40	3.5 (1 - 6)	26.8	8.2 - 45.4
SCLC	5	4	80	4.8 (2 - 10)	21.4	2.5 - 63.2
Prostate	3	2	67	2.0 (1 - 3)	7.9	7.8 - 7.9
Cholangiocarcinoma	2	1	50	3.0	8.4	na
Smal Bowel	2	1	50	5.0	7.7	na
Melanoma	2	2	100	3.5 (3 - 4)	14.3	14.2 - 14.3
Adrenal	2	0	0	0	0	na
Solitary fibrous tumour	2	0	0	0	0	na
Other	10	6	60	3.8 (1 - 8)	12.2	3.1 - 40.5
Total	**100**	**70**	**70**	**4.3 (1-16)**	**13.8**	**2.5 - 65.0**

2a



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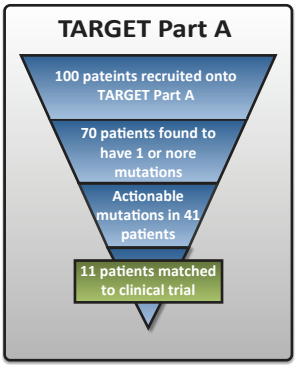

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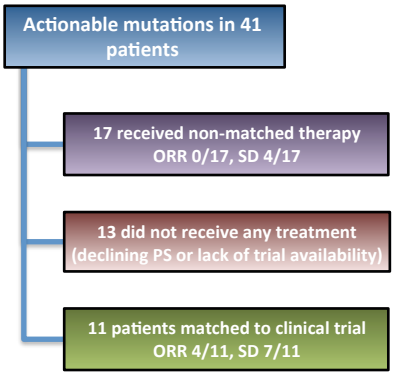

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Patient	Cancer type	Tumour mutation	ctDNA mutation	Actionability*	ctDNA VAF	Clinical trial	Duration on therapy	Best response
TAR-004	NSCLC	EGFR G719S EGFR S768I	EGFR G719S EGFR S768I	1A	2.5% 2.2%	EGFRi DDI study	6 months	SD
TAR-006	NSCLC	EGFR exon 19del	No mutation	1A	na	EGFRi DDI study	8 months	PR
TAR-012	NSCLC	NRAS Q61K	NRAS Q61K	3	19.9%	MEK inhibitor	12 months	PR
TAR-015	Breast	AKT1 E17K	AKT1 E17K	3	1.8%	AKT inhibitor	14 months	SD
TAR-048	CRC	No mutation	FANCA W911fs*31	2C	3.6%	Olaparib and ATR inhibitor	3 months	SD
TAR-051	CRC	KRAS G12S PTEN R130Ter	KRAS G12S PTEN R130Ter	1A	9.4% 7.9%	PI3K beta/delta inhibitor	3 months	SD
TAR-052	Thyroid	MET R970C	MET R970C	2C	43.4%	MET inhibitor	1.5 months	SD
TAR-060	Melanoma	BRAF V600E	BRAF V600E NRAS Q61K	1A	16.3% 7.5%	Pan-RAF inhibitor	4 months	SD
TAR-072	NSCLC	EGFR exon 19del TP53 R175H	EGFR exon 19del TP53 R175H	1A	6.6% 3.7%	EGFRi DDI study	18 months	PR
TAR-078	NSCLC	EGFR exon 19del	EGFR exon 19del	1A	7.0%	EGFRi DDI study	20 months	PR
TAR-098	Adrenal	CTNNB1 D32N	CTNNB1 D32N	3	2.4%	Aurora A Kinase inhibitor	3.5 months	SD

2e

TAR-012

Gene	Chromosome	Protein change	VAF (%)	Mutation	Actionability
NRAS	chr1	p.Q61K	19.9	SNV	3

Copy Number Aberrations	
Amplifications	7p11-p22 (EGFR, ETV1, PDGFS), 7q11-q36 (CDK6, BRAF)
Deletions	None reported