Dissertation

Identification of actionable targets in patients with advanced cancer

submitted by

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DECLARATION

I hereby declare that this dissertation is my own original work and that I have fully acknowledged by name all of those individuals and organizations that have contributed to the research for this dissertation. Due acknowledgement has been made in the text to all other material used. Throughout this dissertation and in all related publications I followed the "Guidelines of the Medical University of Graz on Good Scientific Practice".

Samantha HASENLEITHNER; September 2020

DISCLOSURES

Please note that parts of this thesis have already been accepted for publication.

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To my family: I don’t have to put it in writing here. Nothing but endless love and appreciation for you. You are irreplaceable. To my husband: The name’s Hasenleithner. Dr. Hasenleithner.

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ABBREVIATIONS

ABL1	ABL proto-oncogene 1
ACMG	American College of Medical Genetics
ADCA	Adenocarcinoma
AF	Allele fraction
AMP	Association for Molecular Pathology
ALK	Anaplastic lymphoma kinase
APC	Adenomatous polyposis coli
BAM	Binary alignment map
BCR	Breakpoint cluster region
BRAF	B-Raf proto-oncogene, serine/threonine kinase
BRCA1	BRCA1 DNA repair associated
BRCA2	BRCA2 DNA repair associated
BC	Breast cancer
CanDL	Cancer Driver Log
CGI	Cancer Genome Interpreter
CAP	College of American Pathologists
COSMIC	Catalog of Somatic Mutations in Cancer
ccfDNA	Circulating cell-free DNA
CDKN2A	Cyclin dependent kinase inhibitor 2A
CML	Chronic myelogenous leukemia
cfDNA	Cell-free DNA
ctDNA	Circulating-tumor DNA
ClinGen	Clinical Genome Resource
CIViC	Clinical Interpretation of Variants in Cancer
CRC	Colorectal cancer
CADD	Combined Annotation-Dependent Depletion
CGP	Comprehensive genomic profiling
CNV	Copy number variant
CPIC	Clinical Pharmacogenetics Implementation Consortium
DNA	Deoxyribonucleic acid

EGFR	Epidermal Growth Factor Receptor
ELN	European LeukemiaNet
EMA	European Medicines Agency
ERBB2	Receptor tyrosine-protein kinase erbB-2
ESMO	European Society of Medical Oncology
ER	Estrogen hormone receptor
EU	European Union
eviQ	eviQ Cancer Treatments Online
FBXW7	F-box and WD repeat domain containing 7
FGFR2	Fibroblast growth factor receptor 2
FDA	Food and Drug Administration
GIST	Gastrointestinal stromal tumors
HER2	Human epidermal growth factor receptor 2
HMM	Hidden Markov Models
WfG	IBM Watson for Genomics
IHC	Immunohistochemistry
ICT	Individualized Cancer Treatment
JAX-CKB	JAX Clinical Knowledgebase
KIT	KIT proto-oncogene, receptor tyrosine kinase
KRAS	KRAS proto-oncogene, GTPase
MET	MET proto-oncogene, receptor tyrosine kinase
MSI-H	Microsatellite instability-high
MVLD	Minimal Variant Level Data
MM	Molecular Match
MTB	Molecular tumor board
MTOR	Mechanistic target of rapamycin kinase
mAF	Mutant allele frequency
NCCN	National Comprehensive Cancer Network
NGS	Next-generation sequencing
NICE	National Institute for Health and Care Excellence
NoO	N-of-One
NSCLC	Non-small cell lung cancer

NRAS	NRAS proto-oncogene, GTPase
NTRK	Neurotrophic receptor tyrosine kinase
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
PMDA	Pharmaceuticals and Medical Devices Agency
PolyPhen	Polymorphism Phenotyping
PCM	Precision cancer medicine
PMKB	Precision Medicine Knowledgebase
PR	Progesterone receptor
PD-L1	Programmed death-ligand 1
PFS	Progression-free survival
PD	Progressive disease
PTEN	Phosphatase and tensin homolog
PX	Patient experience
QCI	QIAGEN Clinical Insight
RABGAP1L	RAB GTPase activating protein 1 like
RECIST	Response evaluation criteria in solid tumors
RET	Ret proto-oncogene
ROS1	ROS proto-oncogene 1, receptor tyrosine kinase
SCC	Squamous cell carcinoma
SCNA	Somatic copy number alteration
SNP	Single nucleotide polymorphisms
SNV	Single nucleotide variants
SVM	Support Vector Machines
sWGS	Shallow whole-genome sequencing
TCGA	The Cancer Genome Atlas
TF	Tumor fraction
TMB	Tumor mutational burden
TP53	Tumor protein p53
TSC1	TSC complex subunit 1
TSC2	TSC complex subunit 2
VAF	Variant allele frequency

VCF	Variant call format
VIC	Variant Interpretation for Cancer
VUS	Variant of unknown significance
VEGF	Vascular Endothelial Growth Factor
WHO	World Health Organization

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ZUSAMMENFASSUNG

Präzisionsonkologie ist auf die Umsetzung molekularer Daten in geeignete Therapien für Krebspatienten angewiesen. Mit der wachsenden Komplexität der auf neuen Sequenziertechniken (engl. Next-generation sequencing (NGS)) basierenden Tests hat sich die klinische Interpretation somatischer Genommutationen jedoch zu einer gewaltigen Aufgabe entwickelt. Aus diesem Grund sind präzise onkologische Wissensdatenbanken und in jüngerer Zeit auch klinische Entscheidungshilfen immer nützlicher geworden, um komplexe NGS-Daten zur Identifizierung medikamentös behandelbarer Targets zu annotieren. Diskrepante Varianteninterpretation unter den Open-Source-Knowledgebases hat in jüngster Zeit zu Harmonisierungsbemühungen geführt, und in dieser Hinsicht haben wir versucht, den Leistungsstatus von drei prominenten kommerziellen klinischen Entscheidungsunterstützungs-Tools zu bewerten, nämlich NAVIFY® Mutation Profiler (NAVIFY; Roche), QIAGEN Clinical Insight (QCI™) Interpret (QIAGEN) und CureMatch Bionov™ (CureMatch).

Um den aktuellen Status des jeweiligen Tumorgenoms zu erhalten, analysierten wir zellfreie DNA von Patienten mit metastasierendem Brustkrebs, kolorektalem oder nicht-kleinzelligem Lungenkrebs, indem wir Veränderungen der Anzahl somatischer Kopien evaluierten und parallel dazu ein 77-Gen-Panel einsetzten. Dann bewerteten wir die Übereinstimmung der Ansätze zur Ebenenklassifizierung zwischen NAVIFY und QCI und verglichen die Strategien zur Bestimmung der Aktionsfähigkeit zwischen allen drei Plattformen. Schließlich quantifizierten wir die Ausrichtung der Behandlungsvorschläge über alle Entscheidungs-Tools hinweg. Jede Plattform variierte in ihrer Art der Variantenklassifizierung und der Strategie zur Identifizierung medikamentös behandelbarer Ziele und klinischer Studien, was zu großen Diskrepanzen führte. Sogar die Häufigkeit übereinstimmender handlungsrelevanter Ereignisse bei den Klassifikationen der Stufen I-A oder I-B betrug beim Vergleich von NAVIFY mit QCI, NAVIFY mit CureMatch bzw. CureMatch mit QCI nur 4,3%, 9,5% und 28,4%, und die erhaltenen Behandlungsempfehlungen unterschieden sich drastisch.

Dies ist die erste Studie, bei der die klinische Entscheidungsunterstützungsanalyse durch umfassendes genomisches Profiling der zirkulierenden Tumor-DNA eingesetzt wird, was eine potenzielle klinische Routineanwendung darstellt. Hierin werden detaillierte Beschreibungen von

Diskrepanzen in der Pathogenität, der Aktionsfähigkeit und insbesondere der Ausrichtung des Behandlungs-Matchings gegeben. Diese Analysen zeigen, dass Behandlungsentscheidungen, die auf molekularen Markern basieren, derzeit willkürlich und abhängig von der gewählten Strategie zu sein scheinen. Folglich würden Tumoren mit identischen molekularen Profilen unterschiedlich behandelt, was die vielversprechenden Konzepte der genomisch informierten Medizin in Frage stellt. Unsere Analysen zeigen, wie komplex die Algorithmen für den Behandlungsabgleich sind. Da diese interpretierten Berichte im Mittelpunkt der Diskussionen des Molekularen Tumorboards stehen, sind die Ergebnisse sowohl für Onkologen als auch für Patienten relevant und können die klinische Versorgung stark beeinflussen.

ABSTRACT

Precision oncology relies on the translation of molecular data into suitable therapies for patients with cancer. However, with the growing complexity of next generation sequencing-based tests, clinical interpretation of somatic genomic mutations has evolved into a formidable task. For this reason, precision oncology knowledgebases and, more recently, clinical decision support tools, have become increasingly useful in annotating complex next-generation sequencing data for identifying druggable targets. Discrepant variant interpretation among open-source knowledgebases has led to recent harmonization efforts and in this regard, we sought to evaluate the performance status quo of three prominent commercial clinical decision support tools, i.e. NAVIFY® Mutation Profiler (NAVIFY; Roche), QIAGEN Clinical Insight (QCI™) Interpret (QIAGEN) and CureMatch Bionov™ (CureMatch).

In order to obtain the current status of the respective tumor genome, we analyzed cell-free DNA from patients with metastatic breast, colorectal or non-small cell lung cancer by evaluating somatic copy number alterations and in parallel applied a 77-gene panel. We then assessed the concordance of tier classification approaches between NAVIFY and QCI and compared the strategies to determine actionability among all three platforms. Finally, we quantified the alignment of treatment suggestions across all decision tools. Each platform varied in its mode of variant classification and strategy for identifying druggable targets and clinical trials, which resulted in major discrepancies. Even the frequency of concordant actionable events for tier I-A or tier I-B classifications was only 4.3%, 9.5% and 28.4% when comparing NAVIFY with QCI, NAVIFY with CureMatch, and CureMatch with QCI, respectively and the obtained treatment recommendations differed drastically.

This is the first study employing clinical decision support analysis through comprehensive genomic profiling of circulating tumor DNA, which represents a potential routine clinical application. Herein, detailed descriptions of discrepancies in pathogenicity, actionability and especially alignment of treatment matching are provided. These analyses demonstrate that at present, treatment decisions based on molecular markers appear to be arbitrary and dependent on the chosen strategy. Consequently, tumors with identical molecular profiles would be treated differently, which challenges the promising concepts of genome-informed medicine. Our analyses demonstrate

the complexity of treatment matching algorithms. As these interpreted reports are central to molecular tumor board discussions, the findings are pertinent to both oncologist and patient and may greatly impact clinical care.

1 INTRODUCTION

1.1 A genome worth of data

The Human Genome Project, biology's first large-scale effort that was officially completed nearly two decades ago (1), yielded the sequence of the human genome. Not only did the release of the complete sequence of the human genome lay the foundation for understanding the genetic basis for health and the pathology of human disease, it also has provided the opportunity to redefine various disease paradigms, radically transforming the way we think about both biology and medicine. Now that we have the ability to assay an entire genome worth of data, we have moved from observational science to genomic science, with which we can describe disease on the basis of various digital phenotypes (2). As cancer remains a predominant health threat in developed countries and is essentially a disease of the genome, genomic science has evolved to be central to both cancer research and care. As such, all players in the field, i.e. researchers, government and industry, continue to dedicate significant resources and effort in harvesting genomic data to define digital phenotypes and guide oncology practice (3).

Cancer represents a complex and dynamic disease that affects a multitude of genes and biological pathways, resulting in the disturbance of normal activity at the genomic, transcriptomic, proteomic and epigenomic level. As a result, cancer genome landscapes have been shown to exhibit a completely different set of variation than the germline landscape (4), a consequence derived from a clone of somatic cells that was able to bypass its constrained cellular function. This lack of cellular constraint leads to several uncontrolled processes, including proliferation, invasion of tissue, evasion of the immune system and changes to the local tissue microenvironment (5). The genes that regulate the three core cellular processes of cell fate determination, cell survival and genome maintenance are the main contributors to cancer, known as driver genes (4). Interestingly, of the approximated 20,000 genes to date estimated to comprise the human genome, only roughly 140 of these are estimated to drive cancer (4), although more recent estimates range from 299 genes (6) to 460 cancer driver genes (7), owing to the development of new advanced prediction algorithms. The systematic identification and cataloguing of the driving events across cancer types (8) has expanded on the concept of cancer as merely a tissue-dependent process to that of a genetic disease, making the prospect of drugs that can target cancer-specific molecular aberrations an attractive one (9-13).

1.2 Genome-driven medicine through next-generation sequencing

Our evolving understanding of cancer biology alongside the increasing accessibility to and decreasing cost of genomic sequencing technologies has revealed the promising strategy of genome-driven oncology (14) and, as a consequence, multi-gene sequencing has become a part of daily clinical practice for the detection of genomic alterations to be matched with targeted drugs (15). Clinical applications of genomic sequencing, with next-generation sequencing (NGS) technology at the forefront, not only allow for the identification of potential therapeutic targets, but also have led to improvements in cancer diagnosis and prognosis, decision support and monitoring of disease (16). Initial implementation of clinical-grade NGS typically consisted of targeted sequencing, i.e. the profiling of specific loci known to have clinical relevance and aid in drug selection, for cost reduction purposes. This approach has helped to identify promising gene-drug matches in approximately 1 out of 10 patients who undergo multi-gene sequencing (15,17). However, there is a growing transition from targeted "hotspot" panels to more broad-based approaches (18-20), including whole-genome sequencing (16). With comprehensive genomic profiling (CGP) (Figure 1), the four major classes of genomic alterations, i.e. base substitutions, insertions and deletions, copy number alterations and rearrangements, can be detected in a single NGS assay, making the use of precious clinical samples more efficient.

The increased application of NGS has transformed cancer research, which led to the assembly of large-scale cancer genomics compendia (8) and has resulted in both the characterization and categorization of individual samples at the molecular level for the identification of molecular subtypes (21-23). These efforts in broader genomic characterization of tumors has accelerated the development of novel, effective targeted therapies (24), in turn enabling the use of molecular signatures to inform clinical decisions (25-28). Whether referred to broadly as precision cancer medicine, as precision oncology, or as genomics-driven oncology (29), the application of high-throughput genomic sequencing of tumors is reshaping the standard of clinical care and research in oncology (30).

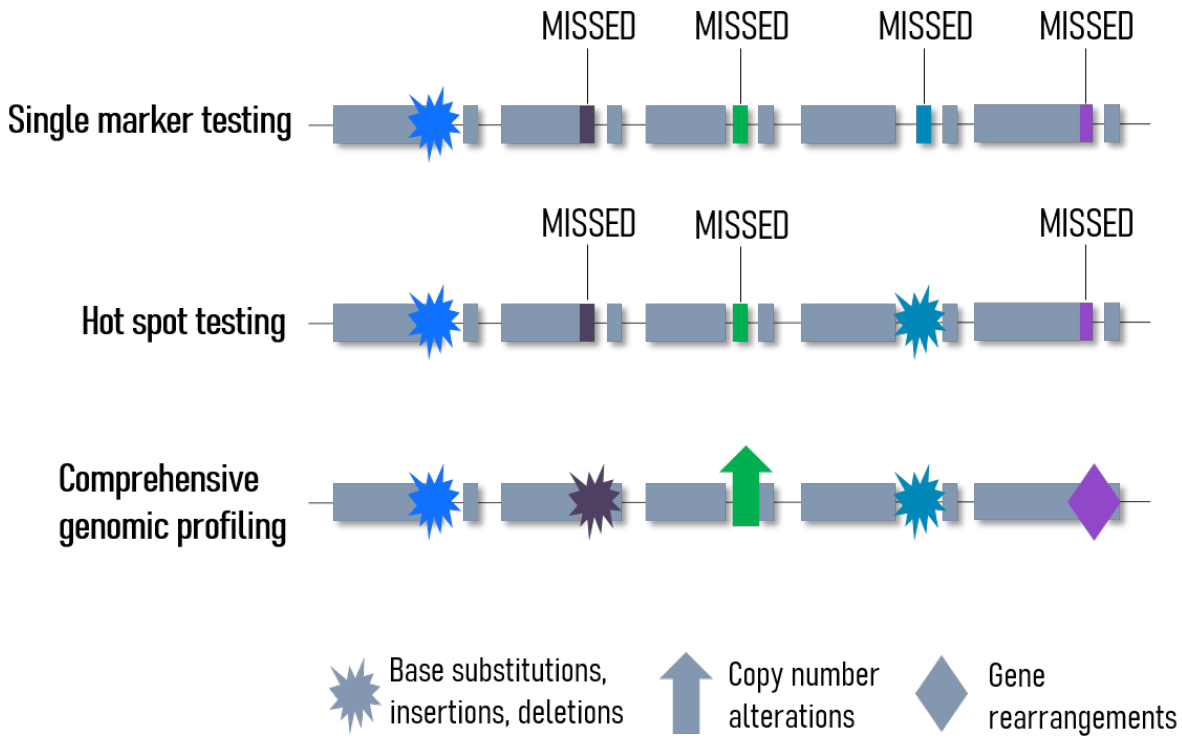

Figure 1. Molecular profiling methods of PCM. Outside of the academic hospital setting, most clinics still predominantly employ single marker testing in their cancer diagnostic workflows. This routine approach is often cancer type-specific and it is able to identify alterations from a pre-specified gene of interest commonly associated with cancer, thus overlooking potential existing mutations in other genes (top). Molecular profiling can also be employed with NGS hot spot panels, which are capable of identifying a set of pre-defined mutations across multiple genes but are unable to detect all classes of alterations (middle). Comprehensive genomic profiling (CGP) is gaining traction as a pan-cancer approach to detecting all four classes of alterations across hundreds of clinically relevant cancer-associated genes (bottom). CGP extends beyond the limited hotspot mutations and includes insertions and deletions, several copy number alterations, such as ERBB2 (HER2) amplification, and an array of fusions and structural rearrangements all from a single sample, which is especially important for limited tissue or DNA samples. The goal of CGP via NGS is to detect therapeutically relevant and targetable genomic alterations that can be used to direct selection of suitable individualized treatment options.

1.3 The precision cancer medicine (PCM) paradigm

The initial concept of personalized medicine can perhaps be tracked back to the first time the phrase "risk factor" was used in the medical literature, stemming from the results of the Framingham heart study that demonstrated risk stratification based on an individual's clinical data and personal health history (31). This example of practicing more personalized or "precise" medicine has been adopted in the field of oncology as well, although the terminology of this paradigm has varied over the past decade, shifting from "personalized oncology" to the more predominant "precision oncology" (Figure 2) (32).

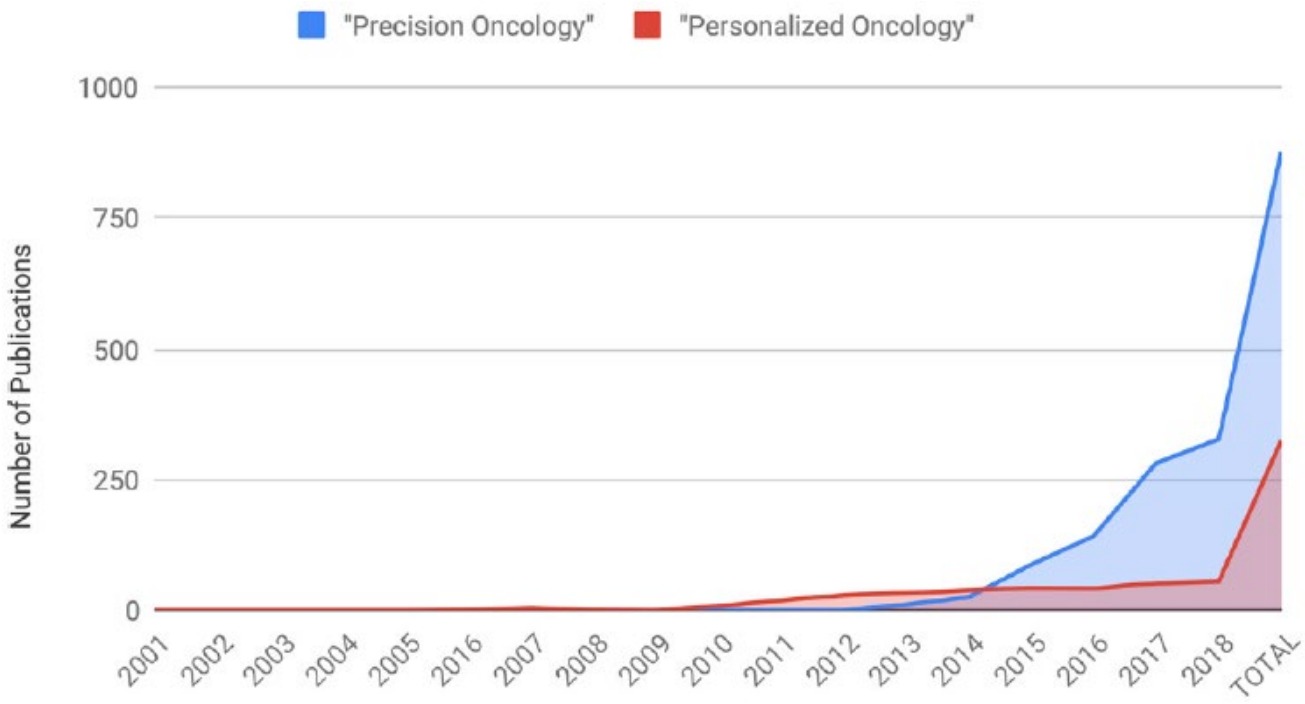

Figure 2. Shifting terminology of precision cancer medicine in the literature. The blue portion of the graph represents the total number of publications which matched the search term "precision oncology" and the red portion are the hits that matched to the search "personalized oncology" on PubMed and Scopus from the years 2000-2018. (32) *[Permission for reprint by BMJ Publishing Group Ltd. (License number: 4900771418803)]*

Counterintuitive to its name, however, there are mixed definitions for what constitutes precision oncology, ranging from targeted therapy, to molecular biomarker-guided tumor profiling, to "omics-guided" tumor profiling via NGS with applications from structural biology to clinical

practice (32). Naturally, as clinical research began to reveal the discrepant genetic alterations between individuals with various types of cancer, it was proposed that such variation may necessitate more personalized therapies selected through the analysis of biomarkers. Indeed, precision cancer medicine (PCM) arose from the accumulation of evidence that targeted agents could be matched to molecular deregulations found in the tumor (33) while simultaneously taking into account other cellular features of an individual's tumor, its microenvironment, and personal traits such as genetics and lifestyle (34).

It was furthermore demonstrated that not every individual patient would derive benefit from targeted agents and thus PCM necessitates the selection of those populations most likely to achieve meaningful clinical benefit (35,36). These recent advances support the obtaining of a cancer patient's genomic "portrait" in order to subsequently match cognate-targeted therapies to the detected genomic alterations, an approach which has already achieved higher response rates in heavily pretreated patients and as well as in those with advanced disease (37-39). Phase I trials have demonstrated that a progression-free survival (PFS) on matched therapy may be better than on prior lines of conventional, approved, unmatched treatments (40,41). Similarly, phase III trials have shown that targeted agents selected via predictive biomarkers demonstrate improvement in response rate and survival (42). There have been several cases in which a response so significant was achieved that the drug underwent expedited review and approval by the Food and Drug Administration (FDA), a so-called breakthrough status, which the FDA publishes in quarterly reports (https://www.fda.gov/drugs/nda-and-bla-approvals/breakthrough-therapy-approvals), as was the case with after phase I testing with the anaplastic lymphoma kinase (ALK) inhibitor ceritinib for patients with non-small cell lung cancer (NSCLC) (37,43). Other drugs receiving breakthrough approval have followed, such as pembrolizumab for the treatment of adult and pediatric patients with metastatic, microsatellite instability-high (MSI-H) or mismatch repair deficient solid tumors (44)—the first approval agnostic of cancer site—or larotrectinib (45) for the treatment of adult and pediatric patients with solid tumors that have a neurotrophic receptor tyrosine kinase (NTRK) gene fusion (https://www.fda.gov/media/97001/download). The integration of genomic analyses into clinical decision-making continues to transform the standard practice of oncology, transitioning from the sole administration of cytotoxic agents to such genome-targeted drugs, leading to improved outcomes for several malignancies (46) and driving the adoption of PCM. As of date, the FDA has approved over 80 targeted drugs for solid and

hematologic malignancies (https://www.mycancergenome.org/content/page/overview-of-targeted-therapies-for-cancer/).

1.3.1 Status quo of PCM

Precision cancer medicine promises to radically individualize patient care, in turn preventing disease, improving survival, and extending healthspan (47). This strategy has already dramatically transformed the prognosis of several formerly deadly cancers. The best example is perhaps the discovery of the aberrant BCR-ABL1 enzyme in chronic myelogenous leukemia (CML), a disease which was previously associated with death after 4 to 5 years, which can now be targeted by the kinase inhibitor imatinib owing to molecular matching, enabling patients to nearly have a normal life expectancy (48). Similarly, KIT inhibitors have proven extremely effective in gastrointestinal stromal tumors (GIST)—a malignancy once known to have minimal to zero response rates—harboring *KIT* mutations, leading to response rates of >80% (48). With such examples, some oncologists may argue that precision oncology is actually already a reality, as demonstrated by the multitude of existing drugs matching to molecular markers, of which selected examples are listed in Table 1.

Table 1. Existing molecular markers matching to approved drugs [*Adapted from* (49) (CC BY 4.0)]

Biomarker	**Drug**	**Tumor context**	**References**
BCR-ABL fusion	Imatinib	Chronic myeloid leukemia	(48)
Estrogen receptor expression	Tamoxifen and aromatase inhibitors	Breast cancer	(50)
HER2 amplification and overexpression	HER2-directed therapies	Breast cancer, Colorectal cancer	(51-54)
ALK and *ROS1* rearrangements	ALK/ROS1 inhibitors	Non-small cell lung cancer	(55,56)
EGFR mutations	EGFR-directed therapies (sensitivity)	Non-small cell lung cancer	(57-59)
KRAS/NRAS/BRAF mutations	EGFR-directed therapies (resistance)	Colorectal cancer	(60-62)

BRCA1/2 mutations, BRCAness	PARP inhibitors, Platinum compounds, mitomycin C	Breast cancer	(63,64)
High PD-L1 expression	PD1-directed therapies	Non-small cell lung cancer	(65)
Tumor mutational burden (TMB)	Immune checkpoint inhibitors	Non-small cell lung cancer	(66)
BRAF mutations	BRAF inhibitors	Melanoma	(67,68)
TSC1/2 mutations, MTOR mutations	mTOR inhibitors	Renal cell carcinoma	(69)
MET exon 14 skipping	MET inhibitors	Various cancers	(70)
MSI-H status	pembrolizumab	Solid tumors	(44)
NTRK fusions	larotrectinib	Solid tumors	(45)

As a result of these proven molecularly-guided successes, the PCM paradigm is beginning to prioritize the incorporation of molecular information for treatment decision-making, rather than focusing solely on tumor location and histology (71). Of course, given the complex microenvironment and spatial heterogeneity of tumors, innovative analyses beyond standard tissue biopsy sequencing are required in order to capture the most relevant molecular alterations which can inform treatment decisions (71).

1.4 The role of liquid biopsy in PCM

For decades, histologic and pathologic analysis in routine oncology has been predominantly performed on tumor tissue obtained from biopsy or resection and thus serves as the primary source of DNA used for current genomic profiling via NGS (72). However, this approach is invasive and does not come without risk, oftentimes not even being feasible due to advanced disease (73). The invasive nature of obtaining tumor tissue along with the increasing understanding of intra- and inter-tumor heterogeneity (74), how a tumor acquires new mutations due to genomic instability or the selective pressure of targeted treatment (75), and the need for serial sampling have made the liquid biopsy a viable alternative and/or supplement to guiding PCM. Circulating cell-free DNA (cfDNA), which contains circulating-tumor DNA (ctDNA) in patients with cancer, can be obtained non-invasively through isolation of plasma from a simple blood draw (76). As ctDNA represents the various tumor clones throughout the body, it is a rich genomic reservoir reflecting the molecular

diversity of the tumor. With a half-life of 16 minutes to several hours (77-79), ctDNA has been shown to provide the most current status of a patient's tumor, enabling the detection of tumor subclones from metastatic lesions (80-84) and is now even being applied prospectively to funnel patients into phase I clinical trials (85). As a non-invasive analyte, cfDNA has a central role in molecular testing, especially when standard tumor biopsies cannot be subjected to sequencing due to insufficient material, which can occur in up to 20-25% of needle biopsies (86,87). This has proven particularly useful given the current widespread clinical application of genomically-guided therapy selection, as profiling of cfDNA may reveal actionable alterations that can offer treatment opportunities for cancer patients (88,89). Molecular profiling of cfDNA optimizes personalized cancer treatment by elucidating the patient's unique genomic profile and thus targeted interventions can be tailored to the patient such that ineffective treatments are avoided (90). The traditional approach of targeting a single resistance mechanism as a result of standard tumor biopsy profiling may lead to mixed clinical responses, as resistant driver subclones may have not been harbored in the original analyzed specimen (91). This reflects the current clinical management of patients whose therapies are based on the characterization of the primary tumor (92), meaning that true tumor-driving events originating in subclones of the primary tumor or metastatic lesions may have been overlooked at the time point of clinical decision-making (93). Thus, regimens selected based on the identification of the most current driver events with the highest variant frequencies are predicted to provide substantial tumor responses (33).

1.5 Identification of actionable targets

Precision oncology is based on the idea that application of high-resolution genomic technologies will improve cancer diagnosis and enable more precise treatment regimens (94). In order to achieve this, resources that collect and categorize tumor-specific alterations are paramount, as these data can be correlated with clinical features for the identification of prognostic or predictive therapy-associated biomarkers (95). These markers enable molecular subtyping of clinical samples which can be used to estimate prognosis, identify somatic events involved in tumor progression and metastasis, as well as detect disturbances in pathways (96). Approaches employing NGS have already begun to comprehensively characterize tumor-specific genomes (97) and multi-parameter efforts, especially The Cancer Genome Atlas (TCGA) network, are underway to achieve even more accurate classifications by generating comprehensive molecular profiles at all levels, i.e. genomic,

transcriptomic, proteomic and epigenomic, for hundreds of tumors (8). These analyses have significantly improved our understanding of molecular aberrations and their functional roles across tumor types (8) and have driven the identification of novel actionable targets through genomic profiling (80).

1.5.1 Annotation and interpretation of genomic profiling results

In order to realize the promise of PCM, i.e. precise and personalized cancer care, the genomic sequence of a patient's tumor must be translated into timely, actionable information that influences clinical course of action and, ideally, this process should be a repeating cycle as tumors evolve under the selective pressure of therapies (Figure 3). Actionable information typically refers to a molecular aberration that can be targeted by an existing approved therapy, but also includes known markers of resistance to therapies or using a molecular profile to match a patient to a recruiting clinical trial.

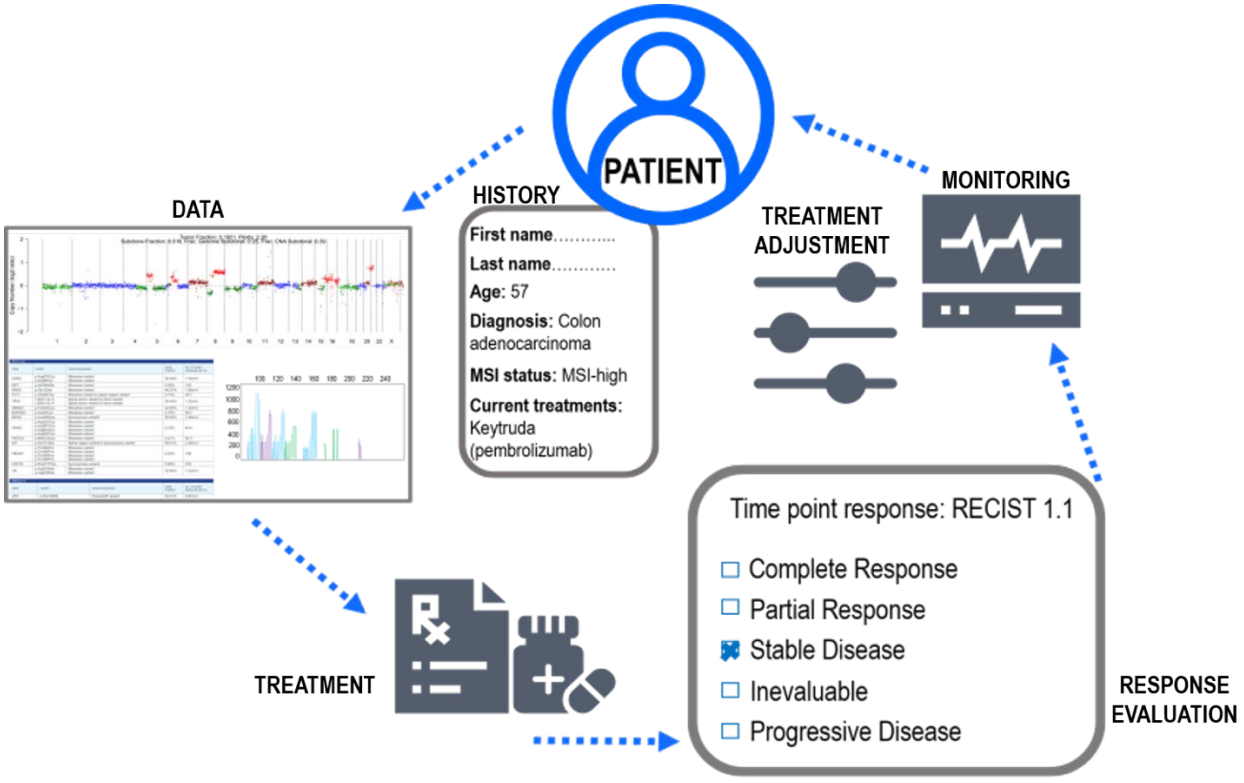

Figure 3. Incorporating molecular data into the treatment matching workflow. Ideally, patient molecular data should be gathered throughout the course of treatment. The oncologist starts with a patient's medical history and may order several molecular profiling tests to help direct a treatment

decision, i.e. by identifying potential drug sensitivity or resistance markers. After a treatment decision is made and a drug is administered, the patient undergoes routine imaging follow-ups to assess tumor response in accordance with the Response evaluation criteria in solid tumors (RECIST) 1.1 criteria. The imaging assessment may be accompanied by further molecular profiling in the case of progressive disease (PD) to identify novel actionable targets which may enable an individualized treatment adjustment.

With the decreasing cost of and increasing access to CGP, which enables the detection of many cancer-related biomarkers from hundreds of genes simultaneously from a single NGS assay (98), academic institutions and clinics have begun to encounter the complexity of genomic actionability in clinical oncology. Previously, employing restrictive hotspot panels for clinical analyses meant that the results were also relatively simple to summarize in a clinical report. Multiplexed sequencing assays have dramatically changed this paradigm, as they may uncover previously unreported variants, thus creating a challenge for genetics specialists and oncologists alike to distinguish between clinically relevant findings and those with no current relevance for clinical care, such as hypothetical gene-drug matches (95). Moreover, most sequencing readouts do not report the detected alterations in a prioritized ranking, further complicating the interpretation of potential clinical actionability (95). To this end, databases for sequence interpretation, variant annotation, and algorithms for prediction purposes and treatment matching have been developed to collect and catalogue cancer-related genomic alterations, making genomically-guided treatment decisions possible (30). These tools are also gaining traction in the consolidated form of clinical decision support tools, which cover all tasks from variant interpretation and annotation to clinical-grade reports citing evidence-based treatment recommendations.

1.5.2 Databases for sequence interpretation

With the rise of large-scale and high-throughput genome sequencing, the amount of genomic data across tumor types is increasing dramatically. The growing wealth of information has necessitated the consolidation of this data into publicly available databases, which has been very much a global effort. These databases range from population databases, cancer-specific databases or germline-specific databases and assist clinical laboratories in the accurate assessment, annotation and prioritization of somatic variants (Table 2). Population databases (Table 2), which provide information regarding frequencies of alternative alleles at a given locus in a geographically distinct

population, are typically used to distinguish assumed polymorphic or benign variants from suspected somatic variants (99). The goal of cancer-specific databases (Table 2) is to provide guidance regarding the incidence and prevalence of specific genomic variants across diverse cancers and subtypes by cross-referencing to other genomic data sources, the literature, biological information, existing targeted therapies, related clinical trials as well as clinical outcome data (99). Germline variant databases house information for determining variants of germline origin, especially those genes associated with cancer predisposition syndromes, and help link somatic variants to well-known germline counterparts, e.g. in *TP53* or *PTEN* (99).

Table 2. Databases for the interpretation of somatic variants [*Adapted from (99), permission for reprint by Elsevier (License number: 4970150229471)]*

Type	Database/catalogue name	URL	Reference
Population and germline variant databases	1000 Genomes Project	http://browser.1000genomes.org	(100)
	Exome Variant Server	http://evs.gs.washington.edu/EVS	
	dbSNP	http://www.ncbi.nlm.nih.gov/snp	(101)
	dbVar	http://www.ncbi.nlm.nih.gov/dbvar	(102)
	ExAC	http://exac.broadinstitute.org	
	gnomAD	http://gnomad.broadinstitute.org/	(103)
	Personalized Genome Project	http://www.personalgenomes.org/	(104)
Cancer-specific variant databases	Catalog of Somatic Mutations in Cancer (COSMIC)	http://cancer.sanger.ac.uk/cosmic	(105)
	My Cancer Genome	http://www.mycancergenome.org	
	cBioPortal	http://www.cbioportal.org	(106)
	Personalized cancer therapy	https://pct.mdanderson.org	
	ClinVar	https://www.ncbi.nlm.nih.gov/clinvar/intro	
	Pediatric Cancer Genome Project	http://explorepcgp.org	(107)
	Intogen	https://www.intogen.org/search	(108)

1.5.2.1 Prediction databases

Given the high-throughput nature of NGS readouts, laboratories frequently encounter difficult-to-annotate variants that are virtually non-existent in the literature or publicly available databases, making it difficult to differentiate between potentially deleterious or potentially well-tolerated variants. For this reason, a multitude of prediction algorithms (Table 3) have been developed to help distinguish which variants warrant further study or more critical evaluation at molecular tumor boards (MTB). Such algorithms are based on a variety of computational methods, e.g. Random Forest, Support Vector Machines (SVM), neural networks and Hidden Markov Models (HMM), and predict the effect of observed variants on protein structure and function, elucidating any potential clinical impact (30). Although each individual algorithm may vary in its prediction approach, they can generally be categorized in two ways: those that predict the impact of a particular missense variant on its downstream protein function and those that predict the impact of a particular variant on subsequent splicing events (99). When making calculations, these algorithms take into account the variant in question and its given context, for example sequence identity and conservation, evolutionary relationships, protein structures at the primary and secondary levels as well as protein stability (30).

Table 3. Algorithms for in silico computational predictions of functional impact *[Adapted from* (99)*, permission for reprint by Elsevier (License number: 4970150229471)*]

Prediction algorithm	**URL**	**Reference**
PolyPhen-2	http://genetics.bwh.harvard.edu/pph2	(109)
SIFT	http://sift.jcvi.org	(110)
MutationAssessor	http://mutationassessor.org	(111)
MutationTaster	http://mutationtaster.org	(112)
PROVEAN	http://provean.jcvi.org/index.php	(113)
Condel	http://bg.upf.edu	(114)
CoVEC	https://sourceforge.net/projects/covec/files	(115)
CADD	http://cadd.gs.washington.edu	(116)
GERP++	http://mendel.stanford.edu/SidowLab/downloads/gerp/index.html	(117)
PhyloP and PhastCons	http://compgen.bscb.cornell.edu/phast	(118)
nsSNPAnalyzer	http://snpanalyzer.uthsc.edu/	(119)
SNPs&GO	http://snps-and-go.biocomp.unibo.it/snps-and-go/	(120)
SNAP2	https://rostlab.org/services/snap2web/	(121)
Panther	http://www.pantherdb.org/tools/csnpScoreForm.jsp	(122)
Human Splicing Finder	http://www.umd.be/HSF3	(123)
MaxEntScan	http:/genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html	(124)

NetGene2	http://www.cbs.dtu.dk/services/NetGene2	(125)
GeneSplicer	http://www.cbcb.umd.edu/software/GeneSplicer/gene_spl.shtml	(126)

1.5.2.2 Public and commercially available oncology knowledgebases

The primary purpose of the abovementioned resources is to assist in the delivery of individualized cancer treatment strategies. However, determining clinical relevance and clinical actionability of detected cancer variants remains a challenge, given the difficulty of distinguishing driver and passenger mutations, lack of standards in the interpretation of genomic variants in clinical practice as well as the lacking clinical evidence which links a particular variant to an observed clinical outcome (99,127,128). In order to address the challenges of translating complex sequencing readouts and scattered molecular information into clinically supportive information, several precision oncology knowledgebases have been developed to guide the use of genomic information for laboratories and clinicians (Table 4). These tools, which consist of publicly curated resources as well as commercially provided information, assist in deciphering variants obtained via NGS to guide treatment decision-making at MTBs.

Table 4. Somatic variant cancer knowledgebases [*Adapted from* (129), (CC BY 4.0)]

Knowledgebase	**Resource availability**	**Provider**	**Content description**	**URL**
My Cancer Genome	Public	Vanderbilt University	Clinical impact of cancer biomarkers	https://www.mycancergenome.org/
ClinVar	Public	National Center for Biotechnology Information (NCBI)	Relationships among human variations and phenotypes	http://www.ncbi.nlm.nih.gov/clinvar/
OncoKB	Public	Memorial Sloan Kettering Cancer Center	Clinical impact of cancer biomarkers	http://oncokb.org/#/
Clinical Interpretation of Variants in Cancer (CIViC)	Public	Washington University School of Medicine (WashU)	Clinical impact of cancer biomarkers	www.civicdb.org
Precision Medicine Knowledgebase (PMKB)	Public	Weill Cornell Medical College	Clinical impact of cancer biomarkers	https://pmkb.weill.cornell.edu/
Cancer Genome Interpreter (CGI)	Public	Institute for Research in Biomedicine, Barcelona, Spain	Clinical impact of cancer biomarkers	https://www.cancergenomeinterpreter.org/home
Personalized Cancer Therapy Database	Public	MD Anderson Cancer Center	Clinical impact of cancer biomarkers	https://pct.mdanderson.org/#/home

MatchMiner	Public	Dana-Farber Cancer Institute	Clinical trial matching in oncology	http://bcb.dfci.harvard.edu/knowledge-systems/
Cancer Driver Log (CanDL)	Public	Ohio State University (OSU) / James Cancer Hospital	Clinical impact of cancer biomarkers	https://candl.osu.edu/
COSMIC Drug Resistance Curation	Public	Wellcome Trust Sanger Institute	Clinical impact of cancer biomarkers	http://cancer.sanger.ac.uk/cosmic/drug_resistance
NCI Clinical Trials	Public	National Cancer Institute of the National Institutes of Health	Listing of clinical trials	www.cancer.gov/about-cancer/treatment/clinical-trials
Drug Gene Interaction Database	Public	The McDonnell Genome Institute	Drug-gene interactions and the druggable genome	http://www.dgidb.org/
HemOnc.org	Public	Public contributors	Interventions and regimens in hematology and oncology	www.hemonc.org/
Genomics of Drug Sensitivity in Cancer	Public	Wellcome Sanger Institute & Massachusetts General Hospital Cancer Center	Drug response data from human cancer cell lines	https://www.cancerrxgene.org/
JAX Clinical Knowledgebase (CKB)	Partially public	The Jackson Laboratory	Clinical impact of cancer biomarkers	https://ckb.jax.org/
Molecular Match (MM)	Commercial	Molecular Match	Clinical trials and therapies in cancer	https://app.molecularmatch.com/
BaseSpace Knowledge Network	Commercial	Illumina	Variant interpretation	https://knowledgenetwork.kn.basespace.illumina.com/bskn/#!/login
Oncomine Knowledgebase Reporter	Commercial	ThermoFisher Scientific	Clinical trials and therapies in cancer	https://www.oncomine.org/resource/login.html

1.5.3 Clinical decision support software solutions for variant interpretation and treatment allocation

The growing and overwhelming amount of genomic data coupled with the compounding number of targeted therapies coming to market has made critical point-of-care decisions for oncologists increasingly challenging. This information overload may lead to knowledge gaps between detecting and treating molecular alterations, such as over- or under-testing of clinical samples and

inconsistencies in treatment decisions, implicating the need for genomic data use to be complemented with clinical education and decision support (130). As a result of the need to combine the complex processes of variant annotation and interpretation and treatment decisions within the context of a specific tumor type, commercial providers have begun to launch a variety of clinical decision support tools that convert molecular information obtained via NGS into a clinical-grade report describing treatment options and clinical trials matched to the patient's genomic profile (Table 5). These company solutions vary across approaches to data curation, variant annotation, variant prioritization, treatment matching strategies, clinical trial matching strategies, end-user functionality, and report generation. For example, IBM Watson launched its AI-driven program, IBM Watson for Genomics, geared towards oncologists to provide them with evidence-based treatment options by mining the meaning and context of unstructured data in clinical reports. These data are then evaluated in the context of patient attributes, professional guidelines, biomarker-based clinical trial options and genomic databases to identify individualized treatment plans. Other tools, such as Roche's NAVIFY Mutation Profiler and QIAGEN's Clinical Insight Interpret, are more straightforward solutions that allow the end user to upload a variant call format (VCF) file and manually adjust assay settings depending on the NGS panel used, correct variant classification as needed and adjust for report generation depending on tumor subtype and patient location.

Table 5. Examples of commercial clinical decision support tools for interpreting NGS data

Software name	**Commercial provider**	**URL**
IBM Watson for Genomics	IBM	https://www.ibm.com/products/watson-for-genomics
N-of-One	Acquired by QIAGEN	http://www.n-of-one.com
NAVIFY Mutation Profiler	Roche Molecular Systems	https://www.navify.com/mutation-profiler/
Qiagen Clinical Insight Interpret	QIAGEN	https://www.qiagen.com/us/products/discovery-and-translational-research/next-generation-sequencing/informatics-and-data/clinical/qiagen-clinical-insight-interpret-for-genereader/?clear=true#resources
Clinical Genomics Workspace	PierianDX	https://www.pieriandx.com/clinical-genomics-software-for-next-generation-sequencing
CureMatch Bionov	CureMatch	https://www.curematch.com/

OncoKDM	OncoDNA	https://www.oncodna.com/en/healthcare-providers/clinical-decision-support-tools/oncokdm/

1.5.4 Guardians of the genome: Clinical and molecular geneticists

The task of providing the most relevant, annotated and prioritized molecular information to oncologists is certainly eased with the utilization of the abovementioned tools and software. These curated repositories of genomic information organize complex data into digestible clinical evidence which can in turn support real-time clinical decision-making (131,132). They can further serve as a basis for educating patients about precision oncology and diverse treatment strategies (131), thus facilitating patient communication and increasing patient activation (133), which are both underrepresented fields in PCM. However, issues with accountability and trustworthiness of oncology knowledgebases pose a challenge for untrained end users and harmonization efforts across cancer-related somatic variant databases and decision support tools have only just recently begun (129,134,135). Although current CGP workflows generate data from well-established genes, they also profile molecular alterations which are currently not understood or those with an unestablished disease-related role (136,137). These points signify the need for individuals with clinical genetic expertise for interpreting the highly complex results from NGS testing, as precision oncology databases alone cannot replace the necessary human insight for aligning treatments to a patient's genomic data and clinical status. The most qualified experts in this regard are clinical and molecular geneticists who have the training to navigate genome browsers, knowledgebases, functional prediction tools and tier classification systems for determining and prioritizing the pathogenicity of variants, both known and unknown. Alongside their ability to summarize interpreted genomic findings in clinical reports for treating oncologists, they have genetic counseling expertise that further enables the transfer of this knowledge directly to the patient in a way that is both understandable and engaging for the patient (138). These reasons warrant central participation of clinical geneticists at MTBs to ensure that oncologists receive and understand the most relevant data for their patient while minimizing exposure to irrelevant information (Figure 4).

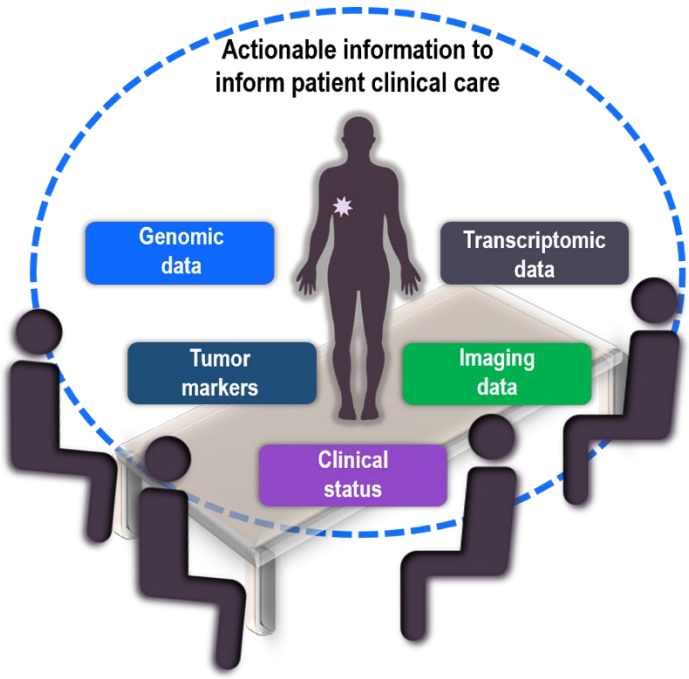

Figure 4. Molecular tumor boards must process and prioritize complex patient-specific data. At a molecular tumor board (MTB), patient-specific information is at the center of discussion by a variety of clinical experts, such as oncologists, pathologists, clinical geneticists, radiologists and bioinformaticians. Here, complex genomic and transcriptomic data is evaluated in the context of co-occurring tumor markers and the patient's most current clinical status and imaging data. The goal of the MTB is to critically evaluate all data at hand such that actionable information is identified and prioritized to inform treatment decisions.

1.6 Analysis of circulating tumor DNA: a service offered by the Institute of Human Genetics

Next to disease of the cardiovascular system, cancer represents the second most frequent cause of death in Austria (139). In 2018, twenty-three new cancer medicines came to the European market, 11 of which contained a new active substance (140), perhaps suggesting a positive trend towards increasing treatment options for patients with cancer. Although indicators suggest that five-year survival rates in Austria for patients with colon, lung, breast and prostate cancer are above averages of other EU countries (141), precision oncology still remains in its infancy not only in Austria, but also worldwide. There are several unanswered questions for all players in the field of PCM, i.e. clinicians, hospitals, providers and payers, that contribute to the slow adoption of precision oncology (Figure 5). Another main contributor hindering precision oncology approaches in local

clinics is the inability to understand and thus implement genomic sequencing results into clinical care, as sequencing reports are often complex and actionable information is not always apparent.

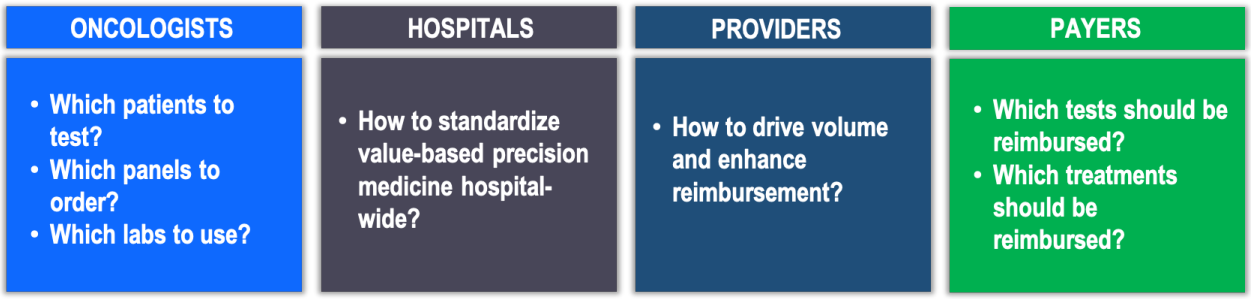

Figure 5. Challenges to implementing precision medicine from 4 perspectives [adapted from Trapelo Health (https://www.trapelohealth.com/)]. The main four perspectives in the improvement of precision medicine come from oncologists, hospitals, providers and payers. Each party is currently attempting to address each of the unique challenges faced on a daily basis in the clinical implementation of precision oncology.

As an institute with infrastructure for high-throughput NGS technology and liquid biopsy and clinical genetics expertise, the Institute of Human Genetics at the Medical University of Graz, Austria began offering a service to local clinicians in 2017 with the goal of expanding the adoption of genome-driven medicine and offering an otherwise difficult-to-access analysis for patients with progressive disease. Oncologists throughout Austria can submit liquid biopsy samples for the analysis of circulating tumor DNA, with options such as individual mutation testing (e.g. standard hotspot testing of *EGFR*, *KRAS*, *PIK3CA*, etc.), panel sequencing through Roche's AVENIO ctDNA Targeted and Expanded panels, and somatic copy number (SCNA) analysis through the established plasma-Seq approach (142). Clinical and molecular geneticists interpret the alterations detected from plasma DNA sequencing and summarize pathogenicity and potential treatment options for the requesting oncologist, with the goal of delivering the most current actionable insight to expand on options for patients who have exhausted all standard lines of treatment (Figure 6).

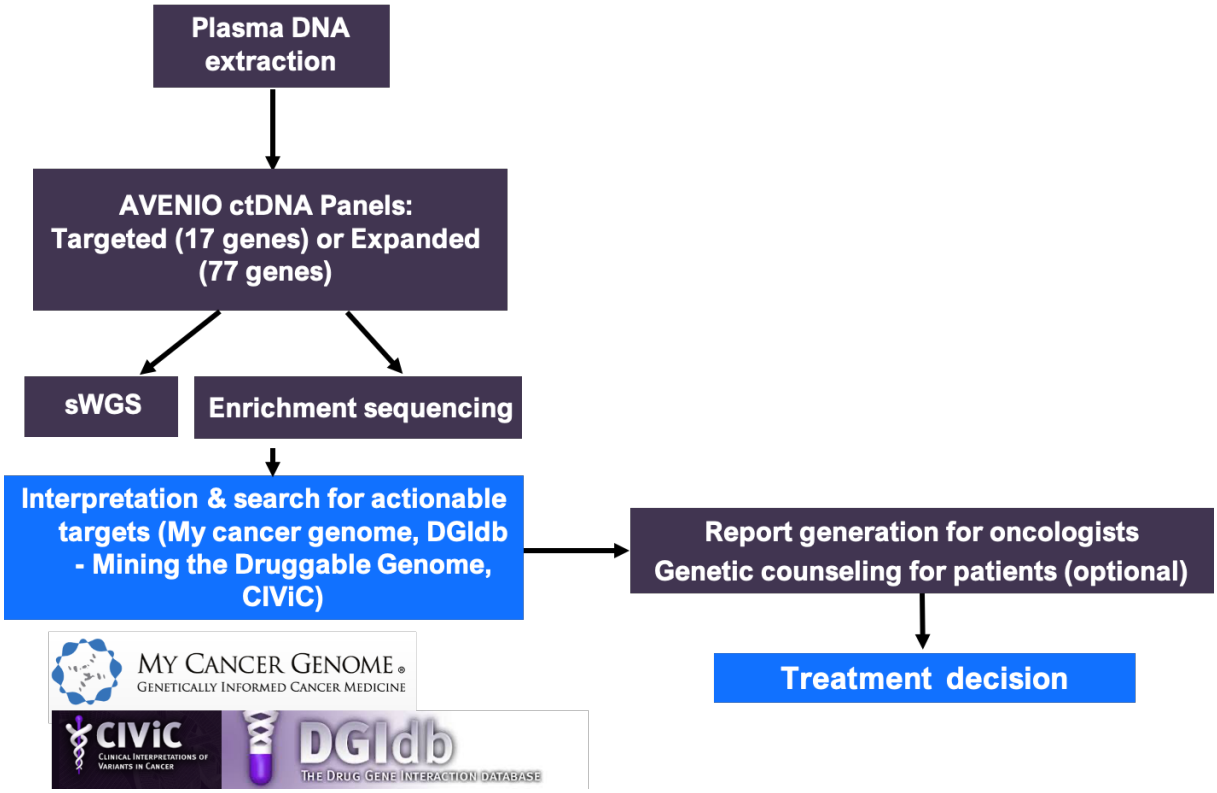

Figure 6. Workflow for analysis of circulating tumor DNA in advanced cancer patients who have exhausted all standard lines of treatment. After plasma DNA extraction, library preparation involves CGP via the AVENIO ctDNA Panels (either Targeted or Expanded). The whole-genome library can be sequenced via sWGS for the identification of focal, clinically relevant SCNAs and the enriched library is sequenced to identify selected genomic alterations from all four classes. At present, the data is interpreted and annotated for pathogenicity and actionability via publicly available variant interpretation databases. This information is compiled into a report for the treating oncologist and, if applicable, the patient is offered genetic counseling if suspected germline variants are detected or if the patient is interested in understanding the results, although these are both rare events. The oncologist reviews the results of the molecular report and decides on a clinical course of action. In addition, an MTB is currently being established in which oncologists, pathologists, human geneticists and bioinformaticians gather to discuss the molecular findings and jointly define therapy recommendations, although this workflow is still in its infancy.

As stated above, analysis of ctDNA provides the opportunity to evaluate the evolving tumor genome throughout the course of therapy, as treatment-related resistance to cancer drugs remains a critical issue when selecting subsequent lines of treatment, which is often observed in patients

with progressive disease. In order to improve this service, we realized that there is an urgency to standardize genome-informed cancer care, as this is crucial for the adoption of precision oncology in Austria.

However, the implementation of precision oncology is complex, involving a cascade of various individual steps that begins with informed consent and, if applicable, genetic counseling (143). A patient's sample, consisting of tissue or blood, is subjected to clinical-grade sequencing. Bioinformatic approaches are then applied to quantify tumor allele fraction (AF) and to detect SCNAs, single nucleotide variants (SNVs), i.e. point mutations, and indels. Subsequently, the detected alterations must be interpreted and annotated for clinical relevance, as these results are then discussed in the setting of an interdisciplinary MTB. The MTB faces the task of identifying druggable targets and disturbed pathways to match driver aberrations with existing drugs. Once a suitable treatment option has been identified, it is aligned with the clinical status and disclosed to the patient. From this precision oncology cascade (Figure 7), we focused on a decisive step for this study, i.e. how to use the complex data obtained from high-throughput sequencing to translate aberrations into appropriate therapies (143).

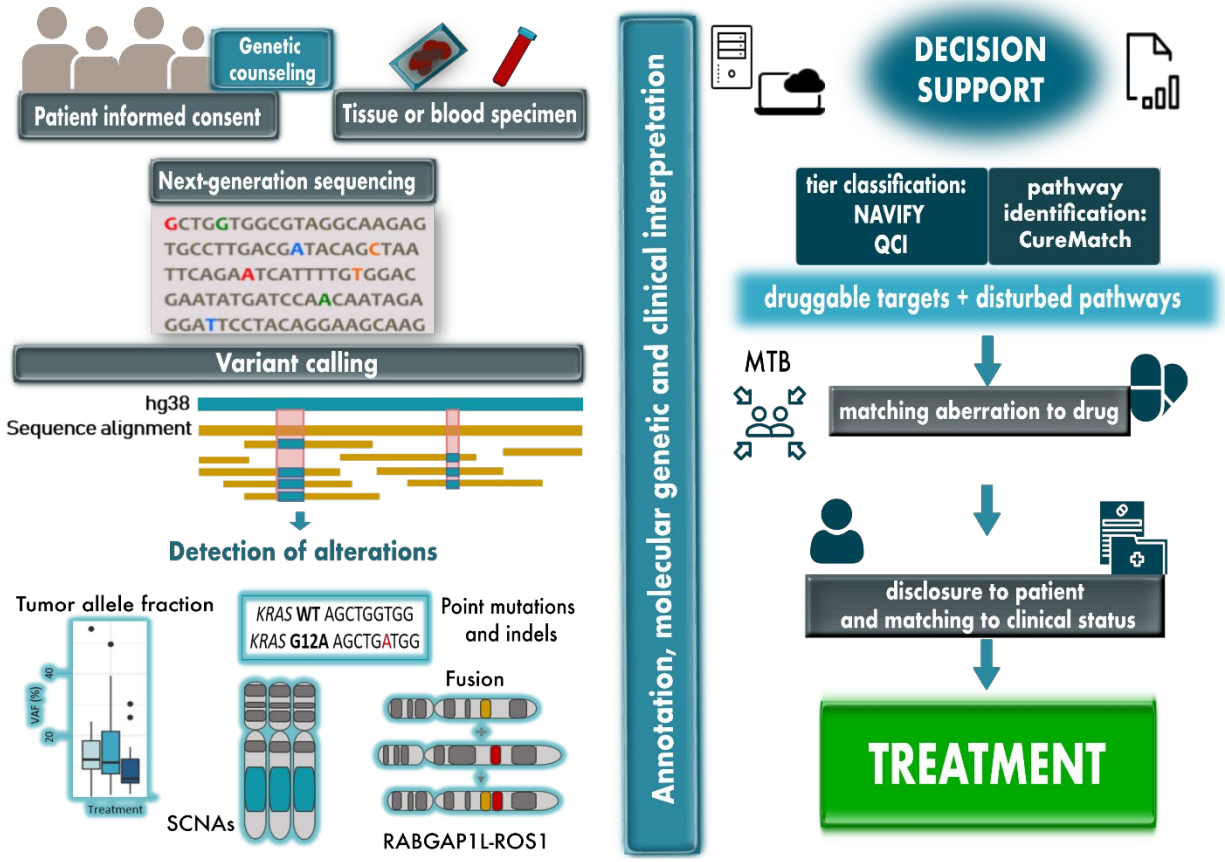

Figure 7. Cascade of individual steps involved in precision oncology [Figure and legend originally published in ESMO Open (Perakis et al., 2020)]. Due to the increasingly extensive sequencing involved in precision oncology, genetic counseling may be required and an informed consent needs to be obtained in every case. Sequencing can be conducted with a tissue sample and/or from blood after isolation of plasma DNA, the latter being the specimen used in our study. Subsequently, various sequencing strategies can be applied to the sample to determine tumor AF and to detect alterations such as SNVs (point mutations) and indels, SCNAs, and fusions. A decisive step in precision oncology is variant annotation, the molecular and clinical interpretation of targets and matching these with drugs. In this study, the data were subjected to interpretation by three clinical decision support tools (NAVIFY, QCI, CureMatch) to obtain treatment possibilities. The multidisciplinary MTB evaluates the data and gives advice to the treating physician. Afterwards, the results are disclosed to the patient and matched to their clinical status and, if applicable, the treatment is started. These last two steps were not within the scope of our retrospective study.

With the increasingly widespread application of clinical therapeutic decision support platforms, it is necessary to evaluate existing commercial products prior to implementation in routine clinical care of advanced cancer patients. In this study, we planned a retrospective comparison of molecular data using three such platforms that specialize in translating genomic profiles into cancer therapy recommendations to help identify advantages, disadvantages, usability and distinguishing features of each individual solution that in turn may influence patient outcomes by identifying actionable targets and other novel clinically relevant information. We sought to ascertain how these tools may support our routine liquid biopsy analyses and assist in the prediction of efficacy of personalized (combination) therapy options for patients with advanced cancers. We sought to elucidate the features of each software such that our local oncologists may become familiar with each platform and how they may potentially help guide the treating clinician in his/her selection of optimized treatments, ultimately leading to an accelerated adoption of precision oncology concepts and improvement of patient clinical outcome.

2 AIMS

We aimed to reconstruct representative molecular profiles (SCNA analysis and mutation panel analysis) from plasma for 48 patients with advanced cancer to comprehensively test 3 commercial clinical decision support tools: the NAVIFY™ Mutation Profiler (Roche), QIAGEN Clinical Insight (QCI™) Interpret for Somatic Cancer (QIAGEN) and CureMatch® Bionov Personalized Therapeutic Decision Support Platform (CureMatch).

Specific aims are:

- To define how many actionable targets can be identified in advanced cancer patients via CGP from liquid biopsy
- To identify differences between advanced colon, breast and lung cancer entities
    - Number/type of actionable targets identified per tumor entity
    - Number of recommendations per tumor entity
    - Number of off-label suggestions per tumor entity
    - Number of clinical trial suggestions per tumor entity
- To describe the alignment of tier designations
- To describe the alignment of druggable targets
- To describe the alignment of treatment recommendations

- How often was an established treatment recommended
- How frequently were off-label recommendations made
- For how many cases could each platform make a recommendation in total
- How well did the treatment recommendations align per patient across platforms

These analyses would prove extremely valuable for increasing oncologists' confidence in and efficiency of employing molecular cancer diagnostics in a routine setting. This will in turn increase clinicians' understanding of the assay strengths, limitations and results of genomics-guided personalized cancer therapy when profiling prospective patients and deciding on evidence-based therapies in the future.

3 MATERIALS AND METHODS

3.1 Ethics and patient recruitment

Recruitment of patients into this study was approved by the Ethics Committee of the Medical University of Graz (approval numbers 21-229 ex 09/10 and 21-227 ex 9/10) and the University Medical Center Groningen (METc approval number METc 2017/217) and conducted according to the Declaration of Helsinki. Patients with metastatic breast cancer (BC) (n=12) and colorectal cancer (CRC) (n=17) were recruited and treated at the Department of Internal Medicine, Division of Oncology, at the Medical University of Graz and patients with stage IIIB and stage IV NSCLC (n=19) were recruited and treated at UCMG at the University of Groningen. Written informed consent was obtained from all patients. Patients were evaluated to have progressive disease in accordance with the response evaluation criteria in solid tumors (RECIST) (144). For each patient, 16 ml of whole blood were collected in 2 PAXgene Blood ccfDNA tubes (Qiagen, Hilden, Germany). When available, protein biomarker expression data was collected for BC patients (estrogen hormone receptor [ER], progesterone receptor [PR] and human epidermal growth factor receptor 2 [HER2] status) and NSCLC patients (programmed death-ligand 1 [PD-L1] status).

3.2 Whole blood centrifugation and plasma DNA isolation

Whole blood was processed by first transferring to 15ml tubes. The blood was then centrifuged as follows: 200×g for 10 min; 10 min at 1600×g; the supernatant was collected and transferred into a

new 15ml tube followed by an additional 1600×g 10 min centrifugation. Plasma was then collected and aliquoted into 2ml Eppendorf tubes and stored at -80°C prior to isolation. Cell-free DNA was isolated from plasma using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. Cell-free DNA was eluted with 90µl nuclease-free water (Qiagen, Hilden, Germany). DNA concentrations were measured using the Qubit™ dsDNA HS Assay kit (Thermo Fisher Scientific, Vienna, Austria) in accordance with the manufacturer's instructions.

3.3 Shallow whole-genome sequencing (sWGS) via plasma-Seq for the identification of somatic copy number alterations (SCNAs)

Whole-genome sequencing libraries were prepared from plasma DNA using our plasma-Seq approach, a shallow whole-genome sequencing strategy described previously in detail (142,145). Libraries were sequenced using either a 150bp single read or 76bp paired-end read strategy on an Illumina MiSeq or NextSeq 550 instrument (Illumina, San Diego, CA, USA), with coverage ranging from 0.1x-0.2x (approximately 5-10 million reads). Analysis of tumor-specific somatic copy number aberrations was performed with an in-house script described previously (142). Focal amplifications can be reliably called down to an AF of 5% with this pipeline (146). In order to estimate the tumor-specific fraction from plasma DNA, alignment (BAM) files were subsequently fed into the ichorCNA algorithm maintained by the BROAD Institute (https://github.com/broadinstitute/ichorCNA). ichorCNA is a probabilistic HMM for the estimation of tumor fraction from shallow whole-genome sequencing (sWGS) data, which yields an estimation that is roughly equivalent to tumor purity from bulk tumor analyses (147).

3.4 Comprehensive genomic profiling via AVENIO ctDNA Expanded Panel

Plasma DNA was subjected to comprehensive genomic profiling via the AVENIO ctDNA Expanded Panel (Roche), an assay specifically designed for the profiling of ctDNA for the identification of genomic aberrations derived from solid tumors. The panel consists of 77 genes covering a total of 192kb, including those currently in the U.S. NCCN Guidelines as well as emerging biomarkers currently being investigated in clinical trials. The comprehensive panel is designed to interrogate the four classes of alterations, i.e. SNVs, indels, selected SCNAs and selected fusions, which are detailed in Supplementary Table 1. Our lab recently validated the AVENIO platform using commercially available highly multiplexed reference standards with

distinct mutations at defined allele frequencies, which enabled variant detection down to a variant allele frequency (VAF) of 0.125% (148).

Libraries were prepared using 10-50ng of plasma DNA and were sequenced 150bp paired-end on an Illumina NextSeq instrument to obtain between 30-40 million paired-end reads per sample. Data analysis was performed using the AVENIO ctDNA Analysis Software (Roche) with customized somatic variant filtration settings (143). Briefly, variants commonly found in germline with an allele frequency ≥ 1% as defined by 1000 Genomes or ExAC were removed along with common single nucleotide polymorphisms (SNPs) as defined by dbSNP. Intron variants except for novel splice site variants, likely germline variants, synonymous variants and copy number alterations (MET, ERBB2, EGFR) with copy number variant (CNV) scores <5 were omitted from analysis. Variants were only kept if the mutant read depth was >10 reads and significant filtered somatic variants were summarized in a VCF file for subsequent analysis via NAVIFY and QCI. For CureMatch analysis, VCF files for each patient were annotated for pathogenicity, i.e. VUS, likely pathogenic or pathogenic, using publicly available databases and summarized in a PDF report for off-site analysis by the company (143).

3.5 Data preparation for decision support submission and analysis

3.5.1 Generation of CureMatch reports for Bionov analysis

All likely pathogenic, pathogenic and VUS variants annotated from the AVENIO ctDNA Expanded output VCF were summarized alongside significant copy number alterations detected by AVENIO (*EGFR*, *MET* and *ERBB2* only), patient clinical information (e.g. age, gender, tumor type), all focal amplifications or deletions detected from plasma-Seq and the representative plasma tumor fraction in a patient-specific PDF report. The report design was structured as a typical NGS genomic report containing the analysis methods and descriptions thereof, descriptions of gene aberrations, including gene name, genomic position and reference genome, coding and amino acid change, variant description, depth allele fraction, classification, etc. Additionally, protein biomarker expression (*ER*, *HER2*, *PGR* expression for BC and *PD-L1* expression for NSCLC), where available, was provided for each patient in the report. An exemplary submitted report is provided in the Appendix. All 48 patient reports were sent to CureMatch for analysis. Report generation from the company was slightly staggered, but all analyses were performed within a couple of months of each other such that there was no critical time lag that could influence the

results, e.g. due to new evidence curation. CureMatch returned their analyses in PDF format for each patient, listing the actionability of each listed genomic aberration as well as the top three 3-drug and 2-drug combinations alongside the top three monotherapies. Each drug listing was accompanied with a proprietary Matching Score given as a percent. Furthermore, drug recommendations were listed along with their genomic target and whether the recommendation was an on-label or off-label indication depending on the tumor context of the patient. A sample report generated by the CureMatch Bionov algorithm is provided in the Appendix.

3.5.2 Analysis with NAVIFY Mutation Profiler

Since the Roche AVENIO Analysis Software generates separate VCF files for SNVs and indels, the data from these VCFs were merged prior to analysis. Assay settings were configured in the NAVIFY Mutation Profiler to reflect the hg38 reference genome, VCF file fields for read depth and allele fraction, the European Union clinical region and the most current Roche, COSMIC, TCGA, ClinVAR, ExAC, dbNSFP and CIViC content. The tumor type was selected for each patient using the "Diagnosis" pull-down menu and the corresponding VCF file was uploaded for each patient. Subsequently, any detected SCNAs or fusions were entered manually into the software describing the structural variant type, whether amplification or deletion for SCNAs, and gene name. After submitting for analysis, datasets were further processed to re-categorize all unclassified variants into one of the AMP tier designations, i.e. tier I-A, I-B, II-C, II-D, III or IV variants. For the majority of these affected variants, the tier given was tier III variant of unknown significance (VUS), as no existing or Roche classification was available. The clinical trial filter powered by MolecularMatch was selected to display all recruiting trials for all sexes and ages within Austria. The final resulting reports with automatically assigned drug recommendations were downloaded as a PDF for each patient. A sample report generated by the NAVIFY Mutation Profiler is provided in the Appendix.

3.5.3 Analysis with QIAGEN Clinical Insight Interpret

VCF files were merged as described above prior to upload to QIAGEN's Clinical Insight Interpret tool. The corresponding tumor type/diagnosis was selected for each patient and as with the NAVIFY platform, focal SCNAs obtained from plasma-Seq and SCNAs and fusions from the AVENIO analysis were entered manually into the software. After the analyses were generated, the

computed pathogenicity classifications automatically assigned to each variant by QCI were kept. In contrast to NAVIFY, the end user must manually select from the list of potential therapies matching to the aberration in question and off-label indications are only provided if the user changes the selected tumor phenotype to "All Cancers". For this study, off-label indications were excluded from the QCI analysis and all tumor type-specific treatments were added to the report, regardless of level of actionability evidence, e.g. 1A, 2D, etc. Clinical trials were filtered by tumor type and location, i.e. Austria. The final resulting reports were downloaded as a PDF for each patient. A sample report generated by Qiagen QCI is provided in the Appendix.

3.6 Analysis of software output and patient reports generated by clinical decision support tools

3.6.1 Comparison of tier classifications between NAVIFY and QCI

In order to facilitate a comparison between the tier classifications assigned by NAVIFY and QCI, the QCI II-C and III likely pathogenic, pathogenic and uncertain significance subclassifications were collapsed into just tier II-C and III. Because NAVIFY has the unique feature that additionally annotates pertinent negative genes, e.g. RAS wildtype status, as well as unknown combinations of alterations, e.g. co-alteration of *BRAF* V600E and *KRAS* G12D, NAVIFY analyses may output a higher number of tier annotations for each sample. For this reason, these annotations were not included for comparison. A side-by-side comparison of the tier designations was performed for each individual variant per patient and assessed for concordance between the two platforms. The frequency of concordant and discordant events was recorded alongside the aberration for each event.

3.6.2 Comparison of identified actionable targets across all three platforms

Concordance in actionability was assessed across all three platforms for each individual variant per patient by performing pairwise comparisons for each software. If the software generated a treatment match for an aberration, this was considered an actionable target. Alterations for which no therapy could be identified were classified as "not actionable". QCI also matches potential chemotherapies to genomic aberrations and such matches were also included in the actionability analyses, although they do not constitute targeted therapies. Concordance between variant tier classifications were not taken into account when assessing the concordance of actionability.

3.6.3 Comparison of matched treatments across all three platforms

Each treatment assigned to each actionable alteration was assessed for degree of overlap across the three decision support tools. For example, in a pairwise comparison, if one platform recommended two drugs, either in combination or as two separate recommendations, and one of these drugs was concordant with a second platform, this was quantified as a one-drug match. When performing a three-way comparison, if one platform matched 3 drugs to a particular patient profile and 2 of these drugs overlapped with the recommendations of the second and third platforms, this was considered a three-way two-drug overlap. All treatments were considered in this analysis, regardless if the drug was a targeted agent or chemotherapy recommendation.

3.6.4 Clinical trial identification in Austria

For NAVIFY and QCI analyses, patient profiles were matched to potentially suitable clinical trials by selecting for country-specific trials in Austria. Age, sex and other trial-specific inclusion criteria were not taken into consideration. The CureMatch algorithm provides trial information as evidence for the recommended combination therapies rather than as a recruitment suggestion for the patient. Although this strategy differs, the number of trials matched to each patient profile were still considered in the summary of clinical trials across each platform.

3.6.5 Statistical analyses

The Student's t-test was used to calculate significant differences between detected molecular features (i.e. number of mutations, focal events, VAFs), submitted markers for analysis and detected actionable targets per tumor type. Correlation analyses between the identified actionable targets and focal events or matching drugs was performed using a Pearson correlation. Statistical analysis was performed using the ggpubr package in R. A P of <0.05 was considered to be statistically significant (143).

4 RESULTS

4.1 Company profiles

4.1.1 NAVIFY® Mutation Profiler (Roche)

In March 2019, Roche launched the NAVIFY Mutation Profiler along with the NAVIFY Therapy Matcher. The NAVIFY Mutation Profiler (NAVIFY) has been designated as an CE-IVD medical device and its cloud-based workflow is meant to help clinicians interpret the actionability of genomic aberrations by processing and annotating molecular profiles (143). The goal of NAVIFY is to streamline the clinical decision and thus reports are simplified to facilitate their integration into an MTB for oncologists. NAVIFY, which takes a standard VCF file as an input, allows customizable assay settings for adjusting for read depth and variant allele frequency thresholds as well as access to the most recent curated content from various databases such as COSMIC, TCGA, CLINVAR, ExAC and Roche's own curated content. Somatic variants were classified in accordance with AMP guidelines (Supplementary Table 2) and, in most cases, the user must manually move variants from the "Unclassified" designation to a new tier in order to proceed to report generation. The NAVIFY Mutation Profiler is just one of the workflow products in the NAVIFY portfolio which seeks to streamline tumor board preparation and discussion, ultimately enabling a holistic view of a patient's data to help decide on the most individualized treatment plan (143).

NAVIFY may be seen as a solution that is ready-to-use and implement in the clinic without the necessity of clinical trials, as only well-established tier I-A, I-B and II-C alterations are actionable. Here, the selection of tumor type is crucial, as indications are designated off-label outside of specific clinical contexts. For example, if the tumor type "Breast Cancer" is selected, ERBB2 status is unknown to the software, subsequently assigning all suitable PIK3CA alterations with an off-label match to fulvestrant and alpelisib, as this combination was FDA-approved for ERBB2-positive breast cancer (143).

4.1.2 QCI Clinical Insight Interpret (Qiagen)

Since 2017, the QIAGEN Clinical Insight (QCI™) for Somatic Cancer tool has offered itself as an integrated clinical decision support solution for the annotation, interpretation and reporting of NGS data. QCI seeks to extract clinical significance and actionability from sequencing data (143). The

platform, which requires a VCF upload, provides evidence that triggers all 28 criteria of the ACMG (American College of Medical Genetics)/AMP (Association for Molecular Pathology) variant interpretation guidelines [Auto-computed ACMG/AMP or AMP/ASCO/CAP(College of American Pathologists) classifications, and access to over 1 million unpublished variant-phenotype relationships from the QIAGEN Knowledge Base; QCI for Oncology enables standardized reporting according to AMP/ASCO (American Society of Clinical Oncology)/CAP guidelines for the interpretation and reporting of somatic variants (99) and provides biological insights based on ACMG guidelines (149); QCI Interpret automatically incorporates diagnostic and predictive markers for drug response criteria supporting tier classifications of the ACMG/AMP and AMP/ASCO/CAP professional guidelines, as well as cytogenetic results, the variant's reported frequency, and geographically matched trials]. Hence, QCI Interpret uses ACMG guidelines to determine pathogenicity and AMP guidelines to determine actionability, but it does not prioritize the alterations or treatment recommendations, thus leaving such decisions to the individual generating the report or to the clinician receiving it. As NAVIFY classifies somatic variants in accordance with AMP guidelines, whereas QCI uses a combination of the ACMG and AMP guidelines for variant classification (Supplementary Table 3), the latter analysis output yielded a more diverse subclassification across tiers, where tier II-C and tier III variants were subclassified into "Likely Pathogenic", "Pathogenic" or "Uncertain" categories. For our analyses, we combined these subclassifications to tier II-C and tier III, respectively, in order to facilitate a comparison between NAVIFY and QCI (143).

QCI Interpret offers a more hybrid solution for researchers and clinicians alike, as it allows the visualization of variant details such as genomic location and nearby similar variants, reported functional impact, and predicted biochemical impact (e.g. CADD, PolyPhen), laboratory observations and effect on protein, features which are predominantly easier to interpret for those in the research community (143).

4.1.3 CureMatch Bionov™ (CureMatch)

CureMatch, Inc. is a San Diego-based digital health company founded in 2015 that focuses on personalized oncology and combination therapy solutions. CureMatch's Decision Support System seeks to guide oncologists in selecting individual customized treatment plans based on comprehensive molecular profiling data by targeting the entire aberrant profile rather than individual targets, including individualized parameters such as mismatch repair status and tumor

mutational burden (143). Their decision support platform, a decision engine that is an expert-based rule system, works by aggregating all known clinical evidence on predictive biomarkers and anti-cancer drugs. The outputs include ranking of alterations and treatments in terms of priority and is specifically geared towards implementing combination therapy and provides combinations of 3-, 2- and 1-drug(s) using a percent matching score. It enables the input of all identified genomic alterations as well as available protein markers relevant for therapy, such as hormone receptor or PD-L1 expression status in BC or NSCLC patients, respectively (143).

Hence, Bionov™ provides a scored and ranked listing of the most well-adapted personalized medicine treatment options. The comprehensive analysis considers commonly used treatments, as well as targeted therapy, immunotherapy, hormone therapy, and newly approved oncology drugs. These options are presented either alone (monotherapy) or in combinations of up to 4 drugs. Known and clinically validated predictive biomarkers for the response to cytotoxic, targeted, hormone or immunotherapy agents are considered. The effect of each drug on the alterations presented by the tumor is considered as follows: a drug is "directly matched" if the altered gene/protein is a demonstrated target of the drug (small-molecule inhibitors or antibodies); a drug is considered "indirectly matched" if the altered gene/protein is related (i.e. located in the same signaling pathway) to the direct target of the drug. For a given combination, the matching score (percentage) corresponds to the concatenation of all matching effects actually assessed in comparison to the maximal matching effect that would have been theoretically obtained if all alterations were actionable. This percentage is later finely tuned using a multi-parametric variable taking into account specific additional molecular and/or clinical criteria (expert-reviewed rule-based system) (143).

In addition to the treatment suggestions, members of the clinical team offer the coordination of a virtual molecular tumor board to discuss the results and dosing strategies with the treating oncologist.

4.2 Metastatic patient cohorts

In total, we analyzed 48 plasma samples with relatively high ctDNA from patients with advanced stage breast cancer (BC) (n=12), colorectal cancer (CRC) (n=17) and non-small-cell lung cancer (NSCLC) (n=19). Median age of the total patient cohort was 61 years (range 48-79) and patients had received a median of 2.5 prior lines of therapy (Supplementary Table 4). Exactly half of the

patients (24/48) were female. Seven patients with NSCLC (37%) had a squamous cell carcinoma (SCC) histology whereas the rest had an adenocarcinoma (ADCA) histology.

4.3 Molecular features of patient samples

All plasma samples were analyzed with a 77-gene panel (AVENIO ctDNA Expanded Panel; Supplementary Table 1) and by plasma-Seq (142) to map SCNAs, including focal events, according to our previous definition (150) and to quantify tumor fraction (TF) with ichorCNA (147). Median ichorCNA-derived TFs were 24.95% (range 10.52-48.93), 14.94% (range 4.07-54.69), and 4.46% (range 1.63-45.42) in patients with BC, CRC, and NSCLC, respectively (Figure 8A) (143). NSCLC plasma samples had also the lowest median ctDNA VAF (6.04%) followed by CRC (11.27%) and BC (12.23%), whereas the lowest median number of mutations was detected in BC (3 variants) compared to NSCLC (4 variants) or CRC (6 variants) (Figure 8A). Differences in the number of detected variants was significant when comparing BC and CRC ($P = 0.03$), but not when comparing NSCLC with BC ($P = 0.14$) or CRC ($P = 0.44$). That patients with NSCLC had, despite our selection, the lowest ctDNA AFs is in agreement with previous reports about varying tumor fractions depending on tumor entity (151,152). From the plasma-Seq data, we observed a median number of 6 focal events in BC patients [range 0-14], 1 in CRC [range 0-5] and 0 in NSCLC [range 0-7], a difference that was statistically significant between BC and both CRC ($P = 0.01$) and NSCLC ($P = 0.004$) but not between CRC and NSCLC ($P = 0.26$) (Figure 8A).

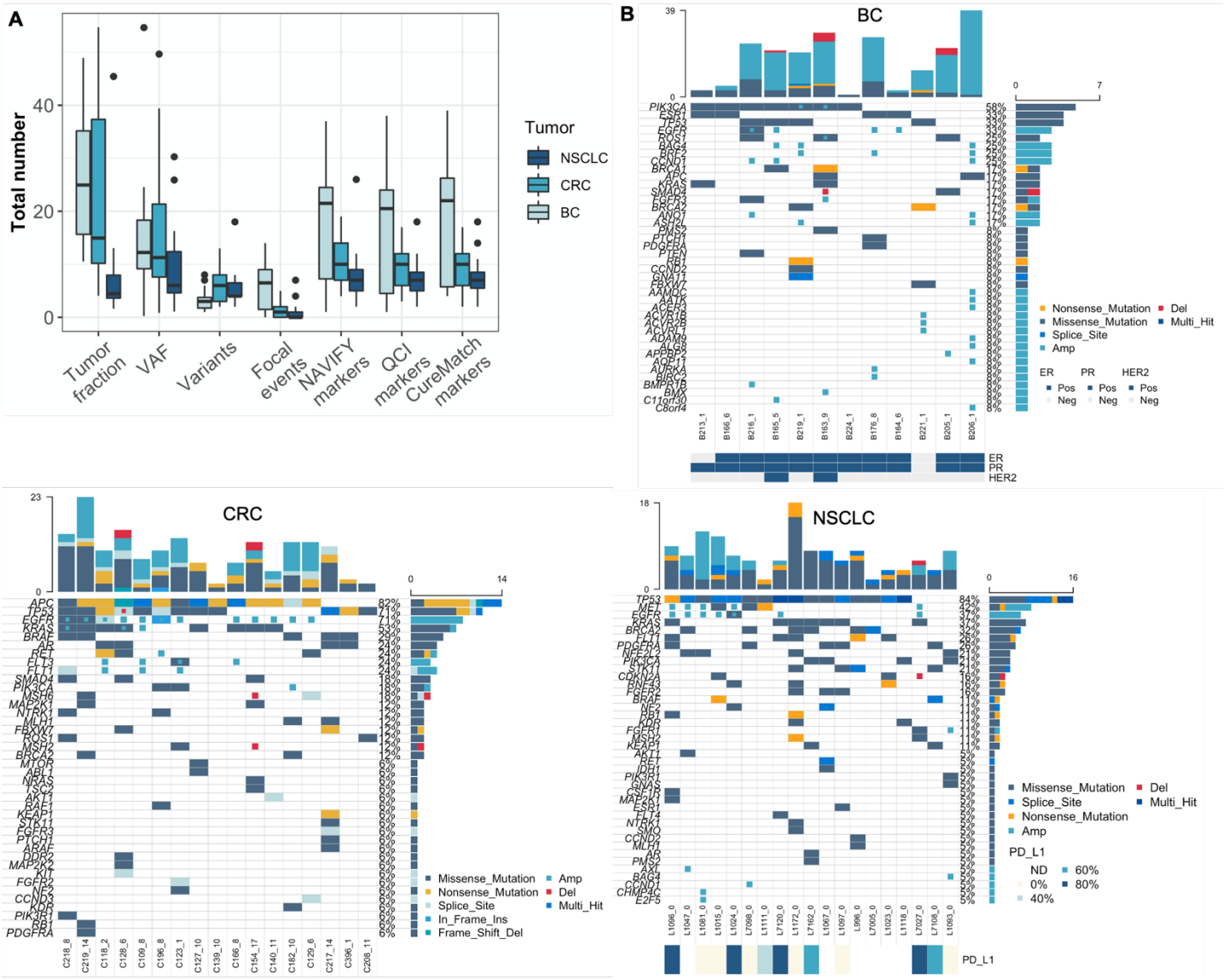

Figure 8. Summary of molecular profiling data across all three patient cohorts [Figure and legend adapted from ESMO Open (Perakis et al., 2020)]

(A) Boxplot showing the tumor fraction (TF) in cfDNA, ctDNA variant allele frequency (VAF), number of variants, and number of focal events detected from cfDNA in BC, CRC and NSCLC patients. Tumor fraction was estimated from shallow whole genome sequencing (sWGS) data using the ichorCNA algorithm and is displayed as a percent. VAF represents the percent allele frequency of all somatic non-synonymous variants detected from plasma DNA. The variants column represents the total number of aberrations detected per patient per cohort, i.e. all somatic non-synonymous SNVs, indels, fusions or focal SCNAs. Focal events represent all focal somatic copy number alterations (SCNAs), i.e. amplifications or deletions, detected from sWGS. In addition, the median number of reported markers, i.e. all alterations, such as non-synonymous single nucleotide

variants (SNVs), SCNAs or protein markers detected by routine clinical methods (BC and NSCLC only) reported to the clinical decision support tool, per tumor type per platform is shown.
(B) Oncoplots showing top 40 genes affected by mutation (i.e. SNV or indel) and/or SCNA identified in plasma DNA for BC (left), CRC (center) and NSCLC (right) patients. SCNAs are represented by either focal amplification (Amp) or focal deletion (Del). For BC patients, hormone receptor status is shown as a clinical feature below the plot for each patient (estrogen receptor, ER; progesterone receptor, PR; human epidermal growth factor receptor 2, HER2) as either positive (Pos) or negative (Neg). Similarly, if evaluated, PD-L1 expression status is shown as a clinical feature below the plot for NSCLC patients as a percent (ND, not detected).

As expected, the most frequently detected mutations for each respective tumor entity were consistent with tissue-derived data in the literature and in public databases. For each patient, we then summarized all genomic and proteomic markers including focal SCNAs, non-synonymous SNVs, indels, potential splice variants and, if applicable, immunohistochemistry (IHC) markers such as programmed death ligand 1 (PD-L1) staining for NSCLC or hormone receptor/HER2 status for BC (Figure 8B). The highest number of markers was obtained for BC patients due to the large number of focal SCNAs detected in plasma and hormone receptor status data, followed by CRC and NSCLC (Figure 8A) (143).

4.4 Clinical decision support tools vary in software features and overall strategy

Our results emphasize the distinct differences of each clinical decision support platform evaluated in this study (Table 6). In brief, NAVIFY employs the most stringent classification of somatic variants in accordance with AMP guidelines (99) and deems only well-established tier I-A, I-B and II-C alterations as actionable. On the other hand, QCI Interpret employs ACMG guidelines (149) to determine pathogenicity of variants and AMP guidelines to determine their actionability. QCI furthermore does not prioritize the alterations or treatment recommendations, thus leaving such decisions to the individual generating the report or to the clinician receiving it (143). Both NAVIFY and QCI report functional as well as predicted biochemical impact (e.g. Combined Annotation-Dependent Depletion (CADD) (116), PolyPhen (Polymorphism Phenotyping; http://genetics.bwh.harvard.edu/pph2/)) and QCI additionally provides observations from other laboratories, the effect of a mutation at the protein level, recorded prognostic outcomes, somatic frequency, as well as an interactive genome browser. The QCI platform requires manual curation

of information by the end user and thus these additional supporting visual aids, especially the detailed explanation of the computed classifications, reported functional impact and effect on protein, aid the interpretation workflow and influence the decision-making process for assigning treatments (143).

Table 6. Summary of decision support tool features [Table originally published in ESMO Open (Perakis et al., 2020)]

	Roche NAVIFY® Mutation Profiler	**QIAGEN QCI™ Interpret**	**CureMatch Bionov™**
Platform	Web application	Web application	A HIPAA and GDPR compliant web-based application available to users
Data input format	VCF	VCF	Annotated patient report (PDF)
Considers clinical characteristics such as prior treatment lines, comorbidities, medical history	No	No	Yes (if provided by the user)
Analysis of SCNAs	Yes, manual entry (segment information optional)	Yes, manual entry	Yes
Variant filtration	VCF filtered on user-defined assay parameters	VCF filtered on user-defined assay parameters	Filtration done in lab prior to submission
Variant classification	AMP guidelines	Mixed ACMG/AMP guidelines	Lab-specific guidelines for annotating variants and determining pathogenicity
Inclusions of VUSs in report	Yes	Yes, but not by default	Yes
Treatment suggestions	Based on individual variants	Based on individual variants	Combination therapies based on entire molecular profile
Recommendation of combination therapies	Yes, only tumor-specific recommendations for established tier I variants	Yes, only tumor-specific recommendations for established tier I variants	Yes
Suggestion of clinical trials	Yes, can adjust for location	Yes, shows currently enrolling studies involving variant	Provides clinical trial information as evidence for the recommended combinations
Variables considered for clinical trial matching	Age; sex; user-defined location; tumor type; molecular alteration; treatment	User-defined location; tumor type; molecular alteration; treatment	

Off-label suggestions	Yes	Yes, but not by default	Yes
Report reviewed by external clinical team	No	No	Yes
Virtual molecular tumor board option	Only in combination with other NAVIFY products in portfolio (NAVIFY Tumor Board)	No	Yes
Estimated time to generate report[a]	30-45 minutes[b]	30-60 minutes[b]	48-72 hours[c]

[a]Estimation is based on our experience only with the data used in this study. Time for report generation varies for each case and is dependent on user experience, the number of aberrations reported and the end user's analysis strategy.
[b]Includes data upload and hands-on time
[c]Vendor estimate of turnaround time for report generation (analysis performed by vendor)

The CureMatch Bionov strategy was included in this study, as the software's strategy differs dramatically from the other two. The Bionov solution involves the ranking and prioritization of alterations and treatments for with the goal of implementing personalized combination therapies, thus targeting the entire aberrant profile rather than simply matching treatments to individual targets. Therapy recommendations are accompanied by a proprietary "Matching Score" ((153), details in company profile), which prioritizes therapies and generates the top three 3-drug and 2-drug combination therapies and top three monotherapies. Another important distinguishing feature is the possibility of incorporating patient-specific history into the analyses, such as prior treatment lines, comorbidities and other pertinent clinical information such as TMB and microsatellite status, mismatch repair status and octreotide scans (143).

4.5 Tier classifications of genomic aberrations differ between NAVIFY and QCI analyses

As both NAVIFY (Version 1.1.0.3d9a34b, Release date: 08/26/2019) and QCI (Version 5.5.20190701) analyses were performed with identical datasets (VCFs, variant call files) and as both apply a tier-based somatic variant classification, we started with a detailed comparison between the two (143). One differentiating feature is that NAVIFY also considers pertinent negative genes in its analysis, i.e. *KRAS*/*NRAS* wildtype status is designated tier I-A in CRC, as well as potentially relevant co-alterations, e.g. co-occurrence of a *KRAS* mutation and *MET* amplification in CRC. For this reason, NAVIFY yielded a higher total number of classifications (551 total classifications) compared to all combined QCI analyses (492 total classifications) (Figure 9) (143).

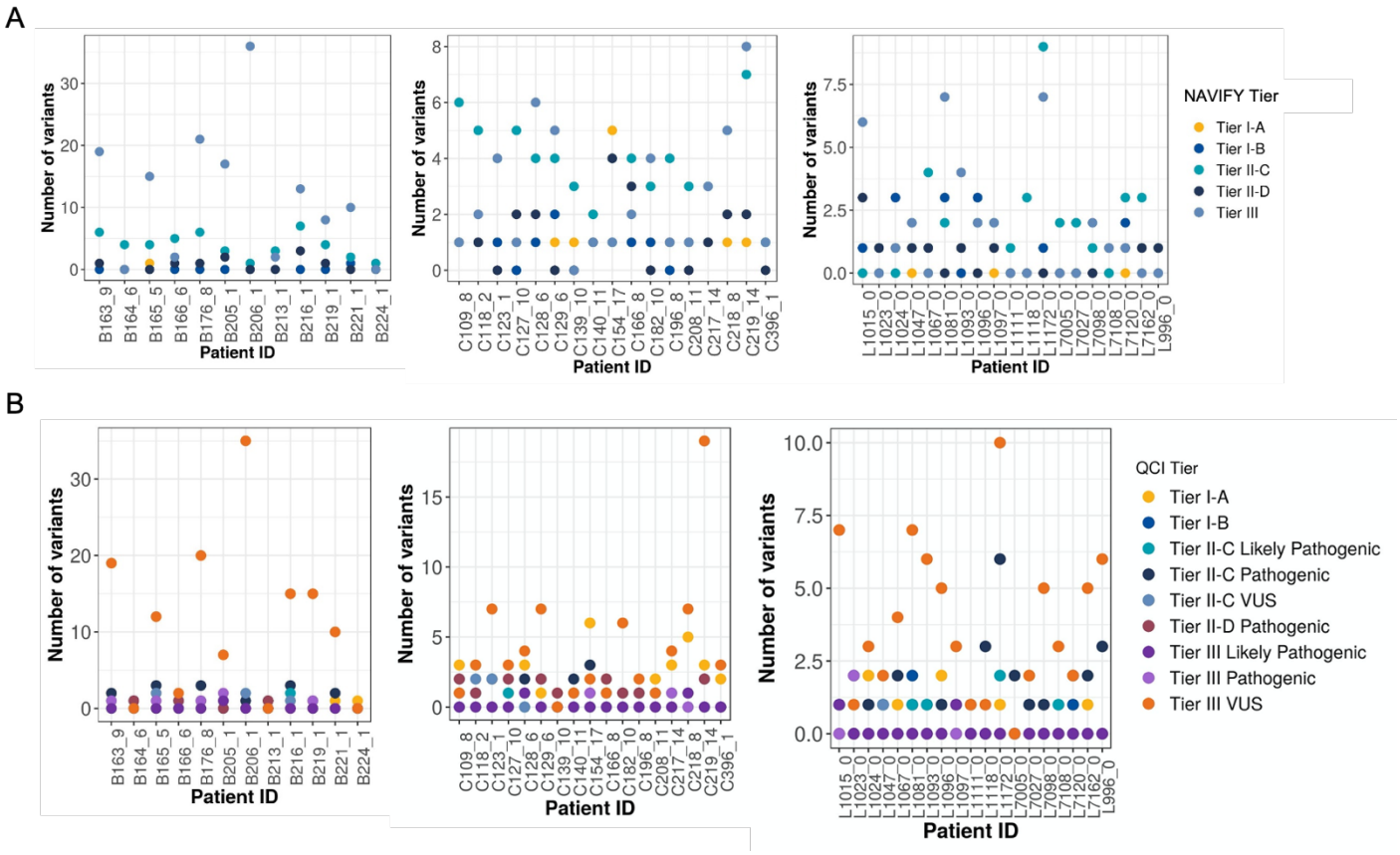

Figure 9. Variant tier designation per patient classified by NAVIFY and QCI [Figure and legend originally published in ESMO Open (Perakis et al., 2020)]

(A) Dot plots illustrating the distribution of the number of variants classified by NAVIFY as either tier I-A, I-B, II-C, II-D or III (VUS: variant of unknown significance) per patient in each cohort (BC, left; CRC, middle; NSCLC, right).

(B) Dot plots illustrating the distribution of the number of variants classified by QCI as either tier I-A, I-B, II-C Pathogenic, II-C VUS, II-D Pathogenic, III Likely Pathogenic, III Pathogenic or III (VUS) per patient in each cohort (BC, left; CRC, middle; NSCLC, right).

For subsequent comparisons, we only considered the 492 total alterations that overlapped between both platforms. From these 492 alterations, 344 (70%) were concordant and 148 (30%) comprised discordant events. Because of their association with relevant therapies, we were particularly interested in tier I classifications. Across all 48 patients, 14 alterations (4.1%) were classified concordantly as tier I-A between NAVIFY and QCI, with QCI demonstrating 47 discordant and unique I-A designations (Figure 10A and 10C) (143).

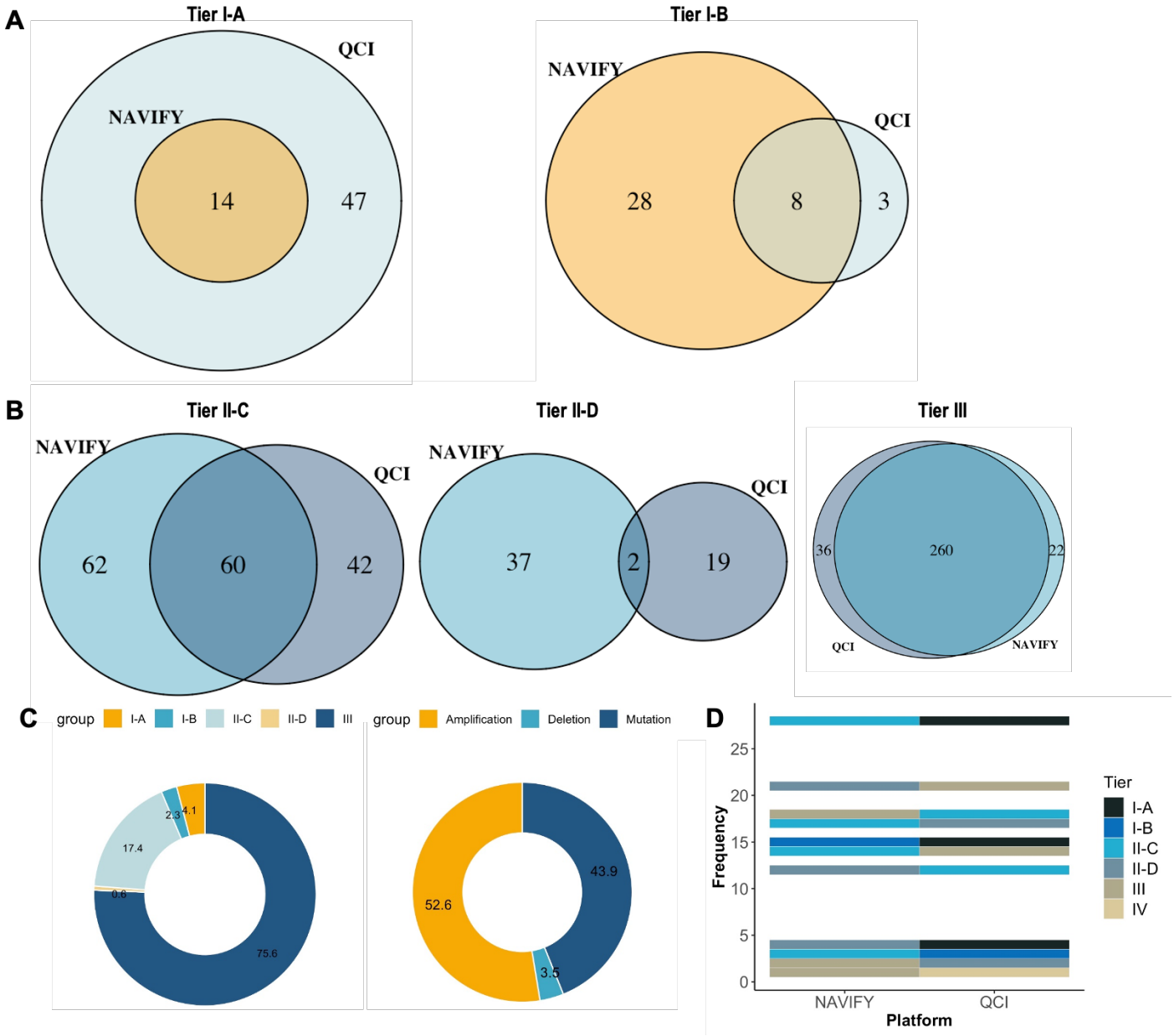

Figure 10. Concordant and discordant classifications of aberrations using NAVIFY and QCI and aberration type-based analysis [Figure and legend adapted from ESMO Open (Perakis et al., 2020)]

(A) Venn diagrams displaying the number of concordant and discordant tier I-A and tier I-B (top) classifications as annotated by NAVIFY and QCI across all 48 patients.

(B) Venn diagrams displaying the number of concordant and discordant tier II-C, tier II-D and tier III classifications as annotated by NAVIFY and QCI across all 48 patients.

(C) Donut plot illustrating composition of concordantly classified variants between NAVIFY and QCI according to tier. Each aberration which was designated the same classification is shown here

and plotted by tier (left). Composition of concordantly classified variants between NAVIFY and QCI according to aberration type (right).
(D) Heatmap showing discordantly classified aberrations between NAVIFY and QCI along with frequency of the discordant classification.

Similarly, only 8 alterations (2.3%) were classified concordantly as tier I-B by both decision support platforms (Figure 10A and 10C). Not surprisingly, all of these tier I concordantly classified alterations consisted of established predictive somatic alterations, such as *BRAF* V600E or *KRAS* G12 and Q61 variants or *ERBB2* and *MET* amplifications (Supplementary Table 5). Tier III VUS designations accounted for the majority of concordant events between platforms, followed by II-C and the least overlap among II-D designations (Figure 10B). When comparing at the aberration type level, it was revealed that the majority of concordant events came from SNVs and amplifications, i.e. 43.9% and 52.6%, respectively (Figure 10C) (143).
63 of the total 148 discordantly classified events (43%) involved tier I alterations. 47 (32%) were classified as tier I-A by one platform (QCI) but differently by the other (NAVIFY), i.e. 15 (10%) as tier I-B and 32 alterations (22%) as tier II-C or tier II-D, indicating that classification of a variant as actionable by one platform but not by another is a frequent event (Figure 10D) (143).

4.6 Analyses from all three platforms led to differences in actionability

We compared side-by-side actionability for each variant across all three platforms, also including the designated tier classifications for NAVIFY and QCI, by labeling the alteration as either actionable or not depending on the ability of the software to match a drug to the alteration. When comparing NAVIFY and QCI, we observed that only 4.3% (21/492 alterations) of events were classified as actionable by both platforms, which included *ERBB2* and *MET* amplifications, *PIK3CA* mutations and one case of a *RABGAP1L-ROS1* fusion (Supplementary Table 6) (143). Discrepancies in actionability were linked to diverse SCNAs and mutations and even included well-established predictive biomarkers. For example, NAVIFY considered *MET* amplifications to be actionable targets, whereas QCI did not (Supplementary Table 6). The latter suggests the importance of the context of tumor type for certain platforms, as QCI only called *MET* amplifications in the NSCLC setting to be druggable whereas NAVIFY listed this as an off-label indication outside of the NSCLC context. Similarly, QCI deemed activating mutations in *KRAS*, *NRAS* or *EGFR* amplifications actionable whereas NAVIFY did not (143).

We then incorporated CureMatch analyses into the comparison. When comparing the concordance of actionability per target for the 492 NAVIFY/QCI alterations with CureMatch, we observed a 66.3% (326/492 alterations) and 80.1% (394/492) concordance with NAVIFY and QCI, respectively. Again, the number of concordant events between two platforms that were actually targetable was minor, i.e. 9.5% (31/326) between NAVIFY and CureMatch and 28.4% (112/394) between QCI and CureMatch (Supplementary Tables 7-8) (143). The general higher targetabilities obtained via QCI analyses compared to NAVIFY is a result of the QCI algorithm, which also recommends suitable cytotoxic regimens for an aberration, although this drug may not be a direct "match", i.e. targeted agent. The NAVIFY matches primarily on targeted therapies and only lists chemotherapy possibilities in conjunction with a targeted agent.

In order to observe the distribution of the number of analyzed markers, targetable aberrations and the number of drug recommendations matching to these, we performed a comparison per patient across each tumor type (Figure 11A-C). There was a statistically significant association with the number of targetable alterations and the number of matching therapies across all three platforms (NAVIFY: Figure 11A, Figure 12, Pearson's R = 0.743, P = 0.02; QCI: Figure 11B, Figure 12, Pearson's R= 0.493, P = 0.003; CureMatch: Figure 11C, Figure 12, Pearson's R=0.766, P <0.001). CureMatch analyses furthermore identified a correlation between the number of actionable targets with the number of focal SCNAs detected via plasma-Seq (Pearson's correlation coefficient R=0.524, P < 0.001) (Figure 12) (143). For both CureMatch and QCI, CRC patients had the highest median number of actionable targets and all platforms identified the highest median number of matching drugs for CRC patients (Figure 11A-C). Interestingly, CureMatch analyses revealed that the actionability of alterations varied within the same gene and/or domain, indicating dependency of targetability on the specific somatic variant reported in CRC and NSCLC patients (Figure 13) (143).

To assess variation in how each platform defines actionability, we calculated the average percent actionability defined by each tool by dividing the total number of actionable targets by the total number of submitted markers. Median overall actionability was highest with CureMatch in all cohorts (Figure 14), whereas due to the stringent classification algorithm, patient genomes had the lowest median percent actionability with NAVIFY (143).

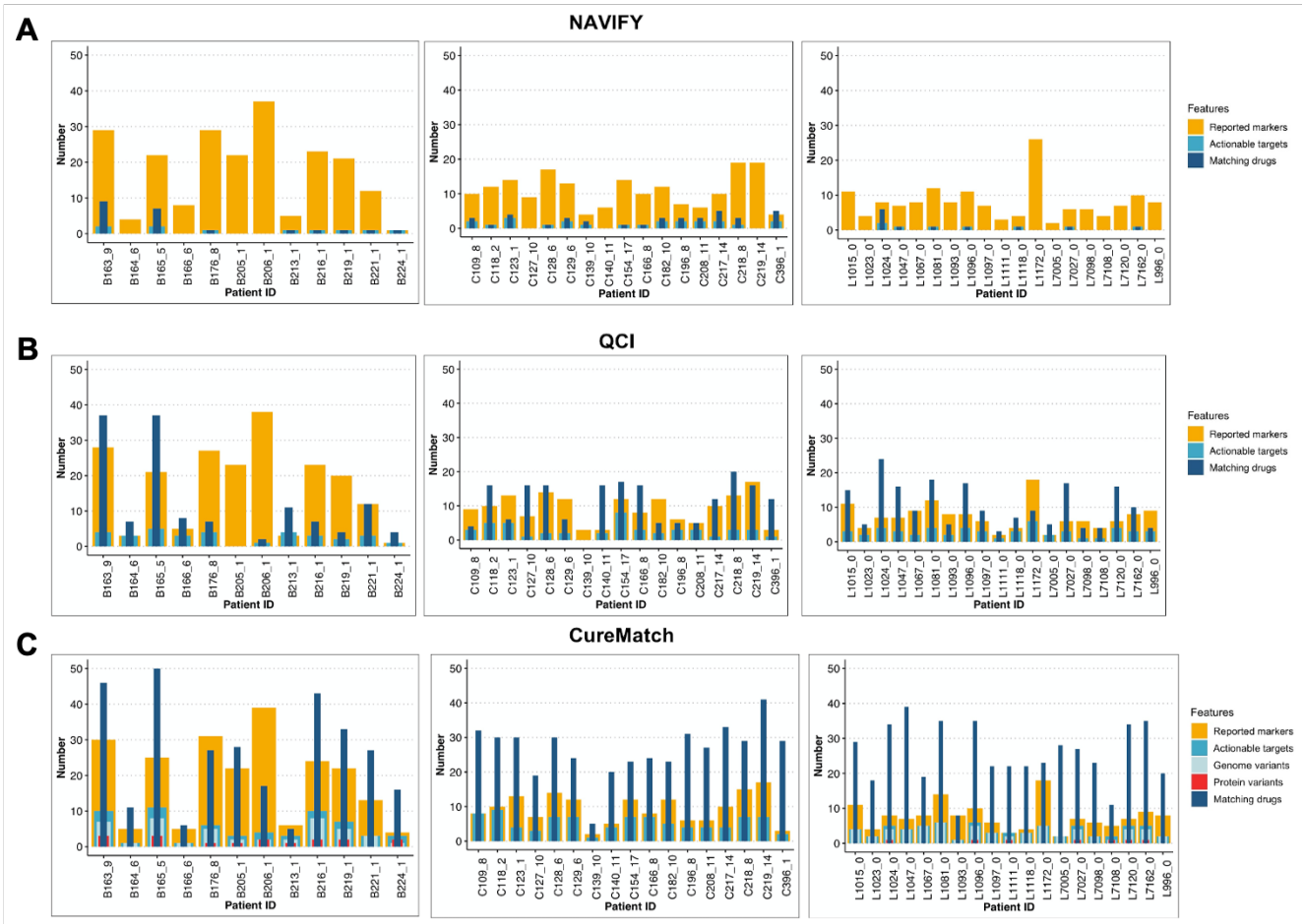

Figure 11. Reported markers, actionability and number of treatment suggestions [Figure and legend originally published in ESMO Open (Perakis et al., 2020)]

(A-C) Overlapping bar charts from NAVIFY (A; top), QCI (B; center), and CureMatch (C; bottom) per patient and per tumor entity (left, BC; center, CRC; right, NSCLC). Reported markers (orange) represent all alterations reported to the clinical decision support tool whereas actionable targets (turquoise) are those alterations which were successfully matched to a therapy. Matching drugs (dark blue) correspond to the total number of drugs identified for each patient's molecular profile. In the CureMatch analysis, the actionable targets in the BC and NSCLC samples could be categorized as either a genome (light blue) or protein variant (red).

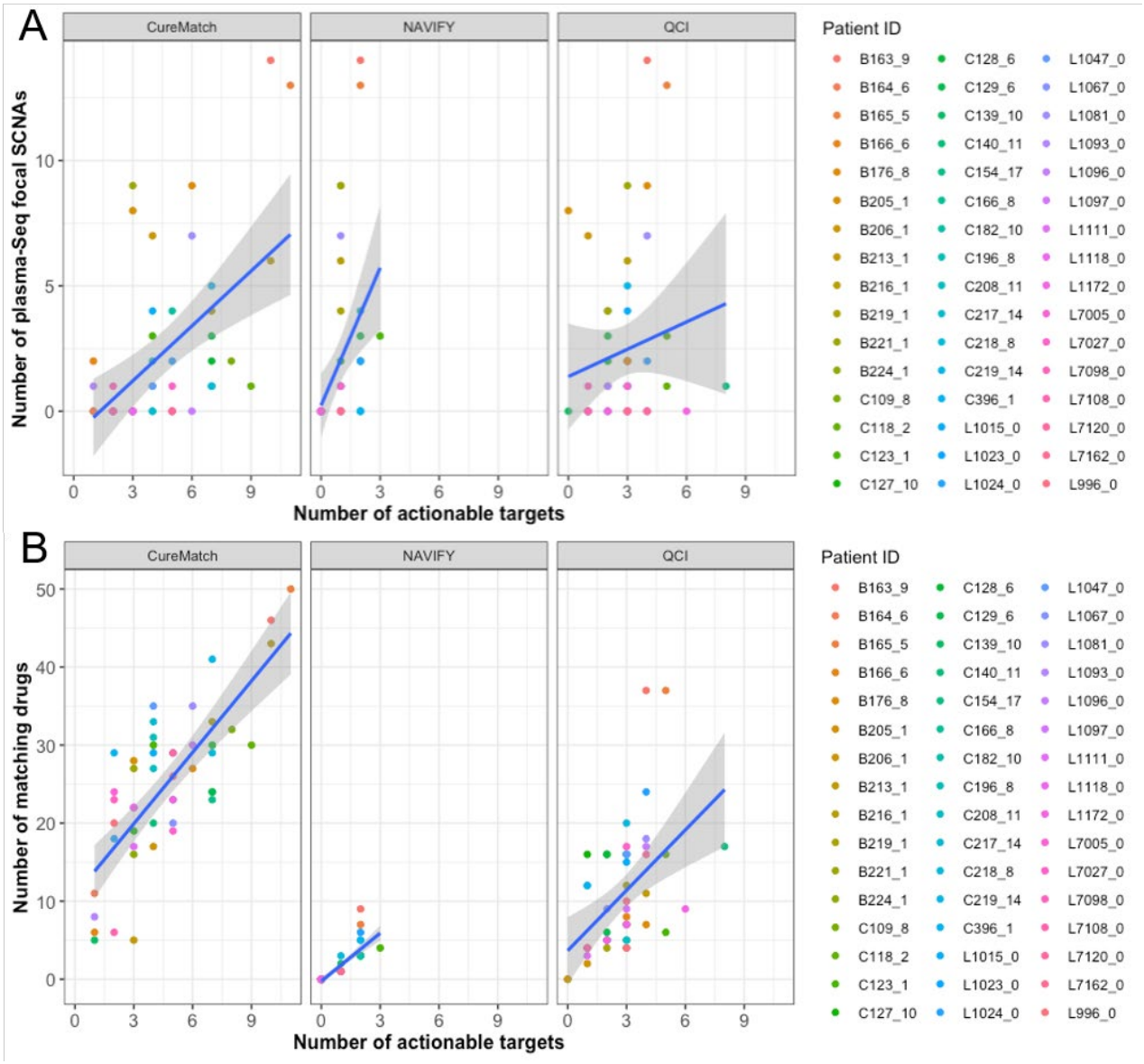

Figure 12. Actionability across focal SCNAs [Figure and legend adapted from ESMO Open (Perakis et al., 2020)]

Correlation of the total number of actionable targets identified by NAVIFY, QCI and CureMatch **(A)** with the number of focal events identified by plasma-Seq (NAVIFY: Pearson's correlation coefficient R= 0.167, P = 0.57; QCI: Pearson's correlation coefficient R= 0.160, P = 0.43; CureMatch: Pearson's correlation coefficient R= 0.524, P <0.001) and with total number of matching drugs identified per patient **(B)** (NAVIFY: Pearson's correlation coefficient R= 0.743, P = 0.02; QCI: Pearson's correlation coefficient R= 0.493, P = 0.003; CureMatch: Pearson's correlation coefficient R= 0.766, P <0.001). For the NAVIFY and QCI analyses, there were several patients for whom either no actionable target or focal event could be identified and are therefore not plotted.

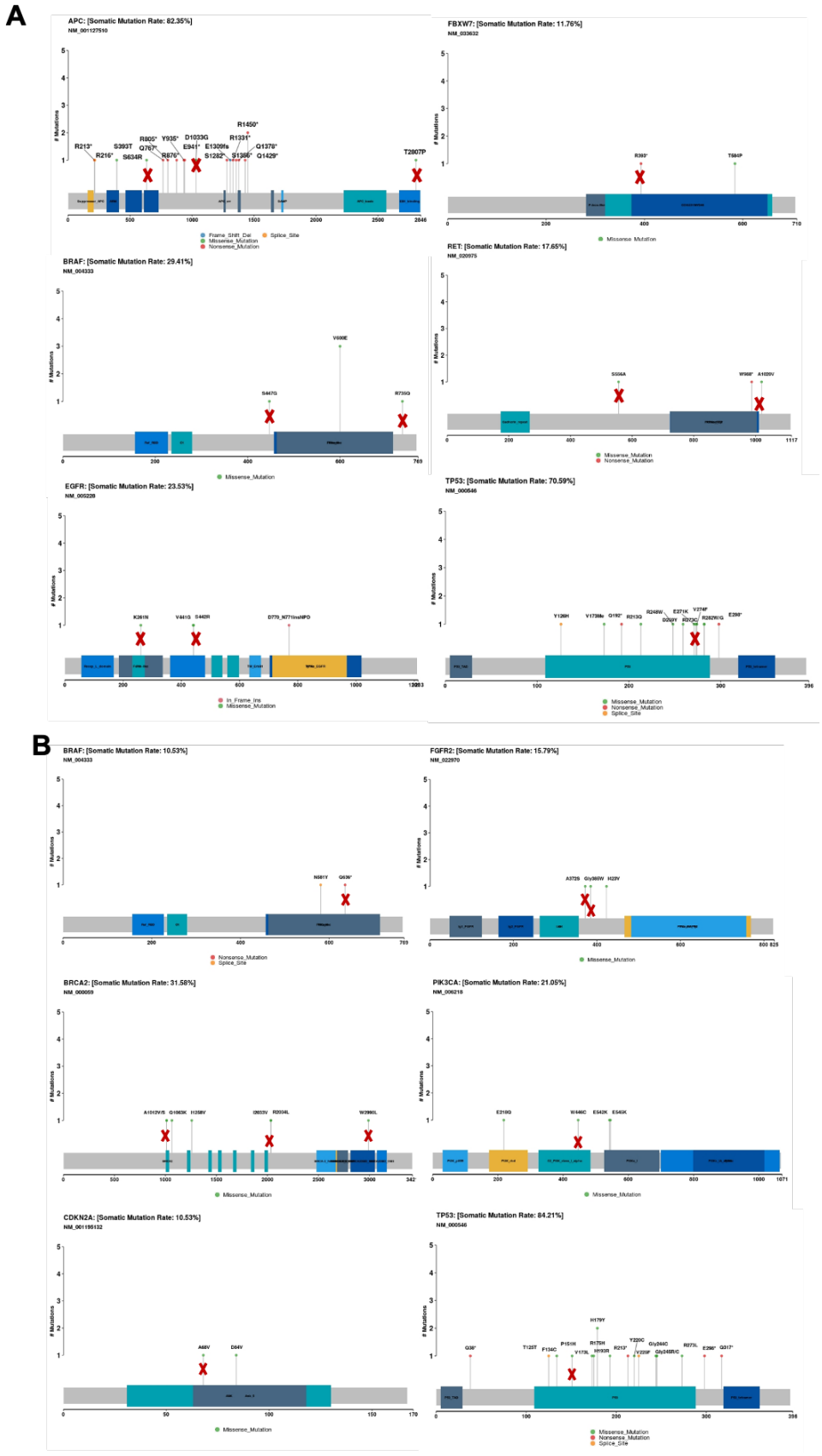
A
APC: [Somatic Mutation Rate: 82.35%]
NM_001127510
FBXW7: [Somatic Mutation Rate: 11.76%]
NM_033632
BRAF: [Somatic Mutation Rate: 29.41%]
NM_004333
RET: [Somatic Mutation Rate: 17.65%]
NM_020975
EGFR: [Somatic Mutation Rate: 23.53%]
NM_005228
TP53: [Somatic Mutation Rate: 70.59%]
NM_000546
B
BRAF: [Somatic Mutation Rate: 10.53%]
NM_004333
FGFR2: [Somatic Mutation Rate: 15.79%]
NM_022970
BRCA2: [Somatic Mutation Rate: 31.58%]
NM_000059
PIK3CA: [Somatic Mutation Rate: 21.05%]
NM_006218
CDKN2A: [Somatic Mutation Rate: 10.53%]
NM_001195132
TP53: [Somatic Mutation Rate: 84.21%]
NM_000546

Figure 13. Actionability across gene domains according to CureMatch Bionov analyses [Figure and legend adapted from ESMO Open (Perakis et al., 2020)]

(A) Lollipop plots showing variable actionability across gene domains for *APC*, *BRAF*, *EGFR*, *FBXW7*, *RET* and *TP53* in CRC.

(B) Variable actionability across gene domains for *BRAF*, *BRCA2*, *CDKN2A*, *FGFR2*, *PIK3CA* and *TP53* in NSCLC. Red crosses indicate that this particular variant was not actionable as determined by pathogenicity and CureMatch Bionov analysis.

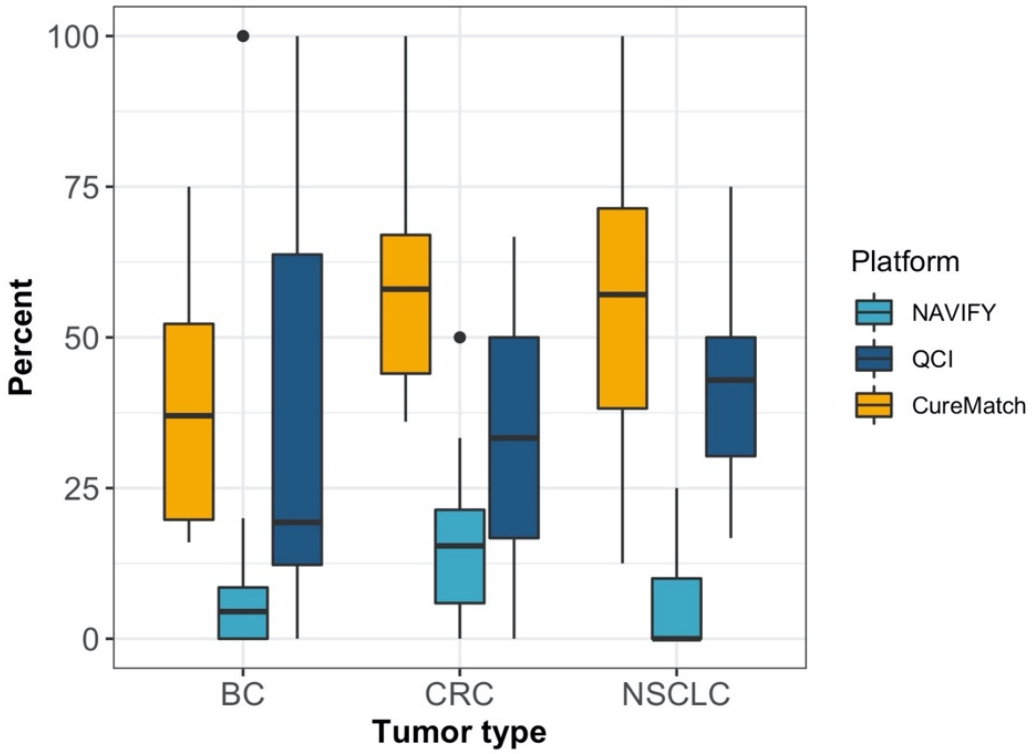

Figure 14. Comparison of average actionability across all platforms [Figure and legend originally published in ESMO Open (Perakis et al., 2020)]. Boxplot showing distribution of average actionability per tumor type per platform.

4.7 Treatment recommendations were identified for the majority of patients but these options varied across platforms

With the NAVIFY algorithm, 29/48 (60%) patients were matched to an existing targeted therapy and for 19 cases (40%), no treatment could be aligned to the patient molecular data (Supplementary Table 9). The majority of these matches had an on compendia designation, meaning that the indication was approved for the aberration in the given tumor type context (Figure 15A). QCI was

able to recommend treatments for 45/48 patients (94%), although these were—as stated above—not necessarily always targeted agents (Supplementary Table 10). When all chemotherapeutic agents recommended by QCI (not including those recommended as a combination therapy with a targeted agent) were removed from the analysis on a per-patient basis, the number of drug recommendations per patient profile was reduced on average by 3.5, 4.6 and 2.8 drugs for BC, CRC and NSCLC patients, respectively (143). CureMatch analyses were able to identify at least 1 biomarker-guided therapy, although in 10 cases, a 3-drug combination could not be identified and 2 cases only had monotherapy suggestions (Supplementary Table 11, Figure 17). The highest number of off-label and on compendia suggestions with this platform were made for BC and NSCLC patients, respectively (Figure 15A).

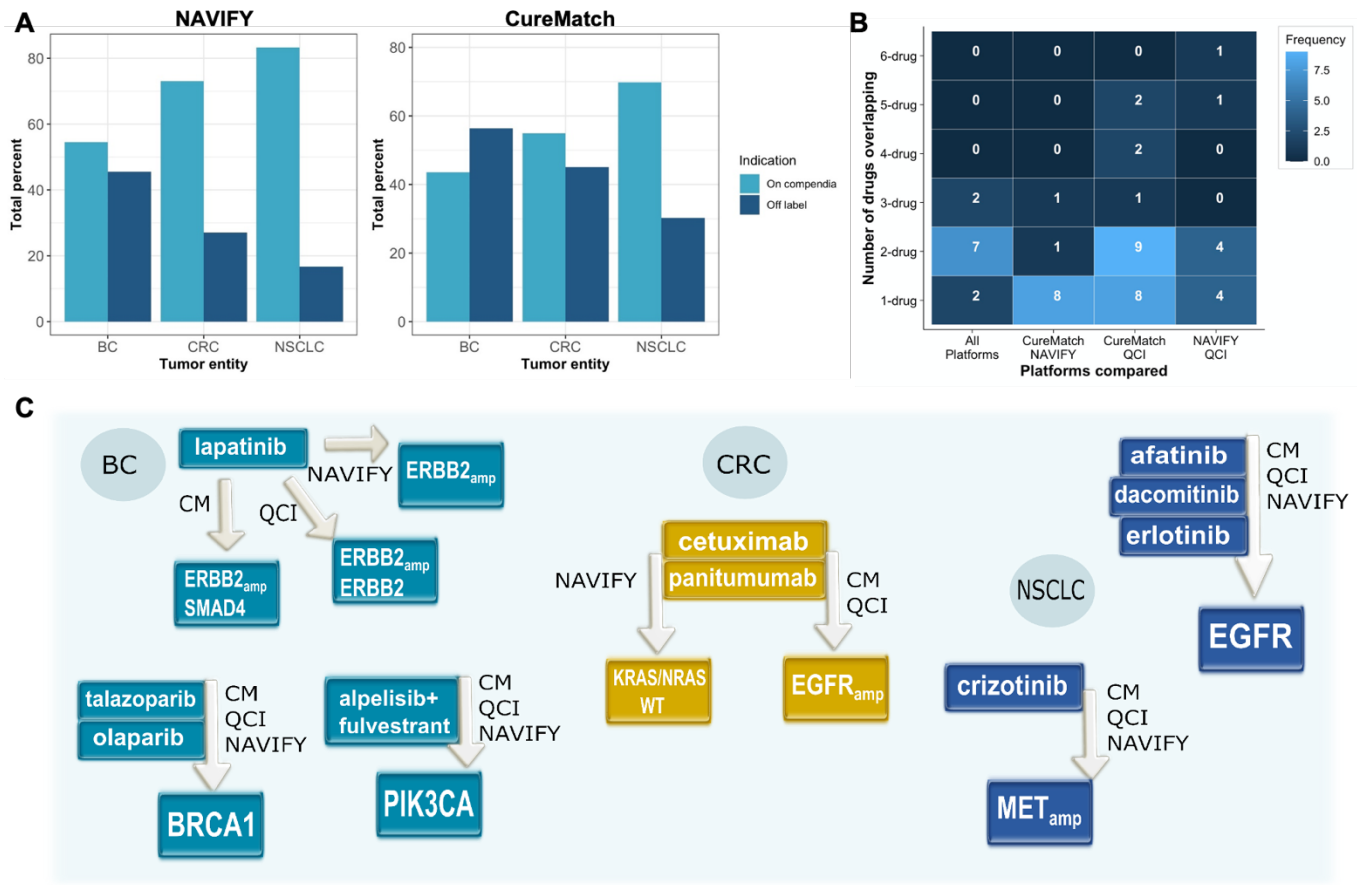

Figure 15. Treatment suggestions per decision support platform [Figure and legend originally published in ESMO Open (Perakis et al., 2020)]

(A) Frequency of on compendia or off-label indications per tumor type for NAVIFY (left) and CureMatch (right).

(B) Frequency of overlapping suggested therapies per platform comparison and per number of drugs. The platform recommendations were compared side-by-side with each other and all three together (x-axis). The number of drugs overlapping from the recommendations is listed on the y-axis. The numbers inside the boxes represent the number of events fitting each category.
(C) Treatment alignment among all 3 platforms was limited to common predictive biomarkers. Here, the most frequently overlapping drugs are shown along with how each decision support platform justified the drug, which sometimes varied (CM, CureMatch; NAVIFY, NAVIFY Mutation Profiler; QCI, QIAGEN Clinical Insight Interpret).

We then assessed to what degree the treatment recommendations align across all 3 platforms by observing the frequency of overlap. We observed, rather surprisingly, very minimal alignment, with CureMatch and QCI aligning most frequently with a two-drug overlap (Figure 15B). There were only 7 instances in which all three platforms agreed on at least 2 drugs for the particular patient. These cases, however, were not surprising, as they pertained to well-known predictive biomarkers and drugs, such as 6 CRC patients with cetuximab/panitumumab recommendations and 1 BC patient harboring a *PIK3CA* alteration matching to a fulvestrant and alpelisib combination (Supplementary Table 12) (143). There were other consistent minor overlaps among all three clinical decision support tools: two cases of a 1-drug alignment with crizotinib or with fulvestrant; a 3-drug alignment of lapatinib, olaparib and talazoparib for *ERBB2* or *BRCA1* alterations; and the recommendation of afatinib, dacomitinib or erlotinib for a single *EGFR* alteration (Figure 15C). Another unexpected finding was that for several cases in which therapy suggestions aligned across platforms, the targetable aberration justifying the given match varied (Supplementary Table 14). In fact, treatment alignment among all three platforms was limited to a few common predictive biomarkers (Figure 15C).

4.8 Identification of suitable recruiting clinical trials via clinical decision support

In the NAVIFY analyses, location-specific clinical trials had a high degree of matching, with 8/12 (67%), 16/17 (94%) and 8/19 (42%) patients qualifying for a biomarker-based clinical trial in Austria in BC, CRC and NSCLC patients, respectively. Some of these BC patients were even eligible for more than 7 trials (Figure 16). In the QCI analyses, roughly half of BC (6/12; 50%) and NSCLC (9/19; 47%) and 15/17 (88%) of CRC patients matched to existing trials in the user-defined country of Austria (Figure 16) (143). The CureMatch algorithm differs in terms of how it identifies

clinical trials. Rather than matching patients into currently recruiting or ongoing clinical trials, this software provides the clinical trial information as evidence for its recommended treatment combinations. In this regard, more CRC cases matched to existing trials compared to BC and NSCLC, with 9/17 patients (53%) of the cohort at least being attributed to 1 match (Figure 16) (143).

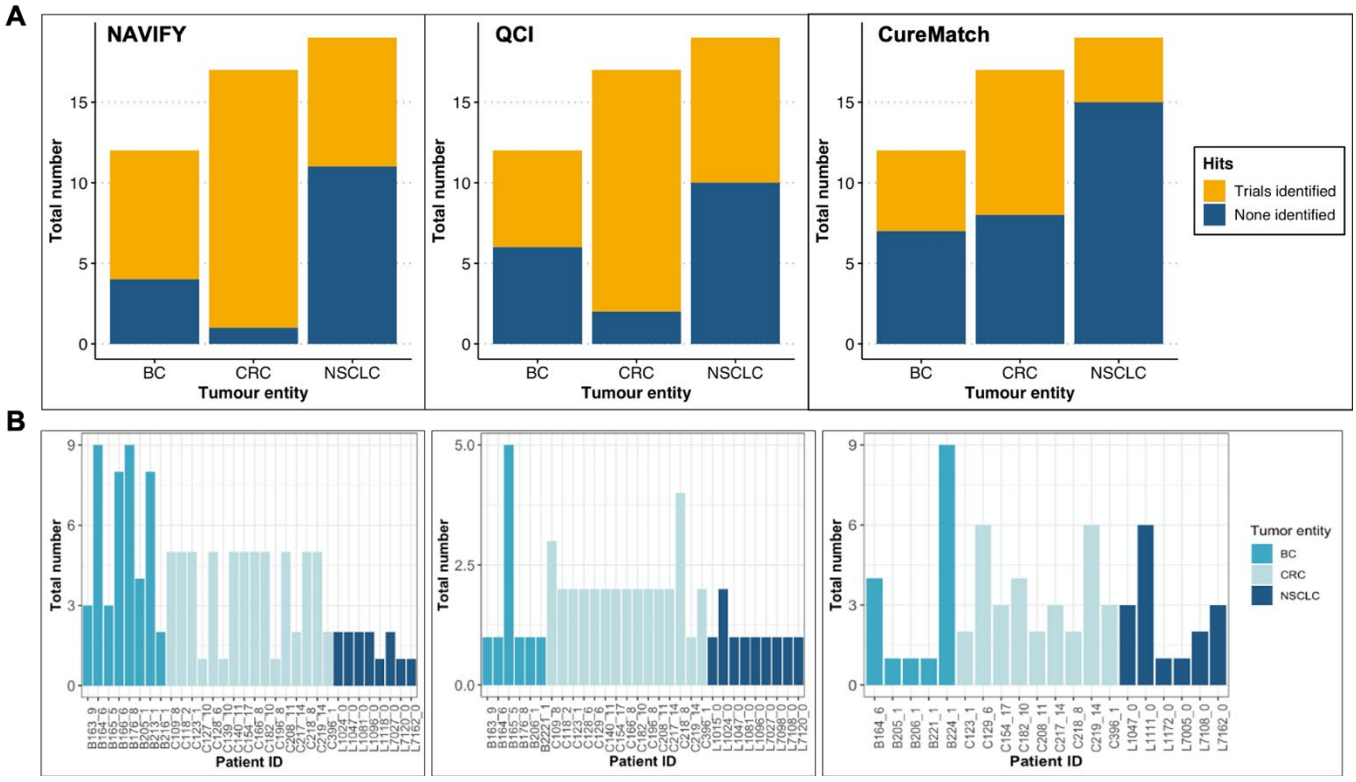

Figure 16. Clinical trials identified per decision support platform [Figure and legend adapted from ESMO Open (Perakis et al., 2020)]

(A) Total number of matching clinical trials per tumor entity for NAVIFY (left), QCI (center), and CureMatch (right).

(B) Bar plots illustrating the total number of clinical trials identified per individual patient matching to suggested therapies (NAVIFY, left; QCI, middle; CureMatch, right).

4.10 CureMatch matching scores

We examined the unique feature of CureMatch, the matching score, further by averaging the top 3 highest and bottom 3 lowest matching scores for each of the regimens. We observed the maximum median "highest" and "lowest" matching score in NSCLC patients (Figure 17). These scores were

summarized across each category to illustrate the distribution of "best fits" per patient and several cases demonstrated similar matching scores regardless of treatment strategy (i.e. combination or monotherapy) (Figure 17) (143).

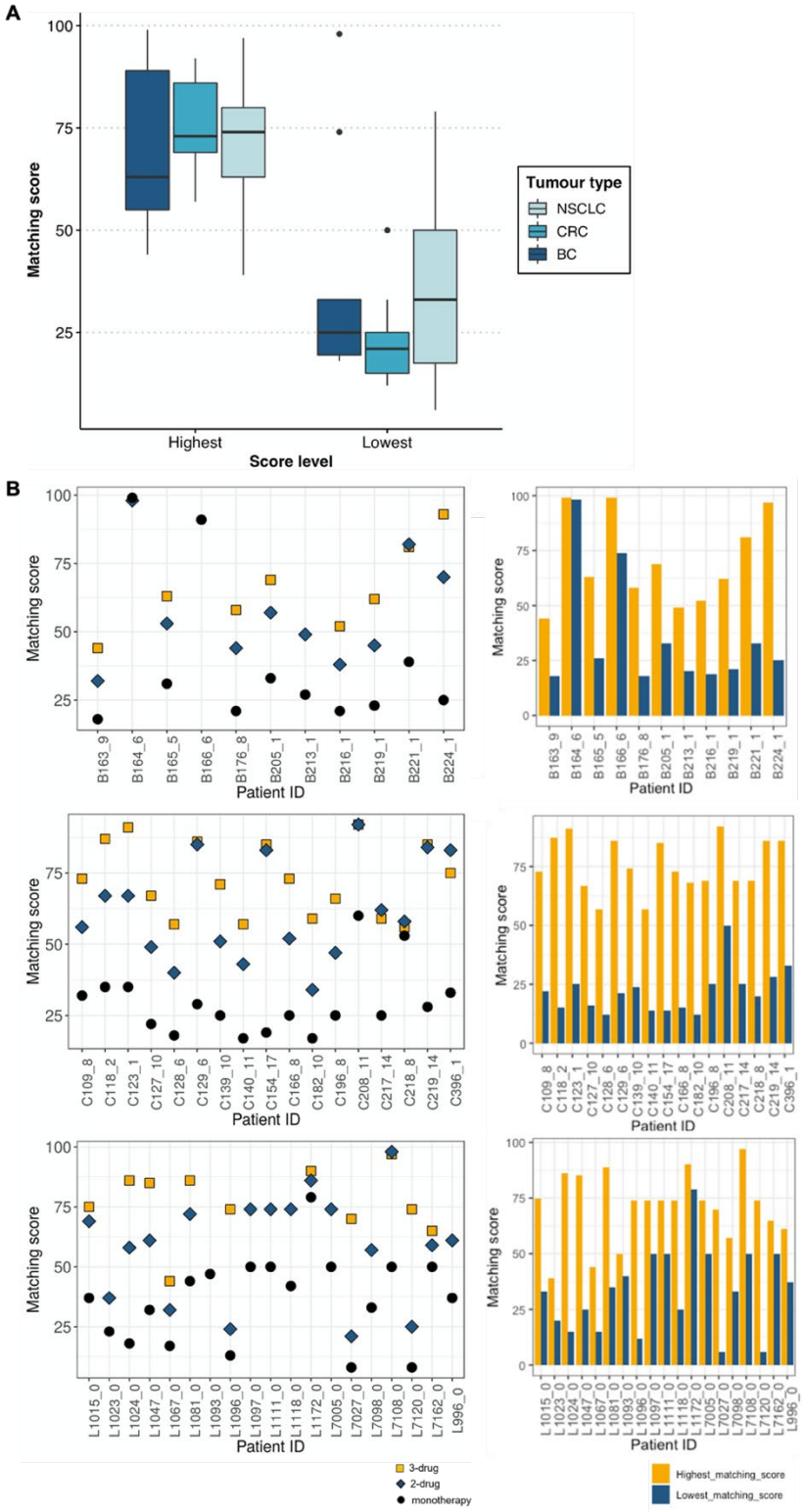

Figure 17. Distribution of matching scores from CureMatch analysis across tumor types and patients [Figure and legend adapted from ESMO Open (Perakis et al., 2020)]
(A) Boxplot showing distribution of the highest and lowest calculated matching scores per patient across tumor types from CureMatch analysis.
(B) Matching scores for the top 3 drug combinations or monotherapies were averaged for each patient. Dot plots (left panel) show the distribution of the averaged matching scores per patient for each 3-drug combination (yellow square), 2-drug combination (dark blue diamond) or monotherapy (black circle) for each cohort (BC, top; CRC, middle; NSCLC, bottom). Bar charts (right panel) demonstrating the highest and lowest calculated matching scores per patient (BC, top; CRC, middle; NSCLC, bottom) according to CureMatch Bionov analysis.

5 DISCUSSION

This study employed comprehensive genomic profiling of cfDNA from patients with common malignancies. cfDNA was selected as an analyte and preferred tool for obtaining the most current snapshot of tumor genome properties at any time during a disease course (80,82,85). From the steps involved in the precision oncology workflow (Figure 7), we focused on a decisive task, i.e. how to use information obtained from high-throughput sequencing to translate aberrations into suitable treatment recommendations for the patient. In this regard, we tested the performance of three commercial clinical decision support tools and observed that each platform has a different approach and philosophy as it relates to variant annotation, identifying actionable targets and matching treatments. Each of these platforms has different strategies and underlying philosophies, which are by themselves all well-justified. Here, analysis of plasma DNA yielded a clear study result, namely that there was high variability regarding annotations for pathogenicity and actionability, results which were similar to comparisons of other oncology knowledge bases (129,154). Our study provides several important novel aspects. First, we included a comparison of the decision support tools from an end user perspective, such as what type of data was required for submission, how it needed to be formatted, how much hands-on time did the end user require and how long on average an analysis ran from data submission to report generation. Furthermore, this study describes to the best of our knowledge for the first time detailed comparisons of actionability and comprehensive listings of all treatment recommendations as well as how they are concordant and discordant and which aberration was involved.

Our results demonstrate the complexity of treatment matching algorithms, which has critical implications for MTBs, meaning that depending on which platform is used in the future, the same somatic alterations will receive different tier classifications, subsequently resulting in different assessments regarding their actionability and furthermore in different treatment alignments. Thus, these findings are pertinent to both oncologist and patient. Our results emphasize that the final treatment decision remains to be made at the discretion of the treating oncologist and, although decision support may accelerate interpretation of rare or complex genomic aberrations, the element of human interpretation cannot be replaced. Importantly, it has also been acknowledged that even national and international consensus in regard to treatment recommendations resulting from MTBs is lacking, and some have begun to critically evaluate the effectiveness of the complex MTB decision-making methodology as well as adherence to the recommendation (155).

Discrepancies in classification guidelines, content sources and defining druggable targets

The discrepancies between NAVIFY and QCI analyses may be attributed to the inherent variation in the AMP or mixed ACMG/AMP guidelines for determining actionability, further affected by the varying content sources used by each software to query therapies, ultimately leading to discrepant treatment matching. For example, QCI considers the following federal agencies and oncology practice guidelines: FDA (Food and Drug Administration), EMA (European Medicines Agency), PMDA (Pharmaceuticals and Medical Devices Agency), ASCO (American Society of Clinical Oncology), NCCN (National Comprehensive Cancer Network), ESMO (European Society for Medical Oncology), CPIC (Clinical Pharmacogenetics Implementation Consortium), CAP (College of American Pathologists), WHO (World Health Organization) and ELN (European LeukemiaNet). NAVIFY similarly queries approved therapies across multiple regulatory agencies, such as FDA, EMA, Swissmedic, NICE (National Institute for Health and Care Excellence), Health Canada, NCCN, ESMO, and eviQ (eviQ Cancer Treatments Online), thus partially differing from the QCI content (143).

Further discrepancies may be related to differences in regional-specific content as well. Our analysis workflow obtained content specifically for the European Union (EU) clinical region, whereas those who may perform identical analyses for the U.S. clinical region may obtain different results. It is also worth noting that curated content is constantly updated as new evidence is accumulated. This indicates that the discrepancies found at the time of this study may no longer be

relevant if new content is available to a given platform such that the classification or treatment suggestion newly aligns for a particular variant. One example to represent this possibility is the approval of encorafenib in combination with cetuximab for the treatment of adult patients with *BRAF* V600E-mutant metastatic CRC. The FDA granted approval for this indication in April 2020, whereas the EMA released its approval for the same identical indication a few months later in June 2020 (143).

Perhaps the most crucial aspect leading to discrepant comparisons are the two distinguishing features of the QCI and CureMatch approach, i.e. that QCI matches chemotherapy agents and that CureMatch identifies downstream druggable targets relative to the aberration in question. These differing algorithms were reflected by the fact that CureMatch and QCI analyses frequently classified *TP53*, *KRAS* and *APC* alterations, typically seen as non-actionable in standard practice, as druggable targets. However, it should be noted that QCI analyses matched various chemotherapy, anti-EGFR (epidermal growth factor receptor) and anti-VEGF (vascular endothelial growth factor) agents to the abovementioned alterations. These treatments thus do not represent targeted therapies, which most likely does not fit into the current paradigm of what is understood to constitute precision oncology by many active in the field. Conversely, the CureMatch strategy does not suggest experimental agents to directly act on these "undruggable targets" (156,157). Rather, it represents a pathway-based method of targeting downstream events of the untargetable pathogenic alteration, an approach which some have investigated previously (158-160). This strategy remains to be investigated further in prospective clinical trials in conjunction with combination therapy regimens.

Lack of alignment in treatment recommendations

The abovementioned distinctions of each clinical decision support tool perhaps render the very minimal alignment found when comparing platform outputs unsurprising. However, the findings illustrate the complexity behind matching druggable targets to existing therapies. This is further accentuated by the fact that pharmaceutical policies may vary from country to country and thus treatment choices may be influenced by geographical location. However, the perhaps rather unexpected outcome of this study was that even for well-established druggable targets, such as *ERBB2* amplifications or *PIK3CA* mutations, the platforms differed in their therapy suggestions. This finding speaks to the need to harmonize clinical and molecular information such that various

users worldwide who query these knowledge bases can access the same interpretations for their data.

Recent and ongoing harmonization efforts to enable uniform clinical interpretation

The high variability of the three clinical decision support tools investigated herein was obvious at each level of assessment, i.e. pathogenicity, actionability, and treatment recommendations. Our observations are further supported by similar evidence described in recently published comparisons of other platforms, which included annotation services such as N-of-One (NoO) and IBM Watson for Genomics (WfG) (154), as well as other knowledgebases (129) such as the Cancer Genome Interpreter Cancer Biomarkers Database (CGI) (161), Clinical Interpretation of Variants in Cancer (CIViC) (162), Jackson Laboratory Clinical Knowledgebase (JAX-CKB) (163), MolecularMatch (MMatch), OncoKB (164) and the Precision Medicine Knowledgebase (PMKB) (165). Our detailed analysis of treatment recommendations and alignment across platforms demonstrates the need for further development and testing of decision support algorithms (16). In this regard, the study by Wagner et al. compared six somatic cancer variant knowledgebases in which they harmonized the variant interpretations which users can access from a freely accessible web interface (search.cancervariants.org) (129). Other tools, such as Variant Interpretation for Cancer (VIC) and the NIH-funded Clinical Genome Resource (ClinGen) effort Minimal Variant Level Data (MVLD) framework (134,166) have also contributed to minimizing bias in the interpretation workflow. Very importantly, these platforms also acknowledge that results must also be further interpreted by human reviewers (143).

At present, our knowledge about how to optimally distinguish druggable targets from hypothetical gene-drug matches is incomplete. Clinical decision support tools, oncology knowledgebases and MTBs across clinics currently lack consensus in the step-wise process of matching NGS readouts to appropriate treatments. This bottleneck in the promising precision oncology pipeline may be overcome by such cooperative and global efforts in harmonization, ideally with drug recommendations aligning to specific geographical locations (143).

Limitations of the study

As our study was retrospective in nature, there are of course several key limitations. It is worth noting that we selected ctDNA samples with rather high ctDNA AFs, and thus a high number of

alterations, in order to facilitate a comparison with each clinical decision support software. It would be interesting to test the informative value of these tools in cases with very low ctDNA AFs, which is frequently an issue in cancer patients (80). Furthermore, our study was not designed to address the optimal time point for performing comprehensive genomic profiling in patients with advanced cancer. Although it is common for clinical studies to select patients with very advanced disease, the extensive tumor heterogeneity both spatially and temporally may be very decisive in terms of response to treatment. Late-stage patients may experience rapid clinical deterioration, which complicates treatment success based on molecular profiling. Thus, it can be hypothesized that personalized cancer treatment might be more effective in earlier lines of treatment.

Another limitation of our analyses is that we disregarded multiple factors that may hinder the implementation of actionable results. For example, clinicians frequently experience difficulties in gaining proper access to drugs, which may especially be accompanied by long approval processes for off-label indications (167). This may particularly be the case for histology-agnostic indications, which are becoming increasingly represented in precision oncology clinical trials (168). Perhaps the most crucial limitation is that our approach solely focused on the current clinical status of the patient. In a true clinical setting, oncologists must consider prior lines of therapy, various comorbidities and general state of health when assigning treatments to their patients. However, this was outside the scope of our study but would be highly interesting to evaluate how clinical decision support may accelerate decisions based on this information.

We also did not evaluate the digitalized MTB component previously available by CureMatch and recently provided by Roche. One attractive aspect of the CureMatch Bionov solution is the offering of an online MTB with every analysis. Oncologists responsible for fine-tuning the patient report and molecular data specialists provide walkthroughs of their analyses, how treatments were justified and even discuss dosing strategies with the client. This is perhaps an appealing option for clinicians seeking second opinions and alternative options for their patients. Subsequent to our study, Roche released an additional product in the NAVIFY portfolio, the NAVIFY Tumor Board. With this feature, outputs from the NAVIFY Mutation Profiler can be imported into the Tumor Board alongside other pertinent clinical details of the patient, e.g. age, comorbidities, previous therapies, etc., to help clinicians visualize and coordinate patient data at MTB discussions. As the NAVIFY Tumor Board was not yet available to us at the time when we conducted our analyses, we were unable to subsequently evaluate how this would influence the matching of treatments to

the molecular profiles we generated. However, a prospective study is now being planned to address this question at our Division of Oncology.

The future of precision oncology: a patient-centric vision

Through in-depth analyses using clinical decision support tools, we observed the complexity of converting NGS data into valid treatment options for patients with common tumor entities. This approach represents only one although major aspect of precision cancer medicine. Precision oncology hypothesizes that anticancer therapy should be personalized to patients in accordance with the molecular profile of their tumors, clinical status, prior lines of treatment and comorbidities. As such, the patient is, in fact, at the center of precision oncology approaches, yet frequently current workflows overlook direct incorporation of the patient into critical decision-making processes. Thus, precision oncology should not just represent genomic medicine and the implementation thereof, but in parallel should also encompass improving the decision-making capacity for each patient. Alongside the urgent questions posed by oncologists, hospitals, providers and payers, the patient may also require additional consultation and clarification to understand the decisions being made as it relates to his or her personalized approach (Figure 18).

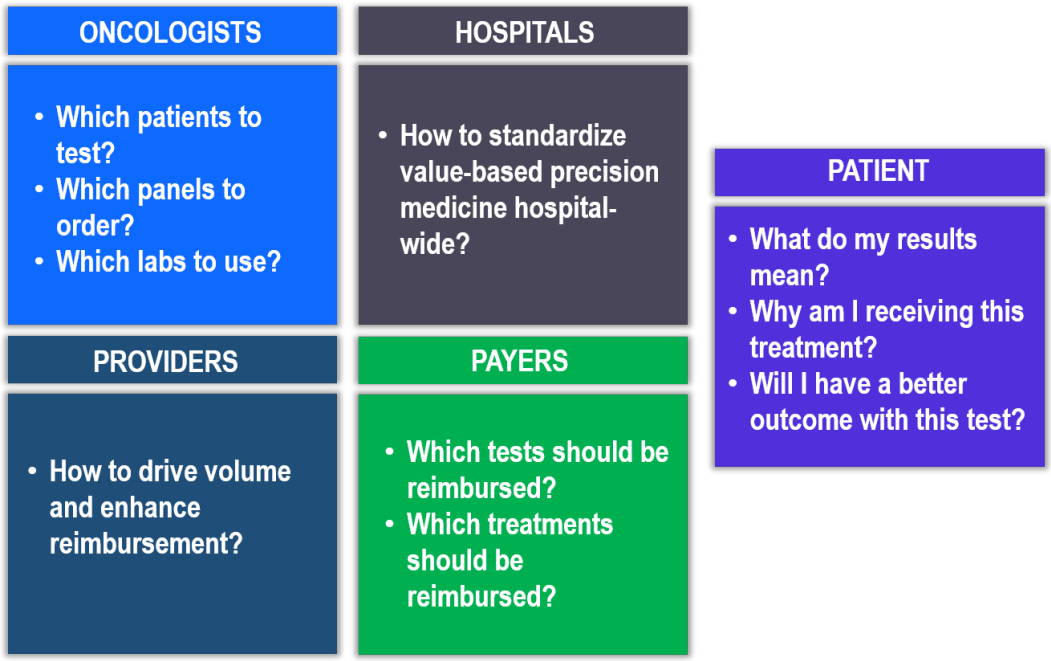

Figure 18. The missing "5th player" affected by decision making in the field of precision oncology [adapted from Trapelo Health (https://www.trapelohealth.com/)]. In addition to oncologists who may question which tests to order, to hospitals focused on standardization of care,

to providers who require reimbursement and to payers who determine which reimbursements are justified, the patient may also have unanswered questions. It would be worthwhile to establish workflows geared towards patient education and personalized consultation such that the patient may better understand the interpretation of his or her molecular testing results which guide treatment decisions. The advantages and caveats to new precision oncology approaches should be communicated clearly to the patient so that he or she may understand how a particular test may potentially influence clinical outcome.

Our in-depth molecular analyses have indeed enabled translational ways of processing the genomic information we generate into both immediate and potential clinical implications, as reflected by the frequent requests from our collaborating clinicians for these analyses. However, through our translational genomics reporting experience, we have observed a rather large delta between our molecular reporting, the treating oncologists who receive the interpreted information and, most importantly, the patient. More specifically, from our experience, we know that clinicians often have difficulties working with complex NGS datasets, even when provided with detailed interpretations from our side, thus necessitating the need for extensive discussions at MTBs. As this complex molecular data ultimately informs treatment decisions, such knowledge is pertinent to the patient who will undergo the selected treatment, although he or she most likely does not have an understanding as to how the selection affecting his or her life and health was even made. In this regard, our research team is interested in novel ways to improve the patient journey. For example, would active participation offer more sense of control? Would patients, family and friends be interested in understanding more about potential downstream intervention opportunities after a cancer diagnosis? Is more knowledge power through being actively involved or is it better to be uninformed, i.e. the patient just wants the best care but does not want to think about the disease constantly when outside of the clinic? Is there a way to simplify and personalize all of the overwhelming amount of oncology-related information out there? In this regard, patient activation, which refers to the willingness, skills and ability to actively manage one's own health care, has already been shown to drive positive health outcomes as well as reduce readmissions and costs of care (133,169,170). Thus, direct and in-depth patient consultation throughout the cancer patient journey may relieve the person affected from unnecessary uncertainties and critical questions may be answered by experts in the field (Figure 19A). In a follow-up study to our clinical decision support analyses, we would like to address this next specific bottleneck in the precision oncology

cascade, i.e. enabling joint decision-making between clinician and patient, through patient activation and engagement.

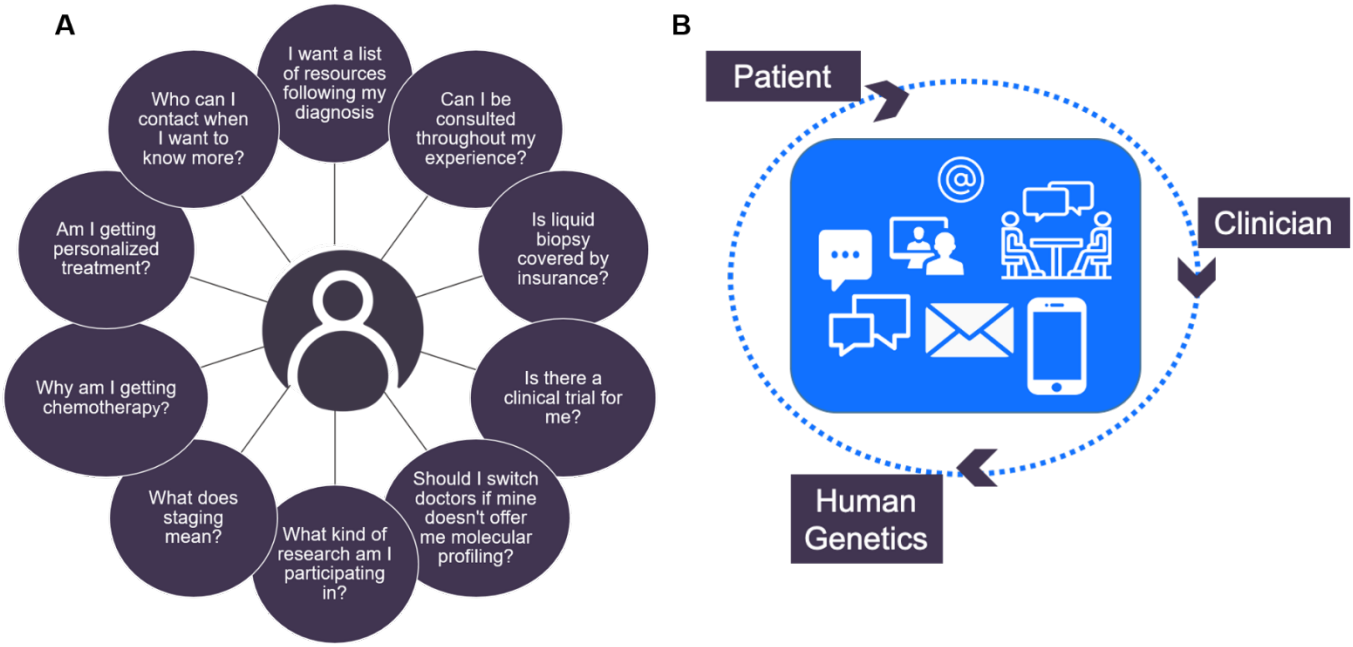

Figure 19. Decreasing patient uncertainty in precision oncology and increasing the use of digitalized communication channels.

(A) At various milestones throughout the patient journey, from diagnosis, to receiving treatment, to participating in research studies, various critical questions may arise that would leave the patient with uncertainty if left unanswered.

(B) One way to circumvent this uncertainty would be to develop a platform that enables digitized patient engagement to supplement in-person genetic consultations. Patients could suggest and evaluate potential interaction channels and should play a central role in the design of a digitalized communication platform that links the patient to all clinicians involved in their cancer care.

We plan to recruit patients with advanced cancer to proactively design the architecture for a digital platform that allows eased and ongoing communication and access between patient, researcher and clinician (Figure 19B). A primary goal is to evaluate novel ways of getting patients and their families involved in extremely complex medical issues, providing them with more sense of control of their patient journey and ultimately impacting the promises of precision medicine. Ideally, if this approach leads to an improvement of precision cancer medicine from the perspective of the patient, our current molecular profiling workflow can be expanded to include report generation for both

clinician *and* patient, the latter who may then undergo genetic consultation of the molecular results to better grasp the reasoning behind treatment regimens selected by the oncology team (Figure 20).

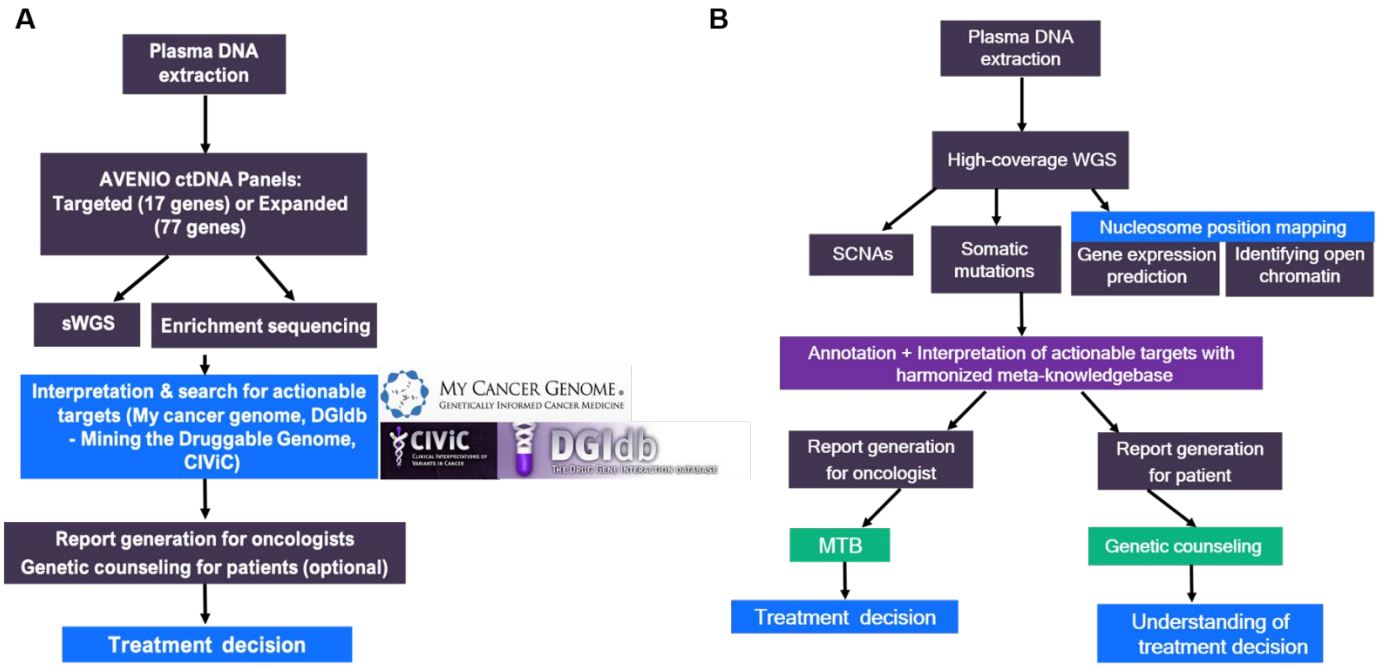

Figure 20. Status quo and potential future of clinical NGS report generation

(A) At present, following plasma DNA extraction, we employ Roche's AVENIO ctDNA panels for sWGS and enrichment sequencing. Subsequently, SCNAs and somatic mutations are called and then interpreted with publicly available databases such as My Cancer Genome and CIVic. These results are summarized in a clinical report and forwarded to the oncologists such that a treatment decision based on molecular profiling can be made. Currently, genetic counseling is not a typical step in this workflow.

(B) In the near future, due to the decreasing costs of WGS, we envision replacing our restricted panel analyses with high-coverage WGS such that we can call SCNAs, somatic mutations and observe nucleosome positioning to predict gene expression and identify regions of open chromatin. Ideally, these data will be interpreted by a harmonized meta-knowledgebase. Subsequently, two types of reports will be generated based on the molecular findings: one interpretation for the treating oncologist, and the other for the patient his or herself. In cases of complex molecular profiles, the clinical report may be discussed at an MTB to derive a treatment decision. It would be encouraged that the patient report be discussed in the setting of a genetic counseling session with the patient (and potentially his or her family) in order to enable a deeper understanding of the

molecular reporting and how it relates to the treatment decision. This type of patient engagement may raise awareness for opportunities in precision oncology care.

The long-term goals of the project are to build stable relationships between clinicians and patients by improving the patient journey and patient experience (PX), in turn strengthening our relationships with the clinicians as well. We envision that our resulting workflow would serve both sides of cancer profiling and monitoring, i.e. the clinician and the patient, helping spread the word about non-invasive methods such as liquid biopsy that have the potential to make a difference and hence will create demand for these services which can be filled on the supply side through established products. We believe this could drive the understanding and adoption of comprehensive molecular profiling, which currently serves as the foundation for precision oncology but is not offered as a routine clinical service at our clinic in Graz.

6 CONCLUSIONS

Precision cancer medicine is still very much in its infancy. Although the application of genomic and molecular analyses to tumor biopsies has indeed begun to improve both diagnosis and treatment of cancers, broad adoption of genomics-driven precision oncology has been held back in the clinic, mostly due to logistical, reimbursement and regulatory issues. These considerations affect all parties involved in the field of precision cancer medicine, i.e. payers, providers, oncologists, hospitals and patients. One current focus of improving the status quo of precision oncology is that treating oncologists may overlook potentially actionable alterations when given complex NGS readouts lacking clear clinical implications and routine molecular tumor board discussions are not always feasible for every patient. This has triggered the development of various clinical decision support tools to help guide clinicians in understanding the results of their genomic testing and the integrated computational algorithms in these tools may help prioritize individual genomic variants to further assist in treatment selection.

In this work, we evaluated three commercial clinical decision support platforms to assess the alignment of clinical reporting when provided with identical molecular profiles from adult patients with metastatic cancer from three common tumor entities. Our approach was able to demonstrate the emerging strategy of sequencing plasma cfDNA to noninvasively detect genomic alterations and tumor heterogeneity from solid tumors in real-time. Comprehensive genomic profiling of

cfDNA from patients with advanced disease may also mitigate procedural costs and delays related to the obtaining and processing of tumor tissue, as time is not a commodity for patients with advanced disease. However, the optimal time point at which a clinician should order genomic sequencing and which assay or analyte, i.e. tumor biopsy or liquid biopsy, best suits his or her clinical question are still subject to ongoing debate.

The clear result of our comparative study of decision support platforms was the uncovering of rather surprising discrepancies when observing how each software designated tier classifications to the variants, how each took a varying approach to defining actionability and how each software matched treatment recommendations. We describe that one of the most important steps within precision oncology, i.e. how to select the most appropriate treatment recommendation based on predictive somatic biomarkers, lacks any standardization and consensus. Patients who have tumors with the same molecular profile would be treated differently depending on which commercial decision support platform is applied. This represents a severe, but yet unrecognized limitation of precision oncology, and may be a contributing factor to the sobering outcomes of many previous reports. However, recent efforts have been initiated to harmonize the genomic and molecular annotations and interpretations across oncology knowledgebases, which is in itself a daunting but imperative task. Still, the concept of clinical decision support and efforts to improve current algorithms should be viewed positively as a step forward to streamlining molecular interpretations and perhaps even identifying alternative treatment solutions for patients who have exhausted all standard lines of care.

It is envisioned that the selection of cancer therapies will be increasingly dictated by molecular analyses to inform individualized treatment plans for patients. Other relevant clinical information such as treatment history, comorbidities and overall status are anticipated to help close the gaps in the understanding of cancer progression. As the field of precision oncology begins to mature, efforts should not forget the importance of integrating sufficient patient communication into their workflows such that patients and their families are better equipped to understand the treatment decision-making process and may have opportunities to contribute to advancing precision cancer care.

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SUPPLEMENTARY TABLES

Supplementary Table 1. List of genes, targeted regions and type of alteration profiled via the AVENIO ctDNA Expanded Kit (Roche) [Table originally published in ESMO Open (Perakis et al., 2020)]

Gene	Seq Target	SNV	Indel*	Fusion**	CNV**
ABL1	Selected Regions	●			
AKT1	Selected Regions	●			
AKT2	Selected Regions	●			
ALK	Selected Regions	●	●	●	
APC	Selected Regions	●	●		
AR	All Coding Regions	●			
ARAF	Selected Regions	●			
BRAF	Selected Regions	●	●		
BRCA1	All Coding Regions	●			
BRCA2	All Coding Regions	●			
CCND1	All Coding Regions	●			
CCND2	All Coding Regions	●			
CCND3	All Coding Regions	●			
CD274	All Coding Regions	●			
CDK4	All Coding Regions	●			
CDK6	Selected Regions	●			
CDKN2A	All Coding Regions	●			
CSF1R	Selected Regions	●			
CTNNB1	Selected Regions	●	●		
DDR2	Selected Regions	●			
DPYD	Selected Regions	●			
EGFR	All Coding Regions	●	●		●
ERBB2	All Coding Regions	●	●		●
ESR1	All Coding Regions	●			

EZH2	Selected Regions	●			
FBXW7	All Coding Regions	●			
FGFR1	Selected Regions	●			
FGFR2	Selected Regions	●		●	
FGFR3	Selected Regions	●		●	
FLT1	Selected Regions	●			
FLT3	Selected Regions	●			
FLT4	Selected Regions	●			
GATA3	Selected Regions	●			
GNA11	Selected Regions	●			
GNAQ	Selected Regions	●			
GNAS	Selected Regions	●			
IDH1	Selected Regions	●			
IDH2	Selected Regions	●			
JAK2	Selected Regions	●			
JAK3	Selected Regions	●			
KDR	Selected Regions	●			
KEAP1	All Coding Regions	●			
KIT	Selected Regions	●	●		
KRAS	All Coding Regions	●			
MAP2K1	Selected Regions	●			
MAP2K2	Selected Regions	●			
MET	All Coding Regions	●	●		●
MLH1	All Coding Regions	●			
MSH2	All Coding Regions	●			
MSH6	All Coding Regions	●			
MTOR	Selected Regions	●			
NF2	All Coding Regions	●			
NFEL2	Selected Regions	●			
NRAS	Selected Regions	●			
NTRK1	Selected Regions	●		●	
PDC1LG2	All Coding Regions	●			
PDGFRA	Selected Regions	●			
PDGFRB	Selected Regions	●			
PIK3CA	Selected Regions	●	●		
PIK3R1	Selected Regions	●			
PMS2	All Coding Regions	●			
PTCH1	Selected Regions	●			
PTEN	All Coding Regions	●	●		
RAF1	Selected Regions	●			
RB1	All Coding Regions	●			
RET	Selected Regions	●		●	

RNF43	Selected Regions	●			
ROS1	Selected Regions	●		●	
SMAD4	All Coding Regions	●			
SMO	All Coding Regions	●			
STK11	All Coding Regions	●			
TP53	All Coding Regions	●			
TERT promoter	Selected Regions	●			
TSC1	Selected Regions	●	●		
TSC2	Selected Regions	●			
UGT1A1***	Selected Regions	●			
VHL	All Coding Regions	●			

All coding regions are based on the longest transcript from Ensembl build 82.
*Indels are limited to variants in a pre-specified list of positions, referred to as "Loci of Interest", except for EGFR exon 19 long deletions, EGFR exon 20 long insertions and MET long insertions, which are not restricted to a pre-defined set of indels.
**Detection of fusions and CNVs are limited to variants in a pre-specified list of positions, referred to as "Loci of Interest" in the AVENIO analysis software.
***UGT1A1*28 allele sequenced but not currently called by the AVENIO analysis software.

Supplementary Table 2. AMP classifications used by NAVIFY Mutation Profiler [Table originally published in ESMO Open (Perakis et al., 2020)]

AMP Tier	Variant significance	Classification rules
Tier IA	Strongest clinical significance	Biomarkers that predict response or resistance AND are approved by drug agency (FDA/EMA/HCSC/Swissmedic) or recommended by medical guidelines based on the region specified and supported by a high level of evidence. Biomarker that has prognostic or diagnostic clinical significance based on medical guidelines for the specified geographic region.
Tier IB	Strong clinical evidence	Biomarkers that predict response or resistance to therapies for a specific type of tumor based on well-powered studies (clinical trials) with consensus from experts in the field. Biomarkers that predict response or resistance AND are recommended by medical guidelines based on the region specified and supported by moderate levels of evidence. Biomarkers that achieved high-clinical prognostic and diagnostic significance based on well-powered studies.
Tier IIC	Potential clinical significance	Biomarkers that predict response or resistance to therapies approved by the FDA or professional societies for a different type of tumor. Biomarkers that serve as inclusion criteria for clinical trials. Multiple small-published studies with some emerging consensus.

Tier IID	Possible clinical significance	Biomarkers that show plausible therapeutic significance based on at least one case report. Biomarkers that show plausible therapeutic diagnostic or prognostic significance based on at least one preclinical study.
Tier III	Unknown or uncertain significance	Not observed at a significant allele frequency across the general population or subpopulations or not well-represented in cancer databases. No published clinical evidence of cancer association.
Tier IV	Benign or likely benign	Observed at significant allele frequencies in the general population or specific subpopulations AND no published evidence of cancer association.

Supplementary Table 3. ACMG/AMP classifications used by QCI Interpret [Table originally published in ESMO Open (Perakis et al., 2020)]

Tier	Subclassification	Level of evidence	Description
IA		Strong clinical significance	FDA-approved therapy Included in professional guidelines
IB		Strong clinical significance	Well-powered studies with consensus from experts in the field
IIC	Likely Pathogenic Variant of Unknown Significance (VUS)	Potential clinical significance	FDA-approved therapies for different tumor types or investigational therapies Multiple small published studies with some consensus
IID	Pathogenic Likely Pathogenic	Potential clinical significance	Preclinical trials or a few case reports without consensus
III	Pathogenic Variant of Unknown Significance (VUS)	Unknown clinical significance	Not observed at a significant allele frequency in the general or specific population databases, or pan-cancer or tumor-specific variant databases No convincing published evidence of cancer association

IV		Benign or likely benign	Observed at a significant allele frequency in the general or specific subpopulation databases No existing published evidence of cancer association

Supplementary Table 4. General patient clinical characteristics. [Table originally published in ESMO Open (Perakis et al., 2020)]

Patient ID	Age	Gender	Primary tumor	Hormone receptor status	PD-L1 expression	Primary tumor mutations	Mutations detected in plasma	Therapy line	Metastatic sites
B166_6	60	Female	BC	ER+/PR+/HER2-	NA	NA	NA	2	liver, bone
B164_6	52	Female	BC	ER+/PR+/HER2-	NA	NA	NA	4	lymph nodes, pleural effusion, liver, bone
B206_1	79	Female	BC	ER+/PR+/HER2-	NA	NA	NA	1	indication of bone metastasis (skull)
B213_1	58	Female	BC	ER-/PR+/HER2-	NA	NA	NA	3	bone, liver,
B219_1	69	Female	BC	ER+/PR+/HER2-	NA	NA	NA	1	bone
B163_9	50	Female	BC	ER+/PR+/HER2+	NA	NA	NA	12	bone
B165_5	48	Female	BC	ER+/PR+/HER2+	NA	NA	NA	3	lung, bone, liver
B176_8	53	Female	BC	ER+/PR+/HER2-	NA	NA	NA	3	lung, bone, liver
B216_1	63	Female	BC	ER+/PR+/HER2-	NA	NA	NA	1	liver
B221_1	46	Female	BC	ER-/PR-/HER2-	NA	NA	NA	2	bone, supraclav. lymph nodes
B224_1	52	Female	BC	ER+/PR+/HER2-	NA	NA	NA	neoadjuvant	regional lymph nodes
B205_1	66	Female	BC	ER+/PR+/HER2-	NA	NA	NA	1	thorac. lymph nodes, pleural carcinosis, bone
C127_10	79	Male	CRC	NA	NA	KRAS G12V,	KRAS G12V	4	liver, lung

Patient ID	Age	Gender	Primary tumor	Hormone receptor status	PD-L1 expression	Primary tumor mutations	Mutations detected in plasma	Therapy line	Metastatic sites
						NRAS WT, BRAF WT			
C109_8	77	Male	CRC	NA	NA	KRAS WT, NRAS WT	KRAS WT, NRAS WT	3	liver, lung
C154_17	71	Male	CRC	NA	NA	KRAS WT, NRAS WT, BRAF WT	NRAS Q61L, NRAS Q61K, NRAS G12D, KRAS G12V	4	liver, lung
C139_10	57	Male	CRC	NA	NA	KRAS WT, NRAS WT, BRAF WT	KRAS WT, NRAS WT, BRAF WT	3	lung, lymph node
C140_11	52	Female	CRC	NA	NA	KRAS A146T, NRAS WT, BRAF WT	KRAS A146T	3	liver, peritoneum
C196_8	70	Male	CRC	NA	NA	KRAS, NRAS, BRAF WT	KRAS, NRAS, BRAF WT	3	liver
C217_14	68	Female	CRC	NA	NA	KRAS WT, BRAF V600E	BRAF V600E	3	lymph nodes, omentum
C128_6	67	Male	CRC	NA	NA	KRAS G12A, NRAS WT, BRAF WT	KRAS G12A	3	liver
C118_2	47	Female	CRC	NA	NA	KRAS G12C	KRAS G12C	4	liver, lung, peritoneum
C166_8	48	Male	CRC	NA	NA	KRAS G12V, NRAS WT, BRAF WT	KRAS G12V	2	liver, lung, bone
C208_11	64	Male	CRC	NA	NA	KRAS WT, NRAS WT, BRAF WT	KRAS WT, NRAS WT, BRAF WT	3	liver, lung, lymph nodes, peritoneum

Patient ID	Age	Gender	Primary tumor	Hormone receptor status	PD-L1 expression	Primary tumor mutations	Mutations detected in plasma	Therapy line	Metastatic sites
C123_1	73	Male	CRC	NA	NA	KRAS WT, NRAS WT	KRAS WT, NRAS WT	4	liver, lung, lymph node, bone
C129_6	66	Male	CRC	NA	NA	KRAS WT, NRAS WT, BRAF WT	KRAS WT, NRAS WT, BRAF WT	3	liver, lung, lymph node
C218_8	63	Male	CRC	NA	NA	KRAS WT	KRAS Q61H, BRAF V600E	6	liver (multiple)
C182_10	45	Female	CRC	NA	NA	KRAS WT, NRAS WT, BRAF WT	KRAS WT, NRAS WT, BRAF WT	4	liver, lung, lymph node
C219_14	61	Male	CRC	NA	NA	KRAS WT, BRAF WT	KRAS Q61H	7	liver (multiple)
C396_1	50	Female	CRC	NA	NA	NA	NA	3	liver, lymph node
L996_0	75	Male	NSCLC	NA	NA	NA	NA	3	
L1015_0	71	Male	NSCLC	NA	0%	NA	NA	3	
L1023_0	68	Male	NSCLC	NA	NA	NA	NA	2	
L1024_0	59	Male	NSCLC	NA	80%	EGFR L858R	EGFR L858R	3	
L1047_0	66	Female	NSCLC	NA	NA	NA	NA	2	
L1067_0	63	Female	NSCLC	NA	NA	KRAS G12C	KRAS G12C	2	
L1093_0	60	Male	NSCLC	NA	0%	EGFR WT, BRAF WT, KRAS WT	EGFR WT, BRAF WT, KRAS WT	2	
L1096_0	53	Female	NSCLC	NA	80%	KRAS G12D	KRAS G12D	2	
L1097_0	55	Female	NSCLC	NA	0%	KRAS G12V	KRAS G21V	2	
L1111_0	59	Male	NSCLC	NA	40%	EGFR WT, BRAF WT, KRAS WT	EGFR WT, BRAF WT, KRAS WT	2	
L1118_0	74	Male	NSCLC	NA	NA	NA	NA	2	
L7005_0	68	Male	NSCLC	NA	NA	NA	NA	2	
L7098_0	78	Male	NSCLC	NA	0%	EGFR WT, BRAF WT, KRAS WT	EGFR WT, BRAF WT, KRAS WT	2	

Patient ID	Age	Gender	Primary tumor	Hormone receptor status	PD-L1 expression	Primary tumor mutations	Mutations detected in plasma	Therapy line	Metastatic sites
L7120_0	54	Male	NSCLC	NA	80%	KRAS G12C	KRAS G12C	1	
L7027_0	61	Female	NSCLC	NA	80%	KRAS Q61L	KRAS Q61L	1	
L7108_0	74	Male	NSCLC	NA	60%	EGFR WT, BRAF WT, KRAS WT	EGFR WT, BRAF WT, KRAS WT	2	
L7162_0	52	Female	NSCLC	NA	60%	KRAS G12V	KRAS G12V	0	
L1172_0	60	Male	NSCLC	NA	0%	EGFR WT, BRAF WT, KRAS WT	KRAS G12V	2	
L1081_0	58	Female	NSCLC	NA	0%	EGFR WT, BRAF WT, KRAS WT	EGFR WT, BRAF WT, KRAS WT	2	

Supplementary Table 5. Frequency of concordance and discordance between designated tier classifications from NAVIFY and QCI [Table originally published in ESMO Open (Perakis et al., 2020)]

Concordant events			
Tier		**Frequency**	**Note**
I-A		14	KRAS G12 or Q61 mutations, NRAS G12 or Q61 mutations, 2 ERBB2 amplifications, 1 EGFR L858R mutation
I-B		8	All EGFR amplifications except for 1 ERBB2 amplification
II-C		60	Diverse genes affected
II-D		2	1 APC splice site mutation, 1 MAP2K1 mutation
III		260	Diverse genes affected
Discordant events			
NAVIFY tier	**QCI tier**	**Frequency**	**Note**
I-B	I-A	15	All are BRAF V600E, KRAS G12, ERBB2 amplification, MET amplification
I-B	II-C	12	All EGFR amplifications in CRC samples
I-B	III	1	EGFR V441G
II-C	I-A	28	KRAS amplification, MET amplification, TP53 mutations, BRCA1 mutation, PIK3CA mutations, KRAS mutation
II-C	I-B	3	ESR1 D538G
II-C	II-D	17	15 of these were mutations in APC in CRC samples, 2 in ESR1
II-C	III	14	Diverse focal amplifications and mutations
II-D	I-A	4	2 TP53 splice site mutations, 1 TP53 point mutation, 1 BRCA2 stop gained
II-D	II-C	12	Majority splice site mutations
II-D	III	21	Diverse genes affected
III	II-C	18	Diverse genes affected
III	II-D	2	ESR1 Y537S
III	IV	1	BRCA1 S1101N mutation

Supplementary Table 6. Frequency of concordance and discordance between actionability status from NAVIFY and QCI [Table originally published in ESMO Open (Perakis et al., 2020)]

Concordant events				
Actionable	**Tier NAVIFY**	**Tier QCI**	**Frequency**	**Aberration type**
No	I-B	III	1	EGFR V441G
No	II-C	I-A	9	1 KRAS amplification, 8 TP53 mutations
No	II-C	II-C	22	Diverse SCNAs and mutations
No	II-C	II-D	15	All APC mutations

No	II-C	III	11	Diverse amplifications and mutations
No	II-D	I-A	2	TP53 mutations
No	II-D	II-C	3	Diverse mutations
No	II-D	II-D	2	Diverse mutations
No	II-D	III	21	Diverse mutations, 1 KDM6A deletion
No	III	II-C	9	3 NFE2L2 mutations, 2 CCND1 amplifications, 1 STK11 mutation, 1 PTEN mutation, 1 EMSY amplification, 1 BIRC2 amplification
No	III	III	259	Diverse SCNAs and mutations
No	III	IV	1	BRCA1 S1101N
Yes	I-A	I-A	3	2 ERBB2 amplifications, 1 EGFR L858R mutation
Yes	I-B	I-A	8	3 BRAF V600E mutations, 5 MET amplifications
Yes	II-C	I-A	5	1 BRCA1 mutation, 4 PIK3CA mutations
Yes	II-C	II-C	5	1 RABGAP1L-ROS1 fusion, 4 PIK3CA mutations

Discordant events

Actionable NAVIFY	**Actionable QCI**	**Tier NAVIFY**	**Tier QCI**	**Frequency**	**Aberration type**
No	Yes	I-A	I-A	11	All KRAS and NRAS mutations
No	Yes	I-B	I-A	7	6 KRAS mutations, 1 ERBB2 amplification
No	Yes	I-B	I-B	8	7 EGFR amplifications, 1 ERBB2 amplification
No	Yes	I-B	II-C	12	All EGFR amplifications
No	Yes	II-C	I-A	5	2 KRAS amplifications, 2 TP53 mutations, 1 KRAS mutation

No	Yes	II-C	I-B	3	All ESR1 D538G mutations
No	Yes	II-C	II-C	33	Diverse SCNAs and mutations, majority TP53 mutations
No	Yes	II-C	II-D	2	ESR1 Y537S/N mutation
No	Yes	II-C	III	3	Diverse pathogenic mutations
No	Yes	II-D	I-A	2	1 TP53 mutation, 1 BRCA2 mutation
No	Yes	II-D	II-C	9	Diverse mutations, majority TP53 mutations
No	Yes	III	II-C	9	Diverse amplifications and mutations
No	Yes	III	II-D	2	Both ESR1 Y537S mutations
Yes	No	II-C	I-A	9	8 MET amplifications and 1 PIK3CA E545K mutation
Yes	No	II-C	II-C	1	MET amplification

Supplementary Table 7. Frequency of concordance and discordance between actionability status from NAVIFY and CureMatch [Table originally published in ESMO Open (Perakis et al., 2020)]

Concordant events			
Actionable	**Tier NAVIFY**	**Frequency**	**Aberration type**
No	I-B	1	EGFR V441G
No	II-C	23	Diverse amplifications and mutations
No	II-D	5	2 MAP2K1 mutations, 1 KDM6A deletion, 1 BRAF mutation, 1 NFE2L2 mutation
No	III	266	Diverse SCNAs and mutations
Yes	I-A	3	2 ERBB2 amplifications, 1 EGFR L858R mutation

Yes		I-B	8	3 BRAF V600E mutations, 5 MET amplifications
Yes		II-C	20	Mostly MET amplifications and PIK3CA mutations, 1 RABGAP1L-ROS1 fusion
Discordant events				
Actionable NAVIFY	**Actionable CM**	**Tier NAVIFY**	**Frequency**	**Aberration type**
No	Yes	I-A	11	All KRAS mutations
No	Yes	I-B	27	Majority EGFR amplifications, 2 ERBB2 amplifications, rest KRAS mutations
No	Yes	II-C	78	Diverse SCNAs and mutations
No	Yes	II-D	34	Diverse splice site mutations and other mutations
No	Yes	III	16	Majority focal amplifications in FLT3 and CCND1

Supplementary Table 8. Frequency of concordance and discordance between actionability status from QCI and CureMatch [Table originally published in ESMO Open (Perakis et al., 2020)]

Concordant events			
Actionable	**Tier QCI**	**Frequency**	**Aberration type**
No	I-A	1	TP53 V274F
No	II-C	11	Diverse amplifications and mutations
No	II-D	1	AKT1 p.K57T
No	III	268	Diverse SCNAs and mutations
No	IV	1	BRCA1 S1101N
Yes	I-A	41	Majority mutations in KRAS, BRAF, PIK3CA, KRAS amplifications, MET amplifications, ERBB2 amplifications
Yes	I-B	8	All EGFR amplifications except for 1

			ERBB2 amplification
Yes	II-C	62	Diverse SCNAs and mutations
Yes	III	1	TP53 T125T
Discordant events			
Actionable QCI	**Actionable CM**	**Tier QCI**	**Frequency**	**Aberration type**
No	Yes	I-A	18	Majority MET amplifications and TP53 mutations
No	Yes	II-C	23	Diverse SCNAs and mutations
No	Yes	II-D	16	All mutations in APC
No	Yes	III	27	Diverse amplifications and mutations
Yes	No	I-B	3	ESR1 D538G
Yes	No	II-C	5	1 PIK3CA amplification and diverse mutations
Yes	No	II-D	4	ESR1 Y537S/N mutations
Yes	No	III	2	ESR1 mutation and CDKN2A mutation

Supplementary Table 9. All treatment suggestions per patient and per aberration from NAVIFY Mutation Profiler analysis [Table originally published in ESMO Open (Perakis et al., 2020)]

Patient_ID	NAVIFY_target	NAVIFY_suggestions	Resistance_associations
C127_10	KRAS c.35G>T p.G12V		cetuximab
			panitumumab
C109_8	KRAS,NRAS WT	cetuximab	
		panitumumab	
	MET amplification	*crizotinib*	
C154_17	MET amplification	*crizotinib*	
C139_10	KRAS,NRAS WT	cetuximab	
		panitumumab	
C140_11	No approved therapies	No approved therapies	No approved therapies
C196_8	KRAS,NRAS WT	cetuximab	
		panitumumab	
	PIK3CA c.3140A>G p.H1047R	*alpelisib+fulvestrant*	
C217_14	KRAS,NRAS WT	cetuximab	
		panitumumab	
	BRAF c.1799T>A p.V600E	bevacizumab+folfoxiri	cetuximab
		cetuximab+irinotecan+vemurafenib	panitumumab
		irinotecan+panitumumab+vemurafenib	

Patient_ID	NAVIFY_target	NAVIFY_suggestions	Resistance_associations
C128_6	MET amplification	*crizotinib*	
C118_2	MET amplification	*crizotinib*	
C166_8	MET amplification	*crizotinib*	
	KRAS c.35G>T p.G12V		cetuximab
			panitumumab
C208_11	KRAS,NRAS WT	cetuximab	
		panitumumab	
	MET amplification	*crizotinib*	
C123_1	KRAS,NRAS WT	cetuximab	
		panitumumab	
	MET amplification	*crizotinib*	
	PIK3CA c.1634A>G p.E545G	*alpelisib+fulvestrant*	
C129_6	KRAS,NRAS WT	cetuximab	
		panitumumab	
C182_10	KRAS,NRAS WT	cetuximab	
		panitumumab	
	MET amplification	*crizotinib*	
C218_8	BRAF c.1799T>A p.V600E	bevacizumab+folfoxiri	cetuximab
		cetuximab+irinotecan+vemurafenib	panitumumab
		irinotecan+panitumumab+vemurafenib	
	KRAS c.183A>T p.Q61H		cetuximab
			panitumumab
C219_14	KRAS c.183A>T p.Q61H		cetuximab
			panitumumab
C396_1	KRAS,NRAS WT	cetuximab	
		panitumumab	
	BRAF c.1799T>A p.V600E	bevacizumab+folfoxiri	cetuximab
		cetuximab+irinotecan+vemurafenib	panitumumab
		irinotecan+panitumumab+vemurafenib	
B163_9	ERBB2 amplification	ado-trastuzumab emtansine	
		hyaluronidase + trastuzumab	
		lapatinib	
		neratinib	
		pertuzumab + trastuzumab	
		trastuzumab	
	BRCA1 c.2263G>T p.E755*	*olaparib*	
		rucaparib	
		talazoparib	
	KRAS p.G12C		*cetuximab*
			panitumumab
B164_6	No approved therapies	No approved therapies	No approved therapies
B165_5	ERBB2 amplification	ado-trastuzumab emtansine	
		hyaluronidase + trastuzumab	
		lapatinib	

Patient_ID	NAVIFY_target	NAVIFY_suggestions	Resistance_associations
		neratinib	
		pertuzumab + trastuzumab	
		trastuzumab	
	PIK3CA c.1634A>C p.E545A	*alpelisib + fulvestrant*	
B166_6	No approved therapies	No approved therapies	No approved therapies
B176_8	MET amplification	*crizotinib*	
B205_1	No approved therapies	No approved therapies	No approved therapies
B206_1	No approved therapies	No approved therapies	No approved therapies
B213_1	PIK3CA c.1624G>A p.E542K	*alpelisib + fulvestrant*	
	KRAS c.35G>T p.G12V		cetuximab
			panitumumab
B216_1	PIK3CA c.3140A>G p.H1047R	*alpelisib + fulvestrant*	
B219_1	PIK3CA c.3140A>G p.H1047R	*alpelisib + fulvestrant*	
B221_1	RABGAP1L-ROS1 fusion	*crizotinib*	
B224_1	PIK3CA c.1624G>A p.E542K	*alpelisib + fulvestrant*	
L996_0	No approved therapies	No approved therapies	No approved therapies
L1015_0	No approved therapies	No approved therapies	No approved therapies
L1023_0	No approved therapies	No approved therapies	No approved therapies
L1024_0	EGFR c.2573T>G p.L858R	afatinib	
		dacomitinib	
		erlotinib	
		gefitinib	
		osimertinib	
	MET amplification	crizotinib	
L1047_0	MET amplification	crizotinib	
L1067_0	KRAS g.34G>T p.G12C		afatinib
			erlotinib
			gefitinib
L1093_0	No approved therapies	No approved therapies	No approved therapies
L1096_0	MET amplification	crizotinib	
	KRAS c.35G>A p.G12D		afatinib
			erlotinib
			gefitinib
L1097_0	KRAS c.35G>T p.G12V		afatinib
			erlotinib
			gefitinib
L1111_0	No approved therapies	No approved therapies	No approved therapies
L1118_0	PIK3CA c.1624G>A p.E542K	*alpelisib + fulvestrant*	
L7005_0	No approved therapies	No approved therapies	No approved therapies
L7027_0	MET amplification	crizotinib	
	KRAS c.182A>T p.Q61L		cetuximab
			panitumumab

Patient_ID	NAVIFY_target	NAVIFY_suggestions	Resistance_associations
L7098_0	No approved therapies	No approved therapies	No approved therapies
L7108_0	No approved therapies	No approved therapies	No approved therapies
L7120_0	KRAS c.34G>T p.G12C		afatinib
			erlotinib
			gefitinib
L1081_0	MET amplification	crizotinib	
L1172_0	KRAS c.35G>T p.G12V		afatinib
			erlotinib
			gefitinib
L7162_0	KRAS c.35G>T p.G12V		afatinib
			erlotinib
			gefitinib
	PIK3CA c.1633G>A p.E545K	*alpelisib + fulvestrant*	

Designated as "other indication"

Supplementary Table 10. All treatment suggestions per patient and per aberration from QCI Interpret analysis [Table originally published in ESMO Open (Perakis et al., 2020)]

Patient ID	QCI target	QCI suggestions	Resistance associations
C127_10	KRAS c.35G>T p.G12V	5-fluorouracil/irinotecan/leucovorin	cetuximab
		5-fluorouracil/leucovorin/oxaliplatin	panitumumab
		aflibercept	
		bevacizumab	
		bevacizumab/erlotinib	
		capecitabine	
		capecitabine/oxaliplatin	
		fluoropyrimidine	
		ipilimumab/nivolumab	
		irinotecan	
		nivolumab	
		ramucirumab	
		regorafenib	
		tipiracil	
C109_8	KRAS amplification		cetuximab
			panitumumab
	MET amplification		cetuximab
			panitumumab
	EGFR amplification	5-fluorouracil/irinotecan/leucovorin	
		cetuximab	
		panitumumab	
	FLT1 amplification	regorafenib	
	FLT3 amplification	regorafenib	
C154_17	KRAS c.35G>A p.G12D	5-fluorouracil/irinotecan/leucovorin	cetuximab
		5-fluorouracil/leucovorin/oxaliplatin	panitumumab
		aflibercept	
		bevacizumab	
		bevacizumab/erlotinib	

Patient ID	QCI target	QCI suggestions	Resistance associations
		capecitabine	
		capecitabine/oxaliplatin	
		fluoropyrimidine	
		ipilimumab/nivolumab	
		irinotecan	
		nivolumab	
		ramucirumab	
		regorafenib	
		tipiracil	
	KRAS c.35G>T p.G12V	5-fluorouracil/irinotecan/leucovorin	cetuximab
		5-fluorouracil/leucovorin/oxaliplatin	panitumumab
		aflibercept	
		bevacizumab	
		bevacizumab/erlotinib	
		capecitabine	
		capecitabine/oxaliplatin	
		fluoropyrimidine	
		ipilimumab/nivolumab	
		irinotecan	
		nivolumab	
		ramucirumab	
		regorafenib	
		tipiracil	
	MET amplification		cetuximab
			panitumumab
	NRAS c.182A>T p.Q61L	aflibercept	
		bevacizumab	cetuximab
		fluoropyrimidine	panitumumab
		regorafenib	
		tipiracil	
	NRAS c.181C>A p.Q61K	aflibercept	
		bevacizumab	cetuximab
		fluoropyrimidine	panitumumab
		regorafenib	
		tipiracil	
	NRAS c.35G>A p.G12D	aflibercept	
		bevacizumab	cetuximab
		fluoropyrimidine	panitumumab
		regorafenib	
		tipiracil	
	EGFR amplification	5-fluorouracil/irinotecan/leucovorin	
		5-fluorouracil/leucovorin/oxaliplatin	
		cetuximab	
		panitumumab	
	MSH2 loss	ipilimumab/nivolumab	
		nivolumab	
		pembrolizumab	
	MAP2K1 c.171G>C p.K57N		cetuximab

Patient ID	QCI target	QCI suggestions	Resistance associations
C139_10	No approved therapies	No approved therapies	No approved therapies
C140_11	KRAS c.436G>A p.A146	5-fluorouracil/irinotecan/leucovorin	cetuximab
		5-fluorouracil/leucovorin/oxaliplatin	panitumumab
		aflibercept	
		bevacizumab	
		bevacizumab/erlotinib	
		capecitabine	
		capecitabine/oxaliplatin	
		fluoropyrimidine	
		ipilimumab/nivolumab	
		irinotecan	
		nivolumab	
		ramucirumab	
		regorafenib	
		tipiracil	
	AKT1 c.49G>A p.E17K		
	EGFR amplification	5-fluorouracil/irinotecan/leucovorin	
		5-fluorouracil/leucovorin/oxaliplatin	
		cetuximab	
		panitumumab	
C196_8	TP53 c.375+2T>G	5-fluorouracil/leucovorin/oxaliplatin	
	EGFR c.2308_2316dupGACAACCCC p.D770_P772dup	cetuximab	
		panitumumab	
	PIK3CA c.3140A>G p.H1047R	5-fluorouracil/leucovorin/oxaliplatin	
		5-fluorouracil/leucovorin	
		cetuximab	
C217_14	BRAF c.1799T>A p.V600E	5-fluorouracil/irinotecan/leucovorin	cetuximab
		5-fluorouracil/leucovorin	panitumumab
		5-fluorouracil/leucovorin/oxaliplatin	vemurafenib
		bevacizumab	
		chemotherapy/5-fluorouracil	
		ipilimumab/nivolumab	
		irinotecan/panitumumab/vemurafenib	
		nivolumab	
		regorafenib	
C128_6	KRAS c.35G>C p.G12A	5-fluorouracil/irinotecan/leucovorin	cetuximab
		5-fluorouracil/leucovorin/oxaliplatin	panitumumab
		aflibercept	
		bevacizumab	
		bevacizumab/erlotinib	
		capecitabine	
		capecitabine/oxaliplatin	
		fluoropyrimidine	
		ipilimumab/nivolumab	
		irinotecan	

Patient ID	QCI target	QCI suggestions	Resistance associations
		nivolumab	
		ramucirumab	
		regorafenib	
		tipiracil	
	MET amplification		cetuximab
			panitumumab
	EGFR amplification	5-fluorouracil/irinotecan/leucovorin	
		cetuximab	
		panitumumab	
C118_2	KRAS c.34G>T p.G12C	5-fluorouracil/irinotecan/leucovorin	cetuximab
		5-fluorouracil/leucovorin/oxaliplatin	panitumumab
		aflibercept	
		bevacizumab	
		bevacizumab/erlotinib	
		capecitabine	
		capecitabine/oxaliplatin	
		fluoropyrimidine	
		ipilimumab/nivolumab	
		irinotecan	
		nivolumab	
		ramucirumab	
		regorafenib	
		tipiracil	
	MET amplification		cetuximab
			panitumumab
	EGFR amplification	5-fluorouracil/irinotecan/leucovorin	
		cetuximab	
		panitumumab	
	FLT1 amplification	regorafenib	
	FLT3 amplification	regorafenib	
	PDGFRB c.3287C>T p.A1096V	regorafenib	
C166_8	KRAS c.35G>T p.G12V	5-fluorouracil/irinotecan/leucovorin	cetuximab
		5-fluorouracil/leucovorin/oxaliplatin	panitumumab
		aflibercept	
		bevacizumab	
		bevacizumab/erlotinib	
		capecitabine	
		capecitabine/oxaliplatin	
		fluoropyrimidine	
		ipilimumab/nivolumab	
		irinotecan	
		nivolumab	
		ramucirumab	
		regorafenib	
		tipiracil	
	MET amplification		cetuximab
			panitumumab

Patient ID	QCI target	QCI suggestions	Resistance associations
	EGFR amplification	5-fluorouracil/irinotecan/leucovorin	
		5-fluorouracil/leucovorin/oxaliplatin	
		cetuximab	
		panitumumab	
	FLT3 amplification	regorafenib	
C208_11	MET amplification		cetuximab
			panitumumab
	TP53 c.811G>A p.E271K	5-fluorouracil/leucovorin/oxaliplatin	
	EGFR amplification	5-fluorouracil/leucovorin/oxaliplatin	
		5-fluorouracil/irinotecan/leucovorin	
		cetuximab	
		panitumumab	
	RET amplification	regorafenib	
C123_1	MET amplification		cetuximab
			panitumumab
	TP53 c.844C>G p.R282G	5-fluorouracil/leucovorin/oxaliplatin	
	EGFR amplification	5-fluorouracil/irinotecan/leucovorin	
		5-fluorouracil/leucovorin/oxaliplatin	
		cetuximab	
		panitumumab	
	FLT1 amplification	regorafenib	
	FLT3 amplification	5-fluorouracil/irinotecan/leucovorin	
		regorafenib	
	PIK3CA c.1634A>G p.E545G	5-fluorouracil/irinotecan/leucovorin	
		5-fluorouracil/leucovorin	
		cetuximab	
C129_6	ERBB2 amplification	lapatinib/trastuzumab	EGFR TKI
			cetuximab
	EGFR amplification	5-fluorouracil/irinotecan/leucovorin	
		5-fluorouracil/leucovorin/oxaliplatin	
		cetuximab	
		panitumumab	
C182_10	MET amplification		cetuximab
			panitumumab
	CCND1 amplification	chemotherapy/5-fluorouracil	
	EGFR amplification	5-fluorouracil/irinotecan/leucovorin	
		5-fluorouracil/leucovorin/oxaliplatin	
		cetuximab	
		panitumumab	
C218_8	BRAF c.1799T>A p.V600E	5-fluorouracil/irinotecan/leucovorin	cetuximab
		5-fluorouracil/leucovorin	panitumumab
		5-fluorouracil/leucovorin/oxaliplatin	vemurafenib
		bevacizumab	
		chemotherapy/5-fluorouracil	
		ipilimumab/nivolumab	
		irinotecan/panitumumab/vemurafenib	
		nivolumab	

Patient ID	QCI target	QCI suggestions	Resistance associations
		panitumumab	
		regorafenib	
	KRAS amplification		cetuximab
			panitumumab
	KRAS c.183A>T p.Q61H	5-fluorouracil/irinotecan/leucovorin	cetuximab
		5-fluorouracil/leucovorin/oxaliplatin	panitumumab
		aflibercept	
		bevacizumab	
		bevacizumab/erlotinib	
		capecitabine	
		capecitabine/oxaliplatin	
		fluoropyrimidine	
		ipilimumab/nivolumab	
		irinotecan	
		nivolumab	
		ramucirumab	
		regorafenib	
		tipiracil	
	EGFR amplification	5-fluorouracil/irinotecan/leucovorin	
		cetuximab	
		panitumumab	
C219_14	BRAF c.1781A>G p.D594G	bevacizumab	cetuximab
		ipilimumab/nivolumab	
		nivolumab	
		panitumumab	
		regorafenib	
	KRAS c.183A>C p.Q61H	5-fluorouracil/irinotecan/leucovorin	cetuximab
		5-fluorouracil/leucovorin/oxaliplatin	panitumumab
		aflibercept	
		bevacizumab	
		bevacizumab/erlotinib	
		capecitabine	
		capecitabine/oxaliplatin	
		fluoropyrimidine	
		ipilimumab/nivolumab	
		irinotecan	
		nivolumab	
		ramucirumab	
		regorafenib	
		tipiracil	
	EGFR amplification	5-fluorouracil/irinotecan/leucovorin	
		cetuximab	
		panitumumab	
	MAP2K1 c.170A>C p.K57T		cetuximab
C396_1	BRAF c.1799T>A p.V600E	5-fluorouracil/irinotecan/leucovorin	cetuximab
		5-fluorouracil/leucovorin	panitumumab
		5-fluorouracil/leucovorin/oxaliplatin	vemurafenib
		bevacizumab	

Patient ID	QCI target	QCI suggestions	Resistance associations
		chemotherapy/5-fluorouracil	
		ipilimumab/nivolumab	
		irinotecan/panitumumab/vemurafenib	
		nivolumab	
		regorafenib	
B163_9	BRCA1 c.2263G>T p.E755*	anthracycline/chemotherapy	
		carboplatin	
		cisplatin	
		olaparib	
		platinum agent	
		talazoparib	
		tamoxifen	
		cisplatin	
	ERBB2 amplification	anastrozole	lapatinib/trastuzumab
		anthracycline	trastuzumab
		anthracycline/chemotherapy	
		aromatase inhibitor	
		aromatase inhibitor/trastuzumab	
		bevacizumab	
		capecitabine	
		capecitabine/docetaxel	
		carboplatin/docetaxel/recombinant human hyaluronidase/trastuzumab	
		cyclophosphamide/docetaxel/doxorubicin	
		carboplatin/docetaxel/doxorubicin/recombinant human hyaluronidase/trastuzumab	
		cyclophosphamide/doxorubicin	
		cyclophosphamide/doxorubicin/paclitaxel/recombinant human hyaluronidase/trastuzumab	
		docetaxel	
		docetaxel/trastuzumab	
		epirubicin	
		exemestane	
		fulvestrant	
		lapatinib	
		lapatinib/letrozole	
		letrozole	
		neratinib	
		paclitaxel	
		paclitaxel/recombinant human hyaluronidase/trastuzumab	
		pertuzumab	
		pertuzumab/trastuzumab	
		recombinant human hyaluronidase/trastuzumab	
		tamoxifen	
		trastuzumab emtansine	
	ERBB2 c.2305G>T p.D769Y	lapatinib	
		neratinib	

Patient ID	QCI target	QCI suggestions	Resistance associations
		pertuzumab	
		pertuzumab/trastuzumab	
		trastuzumab	
		trastuzumab emtansine	
	PIK3CA amplification	alpelisib	
B164_6	ESR1 c.1613A>G p.D538G	raloxifine	5-fluorouracil
		toremifene	anastrozole
		neratinib	capecitabine
			cyclophosphamide
			doxorubicin
			fulvestrant
			gemcitabine
			letrozole
			paclitaxel
			tamoxifen
			trastuzumab
			vinorelbine
	EGFR amplification	anthracycline/chemotherapy	tamoxifen
		lapatinib	
		neratinib	
	ESR1 c.1610A>C p.Y537S	raloxifene	5-fluorouracil
		toremifene	anastrozole
		neratinib	capecitabine
			cyclophosphamide
			dasatinib
			denosumab
			docetaxel
			doxorubicin
			exemestane
			fulvestrant
			gemcitabine
			letrozole
			tamoxifen
			vinorelbine
			zoledronic acid
B165_5	ERBB2 amplification	anastrozole	lapatinib/trastuzumab
		anthracycline	trastuzumab
		anthracycline/chemotherapy	
		aromatase inhibitor	
		aromatase inhibitor/trastuzumab	
		bevacizumab	
		capecitabine	
		capecitabine/docetaxel	
		carboplatin/docetaxel/recombinant human hyaluronidase/trastuzumab	

Patient ID	QCI target	QCI suggestions	Resistance associations
		cyclophosphamide/docetaxel/doxorubicin	
		carboplatin/docetaxel/doxorubicin/recombinant human hyaluronidase/trastuzumab	
		cyclophosphamide/doxorubicin	
		cyclophosphamide/doxorubicin/paclitaxel/recombinant human hyaluronidase/trastuzumab	
		docetaxel	
		docetaxel/trastuzumab	
		epirubicin	
		exemestane	
		fulvestrant	
		lapatinib	
		lapatinib/letrozole	
		letrozole	
		neratinib	
		paclitaxel	
		paclitaxel/recombinant human hyaluronidase/trastuzumab	
		pertuzumab	
		pertuzumab/trastuzumab	
		recombinant human hyaluronidase/trastuzumab	
		tamoxifen	
		trastuzumab emtansine	
	PIK3CA c.1634A>C p.E545A	alpelisib	
		alpelisib/fulvestrant	
		lapatinib/letrozole	
		letrozole	
	EGFR amplification	anthracycline/chemotherapy	tamoxifen
		lapatinib	
		neratinib	
	TP53 c.577C>T p.H193Y	cisplatin	
	BRCA1 s.548G>C p.G183A	anthracycline/chemotherapy	
		carboplatin	
		cisplatin	
		olaparib	
		platinum agent	
		talazoparib	
		tamoxifen	
	MAP2K4 loss		
B166_6	ESR1 c.1613A>G p.D538G	raloxifene	5-fluorouracil
		tamoxifen	anastrozole
		toremifene	capecitabine
		neratinib	cyclophosphamide
			doxorubicin
			fulvestrant
			gemcitabine
			letrozole

Patient ID	QCI target	QCI suggestions	Resistance associations
			paclitaxel
			tamoxifen
			trastuzumab
			vinorelbine
	PIK3CA c.263G>A p.R88Q	alpelisib	
		lapatinib/letrozole	
		letrozole	
	ESR1 c.1610A>C p.Y537S	raloxifene	5-fluorouracil
		toremifene	anastrozole
		neratinib	capecitabine
			cyclophosphamide
			dasatinib
			denosumab
			docetaxel
			doxorubicin
			exemestane
			fulvestrant
			gemcitabine
			letrozole
			tamoxifen
			vinorelbine
			zoledronic acid
B176_8	ESR1 c.1613A>G p.D538G	raloxifene	5-fluorouracil
		tamoxifen	anastrozole
		toremifene	capecitabine
		neratinib	cyclophosphamide
			doxorubicin
			fulvestrant
			gemcitabine
			letrozole
			paclitaxel
			tamoxifen
			trastuzumab
			vinorelbine
	EGFR amplification	anthracycline/chemotherapy	tamoxifen
		lapatinib	
		neratinib	
	ESR1 c.1609T>A p.Y537N	raloxifene	5-fluorouracil
		toremifene	anastrozole
		neratinib	capecitabine
			cyclophosphamide
			dasatinib
			denosumab
			docetaxel
			doxorubicin

Patient ID	QCI target	QCI suggestions	Resistance associations
			exemestane
			fulvestrant
			gemcitabine
			letrozole
			tamoxifen
			vinorelbine
			zoledronic acid
	ESR1 c.1138G>C p.E380Q	fulvestrant	
		raloxifene	
		tamoxifen	
		toremifene	
		neratinib	
B205_1	No approved therapies	No approved therapies	No approved therapies
B206_1	CCND1 amplification	capecitabine	
		neratinib	
B213_1	PIK3CA c.1624G>A p.E542K	alpelisib	
		alpelisib/fulvestrant	
		lapatinib/letrozole	
		letrozole	
	KRAS c.35G>T p.G12V	cyclophosphamide/doxorubicin	
	ESR1 c.1610A>C p.Y537S	neratinib	5-fluorouracil
		raloxifene	anastrozole
		toremifene	capecitabine
			cyclophosphamide
			dasatinib
			denosumab
			docetaxel
			doxorubicin
			exemestane
			fulvestrant
			gemcitabine
			letrozole
			tamoxifen
			vinorelbine
			zoledronic acid
	MSH6 c.1467A>G p.P489P	pembrolizumab	
B216_1	PIK3CA c.3140A>G p.H1047R	alpelisib	
		alpelisib/fulvestrant	
		lapatinib/letrozole	
		letrozole	
	TP53 c.993+T>G	cisplatin	
		everolimus/exemestane	
		letrozole	

Patient ID	QCI target	QCI suggestions	Resistance associations
		paclitaxel	
	TP53 c.641A>C p.H214P	cisplatin	
		everolimus/exemestane	
		letrozole	
		paclitaxel	
B219_1	PIK3CA c.3140A>G p.H1047R	alpelisib	
		alpelisib/fulvestrant	
		lapatinib/letrozole	
		letrozole	
	PIK3CA amplification	alpelisib	
B221_1	BRCA2 c.2T>A p.M1K	anthracycline/chemotherapy	
		carboplatin	
		cisplatin	
		olaparib	
		platinum agent	
		platinum chemotherapy	
		talazoparib	
		tamoxifen	
	RABGAP1L-ROS1 fusion	entrectinib	
	TP53 c.841G>C p.D281H	cisplatin	
		everolimus/exemestane	
		letrozole	
		paclitaxel	
B224_1	No approved therapies	No approved therapies	No approved therapies
L996_0	CDKN2A c.132C>G p.Y44*	EGFR TKI	
	STK11 c.464+1G>A	docetaxel	
		nivolumab	
	TP53 c.733G>T p.G245C	docetaxel	lorlatinib
		ipilimumab/nivolumab	
		nivolumab	
L1015_0	EGFR amplification	EGFR TKI	afatinib
		brigatinib	gefitinib
		carboplatin/paclitaxel	
		cetuximab	
		cisplatin/vinorelbine	
		dacomitinib	
		erlotinib	
		necitumumab	
		osimertinib	
		platinum chemotherapy	
	TP53 c.673G>T p.V225F	ipilimumab/nivolumab	lorlatinib
		docetaxel	
		nivolumab	
	TP53 c.673-1G>C	ipilimumab/nivolumab	lorlatinib
		docetaxel	
		nivolumab	

Patient ID	QCI target	QCI suggestions	Resistance associations
L1023_0	TP53 c.920-1G>T	docetaxel	lorlatinib
		erlotinib	
		ipilimumab/nivolumab	
		nivolumab	
	CDKN2A c.251A>T p.D84V	EGFR TKI	
L1024_0	EGFR c.2573T>G p.L858R	EGFR TKI	erlotinib
		afatinib	gefitinib
		afatinib/cetuximab	gemcitabine
		atezolizumab	lorlatinib
		brigatinib	nivolumab
		carboplatin/gemcitabine	osimertinib
		carboplatin/paclitaxel	paclitaxel
		cisplatin/docetaxel	pembrolizumab
		cisplatin/gemcitabine	pemetrexed
		cisplatin/pemetrexed	ramucirumab
		dacomitinib	
		docetaxel	
		everolimus	
		ipilimumab/nivolumab	
		necitumumab	
		platinum chemotherapy	
	MET amplification	crizotinib	
		gefitinib	
	EGFR amplification	EGFR TKI	
		afatinib	
		brigatinib	
		carboplatin/paclitaxel	
		cetuximab	
		cisplatin/vinorelbine	
		dacomitinib	
		erlotinib	
		gefitinib	
		necitumumab	
		osimertinib	
		platinum chemotherapy	
L1047_0	MET amplification	crizotinib	EGFR TKI
		docetaxel	erlotinib
			gefitinib
			osimertinib
	EGFR amplification	EGFR TKI	
		brigatinib	
		carboplatin/paclitaxel	
		cetuximab	
		cisplatin/vinorelbine	
		dacomitinib	
		erlotinib	
		necitumumab	

Patient ID	QCI target	QCI suggestions	Resistance associations
		osimertinib	
		platinum chemotherapy	
	AXL amplification		gefitinib
			osimertinib
	TP53 c.920-1G>T	docetaxel	lorlatinib
		ipilimumab/nivolumab	
		nivolumab	
L1067_0	KRAS g.34G>T p.G12C	atezolizumab	EGFR TKI
		carboplatin/paclitaxel	afatinib
		cisplatin/vinorelbine	cisplatin
		docetaxel	crizotinib
		ipilimumab/nivolumab	erlotinib
		nivolumab	gefitinib
			osimertinib
			vinorelbine
	TP53 c.730G>T p.G244C	docetaxel	lorlatinib
		erlotinib	
		ipilimumab/nivolumab	
		nivolumab	
L1093_0	FGFR1 amplification	nintedanib	osimertinib
	PIK3CA c.1338G>T p.W446C	EGFR TKI	
		erlotinib	
		everolimus	
		gefitinib	
L1096_0	KRAS c.35G>A p.G12D	atezolizumab	EGFR TKI
		carboplatin/paclitaxel	afatinib
		cisplatin/vinorelbine	cisplatin
		docetaxel	crizotinib
		ipilimumab/nivolumab	erlotinib
		nivolumab	gefitinib
			osimertinib
			vinorelbine
	MET amplification	crizotinib	EGFR TKI
		docetaxel	erlotinib
			gefitinib
			osimertinib
	EGFR amplification	EGFR TKI	afatinib
		brigatinib	gefitinib
		carboplatin/paclitaxel	
		cetuximab	
		cisplatin/vinorelbine	
		dacomitinib	
		erlotinib	
		necitumumab	
		osimertinib	
		platinum chemotherapy	
	TP53 c.637C>T p.R213*	docetaxel	

Patient ID	QCI target	QCI suggestions	Resistance associations
		ipilimumab/nivolumab	
		nivolumab	
L1097_0	KRAS c.35G>T p.G12V	atezolizumab	EGFR TKI
		carboplatin/paclitaxel	afatinib
		cisplatin/vinorelbine	cisplatin
		docetaxel	crizotinib
		ipilimumab/nivolumab	erlotinib
		nivolumab	gefitinib
			osimertinib
			vinorelbine
	STK11 c.503A>G p.H168R	docetaxel	
		nivolumab	
	TP53 c.375G>C p.T125T	docetaxel	lorlatinib
		erlotinib	
		ipilimumab/nivolumab	
		nivolumab	
L1111_0	TP53 c.517G>T p.V173L	docetaxel	lorlatinib
		ipilimumab/nivolumab	
		nivolumab	
L1118_0	PIK3CA c.1624G>A p.E542K	EGFR TKI	
		erlotinib	
		everolimus	
		gefitinib	
	TP53 c.892G>T p.E298*	ipilimumab/nivolumab	lorlatinib
		docetaxel	
		erlotinib	
		nivolumab	
	TP53 c.818G>T p.R273L	ipilimumab/nivolumab	lorlatinib
		docetaxel	
		erlotinib	
		nivolumab	
L7005_0	BRCA2 c.426-1G>T	pembrolizumab	
	TP53 c.733G>C p.G245R	docetaxel	lorlatinib
		erlotinib	
		ipilimumab/nivolumab	
		nivolumab	
L7027_0	KRAS c.182A>T p.Q61L	atezolizumab	EGFR TKI
		cisplatin/vinorelbine	afatinib
		docetaxel	cisplatin
		ipilimumab/nivolumab	erlotinib
		nivolumab	gefitinib
			osimertinib
			vinorelbine
	MET amplification	crizotinib	EGFR TKI
		docetaxel	erlotinib
			gefitinib
			osimertinib

Patient ID	QCI target	QCI suggestions	Resistance associations
	EGFR amplification	EGFR TKI	afatinib
		brigatinib	gefitinib
		carboplatin/paclitaxel	
		cetuximab	
		cisplatin/vinorelbine	
		dacomitinib	
		erlotinib	
		necitumumab	
		osimertinib	
		platinum chemotherapy	
L7098_0	TP53 c.535C>T p.H179Y	docetaxel	lorlatinib
		erlotinib	
		ipilimumab/nivolumab	
		nivolumab	
	MET c.2809G>A p.V937I		
L7108_0	BRAF c.1741A>T p.N581Y	dabrafenib	
		platinum chemotherapy	
		sorafenib	
		vemurafenib	
L7120_0	KRAS c.34G>T p.G12C	atezolizumab	EGFR TKI
		carboplatin/paclitaxel	afatinib
		cisplatin/vinorelbine	cisplatin
		docetaxel	crizotinib
		ipilimumab/nivolumab	erlotinib
		nivolumab	gefitinib
			osimertinib
			vinorelbine
	EGFR amplification	EGFR TKI	afatinib
		brigatinib	gefitinib
		carboplatin/paclitaxel	
		cetuximab	
		cisplatin/vinorelbine	
		dacomitinib	
		erlotinib	
		necitumumab	
		osimertinib	
		platinum chemotherapy	
	TP53 c.401T>G p.F134C	ipilimumab/nivolumab	lorlatinib
		docetaxel	
		nivolumab	
	TP53 c.112C>T p.Q38*	ipilimumab/nivolumab	lorlatinib
		docetaxel	
		nivolumab	
L1081_0	MET amplification	crizotinib	EGFR TKI
		docetaxel	erlotinib
			gefitinib
			osimertinib
	EGFR amplification	EGFR TKI	afatinib

Patient ID	QCI target	QCI suggestions	Resistance associations
		brigatinib	gefitinib
		carboplatin/paclitaxel	
		cetuximab	
		cisplatin/vinorelbine	
		dacomitinib	
		erlotinib	
		necitumumab	
		osimertinib	
		platinum chemotherapy	
	ERBB2 amplification	EGFR TKI	erlotinib
		afatinib	osimertinib
		dacomitinib	
		lapatinib	
		pertuzumab/trastuzumab	
		platinum chemotherapy	
	TP53 c.535C>T p.H179Y	docetaxel	lorlatinib
		ipilimumab/nivolumab	
		nivolumab	
L1172_0	KRAS c.35G>T p.G12V	atezolizumab	EGFR TKI
		carboplatin/paclitaxel	afatinib
		cisplatin/vinorelbine	cisplatin
		docetaxel	crizotinib
		ipilimumab/nivolumab	erlotinib
		nivolumab	gefitinib
			osimertinib
			vinorelbine
	TP53 c.949C>T p.Q317*	ipilimumab/nivolumab	
		docetaxel	
		erlotinib	
		nivolumab	
	TP53 c.659A>G p.Y220C	ipilimumab/nivolumab	
		docetaxel	
		erlotinib	
		nivolumab	
	TP53 c.590T>G p.V197G	ipilimumab/nivolumab	
		docetaxel	
		erlotinib	
		nivolumab	
	TP53 c.578A>G p.H193R	ipilimumab/nivolumab	
		docetaxel	
		erlotinib	
		nivolumab	
	TP53 c.452C>A p.P151H	ipilimumab/nivolumab	
		docetaxel	
		erlotinib	
		nivolumab	
L7162_0	KRAS c.35G>T p.G12V	atezolizumab	EGFR TKI
		carboplatin/paclitaxel	afatinib

Patient ID	QCI target	QCI suggestions	Resistance associations
		cisplatin/vinorelbine	cisplatin
		docetaxel	crizotinib
		ipilimumab/nivolumab	erlotinib
		nivolumab	gefitinib
			osimertinib
			vinorelbine
	PIK3CA c.1633G>A p.E545K	EGFR TKI	
		erlotinib	
		everolimus	
		gefitinib	
	TP53 c.524G>A p.R175H	docetaxel	lorlatinib
		erlotinib	
		ipilimumab/nivolumab	
		nivolumab	

Supplementary Table 11. All treatment suggestions per patient profile from CureMatch Bionov analysis [Table originally published in ESMO Open (Perakis et al., 2020)]

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
C127_10	3	binimetinib	67	KRAS	MAP2K1,MAP2K2	Off-label
		sulindac		APC	PTGS1,PTGS2	Off-label
		ziv-aflibercept		TP53	FLT1,KDR	On compendia
	3	binimetinib	67	TP53	FLT1,KDR	On compendia
		sulindac		KRAS	MAP2K1,MAP2K2	Off-label
		bevacizumab		APC	PTGS1,PTGS2	Off-label
	3	binimetinib	67	KRAS	MAP2K1,MAP2K2	Off-label
		sulindac		TP53	FLT1,KDR	On compendia
		regorafenib		APC	PTGS1,PTGS2	Off-label
	2	binimetinib	49	KRAS	MAP2K1,MAP2K2	Off-label
		ziv-aflibercept		TP53	FLT1,KDR	On compendia
	2	bevacizumab	49	TP53	FLT1,KDR	On compendia
		binimetinib		KRAS	MAP2K1,MAP2K2	Off-label
	2	binimetinib	49	KRAS	MAP2K1,MAP2K2	On compendia
		regorafenib		TP53	FLT1,KDR	On compendia
	1	ziv-aflibercept	25	TP53	FLT1,KDR	On compendia
	1	trametinib	25	KRAS	MAP2K1,MAP2K2	On compendia
	1	cobimetinib	16	KRAS	MAP2K1,MAP2K2	Off-label
C109_8	3	binimetinib	73	KRAS	MAP2K1,MAP2K2	On compendia
		cabozantinib		TP53, FLT3, FLT1,MET	FLT1,KDR	Off-label
		cetuximab		EGFR		On-compendia
	3	cabozantinib	73	TP53, FLT3, FLT1,MET	FLT1,KDR	Off-label
		cetuximab		EGFR		On-compendia
		trametinib		KRAS	MAP2K1,MAP2K2	On-compendia
	3	binimetinib	73	KRAS	MAP2K1,MAP2K2	On-compendia
		cabozantinib		TP53, FLT3, FLT1,MET		Off-label
		panitumumab		EGFR		On-compendia
	2	binimetinib	57	KRAS	MAP2K1,MAP2K2	On-compendia
		cabozantinib		TP53, FLT3, FLT1,MET	FLT1,KDR	Off-label
	2	cabozantinib	57	TP53, FLT3, FLT1,MET	FLT1,KDR	Off-label
		trametinib		KRAS	MAP2K1,MAP2K2	On-compendia

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
	2	binimetinib	54	KRAS	MAP2K1,MAP2K2	On-compendia
		vandetanib		EGFR,TP53,FLT3,FLT1	FLT1,KDR	Off-label
	1	cabozantinib	45	TP53, FLT3, FLT1,MET	FLT1,KDR	Off-label
	1	nintedanib	29	TP53, FLT3, FLT1	FLT1,KDR	Off-label
	1	sorafenib	22	TP53,FLT3,FLT1	FLT1,KDR	Off-label
C154_17	3	binimetinib	85	KRAS,NRAS	MAP2K1,MAP2K2	On compendia
		cetuximab		EGFR		On compendia
		pembrolizumab		MSH2/MSH6		On compendia
	3	binimetinib	85	KRAS,NRAS	MAP2K1,MAP2K2	On compendia
		panitumumab		EGFR		On compendia
		pembrolizumab		MSH2/MSH6		On compendia
	3	cetuximab	85	EGFR		On compendia
		pembrolizumab		MSH2/MSH6		On compendia
		trametinib		KRAS,NRAS	MAP2K1,MAP2K2	On compendia
	2	binimetinib	83	KRAS,NRAS	MAP2K1,MAP2K2	On compendia
		pembrolizumab		MSH2/MSH6		On compendia
	2	pembrolizumab	83	MSH2/MSH6		On compendia
		trametinib		KRAS,NRAS	MAP2K1,MAP2K2	On compendia
	2	binimetinib	83	KRAS,NRAS	MAP2K1,MAP2K2	On compendia
		nivolumab		MSH2/MSH6		On compendia
	1	binimetinib	21	KRAS,NRAS	MAP2K1,MAP2K2	On compendia
	1	trametinib	21	KRAS,NRAS	MAP2K1,MAP2K2	On compendia
	1	cobimetinib	14	KRAS,NRAS	MAP2K1,MAP2K2	Off-label
C139_10	3	sulindac	74	APC	PTGS1,PTGS2	Off-label
		regorafenib		APC	WNT	On compendia
		mebendazole		APC	WNT	Off-label
	3	sulindac	74	APC	PTGS1,PTGS2	Off-label
		sorafenib		APC	WNT	Off-label
		mebendazole		APC	WNT	Off-label

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
	3	celecoxib	64	APC	PTGS2	Off-label
		regorafenib		APC	WNT	On compendia
		mebendazole		APC	WNT	Off-label
	2	regorafenib	52	APC	WNT	On compendia
		sulindac		APC	PTGS1,PTGS2	Off-label
	2	sorafenib	52	APC	WNT	Off-label
		sulindac		APC	PTGS1,PTGS2	Off-label
	2	sulindac	49	APC	PTGS1,PTGS2	Off-label
		mebendazole		APC	WNT	Off-label
	1	sulindac	28	APC	PTGS1,PTGS2	Off-label
	1	sorafenib	24	APC	WNT	Off-label
	1	regorafenib	24	APC	WNT	On compendia
C140_11	3	binimetinib	57	KRAS	MAP2K1,MAP2K2	On compendia
		cetuximab		EGFR		On compendia
		sulindac		APC	PTGS1,PTGS2	Off-label
	3	binimetinib	57	KRAS	MAP2K1,MAP2K2	On compendia
		panitumumab		EGFR		On compendia
		sulindac		APC	PTGS1,PTGS2	Off-label
	3	cetuximab	57	EGFR		On compendia
		sulindac		APC	PTGS1,PTGS2	Off-label
		trametinib		KRAS	MAP2K1,MAP2K2	On compendia
	2	binimetinib	43	KRAS	MAP2K1,MAP2K2	On compendia
		cetuximab		EGFR		On compendia
	2	binimetinib	43	KRAS	MAP2K1,MAP2K2	On compendia
		panitumumab		EGFR		On compendia
	2	cetuximab	43	EGFR		On compendia
		trametinib		KRAS	MAP2K1,MAP2K2	On compendia
	1	binimetinib	19	KRAS	MAP2K1,MAP2K2	On compendia
	1	trametinib	19	KRAS	MAP2K1,MAP2K2	On compendia
	1	regorafenib	14	APC	WNT	On compendia
C196_8	3	cetuximab	69	EGFR		On compendia
		copanlisib		PIK3CA		Off-label
		vandetanib		EGFR,TP53	FLT1,KDR	Off-label

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
	3	cetuximab	64	EGFR		On compendia
		temsirolimus		PIK3CA	MTOR	Off-label
		vandetanib		EGFR,TP53	FLT1,KDR	Off-label
	3	cetuximab	64	EGFR		On compendia
		sirolimus		PIK3CA	MTOR	Off-label
		vandetanib		EGFR,TP53	FLT1,KDR	Off-label
	2	cetuximab	50	EGFR		On compendia
		vandetanib		EGFR,TP53	FLT1,KDR	Off-label
	2	cetuximab	49	EGFR		On compendia
		copanlisib		PIK3CA		Off-label
	2	cetuximab	43	EGFR		On compendia
		ziv-aflibercept		TP53	FLT1,KDR	On compendia
	1	cetuximab	25	EGFR		On compendia
	1	panitumumab	25	EGFR		On compendia
	1	necitumumab	25	EGFR		Off-label
C217_14	3	dabrafenib	69	BRAF		On compendia
		trametinib		BRAF	MAP2K1,MAP2K2	On compendia
		vandetanib		EGFR, TP53	FLT1,KDR	Off-label
	3	cetuximab	58	EGFR		On compendia
		encorafenib		BRAF		On compendia
		binimetinib		BRAF	MAP2K1,MAP2K2	On compendia
	3	cetuximab	49	EGFR		On compendia
		irinotecan		-		On compendia
		vemurafenib		BRAF		On compendia
	2	dabrafenib	62	BRAF		On compendia
		vandetanib		EGFR,TP53	FLT1,KDR	Off-label
	2	vandetanib	62	EGFR, TP53	FLT1,KDR	Off-label
		vemurafenib		BRAF		On compendia
	2	trametinib	62	BRAF	MAP2K1,MAP2K2	On compendia
		vandetanib		EGFR, TP53	FLT1,KDR	Off-label
	1	binimetinib	25	BRAF	MAP2K1,MAP2K2	On compendia
	1	vemurafenib	25	BRAF		On compendia
	1	dabrafenib	25	BRAF		On compendia

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
C128_6	3	binimetinib	57	KRAS	MAP2K1,MAP2K2	Off-label
		cabozantinib		TP53,MET	FLT1,KDR	Off-label
		cetuximab		EGFR		On compendia
	3	cabozantinib	57	TP53,MET	FLT1,KDR	Off-label
		cetuximab		EGFR		On compendia
		trametinib		KRAS	MAP2K1,MAP2K2	Off-label
	3	binimetinib	57	KRAS	MAP2K1,MAP2K2	Off-label
		cabozantinib		TP53,MET	FLT1,KDR	Off-label
		panitumumab		EGFR		On compendia
	2	binimetinib	41	KRAS	MAP2K1,MAP2K2	Off-label
		cabozantinib		TP53,MET	FLT1,KDR	Off-label
	2	cabozantinib	41	TP53,MET	FLT1,KDR	Off-label
		trametinib		KRAS	MAP2K1,MAP2K2	Off-label
	2	binimetinib	37	KRAS	MAP2K1,MAP2K2	Off-label
		vandetanib		EGFR,TP53	FLT1,KDR	Off-label
	1	cabozantinib	29	TP53,MET	FLT1,KDR	Off-label
	1	binimetinib	12	KRAS	MAP2K1,MAP2K2	Off-label
	1	trametinib	12	KRAS	MAP2K1,MAP2K2	Off-label
C118_2	3	binimetinib	87	KRAS	MAP2K1,MAP2K2	On compendia
		cabozantinib		TP53,MET,RET,FLT1,FLT3	FLT1,KDR	Off-label
		cetuximab		EGFR		On compendia
	3	cabozantinib	87	TP53,MET,RET,FLT1,FLT3	FLT1,KDR	Off-label
		cetuximab		EGFR		On compendia
		trametinib		KRAS	MAP2K1,MAP2K2	On compendia
	3	binimetinib	87	KRAS	MAP2K1,MAP2K2	On compendia
		cabozantinib		TP53,MET,RET,FLT1,FLT3	FLT1,KDR	Off-label
		panitumumab		EGFR		On compendia
	2	binimetinib	69	KRAS	MAP2K1,MAP2K2	On compendia
		cabozantinib		TP53,MET,RET,FLT1,FLT3	FLT1,KDR	Off-label
	2	cabozantinib	69	TP53,MET,RET,FLT1,FLT3	FLT1,KDR	Off-label
		trametinib		KRAS	MAP2K1,MAP2K2	On compendia
	2	binimetinib	64	KRAS	MAP2K1,MAP2K2	On compendia
		vandetanib		EGFR,TP53,RET,FLT1,FLT3	FLT1,KDR	Off-label

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
	1	cabozantinib	54	TP53,MET,RET,FLT1,FLT3	FLT1,KDR	Off-label
	1	regorafenib	35	TP53,RET,FLT1	FLT1,KDR	On compendia
	1	binimetinib	15	KRAS	MAP2K1,MAP2K2	On compendia
C166_8	3	binimetinib	73	KRAS	MAP2K1,MAP2K2	On compendia
		cabozantinib		FLT1,FLT3,MET		Off-label
		cetuximab		EGFR		On compendia
	3	binimetinib	73	KRAS	MAP2K1,MAP2K2	On compendia
		cabozantinib		FLT1,FLT3,MET		Off-label
		panitumumab		EGFR		On compendia
	3	cabozantinib	73	FLT1,FLT3,MET		Off-label
		cetuximab		EGFR		On compendia
		trametinib		KRAS	MAP2K1,MAP2K2	On compendia
	2	binimetinib	54	KRAS	MAP2K1,MAP2K2	Off-label
		cabozantinib		FLT1,FLT3,MET		Off-label
	2	cabozantinib	54	FLT1,FLT3,MET		Off-label
		trametinib		KRAS	MAP2K1,MAP2K2	Off-label
	2	cabozantinib	49	FLT1,FLT3,MET		Off-label
		cobimetinib		KRAS	MAP2K1,MAP2K2	Off-label
	1	cabozantinib	40	FLT1,FLT3,MET		Off-label
	1	nintedanib	20	FLT1,FLT3		Off-label
	1	binimetinib	15	KRAS	MAP2K1,MAP2K2	On compendia
C208_11	3	afatinib	92	EGFR		Off-label
		cabozantinib		TP53,MET,RET	FLT1,KDR	Off-label
		cetuximab		EGFR		On compendia
	3	cabozantinib	92	TP53,MET,RET	FLT1,KDR	Off-label
		cetuximab		EGFR		On compendia
		erlotinib		EGFR		Off-label
	3	cabozantinib	92	TP53,MET,RET	FLT1,KDR	Off-label
		cetuximab		EGFR		On compendia
		lapatinib		EGFR		On compendia
	2	cabozantinib	92	TP53,MET,RET	FLT1,KDR	Off-label
		cetuximab		EGFR		On compendia
	2	cabozantinib	92	TP53,MET,RET	FLT1,KDR	Off-label

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
		panitumumab		EGFR		On compendia
	2	afatinib	92	EGFR		Off-label
		cabozantinib		TP53,MET,RET	FLT1,KDR	Off-label
	1	cabozantinib	68	TP53,MET,RET	FLT1,KDR	Off-label
	1	vandetanib	63	EGFR,TP53,RET	FLT1,KDR	Off-label
	1	ibrutinib	50	EGFR,RET		Off-label
C123_1	3	cabozantinib	91	TP53,MET	FLT1,KDR	Off-label
		cetuximab		EGFR		On compendia
		copanlisib		PIK3CA		Off-label
	3	cabozantinib	91	TP53,MET	FLT1,KDR	Off-label
		copanlisib		PIK3CA		Off-label
		panitumumab		EGFR		On compendia
	3	cabozantinib	91	TP53,MET	FLT1,KDR	Off-label
		copanlisib		PIK3CA		Off-label
		lapatinib		EGFR		On compendia
	2	cabozantinib	67	TP53,MET	FLT1,KDR	Off-label
		cetuximab		EGFR		On compendia
	2	cabozantinib	67	TP53,MET	FLT1,KDR	Off-label
		panitumumab		EGFR		On compendia
	2	cabozantinib	67	TP53,MET	FLT1,KDR	Off-label
		lapatinib		EGFR		On compendia
	1	cabozantinib	43	TP53,MET	FLT1,KDR	Off-label
	1	vandetanib	38	EGFR,TP53	FLT1,KDR	Off-label
	1	cetuximab	25	EGFR		On compendia
C129_6	3	carfilzomib	86	CCNE1	proteasome	Off-label
		lapatinib		EGFR,ERBB2		On compendia
		pembrolizumab		MSH6		On compendia
	3	cetuximab	86	EGFR		On compendia
		ibrutinib		EGFR,ERBB2		Off-label
		pembrolizumab		MSH6		On compendia
	3	cetuximab	86	EGFR		On compendia
		lapatinib		EGFR,ERBB2		On compendia

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
		pembrolizumab		MSH6		On compendia
	2	lapatinib	85	EGFR,ERBB2		On compendia
		pembrolizumab		MSH6		On compendia
	2	afatinib	85	EGFR,ERBB2		Off-label
		pembrolizumab		MSH6		On compendia
	2	ibrutinib	85	EGFR,ERBB2		Off-label
		pembrolizumab		MSH6		On compendia
	1	lapatinib	33	EGFR,ERBB2		On compendia
	1	afatinib	33	EGFR,ERBB2		Off-label
	1	neratinib	21	EGFR,ERBB2		Off-label
C182_10	3	cabozantinib	68	MET		Off-label
		cetuximab		EGFR		On compendia
		everolimus		CCND1,PIK3CA	MTOR	Off-label
	3	cabozantinib	58	MET		Off-label
		cetuximab		EGFR		On compendia
		palbociclib		CCND1	CDK4/6	Off-label
	3	cabozantinib	50	MET		Off-label
		cetuximab		EGFR		On compendia
		regorafenib		APC	WNT	Off-label
	2	cetuximab	49	EGFR		On compendia
		everolimus		CCND1,PIK3CA	MTOR	Off-label
	2	cabozantinib	39	MET		Off-label
		cetuximab		EGFR		On compendia
	2	cetuximab	31	EGFR		On compendia
		regorafenib		APC	WNT	On compendia
	1	cetuximab	20	EGFR		On compendia
	1	palbociclib	20	CCND1	CDK4/6	Off-label
	1	regorafenib	12	APC	WNT	On compendia
C218_8	3	dabrafenib	69	BRAF		On compendia
		trametinib		BRAF,KRAS	MAP2K1,MAP2K2	On compendia

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
		vandetanib		EGFR,TP53,FLT1,FLT3	FLT1,KDR	Off-label
	3	cetuximab	61	EGFR		On compendia
		dabrafenib		BRAF		On compendia
		trametinib		BRAF,KRAS	MAP2K1,MAP2K2	On compendia
	3	cetuximab	39	EGFR		On compendia
		irinotecan		-		On compendia
		vemurafenib		BRAF		On compendia
	2	trametinib	64	BRAF,KRAS	MAP2K1,MAP2K2	On compendia
		vandetanib		EGFR,TP53,FLT1,FLT3	FLT1,KDR	Off-label
	2	binimetinib	59	BRAF,KRAS	MAP2K1,MAP2K2	On compendia
		vandetanib		EGFR,TP53,FLT1,FLT3	FLT1,KDR	Off-label
	2	vandetanib	50	EGFR,TP53,FLT1,FLT3	FLT1,KDR	Off-label
		vemurafenib		BRAF		On compendia
	1	binimetinib	30	BRAF,KRAS	MAP2K1,MAP2K2	On compendia
	1	dabrafenib	20	BRAF		On compendia
	1	vemurafenib	20	BRAF		On compendia
C219_14	3	afatinib	86	EGFR,ERBB3	ERBB2	Off-label
		palbociclib		CDK4		Off-label
		pembrolizumab		PMS2		On compendia
	3	afatinib	85	EGFR,ERBB3	ERBB2	Off-label
		pembrolizumab		PMS2		Off-label
		trametinib		KRAS		On compendia
	3	pembrolizumab	84	PMS2		On compendia
		trametinib		KRAS	MAP2K1,MAP2K2	On compendia
		vandetanib		EGFR,TP53	FLT1,KDR	Off-label
	2	afatinib	84	EGFR,ERBB3	ERBB2	Off-label
		pembrolizumab		PMS2		On compendia
	2	lapatinib	84	EGFR,ERBB3	ERBB2	Off-label
		pembrolizumab		PMS2		On compendia

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
	2	ibrutinib	84	EGFR,ERBB3	ERBB2	Off-label
		pembrolizumab		PMS2		On compendia
	1	afatinib	28	EGFR,ERBB3	ERBB2	Off-label
	1	lapatinib	28	EGFR,ERBB3	ERBB2	On compendia
	1	ibrutinib	28	EGFR,ERBB3	ERBB2	Off-label
C396_1	3	dabrafenib	86	BRAF		On compendia
		trametinib		BRAF	MAP2K1,MAP2K2	On compendia
		vandetanib		EGFR,TP53	FLT1,KDR	Off-label
	3	cetuximab	73	EGFR		On compendia
		encorafenib		BRAF		On compendia
		binimetinib		BRAF	MAP2K1,MAP2K2	On compendia
	3	cetuximab	65	EGFR		On compendia
		irinotecan		-		On compendia
		vemurafenib		BRAF		On compendia
	2	dabrafenib	83	BRAF		On compendia
		vandetanib		EGFR,TP53	FLT1,KDR	Off-label
	2	vandetanib	83	EGFR,TP53	FLT1,KDR	Off-label
		vemurafenib		BRAF		On compendia
	2	trametinib	83	BRAF	MAP2K1,MAP2K2	On compendia
		vandetanib		EGFR,TP53	FLT1,KDR	Off-label
	1	binimetinib	33	BRAF	MAP2K1,MAP2K2	On compendia
	1	vemurafenib	33	BRAF		On compendia
	1	dabrafenib	33	BRAF		On compendia
B163_9	3	brigatinib	44	ERBB2,FGFR3,SMAD4	EGFR,ERBB2,MAP2K1,MAP2K2	Off-label
		fulvestrant		ESR1		On compendia
		olaparib		BRCA1	PARP1,PARP2	On compendia
	3	brigatinib	44	ERBB2,FGFR3,SMAD4	EGFR,ERBB2,MAP2K1,MAP2K2	Off-label
		fulvestrant		ESR1		On compendia
		talazoparib		BRCA1	PARP1,PARP2	On compendia
	3	brigatinib	44	ERBB2,FGFR3,SMAD4	EGFR,ERBB2,MAP2K1,MAP2K2	Off-label

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
		olaparib		BRCA1	PARP1,PARP2	On compendia
		tamoxifen		ESR1		On compendia
	2	brigatinib	32	ERBB2,FGFR3,SMAD4	EGFR,ERBB2,MAP2K1,MAP2K2	Off-label
		fulvestrant		ESR1		On compendia
	2	brigatinib	32	ERBB2,FGFR3,SMAD4	EGFR,ERBB2,MAP2K1,MAP2K2	Off-label
		tamoxifen		ESR1		On compendia
	2	brigatinib	32	ERBB2,FGFR3,SMAD4	EGFR,ERBB2,MAP2K1,MAP2K2	Off-label
		toremifene		ESR1		On compendia
	1	brigatinib	19	ERBB2,FGFR3,SMAD4	EGFR,ERBB2,MAP2K1,MAP2K2	Off-label
	1	lapatinib	18	ERBB2,SMAD4	EGFR,ERBB2,MAP2K1,MAP2K2	On compendia
	1	ibrutinib	18	ERBB2,SMAD4	EGFR,ERBB2,MAP2K1,MAP2K2	Off-label
B164_6	3	NA	NA	NA	NA	NA
	3	NA	NA	NA	NA	NA
	3	NA	NA	NA	NA	NA
	2	lapatinib	98	EGFR		On compendia
		panitumumab		EGFR		Off-label
	2	lapatinib	98	EGFR		On compendia
		necitumumab		EGFR		Off-label
	2	erlotinib	98	EGFR		Off-label
		cetuximab		EGFR		Off-label
	1	lapatinib	99	EGFR		On compendia
	1	panitumumab	99	EGFR		Off-label
	1	cetuximab	99	EGFR		Off-label

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
B165_5	3	everolimus	63	CCND1,PIK3CA	CDK4,CDK6,MTOR	On compendia
		ibrutinib		EGFR,ERBB2,YES1		Off-label
		lenvatinib		FGFR1,FGFR2,TP53	FLT1,KDR,VEGFA	Off-label
	3	fulvestrant	63	ESR1		On compendia
		ibrutinib		EGFR,ERBB2,YES1		Off-label
		lenvatinib		FGFR1,FGFR2,TP53	FLT1,KDR,VEGFA	Off-label
	3	ibrutinib	63	EGFR,ERBB2,YES1		Off-label
		lenvatinib		FGFR1,FGFR2,TP53	FLT1,KDR,VEGFA	Off-label
		tamoxifen		ESR1		On compendia
	2	ibrutinib	54	EGFR,ERBB2,YES1		Off-label
		lenvatinib		FGFR1,FGFR2,TP53	FLT1,KDR,VEGFA	Off-label
	2	ibrutinib	53	EGFR,ERBB2,YES1		Off-label
		nintedanib		FGFR1,FGFR2,TP53	FLT1,KDR,VEGFA	Off-label
	2	erdafitinib	52	FGFR1,FGFR2		Off-label
		ibrutinib		EGFR,ERBB2,YES1		Off-label
	1	brigatinib	35	EGFR,ERBB2,FGFR1,FGRF2,YES1		Off-label
	1	ibrutinib	31	EGFR,ERBB2,YES1		Off-label
	1	fostamatinib	26	FGFR1,TP53,YES1	FLT1,KDR,VEGFA	Off-label
B166_6	3	NA	NA	NA	NA	NA
	3	NA	NA	NA	NA	NA
	3	NA	NA	NA	NA	NA
	2	NA	NA	NA	NA	NA
	2	NA	NA	NA	NA	NA
	2	NA	NA	NA	NA	NA

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
	1	copanlisib	99	PIK3CA		On compendia
	1	alpelisib	99	PIK3CA		On compendia
	1	everolimus	74	PIK3CA		Off-label
B176_8	3	brigatinib	58	AURKA,EGFR,FGFR1		Off-label
		cabozantinib		MET		Off-label
		glasdegib		PTCH1	SMO	Off-label
	3	brigatinib	58	AURKA,EGFR,FGFR1		Off-label
		cabozantinib		MET		Off-label
		sonidegib		PTCH1	SMO	Off-label
	3	brigatinib	58	AURKA,EGFR,FGFR1		Off-label
		cabozantinib		MET		Off-label
		vismodegib		PTCH1	SMO	Off-label
	2	brigatinib	46	AURKA,EGFR,FGFR1		Off-label
		cabozantinib		MET		Off-label
	2	brigatinib	46	AURKA,EGFR,FGFR1		Off-label
		crizotinib		MET		Off-label
	2	brigatinib	41	AURKA,EGFR,FGFR1		Off-label
		glasdegib		PTCH1	SMO	Off-label
	1	brigatinib	28	AURKA,EGFR,FGFR1		Off-label
	1	lapatinib	18	EGFR		On compendia
	1	panitumumab	18	EGFR		Off-label
B205_1	3	fulvestrant	69	ESR1		On compendia
		lapatinib		SMAD4	EGFR,ERBB2,MAP2K1,MAP2K2	On compendia
		ribociclib		CDKN2A	CDK4	On compendia
	3	lapatinib	69	SMAD4	EGFR,ERBB2,MAP2K1,MAP2K2	On compendia
		ribociclib		CDKN2A	CDK4	On compendia
		tamoxifen		ESR1		On compendia
	3	lapatinib	69	SMAD4	EGFR,ERBB2,MAP2K1,MAP2K2	On compendia
		ribociclib		CDKN2A	CDK4	On compendia
		toremifene		ESR1		On compendia

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
	2	fulvestrant	57	ESR1		On compendia
		ribociclib		CDKN2A	CDK4	On compendia
	2	ribociclib	57	CDKN2A	CDK4	On compendia
		tamoxifen		ESR1		On compendia
	2	ribociclib	57	CDKN2A	CDK4	On compendia
		toremifene		ESR1		On compendia
	1	fulvestrant	33	ESR1		On compendia
	1	tamoxifen	33	ESR1		On compendia
	1	toremifene	33	ESR1		On compendia
B206_1	3	fulvestrant	60	ESR1		On compendia
		mifepristone		PGR		Off-label
		ribociclib		CCND1	CDK4,CDK6,MTOR	On compendia
	3	mifepristone	60	PGR		Off-label
		ribociclib		CCND1	CDK4,CDK6,MTOR	On compendia
		tamoxifen		ESR1		On compendia
	3	mifepristone	60	PGR		Off-label
		ribociclib		CCND1	CDK4,CDK6,MTOR	On compendia
		toremifene		ESR1		On compendia
	2	fulvestrant	47	ESR1		On compendia
		ribociclib		CCND1	CDK4,CDK6,MTOR	On compendia
	2	ribociclib	47	CCND1	CDK4,CDK6,MTOR	On compendia
		tamoxifen		ESR1		On compendia
	2	ribociclib	47	CCND1	CDK4,CDK6,MTOR	On compendia
		toremifene		ESR1		On compendia
	1	fulvestrant	28	ESR1		On compendia
	1	tamoxifen	28	ESR1		On compendia
	1	toremifene	28	ESR1		On compendia
B213_1	3	NA	NA	NA	NA	NA
	3	NA	NA	NA	NA	NA

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
	3	NA	NA	NA	NA	NA
	2	binimetinib	49	KRAS	MAP2K1,MAP2K2	Off-label
		mifepristone		PGR		Off-label
	2	binimetinib	49	KRAS	MAP2K1,MAP2K2	Off-label
		ulipristal		PGR		Off-label
	2	mifepristone	49	PGR		Off-label
		trametinib		KRAS	MAP2K1,MAP2K2	Off-label
	1	binimetinib	30	KRAS	MAP2K1,MAP2K2	Off-label
	1	trametinib	30	KRAS	MAP2K1,MAP2K2	Off-label
	1	mifepristone	20	PGR		Off-label
B216_1	3	everolimus	52	CCND1,PIK3CA	CDK4,CDK6,MTOR	On compendia
		fostamatinib		FGFR3,TP53	FLT1,KDR,VEGFA	Off-label
		lapatinib		EGFR		On compendia
	3	fostamatinib	52	FGFR3,TP53	FLT1,KDR,VEGFA	Off-label
		fulvestrant		ESR1		On compendia
		lapatinib		EGFR		On compendia
	3	fostamatinib	52	FGFR3,TP53	FLT1,KDR,VEGFA	Off-label
		lapatinib		EGFR		On compendia
		tamoxifen		ESR1		On compendia
	2	fostamatinib	38	FGFR3,TP53	FLT1,KDR,VEGFA	Off-label
		lapatinib		EGFR		On compendia
	2	everolimus	38	CCND1,PIK3CA	CDK4,CDK6,MTOR	Off-label
		fostamatinib		FGFR3,TP53	FLT1,KDR,VEGFA	On compendia
	2	fostamatinib	38	FGFR3,TP53	FLT1,KDR,VEGFA	Off-label
		fulvestrant		ESR1		On compendia
	1	fostamatinib	23	FGFR3,TP53	FLT1,KDR,VEGFA	Off-label
	1	vandetanib	21	EGFR,TP53	FLT1,KDR,VEGFA	Off-label
	1	lenvatinib	19	FGFR3,TP53	FLT1,KDR,VEGFA	Off-label
B219_1	3	copanlisib	62	PIK3CA		Off-label
		fostamatinib		FGFR1,TP53	FLT1,KDR,VEGFA	Off-label
		fulvestrant		ESR1		On compendia

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
	3	alpelisib	62	PIK3CA		Off-label
		fostamatinib		FGFR1,TP53	FLT1,KDR,VEGFA	Off-label
		fulvestrant		ESR1		On compendia
	3	copanlisib	62	PIK3CA		Off-label
		fostamatinib		FGFR1,TP53	FLT1,KDR,VEGFA	Off-label
		tamoxifen		ESR1		On compendia
	2	fostamatinib	45	FGFR1,TP53	FLT1,KDR,VEGFA	Off-label
		fulvestrant		ESR1		On compendia
	2	fostamatinib	45	FGFR1,TP53	FLT1,KDR,VEGFA	Off-label
		tamoxifen		ESR1		On compendia
	2	fostamatinib	45	FGFR1,TP53	FLT1,KDR,VEGFA	Off-label
		toremifene		ESR1		On compendia
	1	fostamatinib	27	FGFR1,TP53	FLT1,KDR,VEGFA	Off-label
	1	lenvatinib	21	FGFR1,TP53	FLT1,KDR,VEGFA	Off-label
	1	nintedanib	21	FGFR1,TP53	FLT1,KDR,VEGFA	Off-label
B221_1	3	axitinib	81	TP53	FLT1,KDR,VEGFA	Off-label
		brigatinib		RABGAP1L-ROS1		Off-label
		olaparib		BRCA2	PARP1,PARP2	On compendia
	3	axitinib	81	TP53	FLT1,KDR,VEGFA	Off-label
		brigatinib		RABGAP1L-ROS1		Off-label
		talazoparib		BRCA2	PARP1,PARP2	On compendia
	3	axitinib	81	TP53	FLT1,KDR,VEGFA	Off-label
		ceritinib		RABGAP1L-ROS1		Off-label
		olaparib		BRCA2	PARP1,PARP2	On compendia
	2	cabozantinib	82	RABGAP1L-ROS1, TP53	FLT1,KDR,VEGFA	Off-label
		olaparib		BRCA2	PARP1,PARP2	On compendia
	2	cabozantinib	82	RABGAP1L-ROS1, TP53	FLT1,KDR,VEGFA	Off-label
		talazoparib		BRCA2	PARP1,PARP2	On compendia
	2	cabozantinib	82	RABGAP1L-ROS1, TP53	FLT1,KDR,VEGFA	Off-label
		niraparib		BRCA2	PARP1,PARP2	Off-label
	1	cabozantinib	50	TP53	FLT1,KDR,VEGFA	Off-label
	1	olaparib	33	BRCA2	PARP1,PARP2	On compendia
	1	talazoparib	33	BRCA2	PARP1,PARP2	On compendia

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
B224_1	3	copanlisib	97	PIK3CA		Off-label
		mifepristone		PGR		Off-label
		fulvestrant		CYP19A1	ESR1	On compendia
	3	copanlisib	97	PIK3CA		Off-label
		tamoxifen		CYP19A1	ESR1	On compendia
		mifepristone		PGR		Off-label
	3	exemestane	85	CYP19A1		On compendia
		everolimus		PIK3CA	MTOR	On compendia
		mifepristone		PGR		Off-label
	2	copanlisib	74	PIK3CA		Off-label
		fulvestrant		CYP19A1	ESR1	On compendia
	2	copanlisib	74	PIK3CA		Off-label
		tamoxifen		CYP19A1	ESR1	On compendia
	2	exemestane	61	CYP19A1		On compendia
		everolimus		PIK3CA	MTOR	On compendia
	1	fulvestrant	25	CYP19A1	ESR1	On compendia
	1	tamoxifen	25	CYP19A1	ESR1	On compendia
	1	toremifene	25	CYP19A1	ESR1	On compendia
L996_0	3	NA	NA	NA	NA	NA
	3	NA	NA	NA	NA	NA
	3	NA	NA	NA	NA	NA
	2	cabozantinib	61	TP53	FLT1, KDR, VEGFA	on compendia
		everolimus	61	STK11	MTOR	off-label
	2	cabozantinib	61	TP53	FLT1, KDR, VEGFA	on compendia
		sirolimus	61	STK11	MTOR	off-label
	2	cabozantinib	61	TP53	FLT1, KDR, VEGFA	on compendia
		temsirolimus	61	STK11	MTOR	off-label

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
	1	everolimus	37	STK11	MTOR	off-label
	1	sirolimus	37	STK11	MTOR	off-label
	1	temsirolimus	37	STK11	MTOR	off-label
L1015_0	3	abemaciclib	75	CDKN2A	CDK4	off-label
		cetuximab		EGFR		on compendia
		vandetanib		EGFR, TP53	FLT1,KDR,VEGFA	on compendia
	3	cetuximab	75	EGFR		on compendia
		palbociclib		CDKN2A	CDK4	off-label
		vandetanib		EGFR, TP53	FLT1,KDR,VEGFA	on compendia
	3	cetuximab	75	EGFR		on compendia
		ribociclib		CDKN2A	CDK4	off-label
		vandetanib		TP53	FLT1,KDR,VEGFA	on compendia
	2	abemaciclib	69	CDKN2A	CDK4	off-label
		vandetanib		EGFR, TP53	FLT1,KDR,VEGFA	on compendia
	2	palbociclib	69	CDKN2A	CDK4	off-label
		vandetanib		EGFR, TP53	FLT1,KDR,VEGFA	on compendia
	2	ribociclib	69	CDKN2A	CDK4	off-label
		vandetanib		EGFR, TP53	FLT1,KDR,VEGFA	on compendia
	1	vandetanib	45	EGFR, TP53	FLT1,KDR,VEGFA	on compendia
	1	afatinib	33	EGFR		on compendia
	1	cetuximab	33	EGFR		on compendia
L1023_0	3	NA	NA	NA	NA	NA
	3	NA	NA	NA	NA	NA
	3	NA	NA	NA	NA	NA
	2	cabozantinib	39	TP53	FLT1, KDR, VEGFA	on compendia
		sulindac		RNF43	PTGS1,PTGS2	off-label
	2	regorafenib	39	TP53	FLT1, KDR, VEGFA	off-label

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
		sulindac		RNF43	PTGS1,PTGS2	off-label
	2	sorafenib	34	TP53	FLT1, KDR, VEGFA	off-label
		sulindac		RNF43	PTGS1,PTGS2	off-label
	1	cabozantinib	25	TP53	FLT1, KDR, VEGFA	on compendia
	1	regorafenib	25	TP53	FLT1, KDR, VEGFA	off-label
	1	sorafenib	20	TP53	FLT1, KDR, VEGFA	off-label
L1024_0	3	afatinib	86	EGFR		on compendia
		atezolizumab		CD274		on compendia
		cabozantinib		MET, TP53	FLT1,KDR,VEGFA	on compendia
	3	atezolizumab	86	CD274		on compendia
		cabozantinib		MET, TP53	FLT1,KDR,VEGFA	on compendia
		erlotinib		EGFR		on compendia
	3	atezolizumab	86	CD274		on compendia
		cabozantinib		MET, TP53	FLT1,KDR,VEGFA	on compendia
		dacomitinib		EGFR		on compendia
	2	afatinib	69	EGFR		on compendia
		atezolizumab		CD274		on compendia
	2	atezolizumab	69	CD274		on compendia
		dacomitinib		EGFR		on compendia
	2	afatinib	35	EGFR		on compendia
		cabozantinib		MET, TP53	FLT1,KDR,VEGFA	on compendia
	1	afatinib	20	EGFR		on compendia
	1	dacomitinib	20	EGFR		on compendia
	1	cabozantinib	15	MET, TP53	FLT1,KDR,VEGFA	on compendia
1047_0	3	afatinib	85	EGFR		on compendia
		cabozantinib		MET,TP53	FLT1,KDR,VEGFA	on compendia
		carboplatin		BRCA2	DNA damage, PARP1, PARP2	on compendia
	3	afatinib	85	EGFR		on compendia
		cabozantinib		MET,TP53	FLT1,KDR,VEGFA	on compendia
		niraparib		BRCA2		off-label
	3	afatinib	85	EGFR		on compendia
		cabozantinib		MET,TP53	FLT1,KDR,VEGFA	on compendia
		olaparib		BRCA2	DNA damage, PARP1, PARP2	off-label

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
	2	afatinib	61	EGFR		on compendia
		cabozantinib		MET,TP53	FLT1,KDR,VEGFA	on compendia
	2	cabozantinib	61	MET,TP53	FLT1,KDR,VEGFA	on compendia
		erlotinib		EGFR		on compendia
	2	cabozantinib	61	MET,TP53	FLT1,KDR,VEGFA	on compendia
		cetuximab		EGFR		on compendia
	1	cabozantinib	37	MET,TP53	FLT1,KDR,VEGFA	on compendia
	1	vandetanib	33	EGFR, TP53	FLT1,KDR,VEGFA	on compendia
	1	carboplatin	25	BRCA2	DNA damage, PARP1, PARP2	on compendia
L1067_0	3	cabozantinib	44	TP53	FLT1, KDR, VEGFA	on compendia
		ivosidenib		IDH1		off-label
		trametinib		KRAS	MAP2K1,MAP2K2	on compendia
	3	axitinib	44	TP53	FLT1, KDR, VEGFA	off-label
		ivosidenib		IDH1		off-label
		trametinib		KRAS	MAP2K1,MAP2K2	on compendia
	3	binimetinib	44	KRAS	MAP2K1,MAP2K2	off-label
		cabozantinib		TP53	FLT1, KDR, VEGFA	on compendia
		ivosidenib		IDH1		off-label
	2	ivosidenib	34	IDH1		off-label
		trametinib		KRAS	MAP2K1,MAP2K2	on compendia
	2	binimetinib	34	KRAS	MAP2K1,MAP2K2	off-label
		ivosidenib		IDH1		off-label
	2	cabozantinib	29	TP53	FLT1, KDR, VEGFA	on compendia
		ivosidenib		IDH1		off-label
	1	ivosidenib	20	IDH1		off-label
	1	trametinib	15	KRAS	MAP2K1,MAP2K2	on compendia
	1	binimetinib	15	KRAS	MAP2K1,MAP2K2	off-label
L1093_0	3	NA	NA	NA	NA	NA
	3	NA	NA	NA	NA	NA

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
	3	NA	NA	NA	NA	NA
	2	NA	NA	NA	NA	NA
	2	NA	NA	NA	NA	NA
	2	NA	NA	NA	NA	NA
	1	erdafitinib	50	FGFR1		off-label
	1	fostamatinib	50	FGFR1		off-label
	1	lenvatinib	40	FGFR1		off-label
L1096_0	3	atezolizumab	74	CD274		on compendia
		trametinib		KRAS, MAP2K1	MAP2K1,MAP2K2	on compendia
		vandetanib		EGFR,TP53	FLT1,KDR,VEGFA	on compendia
	3	durvalumab	74	CD274		on compendia
		trametinib		KRAS, MAP2K1	MAP2K1,MAP2K2	on compendia
		vandetanib		EGFR,TP53	FLT1,KDR,VEGFA	on compendia
	3	nivolumab	74	CD274	PDCD1	on compendia
		trametinib		KRAS, MAP2K1	MAP2K1,MAP2K2	on compendia
		vandetanib		EGFR,TP53	FLT1,KDR,VEGFA	on compendia
	2	trametinib	25	KRAS, MAP2K1	MAP2K1,MAP2K2	on compendia
		vandetanib		EGFR,TP53	FLT1,KDR,VEGFA	on compendia
	2	binimetinib	25	KRAS, MAP2K1	MAP2K1,MAP2K2	off-label
		vandetanib		EGFR,TP53	FLT1,KDR,VEGFA	on compendia
	2	cobimetinib	25	KRAS, MAP2K1	MAP2K1,MAP2K2	off-label
		vandetanib		EGFR,TP53	FLT1,KDR,VEGFA	on compendia
	1	trametinib	14	KRAS, MAP2K1	MAP2K1,MAP2K2	on compendia
	1	binimetinib	14	KRAS, MAP2K1	MAP2K1,MAP2K2	off-label
	1	cabozantinib	12	MET, TP53	FLT1,KDR,VEGFA	on compendia
L1097_0	3	NA	NA	NA	NA	NA

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
	3	NA	NA	NA	NA	NA
	3	NA	NA	NA	NA	NA
	2	lenvatinib	74	FGFR2, TP53	FLT1,KDR,VEGFA	off-label
		trametinib		KRAS	MAP2K1,MAP2K2	on compendia
	2	nintedanib	74	FGFR2, TP53	FLT1,KDR,VEGFA	off-label
		trametinib		KRAS	MAP2K1,MAP2K2	on compendia
	2	regorafenib	74	FGFR2, TP53	FLT1,KDR,VEGFA	off-label
		trametinib		KRAS	MAP2K1,MAP2K2	on compendia
	1	lenvatinib	50	FGFR2, TP53	FLT1,KDR,VEGFA	off-label
	1	nintedanib	50	FGFR2, TP53	FLT1,KDR,VEGFA	off-label
	1	regorafenib	50	FGFR2, TP53	FLT1,KDR,VEGFA	off-label
L1111_0	3	NA	NA	NA	NA	NA
	3	NA	NA	NA	NA	NA
	3	NA	NA	NA	NA	NA
	2	atezolizumab	74	CD274		on compendia
		cabozantinib		MET, TP53	FLT1, KDR, VEGFA	on compendia
	2	cabozantinib	74	MET, TP53	FLT1, KDR, VEGFA	on compendia
		durvalumab		CD274		on compendia
	2	cabozantinib	74	MET, TP53	FLT1, KDR, VEGFA	on compendia
		nivolumab		CD274	PDCD1	on compendia
	1	atezolizumab	50	CD274	PDCD1	on compendia
	1	durvalumab	50	CD274	PDCD1	on compendia
	1	nivolumab	50	CD274	PDCD1	on compendia

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
L1118_0	3	NA	NA	NA	NA	NA
	3	NA	NA	NA	NA	NA
	3	NA	NA	NA	NA	NA
	2	alpelisib	74	PIK3CA		off-label
		cabozantinib		TP53	FLT1,KDR,VEGFA	on compendia
	2	cabozantinib	74	TP53	FLT1,KDR,VEGFA	on compendia
		copanlisib		PIK3CA		off-label
	2	alpelisib	74	PIK3CA		off-label
		axitinib		TP53	FLT1,KDR,VEGFA	off-label
	1	alpelisib	50	PIK3CA		off-label
	1	copanlisib	50	PIK3CA		off-label
	1	cabozantinib	50	TP53	FLT1,KDR,VEGFA	on compendia
L7005_0	3	NA	NA	NA	NA	NA
	3	NA	NA	NA	NA	NA
	3	NA	NA	NA	NA	NA
	2	cabozantinib	74	TP53	FLT1,KDR,VEGFA	on compendia
		carboplatin		BRCA2	DNA damage, PARP1,PARP2	on compendia
	2	cabozantinib	74	TP53	FLT1,KDR,VEGFA	on compendia
		niraparib		BRCA2	PARP1,PARP2	off-label
	2	cabozantinib	74	TP53	FLT1,KDR,VEGFA	on compendia
		olaparib		BRCA2	PARP1,PARP2	off-label

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
	1	carboplatin	50	BRCA2	DNA damage, PARP1,PARP2	on compendia
	1	niraparib	50	BRCA2	PARP1,PARP2	off-label
	1	olaparib	50	BRCA2	PARP1,PARP2	off-label
L7027_0	3	afatinib	70	EGFR		on compendia
		atezolizumab		CD274		on compendia
		trametinib		KRAS	MAP2K1, MAP2K2	on compendia
	3	afatinib	70	EGFR		on compendia
		durvalumab		CD274	MAP2K1, MAP2K2	on compendia
		trametinib		KRAS		on compendia
	3	afatinib	70	EGFR		on compendia
		nivolumab		CD274		on compendia
		trametinib		KRAS	MAP2K1, MAP2K2	on compendia
	2	cabozantinib	21	MET		on compendia
		trametinib		KRAS	MAP2K1, MAP2K2	on compendia
	2	binimetinib	21	KRAS	MAP2K1, MAP2K2	off-label
		cabozantinib		MET		on compendia
	2	binimetinib	21	KRAS	MAP2K1, MAP2K2	off-label
		vandetanib		EGFR		on compendia
	1	trametinib	9	KRAS	MAP2K1, MAP2K2	on compendia
	1	binimetinib	9	KRAS	MAP2K1, MAP2K2	off-label
	1	cobimetinib	6	KRAS	MAP2K1, MAP2K2	off-label
L7098_0	3	NA	NA	NA	NA	NA
	3	NA	NA	NA	NA	NA
	3	NA	NA	NA	NA	NA
	2	abemaciclib	57	CCND1	CDK4,CDK6,MTOR	off-label
		cabozantinib		TP53	FLT1,KDR,VEGFA	on compendia
	2	cabozantinib	57	TP53	FLT1,KDR,VEGFA	on compendia

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
		palbociclib		CCND1	CDK4,CDK6,MTOR	off-label
	2	cabozantinib	57	TP53	FLT1,KDR,VEGFA	on compendia
		ribociclib		CCND1	CDK4,CDK6,MTOR	off-label
	1	abemaciclib	33	CCND1	CDK4,CDK6,MTOR	off-label
	1	palbociclib	33	CCND1	CDK4,CDK6,MTOR	off-label
	1	ribociclib	33	CCND1	CDK4,CDK6,MTOR	off-label
L7108_0	3	atezolizumab	97	CD274		on compendia
		dabrafenib		BRAF		on compendia
		trametinib		BRAF	MAP2K1,MAP2K2	on compendia
	3	dabrafenib	97	BRAF		on compendia
		durvalumab		CD274		on compendia
		trametinib		BRAF	MAP2K1,MAP2K2	on compendia
	3	dabrafenib	97	BRAF		on compendia
		nivolumab		CD274	PDCD1	on compendia
		trametinib		BRAF	MAP2K1,MAP2K2	on compendia
	2	atezolizumab	98	CD274		on compendia
		dabrafenib		BRAF		on compendia
	2	dabrafenib	98	BRAF		on compendia
		durvalumab		CD274		on compendia
	2	dabrafenib	98	BRAF		on compendia
		nivolumab		CD274	PDCD1	on compendia
	1	atezolizumab	50	CD274		on compendia
	1	durvalumab	50	CD274		on compendia
	1	nivolumab	50	CD274	PDCD1	on compendia
L7120_0	3	atezolizumab	74	CD274	PDCD1	on compendia
		trametinib		KRAS	MAP2K1,MAP2K2	on compendia
		vandetanib		EGFR,TP53	FLT1,KDR,VEGFA	on compendia
	3	durvalumab	74	CD274	PDCD1	on compendia
		trametinib		KRAS	MAP2K1,MAP2K2	on compendia
		vandetanib		EGFR,TP53	FLT1,KDR,VEGFA	on compendia
	3	nivolumab	74	CD274	PDCD1	on compendia
		trametinib		KRAS	MAP2K1,MAP2K2	on compendia
		vandetanib		EGFR,TP53	FLT1,KDR,VEGFA	on compendia

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
	2	trametinib	26	KRAS	MAP2K1,MAP2K2	on compendia
		vandetanib		EGFR,TP53	FLT1,KDR,VEGFA	on compendia
	2	binimetinib	26	KRAS	MAP2K1,MAP2K2	off-label
		vandetanib		EGFR,TP53	FLT1,KDR,VEGFA	on compendia
	2	cobimetinib	23	KRAS	MAP2K1,MAP2K2	off-label
		vandetanib		EGFR,TP53	FLT1,KDR,VEGFA	on compendia
	1	trametinib	9	KRAS	MAP2K1,MAP2K2	on compendia
	1	binimetinib	9	KRAS	MAP2K1,MAP2K2	off-label
	1	cabozantinib	6	TP53	FLT1,KDR,VEGFA	on compendia
L1081_0	3	afatinib	89	EGFR, ERBB2		on compendia
		bosutinib		HCK,SRC		off-label
		cabozantinib		MET,TP53	FLT1,KDR,VEGFA	on compendia
	3	bosutinib	89	HCK,SRC		off-label
		cabozantinib		MET,TP53		on compendia
		lapatinib		EGFR, ERBB2		off-label
	3	cabozantinib	81	MET,TP53	FLT1,KDR,VEGFA	on compendia
		cetuximab		EGFR		on compendia
		ibrutinib		EGFR,ERBB2,HCK,SRC		off-label
	2	cabozantinib	80	MET,TP53	FLT1,KDR,VEGFA	on compendia
		ibrutinib		EGFR,ERBB2,HCK,SRC		off-label
	2	crizotinib	71	MET		on compendia
		ibrutinib		EGFR,ERBB2,HCK,SRC		off-label
	2	afatinib	65	EGFR,ERBB2		on compendia
		bosutinib		HCK,SRC		off-label
	1	ibrutinib	56	EGFR,ERBB2,HCK,SRC		off-label
	1	vandetanib	40	EGFR,HCK,SRC,TP53	FLT1,KDR,VEGFA	on compendia
	1	brigatinib	35	EGFR,ERBB2,HCK,SRC		on compendia
L1172_0	3	atezolizumab	90	Immunotherapy via MSH2		on compendia
		cabozantinib		TP53	FLT1,KDR,VEGFA	on compendia
		trametinib		KRAS	MAP2K1,MAP2K2	on compendia
	3	cabozantinib	90	TP53	FLT1,KDR,VEGFA	on compendia
		durvalumab		Immunotherapy via MSH2		on compendia
		trametinib		KRAS	MAP2K1,MAP2K2	on compendia

Patient ID	Drug combo	Drugs	% Match	Targets involved	Pathway involved	Indication
	3	cabozantinib	90	TP53	FLT1,KDR,VEGFA	on compendia
		nivolumab		Immunotherapy via MSH2		on compendia
		trametinib		KRAS	MAP2K1,MAP2K2	on compendia
	2	atezolizumab	86	Immunotherapy via MSH2		on compendia
		trametinib		KRAS	MAP2K1,MAP2K2	on compendia
	2	durvalumab	86	Immunotherapy via MSH2		on compendia
		trametinib		KRAS	MAP2K1,MAP2K2	on compendia
	2	nivolumab	86	Immunotherapy via MSH2		on compendia
		trametinib		KRAS	MAP2K1,MAP2K2	on compendia
	1	atezolizumab	79	Immunotherapy via MSH2		on compendia
	1	durvalumab	79	Immunotherapy via MSH2		on compendia
	1	nivolumab	79	Immunotherapy via MSH2		on compendia
L7162_0	3	atezolizumab	65	CD274	PDCD1	on compendia
		carboplatin		BRCA2	DNA damage, PARP1,PARP2	on compendia
		trametinib		KRAS	MAP2K1,MAP2K2	on compendia
	3	atezolizumab	65	CD274	PDCD1	on compendia
		niraparib		BRCA2	PARP1,PARP2	off-label
		trametinib		KRAS	MAP2K1,MAP2K2	on compendia
	3	atezolizumab	65	CD274	PDCD1	on compendia
		olaparib		BRCA2	PARP1,PARP2	off-label
		trametinib		KRAS	MAP2K1,MAP2K2	on compendia
	2	atezolizumab	59	CD274	PDCD1	on compendia
		carboplatin		BRCA2	DNA damage, PARP1,PARP2	on compendia
	2	atezolizumab	59	CD274	PDCD1	on compendia
		niraparib		BRCA2	PARP1,PARP2	off-label
	2	atezolizumab	59	CD274	PDCD1	on compendia
		olaparib		BRCA2	PARP1,PARP2	off-label
	1	atezolizumab	50	CD274	PDCD1	on compendia
	1	durvalumab	50	CD274	PDCD1	on compendia
	1	nivolumab	50	CD274	PDCD1	on compendia

Supplementary Table 12. Overlapping drugs from treatment recommendations across all three platforms [Table originally published in ESMO Open (Perakis et al., 2020)]

Patient ID	CM, NAVIFY	NAVIFY, QCI	CM, QCI	Total drugs CM	Total drugs NAVIFY	Total drugs QCI
C127_10	None	None	**1 drug overlap** bevacizumab	7	0	14
C109_8	**2 drug overlap** cetuximab panitumumab	**2 drug overlap** cetuximab panitumumab	**2 drug overlap** cetuximab panitumumab	8	3	6
C154_17	None	None	**4 drug overlap** nivolumab cetuximab panitumumab pembrolizumab	7	1	17
C139_10	None	None	None	5	2	0
C140_11	None	None	**2 drug overlap** cetuximab panitumumab	6	0	16
C196_8	**2 drug overlap** cetuximab panitumumab	**2 drug overlap** cetuximab panitumumab	**2 drug overlap** cetuximab panitumumab	8	4	5
C217_14	**1 drug overlap** cetuximab	None	None	8	8	11
C128_6	None	None	**2 drug overlap** cetuximab panitumumab	6	1	16
C118_2	None	None	**3 drug overlap** cetuximab panitumumab regorafenib	7	1	16
C166_8	None	None	**2 drug overlap** cetuximab panitumumab	7	1	16
C208_11	**2 drug overlap** cetuximab panitumumab	**2 drug overlap** cetuximab panitumumab	**2 drug overlap** cetuximab panitumumab	8	3	7
C123_1	**2 drug overlap** cetuximab panitumumab	**2 drug overlap** cetuximab panitumumab	**2 drug overlap** cetuximab panitumumab	6	3	7
C129_6	**2 drug overlap** cetuximab panitumumab	**2 drug overlap** cetuximab panitumumab	**2 drug overlap** cetuximab panitumumab	6	2	8

Patient ID	CM, NAVIFY	NAVIFY, QCI	CM, QCI	Total drugs CM	Total drugs NAVIFY	Total drugs QCI
C182_10	**2 drug overlap** cetuximab panitumumab	**2 drug overlap** cetuximab panitumumab	**2 drug overlap** cetuximab panitumumab	5	3	6
C218_8	None	None	**2 drug overlap** irinotecan cetuximab	7	6	18
C219_14	None	None	None	7	0	17
C396_1	**1 drug overlap** cetuximab	None	None	8	8	11
B163_9	**3 drug overlap** lapatinib olaparib talazoparib	**5 drug overlap** lapatinib neratinib trastuzumab olaparib talazoparib	**5 drug overlap** olaparib talazoparib tamoxifen fulvestrant lapatinib	8	9	28
B164_6	None	None	**1 drug overlap** lapatinib	5	0	6
B165_5	None	**2 drug overlap** lapatinib neratinib	**2 drug overlap** fulvestrant tamoxifen	8	8	27
B166_6	None	None	**1 drug overlap** alpelisib	3	0	7
B176_8	**1 drug overlap** crizotinib	None	**1 drug overlap** lapatinib	8	1	7
B205_1	None	None	None	5	0	0
B206_1	None	None	None	5	0	2
B213_1	None	**2 drug overlap** alpelisib fulvestrant	None	5	2	10
B216_1	**1 drug overlap** fulvestrant	**2 drug overlap** alpelisib fulvestrant	**1 drug overlap** fulvestrant	7	2	8
B219_1	**2 drug overlap** alpelisib fulvestrant	**2 drug overlap** alpelisib fulvestrant	**2 drug overlap** alpelisib fulvestrant	8	2	4
B221_1	None	None	**2 drug overlap** olaparib talazoparib	7	1	13
B224_1	**1 drug overlap**	None	None	7	2	0

Patient ID	CM, NAVIFY	NAVIFY, QCI	CM, QCI	Total drugs CM	Total drugs NAVIFY	Total drugs QCI
	fulvestrant					
L996_0	None	None	None	5	0	4
L1015_0	None	None	**1 drug combination** cetuximab	6	0	15
L1023_0	None	None	None	4	0	5
L1024_0	**3 drug overlap** afatinib dacomitinib erlotinib	**6 drug overlap** afatinib dacomitinib erlotinib gefitinib osimertinib crizotinib	**4 drug overlap** afatinib atezolizumab dacomitinib erlotinib	5	6	24
L1047_0	None	**1 drug overlap** crizotinib	**2 drug overlap** cetuximab erlotinib	8	1	16
L1067_0	None	None	None	6	0	9
L1093_0	None	None	None	3	0	5
L1096_0	None	**1 drug overlap** crizotinib	**2 drug overlap** atezolizumab nivolumab	7	1	17
L1097_0	None	None	None	4	0	9
L1111_0	None	None	**1 drug overlap** nivolumab	4	0	3
L1118_0	**1 drug overlap** alpelisib	None	None	5	2	7
L7005_0	None	None	None	4	0	5
L7027_0	None	**1 drug overlap** crizotinib	**2 drug overlap** atezolizumab nivolumab	9	1	17
L7098_0	None	None	None	4	0	4
L7108_0	None	None	**1 drug overlap** dabrafenib	5	0	4
L7120_0	None	None	**2 drug overlap** atezolizumab nivolumab	7	0	16
L1081_0	**1 drug overlap** crizotinib	**1 drug overlap** crizotinib	**5 drug overlap** crizotinib brigatinib cetuximab	9	1	20

Patient ID	CM, NAVIFY	NAVIFY, QCI	CM, QCI	Total drugs CM	Total drugs NAVIFY	Total drugs QCI
			afatinib lapatinib			
L1172_0	None	None	**2 drug overlap** atezolizumab nivolumab	5	0	9
L7162_0	None	None	**2 drug overlap** atezolizumab nivolumab	7	2	12

Supplementary Table 13. Overlapping drug recommendations have varying justifications based on target [Table originally published in ESMO Open (Perakis et al., 2020)]

Drug		CureMatch	NAVIFY	QCI
bevacizumab				
	Target	APC		KRAS
	Pathway (if applicable)	PTGS1,PTGS2		
cetuximab/panitumumab				
	Target	EGFR amp	KRAS/NRAS WT status	EGFR amp
	Pathway (if applicable)			
nivolumab				
	Target	MSH2/MSH6 loss		KRAS
	Pathway (if applicable)			
pembrolizumab				
	Target	MSH2/MSH6 loss		MSH2 loss
	Pathway (if applicable)			
regorafenib				
	Target	TP53,RET, FLT1 amp		FLT1 amp, FLT3 amp
	Pathway (if applicable)	FLT1,KDR		
lapatinib				
	Target	ERBB2 amp, SMAD4	ERBB2 amp	ERBB2 amp, ERBB2 mutation
	Pathway (if applicable)	EGFR,ERBB2,MAP2K1,MAP2K2		
fulvestrant				
	Target	ESR1 protein	PIK3CA mutation	ERBB2 amp
	Pathway (if applicable)			

Drug		CureMatch	NAVIFY	QCI
	Target			
	Pathway (if applicable)			
atezolizumab				
	Target	CD274 (protein)		KRAS
	Pathway (if applicable)	PDCD1		
nivolumab				
	Target	CD274 (protein)		KRAS
	Pathway (if applicable)	PDCD1		
brigatinib				
	Target	EGFR amp, ERBB2 amp, HCK amp, SRC amp		
	Pathway (if applicable)			

APPENDIX

Attachment 1. Exemplary report submitted to the company CureMatch for Bionov analysis

SAMPLE ID	C118_2
TUMOR ENTITY	Colon
PATIENT GENDER	Female
PATIENT AGE AT SAMPLE COLLECTION	47
ANALYSIS METHODS	mFAST-SeqS, plasma-Seq, iChorCNA, Avenio ctDNA Expanded Kit
iChorCNA % TUMOR FRACTION	34.94
mFAST-SeqS GENOME-WIDE Z-SCORE	Not analyzed

SUMMARY OF ALTERATIONS

AVENIO RESULTS

GENE	*RET*
GENOMIC POSITION (hg38)	chr10:43124907
TRANSCRIPT	ENST00000340058.5
CODING CHANGE	c.2964G>A
AMINO ACID CHANGE	p.Trp988*
VARIANT DESCRIPTION	Stop gained
ALLELE FRACTION %	1.48
VARIANT DEPTH	65
UNIQUE DEPTH	4391
MUTANT MOLECULES PER ML	1620
EXAC OVERALL FREQUENCY %	not listed
DBSNP ID	not listed
COSMIC ID	not listed
LEADING THREE REPORTED PRIMARY TISSUES (COSMIC)	not listed
LEADING THREE REPORTED PRIMARY CANCER TYPES	not listed
CLASSIFICATION (HUMAN GENETICS GRAZ)	pathogenic

GENE	*KRAS*
GENOMIC POSITION (hg38)	chr12:25245351
TRANSCRIPT	ENST00000256078.8
CODING CHANGE	c.34G>T
AMINO ACID CHANGE	p.Gly12Cys
VARIANT DESCRIPTION	Missense variant
ALLELE FRACTION %	57.42
VARIANT DEPTH	3537
UNIQUE DEPTH	6160
MUTANT MOLECULES PER ML	62700

EXAC OVERALL FREQUENCY %	0.00198
DBSNP ID	rs121913530
COSMIC ID	COSM1140136, COSM516
LEADING THREE REPORTED PRIMARY TISSUES (COSMIC)	lung, large intestine, pancreas
LEADING THREE REPORTED PRIMARY CANCER TYPES	LUAD, COAD, READ
CLASSIFICATION (HUMAN GENETICS GRAZ)	pathogenic

GENE	*TP53*
GENOMIC POSITION (hg38)	chr17:7673728
TRANSCRIPT	ENST00000269305.8
CODING CHANGE	c.892G>T
AMINO ACID CHANGE	p.Glu298*
VARIANT DESCRIPTION	Stop gained
ALLELE FRACTION %	66
VARIANT DEPTH	3181
UNIQUE DEPTH	4820
MUTANT MOLECULES PER ML	72000
EXAC OVERALL FREQUENCY %	not listed
DBSNP ID	rs201744589
COSMIC ID	COSM10710, COSM121080, COSM1646820, COSM3723940
LEADING THREE REPORTED PRIMARY TISSUES (COSMIC)	lung, upper aerodigestive tract, oesophagus
LEADING THREE REPORTED PRIMARY CANCER TYPES	LUSC, HNSC, LUAD
CLASSIFICATION (HUMAN GENETICS GRAZ)	pathogenic

GENE	*APC*
GENOMIC POSITION (hg38)	chr5:112838399
TRANSCRIPT	ENST00000257430.8
CODING CHANGE	c.2805C>A
AMINO ACID CHANGE	p.Tyr935*
VARIANT DESCRIPTION	Stop gained
ALLELE FRACTION %	34.08
VARIANT DEPTH	1740
UNIQUE DEPTH	5106
MUTANT MOLECULES PER ML	37200
EXAC OVERALL FREQUENCY %	not listed
DBSNP ID	rs137854575
COSMIC ID	COSM19031
LEADING THREE REPORTED PRIMARY TISSUES (COSMIC)	large intestine

LEADING THREE REPORTED PRIMARY CANCER TYPES	COAD
CLASSIFICATION (HUMAN GENETICS GRAZ)	pathogenic

GENE	*EGFR*
GENOMIC POSITION (hg38)	chr7:55174047
TRANSCRIPT	ENST00000275493.6
CODING CHANGE	c.2184+4A>T
AMINO ACID CHANGE	
VARIANT DESCRIPTION	Splice region variant & Intron variant
ALLELE FRACTION %	0.19
VARIANT DEPTH	17
UNIQUE DEPTH	9004
MUTANT MOLECULES PER ML	206
EXAC OVERALL FREQUENCY %	not listed
DBSNP ID	not listed
COSMIC ID	not listed
LEADING THREE REPORTED PRIMARY TISSUES (COSMIC)	not listed
LEADING THREE REPORTED PRIMARY CANCER TYPES	not listed
CLASSIFICATION (HUMAN GENETICS GRAZ)	pathogenic

GENE	*AR*
GENOMIC POSITION (hg38)	chrX:67545978
TRANSCRIPT	ENST00000374690.7
CODING CHANGE	c.832G>A
AMINO ACID CHANGE	p.Ala278Thr
VARIANT DESCRIPTION	Missense variant
ALLELE FRACTION %	0.31
VARIANT DEPTH	31
UNIQUE DEPTH	9896
MUTANT MOLECULES PER ML	342
EXAC OVERALL FREQUENCY %	not listed
DBSNP ID	not listed
COSMIC ID	not listed
LEADING THREE REPORTED PRIMARY TISSUES (COSMIC)	not listed
LEADING THREE REPORTED PRIMARY CANCER TYPES	not listed
CLASSIFICATION (HUMAN GENETICS GRAZ)	UV

GENE AMPLIFICATION	**GENOMIC SEGMENT (hg38)**	**CNV SCORE**
MET	chr7:37033703-148811780	15.6710453

EGFR	chr7:37033703-148811780	15.84463978

PLASMA-SEQ RESULTS

GENE	CHROMOSOME	START (hg19)	END (hg19)	SEGMENT LOG2	SIZE (MB)	TYPE
FLT1, FLT3	13	26919024	29399845	1.875	2.48	AMP

METHODS

1) **mFAST-SeqS**[1]
   mFAST-SeqS is a fast and cost-effective prescreening method for the identification of plasma samples with increased amounts of ctDNA without a priori knowledge of alterations in the respective tumor genome. With this approach, samples suitable for further extensive qualitative analysis can be selected by estimating ctDNA fraction via the calculated genome-wide z-score.
2) **Plasma-Seq**[2]
   Copy number analysis based on the quantification of sequence information throughout the genome through whole-genome sequencing. Identification of focal events from segmented copy number data. Criteria for a focal event: segment size <20Mb; log2 ratios > 0.2 or <-0.2; <100 genes in the segment; Difference in log2 ratio to adjacent segment > 0.2 if known tumor driver gene affected or > 0.58 in unknown tumor driver genes.
   Limitations
   For detection of copy number aberrations and point mutations, a tumor-specific DNA content of at least 5-10% in the plasma is necessary.
3) **iChorCNA**[3] **for estimating tumor fraction**
   ichorCNA uses a probabilistic model, implemented as a hidden Markov model (HMM), to simultaneously segment the genome, predict large-scale copy number alterations, and estimate the tumor fraction of a ultra-low-pass whole genome sequencing sample (ULP-WGS).
4) **Avenio ctDNA Expanded Kit**
   The AVENIO ctDNA Expanded Kit is a next-generation sequencing (NGS) liquid biopsy assay with a 77 gene panel containing genes in U.S. National Comprehensive Cancer Network (NCCN) Guidelines[4] and emerging cancer biomarkers. The Expanded Kit is a pan-cancer assay that is especially optimized for lung cancer and colorectal cancer (CRC). Analytical performance supported by integrated digital error suppression (iDES) strategies combining molecular barcodes with in silico error suppression techniques.[5]

DEFINITIONS

1) **Focal events**[6]

Focal amplifications were defined as follows: segment <20 Mb; log2-ratio >0,2; segment should contain at least 1 but not more than 100 genes; log2-ratio must be 0.2 higher than weighted mean of the log2-ratios of neighboring 20Mb on both sides if it harbors a known tumor-related driver genes; log2-ratio must be 0.58 higher than weighted mean of the log2-ratios of neighboring 20Mb on both sides if it does not contain a known tumor driver gene; segment should not contain segmental duplications in >50% of its size; segment should not overlap with known entries in DGVar.

Focal deletions were defined as follows: segment should be <20 Mb; log2-ratio must be lower than -0.2; segment should contain at least 1 but not more than 100 genes; log2-ratio must be 0.2 lower

than weighted mean of the log2-ratios of neighbouring 20 Mb on both the sides; segment should not contain segmental duplications in >50% of its size; segment should not overlap with known entries in DGVar.

ROCHE AVENIO ctDNA EXPANDED PANEL ASSAY TARGETS (192kb, 77 genes)

Gene	Seg Target	SNV	Indel*	Fusion**	CNV**
ABL1	Selected Regions	●			
AKT1	Selected Regions	●			
AKT2	Selected Regions	●			
ALK	Selected Regions	●	●	●	
APC	Selected Regions	●	●		
AR	All Coding Regions	●			
ARAF	Selected Regions	●			
BRAF	Selected Regions	●	●		
BRCA1	All Coding Regions	●			
BRCA2	All Coding Regions	●			
CCND1	All Coding Regions	●			
CCND2	All Coding Regions	●			
CCND3	All Coding Regions	●			
CD274	All Coding Regions	●			
CDK4	All Coding Regions	●			
CDK6	Selected Regions	●			
CDKN2A	All Coding Regions	●			
CSF1R	Selected Regions	●			
CTNNB1	Selected Regions	●	●		
DDR2	Selected Regions	●			
DPYD	Selected Regions	●			
EGFR	All Coding Regions	●	●		●
ERBB2	All Coding Regions	●	●		●
ESR1	All Coding Regions	●			
EZH2	Selected Regions	●			
FBXW7	All Coding Regions	●			

Gene	Seg Target	SNV	Indel*	Fusion**	CNV**
FGFR1	Selected Regions	●			
FGFR2	Selected Regions	●		●	
FGFR3	Selected Regions	●		●	
FLT1	Selected Regions	●			
FLT3	Selected Regions	●			
FLT4	Selected Regions	●			
GATA3	Selected Regions	●			
GNA11	Selected Regions	●			
GNAQ	Selected Regions	●			
GNAS	Selected Regions	●			
IDH1	Selected Regions	●			
IDH2	Selected Regions	●			
JAK2	Selected Regions	●			
JAK3	Selected Regions	●			
KDR	Selected Regions	●			
KEAP1	All Coding Regions	●			
KIT	Selected Regions	●	●		
KRAS	All Coding Regions	●			
MAP2K1	Selected Regions	●			
MAP2K2	Selected Regions	●			
MET	All Coding Regions	●	●		●
MLH1	All Coding Regions	●			
MSH2	All Coding Regions	●			
MSH6	All Coding Regions	●			
MTOR	Selected Regions	●			
NF2	All Coding Regions	●			
NFEL2	Selected Regions	●			
NRAS	Selected Regions	●			
NTRK1	Selected Regions	●		●	
PDC1LG2	All Coding Regions	●			
PDGFRA	Selected Regions	●			
PDGFRB	Selected Regions	●			
PIK3CA	Selected Regions	●	●		
PIK3R1	Selected Regions	●			

Gene	Seg Target	SNV	Indel*	Fusion**	CNV**
PMS2	All Coding Regions	●			
PTCH1	Selected Regions	●			
PTEN	All Coding Regions	●	●		
RAF1	Selected Regions	●			
RB1	All Coding Regions	●			
RET	Selected Regions	●		●	
RNF43	Selected Regions	●			
ROS1	Selected Regions	●		●	
SMAD4	All Coding Regions	●			
SMO	All Coding Regions	●			
STK11	All Coding Regions	●			
TP53	All Coding Regions	●			
TERT promoter	Selected Regions	●			
TSC1	Selected Regions	●	●		
TSC2	Selected Regions	●			
UGT1A1***	Selected Regions	●			
VHL	All Coding Regions	●			

All coding regions are based on the longest transcript from Ensembl build 82.
*Indels are limited to variants in a pre-specified list of positions, referred to as "Loci of Interest", except for EGFR exon 19 long deletions, EGFR exon 20 long insertions and MET long insertions, which are not restricted to a pre-defined set of indels.
**Detection of fusions and CNVs are limited to variants in a pre-specified list of positions, referred to as "Loci of Interest" in the AVENIO analysis software.
***UGT1A1*28 allele sequenced but not currently called by the AVENIO analysis software.

REFERENCES

1. Belic, J. et al. Rapid identification of plasma DNA samples with increased ctDNA levels by a modified FAST-SeqS approach. (2015) Clin. Chem. 61, 838–849.
2. Heitzer, E. et al. Tumor associated copy number changes in the circulation of patients with prostate cancer identified through whole-genome sequencing. (2013) Genome Med. 5, 30.
3. Adalsteinsson, Ha, Freeman, et al. Scalable whole-exome sequencing of cell-free DNA reveals high concordance with metastatic tumors. (2017) Nature Communications Nov 6;8(1):1324.)
4. National Comprehensive Cancer Network. October 15, 2016.
5. Newman AM, Bratman SV, To J, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. (2014) Nature Medicine20(5):548–554 doi:10.1038/nm.3519.
6. Ulz, P. et al. Whole-genome plasma sequencing reveals focal amplifications as a driving force in metastatic prostate cancer. (2016) Nat. Commun. 7, 12008.

PreciGENE™

Personalized Combination Therapy®

CureMatch ID	**CM_01287**
Age	66
Gender	Male
Program Version	PEMSOL v2.3 on 6/1/2019
Report Date	6/3/2019
Diagnosis	Colon Cancer
Sample Type	ROCHE Avenio ctDNA NGS liquid biopsy (77 genes panel)

Thank you for choosing CureMatch.

This report provides a ranking of the top treatment options that are personalized for each individual patient, using the molecular profile of a patient's tumor and proprietary databases and algorithms developed by CureMatch.

OVERVIEW

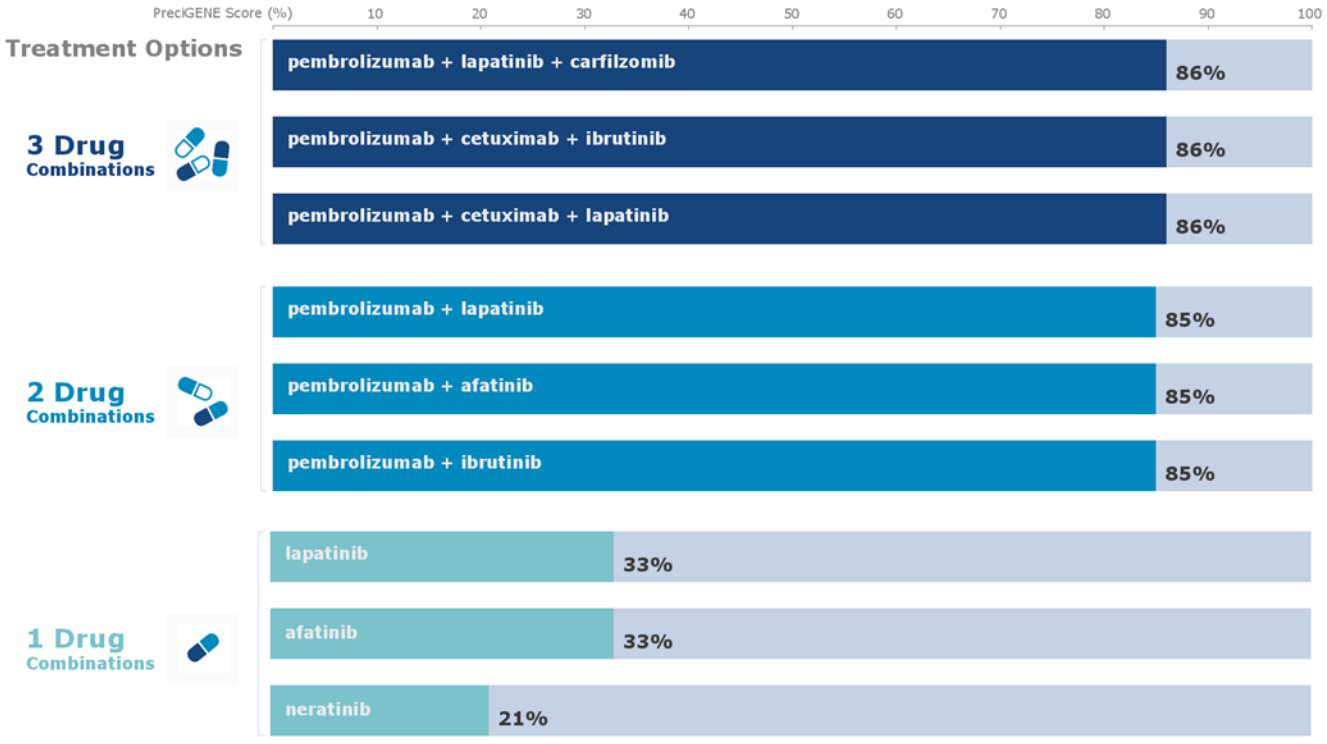
Below is an overview of the results of the CureMatch analysis. The graphic at the bottom provides a snapshot of all possible combinations of 1, 2 or 3 drug(s) that were considered in the analysis, ordered by descending PreciGENE Score. Definitions of these terms and the details on the treatment options are provided on subsequent pages of the report.

6 ACTIONABLE MARKERS

24 MATCHING DRUGS

2324 RELEVANT COMBINATIONS

4.5 M COMBINATIONS CONSIDERED



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DESCRIPTION OF MARKERS

Detailed description of actionable markers is given in subsequent pages of the report.

MARKERS OF KNOWN SIGNIFICANCE

GENOME VARIANTS

MARKER	DESCRIPTION	ACTIONABLE
APC	R876*	**Yes**
APC	S1356*	**Yes**
CCND3	c.575-1G>C	**Yes**
CCNE1	amplification	**Yes**
EGFR	amplification	**Yes**
ERBB2	amplification	**Yes**
MSH6	c.3646+4A>C	**Yes**

PROTEIN VARIANTS

MARKER	DESCRIPTION	ACTIONABLE
None		

REPORTED POLYMORPHISMS

MARKER	DESCRIPTION	ACTIONABLE
None		

MARKERS OF UNKNOWN SIGNIFICANCE

MARKER	DESCRIPTION	ACTIONABLE
RET	A1020V	No
CDC6	amplification	No
GRB7	amplification	No
SETDB1	amplification	No
STARD3	amplification	No

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TOP RANKED COMBINATION THERAPIES USING A MAXIMUM OF 3 DRUGS

PRECIGENE SCORE	DRUGS	TARGETING DESCRIPTION	INDICATIONS & RECOMMENDATIONS	
			ON COMPENDIA	OFF-LABEL
86%	**carfilzomib**	CCNE1 via proteasome pathway		✓
	lapatinib	EGFR ERBB2	✓	
	pembrolizumab	Immunotherapy targeting MSH6	✓	
	Sulindac (NSAID) or celecoxib (NSAID) may be added to this combination. ***FDA black-box warning:*** **- lapatinib**: hepatotoxicity ***Drug-drug interactions:*** There may be drug-drug interactions that are not listed here. Administering drug combinations is at the discretion of the physician. ***Examples of existing clinical trials or publications using similar drug(s):*** While clinical trials using these drugs alone or in combination with other drugs exist, no clinical trial testing this exact association of drugs can be found.			
86%	**cetuximab**	EGFR	✓	
	ibrutinib	EGFR ERBB2		✓
	pembrolizumab	Immunotherapy targeting MSH6	✓	
	Sulindac (NSAID) or celecoxib (NSAID) may be added to this combination. ***FDA black-box warning:*** **- cetuximab**: serious infusion reactions and cardiopulmonary arrest ***Drug-drug interactions:*** There may be drug-drug interactions that are not listed here. Administering drug combinations is at the discretion of the physician. ***Examples of existing clinical trials or publications using similar drug(s):*** While clinical trials using these drugs alone or in combination with other drugs exist, no clinical trial testing this exact association of drugs can be found.			
86%	**cetuximab**	EGFR	✓	
	lapatinib	EGFR ERBB2	✓	
	pembrolizumab	Immunotherapy targeting MSH6	✓	
	Sulindac (NSAID) or celecoxib (NSAID) may be added to this combination. ***FDA black-box warning:*** **- cetuximab**: serious infusion reactions and cardiopulmonary arrest **- lapatinib**: hepatotoxicity ***Drug-drug interactions:*** There may be drug-drug interactions that are not listed here. Administering drug combinations is at the discretion of the physician. ***Examples of existing clinical trials or publications using similar drug(s):*** While clinical trials using these drugs alone or in combination with other drugs exist, no clinical trial testing this exact association of drugs can be found.			

Detailed description of drugs used in the presented combinations is given on subsequent pages.

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TOP RANKED COMBINATION THERAPIES USING A MAXIMUM OF 2 DRUGS

PRECIGENE SCORE	DRUGS	TARGETING DESCRIPTION	INDICATIONS & RECOMMENDATIONS	
			ON COMPENDIA	OFF-LABEL
85%	**lapatinib**	EGFR ERBB2	✓	
	pembrolizumab	Immunotherapy targeting MSH6	✓	
	Sulindac (NSAID) or celecoxib (NSAID) may be added to this combination. ***FDA black-box warning:*** **- lapatinib**: hepatotoxicity ***Drug-drug interactions:*** There may be drug-drug interactions that are not listed here. Administering drug combinations is at the discretion of the physician. ***Examples of existing clinical trials or publications using similar drug(s):*** While clinical trials using these drugs alone or in combination with other drugs exist, no clinical trial testing this exact association of drugs can be found.			
85%	**afatinib**	EGFR ERBB2		✓
	pembrolizumab	Immunotherapy targeting MSH6	✓	
	Sulindac (NSAID) or celecoxib (NSAID) may be added to this combination. ***Drug-drug interactions:*** There may be drug-drug interactions that are not listed here. Administering drug combinations is at the discretion of the physician. ***Examples of existing clinical trials or publications using similar drug(s):*** - NCT03157089: testing afatinib in combination with pembrolizumab in patients with squamous cell carcinoma of the lung (Active, not recruiting; Boehringer Ingelheim) - NCT02364609: pembrolizumab and afatinib in patients with non-small cell lung cancer with resistance to erlotinib (Active, not recruiting; University of California, Davis)			
85%	**ibrutinib**	EGFR ERBB2		✓
	pembrolizumab	Immunotherapy targeting MSH6	✓	
	Sulindac (NSAID) or celecoxib (NSAID) may be added to this combination. ***Drug-drug interactions:*** There may be drug-drug interactions that are not listed here. Administering drug combinations is at the discretion of the physician. ***Examples of existing clinical trials or publications using similar drug(s):*** - NCT03332498: pembrolizumab in combination with ibrutinib for advanced, refractory colorectal cancers (Recruiting; H. Lee Moffitt Cancer Center and Research Institute) - NCT03153202: study to evaluate the safety and preliminary efficacy of ibrutinib and pembrolizumab in patients with chronic lymphocytic leukemia (cll) or mantle cell lymphoma (mcl) (Recruiting; Joshua Brody, Icahn School of Medicine at Mount Sinai) - NCT03021460: pembrolizumab and ibrutinib in treating patients with stage iii-iv melanoma that cannot be removed by surgery (Recruiting; Mayo Clinic) - NCT02950220: pembrolizumab and ibrutinib in treating patients with relapsed or refractory non-hodgkin lymphoma (Active, not recruiting; Kami Maddocks, Ohio State University Comprehensive Cancer Center)			

Detailed description of drugs used in the presented combinations is given on subsequent pages.

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TOP RANKED COMBINATION THERAPIES USING A MAXIMUM OF 1 DRUG

PRECIGENE SCORE	DRUGS	TARGETING DESCRIPTION	INDICATIONS & RECOMMENDATIONS	
			ON COMPENDIA	OFF-LABEL
33%	lapatinib	EGFR ERBB2	✓	
	Sulindac (NSAID) or celecoxib (NSAID) may be added to this combination. **This output was made with the dev version.** ***FDA black-box warning:*** **- lapatinib**: hepatotoxicity ***Drug-drug interactions:*** There may be drug-drug interactions that are not listed here. Administering drug combinations is at the discretion of the physician.			
33%	afatinib	EGFR ERBB2		✓
	Sulindac (NSAID) or celecoxib (NSAID) may be added to this combination. **This output was made with the dev version.** ***Drug-drug interactions:*** There may be drug-drug interactions that are not listed here. Administering drug combinations is at the discretion of the physician.			
21%	neratinib	EGFR ERBB2		✓
	Sulindac (NSAID) or celecoxib (NSAID) may be added to this combination. **This output was made with the dev version.** ***Drug-drug interactions:*** There may be drug-drug interactions that are not listed here. Administering drug combinations is at the discretion of the physician.			

Detailed description of drugs used in the presented combinations is given on subsequent pages.

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ACTIONABLE ALTERATIONS DESCRIPTION

MARKER	DESCRIPTION OF PATHOGENECITY AND ACTIONABILITY
APC R876* S1356*	• APC acts as a tumor suppressor that acts as an antagonist of the Wnt signaling pathway. Abnormal activation of this pathway through loss of the APC protein function contributes to cancer progression. APC also associates with beta-catenin which helps control expression of particular genes and promotes cell growth, division, and maturation. Mutations in this gene are responsible for development of colorectal cancer, gastric cancer, FAP, and desmoid tumors. It controls how often a cell divides, how cells move towards or away from tissues, cell adhesion, transcriptional activation, and apoptosis. Mutations or defects in the gene cause diseases such as familial adenomatous polyposis that progress to becoming malignant. Truncated protein products are usually an indicator of disease-oriented mutations caused by a mutation of the APC gene. [1][2][3]
CCND3 c.575-1G>C	• CCND3 regulates CDK kinases. Similar to CCND1 and CCND2, this protein interacts with the tumor suppressor protein Rb, and plays a role in its phosphorylation. Cells lacking D cyclins, including cyclin D3 coded by the CCND3 gene, demonstrate less susceptibility to oncogenic transformation. [4][5][6][7]
CCNE1 amplification	• CCNE1 functions as a regulators of CDK kinases, regulating the cell cycle. This cyclin forms a complex with and functions as a regulatory subunit of CDK2, whose activity is required for cell cycle G1/S transition. This protein accumulates at the G1-S phase boundary and is degraded as cells progress through S phase. Amplification and overexpression of this gene has been observed in many tumors, which results in chromosome instability, and thus may contribute to tumorigenesis. [8] There are no therapies targeting directly CCNE1. However, CCNE1 activating alterations confer sensitivity to proteasome inhibitors, such as bortezomib, carfilzomib or ixazomib. [9][10]
EGFR amplification	• EGFR encodes the epidermal growth factor receptor, a cell surface protein that binds to different epidermal growth factors. The binding of the protein to its ligands, further dimerization and tyrosine autophosphorylation, leads to cell proliferation. Amplification and activating mutations of EGFR have been shown to be driving events in many cancer types, such as non-small cell lung cancer, glioblastoma and basal-like breast cancers. [11][12][13] • EGFR activating alterations can be targeted by EGFR small tyrosine kinase inhibitors or monoclonal antibodies. [14]
ERBB2 amplification	• ERRB2, a member of the epidermal growth factor (EGF) receptor family of receptor tyrosine kinases, binds tightly to other ligand-bound EGF receptor family members to form a heterodimer, stabilizing ligand binding and enhancing kinase-mediated activation of downstream signaling pathways. Amplification and/or overexpression of this gene has been reported in numerous cancers, including breast and ovarian tumors. [15][16][17] • ERBB2 activating alterations can be targeted by monoclonal antibodies (e.g. trastuzumab, pertuzumab) or small tyrosine kinase inhibitors (e.g. lapatinib, afatinib, neratinib).
MSH6 c.3646+4A>C	• A member of the DNA mismatch repair MutS family, MSH6 helps in the recognition of mismatched nucleotides prior to their repair. It heterodimerizes with MSH2 to form a mismatch recognition complex that functions as a bidirectional molecular switch that exchanges ADP and ATP as DNA mismatches are bound and dissociated. Mutations in this gene may be associated with hereditary nonpolyposis colon cancer, colorectal cancer, and endometrial cancer. Transcripts variants encoding different isoforms have been described. [18] Recruited on chromatin in G1 and early S phase via its PWWP domain that specifically binds trimethylated Lys-36 of histone H3 (H3K36me3): early recruitment to chromatin to be replicated allowing a quick identification of mismatch repair to initiate the DNA mismatch repair reaction. [19]

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DESCRIPTION OF DRUGS

DRUG	DESCRIPTION OF TARGETING
CETUXIMAB	• Cetuximab (ERBITUX®) is an epidermal growth factor receptor (EGFR) antagonist indicated for treatment of K-Ras wild-type / EGFR-expressing / metastatic colorectal cancer as determined by FDA approved tests. • in combination with FOLFIRI for first-line treatment; • in combination with irinotecan in patients who are refractory to irinotecan-based chemotherapy; • as a single agent in patients who have failed oxaliplatin- and irinotecan-based chemotherapy or who are intolerant to irinotecan. [20] • Cetuximab has been given to patients over 1 year old. [20]
PEMBROLIZUMAB	• Pembrolizumab (KEYTRUDA®) is a programmed death receptor-1 (PD-1)-blocking antibody indicated in: microsatellite Instability-High Cancer for the treatment of adult and pediatric patients with unresectable or metastatic, microsatellite instability-high (MSI-H) or mismatch repair deficient solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options, or colorectal cancer that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan.[21] • Pembrolizumab has been given to patients over 2 years old. [21]
PANITUMUMAB	• Panitumumab (VECTIBIX®) is an epidermal growth factor receptor (EGFR) antagonist indicated for the treatment of wild-type KRAS (exon 2) metastatic colorectal cancer (mCRC) in combination with FOLFOX for first-line treatment; or as monotherapy following disease progression after prior treatment with fluoropyrimidine, oxaliplatin, and irinotecan-containing chemotherapy. Panitumumab is not indicated for the treatment of patients with RAS-mutant mCRC or for whom RAS mutation status is unknown. [22] • Panitumumab has been given to patients over 2 years old. [22]
CARFILZOMIB	• Carfilzomib (KYPROLIS®) is a proteasome inhibitor that is indicated: In combination with dexamethasone or with lenalidomide plus dexamethasone for the treatment of patients with relapsed or refractory multiple myeloma who have received one to three lines of therapy. As a single agent for the treatment of patients with relapsed or refractory multiple myeloma who have received one or more lines of therapy. [23]
LAPATINIB	• Lapatinib (TYKERB®) is a kinase inhibitor indicated in combination with capecitabine, for the treatment of patients with advanced or metastatic breast cancer whose tumors overexpress human epidermal growth factor receptor 2 (HER2) and who have received prior therapy including an anthracycline, a taxane, and trastuzumab; letrozole for the treatment of postmenopausal women with hormone receptor-positive metastatic breast cancer that overexpresses the HER2 receptor for whom hormonal therapy is indicated. [24]
IBRUTINIB	• Ibrutinib (IMBRUVICA®) is a kinase inhibitor indicated for the treatment of patients with mantle cell lymphoma (MCL) who have received at least one prior therapy; chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL); chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL) with 17p deletion; and Waldenström's macroglobulinemia (WM). [25]
AFATINIB	• Afatinib (GILOTRIF®) is a kinase inhibitor indicated for first-line treatment of patients with metastatic non-small cell lung cancer (NSCLC) whose tumors have epidermal growth factor receptor (EGFR) exon 19 deletions or exon 21 substitutions (e.g. L858R) as detected by an FDA-approved test; or treatment of patients with metastatic squamous NSCLC progressing after platinum-based chemotherapy. [26]
NERATINIB	Neratinib (NERLYNX®) is a kinase inhibitor indicated for the extended adjuvant treatment of adult patients with early stage HER2-overexpressed/amplified breast cancer, to follow adjuvant trastuzumab-based therapy. [27]

Abbreviations:

FDA = Food and Drug Administration;

IC50 = half maximal inhibitory concentration, expressed in nanomolar concentration of the compound

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DEFINITIONS

Actionable Markers

An alteration that has a known response to an existing FDA-approved therapy (on compendia or off-label).

Combinations analyzed

Number of combinations of 1, 2, or 3 drugs that were analyzed by CureMatch, specifically for the individual patient.

Matching Drugs Considered

Total number of drugs that were found to be relevant to the patient based on their molecular profile, and that were considered in the CureMatch analysis.

On Compendia

Drug indicated by the U.S. Food and Drug Administration (FDA) or recommended by organizations/consortia providing guidance in clinical oncology practice for the pathology presented by the patient.

PreciGENE Score

The PreciGENE Score reflects the degree to which a therapy or combination of therapies matches a patient's biomarker profile. It is represented by a percentage and may be used to compare potential treatment regimens. The PreciGENE Score does not represent the percent chance of response.

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IMPORTANT TERMS

DISCLAIMER

Drugs are listed based on their potential to target tumor alterations. Risk of drug-drug interactions and dose adjustments need to be considered by the physician. All decisions regarding drug and therapy choices should be made by the physician. These results are not meant to substitute for medical advice.

TREATMENT DECISIONS ARE THE RESPONSIBILITY OF THE PHYSICIAN

Decisions regarding patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, such as patient and family history, physical examinations, information from other diagnostics tests, and patient preferences, and the standard of care in a given community. A treating physician's decisions should not be based on a single test, such as the information contained herein.

SCOPE

CureMatch makes no guarantees as to clinical benefit. CureMatch makes no promises or guarantees that a particular drug or drug combination will be safe or effective in the treatment of disease in any patient. CureMatch also makes no guarantees that a drug with potential lack of clinical benefit will in fact provide no clinical benefit.

CureMatch and its employees do not provide medical advice or medical services or endorse or select any healthcare provider or medical treatment. CureMatch assumes no liability or responsibility for information contained in the report provided, including but not limited to the efficacy of any listed treatment. The information provided is intended solely for use by a treating physician who has reviewed and understands all sections within this report, including possible limitations of the services provided by CureMatch. Making the decision to receive any medical procedure or treatment is based on the treating physician and patient's own research and advice from independent medical personnel.

POPULATION & INDIVIDUAL SPECIFIC CONSIDERATIONS

CureMatch's decision support system may not be optimized for specific populations such as children under 18 years old, pregnant or nursing women, certain ethnic groups, patients with comorbidities, and elderly patients. Furthermore, as all substances may be dangerous if misused or used at inappropriate doses, additional precautions should be taken in the case of particular clinical situations such as those involving elderly patients, intolerant/allergic patients or patients presenting comorbidities. Such considerations remain at the physician's discretion, as the physician is able to evaluate and monitor the optimal use of therapeutic drugs with respect to the patient's situation.

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Roche

Case ID
C129-6-no-COSMIC

Patient MRN
not specified

Sample collection date
not specified

Sample receipt date
not specified

Patient name
not specified

Patient sex
not specified

Diagnosis
Colorectal cancer

Sample site
not specified

Ordering physician
not specified

Patient DOB
not specified

Patient ethnicity
not specified

Sample type
not specified

Tumor purity
not specified

Ordering institution
not specified

Report summary

(8 clinically significant variants & combinations, 2 relevant therapies, 5 clinical trials)

KRAS wildtype, NRAS wildtype

EGFR amplification

ERBB2 amplification

CCNE1 amplification

APC p.R876*

APC p.S1356*

MSH6 c.3646+4A>C

CCND3 c.575-1G>C

KRAS wildtype, NRAS wildtype — Tier I-A

Therapies approved/guidelines-recommended in: **Colorectal cancer**

- **cetuximab**
- **panitumumab**

EGFR amplification — Tier I-B

no approved therapies

ERBB2 amplification — Tier I-B

no approved therapies

CCNE1 amplification — Tier II-C

no approved therapies

APC p.R876* — VAF **54%** — RD **988** — Tier II-C

no approved therapies

APC p.S1356* — VAF **0.84%** — RD **14** — Tier II-C

no approved therapies

MSH6 c.3646+4A>C VAF **13%** RD **113** Tier II-D

no approved therapies

CCND3 c.575-1G>C VAF **1.3%** RD **24** Tier II-D

no approved therapies

Clinical trials from MolecularMatch

Selected trials recruiting: **all sexes, all ages** within **Austria**

NCT03869892 Phase **3**

KRAS

Phase III Study in First-line Treatment of Patients With Metastatic Colorectal Cancer Who Are Not Candidate for Intensive Therapy.

NCT03693170 Phase **2**

EGFR

Encorafenib, Binimetinib and Cetuximab in Subjects With Previously Untreated BRAF-mutant ColoRectal Cancer

NCT03101475 Phase **2**

EGFR

Synergism of Immunomodulation and Tumor Ablation

NCT01703390 Phase **2**

EGFR

Biomarker Directed Treatment in Metastatic Colorectal Cancer

NCT01166035 Phase **1, 2**

EGFR

Lenalidomide and Cetuximab in Patients With Advanced Solid Tumors

End of findings section

KRAS wildtype, NRAS wildtype

Tier I-A

Gene clinical summary
Oncogenic KRAS missense mutations are found in cancers, including pancreatic, colorectal and lung cancer (PMID: 28666118). Hotspot mutations result in loss of GTPase function and net downstream activation (PMID: 26815308). Germline mutations in KRAS are associated with RASopathies, which confer increased cancer risk (PMID: 28957739). Currently, no effective RAS inhibitors have been developed, however emerging drugs target codon 12 and the hypervariable domain, as well as downstream effectors of RAS and regulators of RAS membrane association and activity (PMID: 25878363). Other strategies under investigation target metabolic pathways to treat KRAS-mutant tumors (PMID: 28647837). Additionally, studies have reported that KRAS activation leads to resistance to EGFR tyrosine kinase inhibitors (PMID:24758538).

Oncogenic NRAS missense mutations are found in cancers, including melanoma, colorectal, and thyroid cancer (PMID: 28666118). Hotspot mutations result in loss of GTPase function and net downstream activation (PMID: 26815308). Germline mutations in NRAS are associated with RASopathies, which confer increased cancer risk (PMID: 28957739). Currently, no effective RAS inhibitors have been developed, however emerging drugs target codon 12 and the hypervariable domain, as well as downstream effectors of RAS and regulators of RAS membrane association and activity (PMID: 25878363). Additionally, studies have reported that NRAS activation leads to resistance to EGFR tyrosine kinase inhibitors (PMID: 27279914) and anti-EGFR antibodies (PMID: 24758538).

Variant group clinical summary
The monoclonal anti-EGFR antibodies cetuximab and panitumumab are approved and recommended for certain patients with colorectal cancer harboring wild type KRAS and NRAS in a combination regimen (FDA, EMA, Health Canada, Swissmedic, NCCN, ESMO, NICE) or as monotherapy after failure of chemotherapy (FDA, EMA, Health Canada, Swissmedic).

Gene biological summary
KRAS is a member of the RAS GTPase superfamily and is a key regulator of the RAS/RAF/MEK/ERK pathway as well as the downstream PI3K/AKT/MTOR pathway, regulating cell survival, proliferation and migration. RAS proteins cycle between an inactive GDP-bound and an active GTP-bound state in response to receptor tyrosine kinase activation. Downstream effectors are recruited to the membrane and activated by RAS (PMID: 28666118). Important domains in KRAS include the catalytic domain (residues 1-166) and the hypervariable domain (residues 167-188) (PMID: 28597297).

NRAS is a member of the RAS GTPase superfamily and is a key regulator of the RAS/RAF/MEK/ERK pathway and the downstream PI3K/AKT/MTOR pathway, regulating cell survival, proliferation and migration. RAS proteins cycle between an inactive GDP-bound and an active GTP-bound state in response to receptor tyrosine kinase activation. Downstream effectors are recruited to the membrane and activated by RAS (PMID: 28666118). Important domains in NRAS include the catalytic domain (residues 1-166) and the hypervariable domain (residues 167-189) (PMID: 28597297).

Variant group functional summary
KRAS-NRAS wild type indicates that no mutation in codons 12, 13, 59, 61, 117 and 146 have been detected within the KRAS or NRAS genes.

EGFR amplification

Tier I-B

Gene clinical summary
Alterations in EGFR are found in cancers, including lung, breast, and colorectal cancer (PMID: 27461822) (PMID: 26996617). Activating deletions, indels, missense mutations and amplifications result in increased receptor activity, leading to to cellular transformation (PMID: 16377102). Multiple tyrosine kinase inhibitors and monoclonal anti-EGFR antibody therapies targeting activated EGFR have been approved and others are under clinical investigation (PMID: 27660463) (PMID: 21356164).

Variant group clinical summary
In multiple clinical studies of patients with colorectal cancer treated with EGFR antibody therapy (cetuximab, panitumumab, or cetuximab/bevacizumab combination therapy), EGFR amplification was associated with clinical response and improved overall and/or progression-free survival (PMID: 27879995) (PMID: 17664472) (PMID: 28025786) (PMID: 17974556) (PMID: 18577988) (PMID: 15863375) (PMID: 19712476) (PMID: 17940504) (PMID: 18794099).

Patients with solid tumors harboring EGFR amplifications match inclusion criteria for clinical trials, including trials with HER inhibitors (NCT01953926).

Gene biological summary
EGFR (HER1) is a tyrosine kinase receptor which responds to growth factor ligands to activate the RAS/RAF/MEK and PI3K/AKT/MTOR pathways, which regulate cell proliferation, differentiation, growth, and apoptosis (PMID: 11350724). Important domains in EGFR include the extracellular ligand binding domain (residues 25-645), the tyrosine kinase domain (residues 688-979), and the C-terminal tail (residues 980-1210) (PMID: 20388509) (UniProt.org) (PMID: 17318210).

Variant group functional summary
EGFR amplification indicates an increase in genomic copy number of the EGFR gene. EGFR amplifications result in overexpression and increased EGFR activity, and are associated with acquired drug resistance (PMID: 21430269) (PMID: 8058336).

ERBB2 amplification

Tier I-B

Gene clinical summary
Oncogenic ERBB2 alterations, such as amplification and activating mutations are implicated in several cancers including breast, lung and gastric cancer (PMID: 17471238). Several approved monoclonal antibodies and small molecule inhibitors target ERBB2 and other EGFR family members (PMID: 27596216). Emerging therapies include tyrosine kinase inhibitors, antibody-drug conjugates, immunotherapies, and other PI3K pathway inhibitors (PMID: 27249772).

Variant group clinical summary

No therapies are approved or recommended for colorectal cancer based on ERBB2 mutation status (FDA, EMA, Health Canada, Swissmedic, NCCN Guidelines for Colon and Rectal Cancer, ESMO, NICE).

ERBB2 amplification is highly correlated with ERBB2 overexpression in HER2-positive colorectal cancer (PMID: 26449765). In a phase II clinical basket trial, 18 of 57 patients with HER2-amplified colorectal cancer had an objective response to treatment with pertuzumab and trastuzumab (PMID: 30857956). In another phase II trial, upon treatment with lapatinib and trastuzumab, 10 of 33 HER2-positive colorectal cancer patients had an objective response (AACR; Cancer Res 2017;77(13 Suppl):Abstract nr CT005). In a clinical case study, a patient with metastatic colorectal adenocarcinoma harboring ERBB2 amplification demonstrated clinical benefit with stable disease in the primary tumor for 7 months following treatment with the antibody-drug conjugate ado-trastuzumab emtansine (PMID: 28040715).

In additional colorectal cancer studies, ERBB2 amplification was associated with resistance to the anti-EGFR antibodies cetuximab and panitumumab. ERBB2 amplification in 13 of 233 patients was associated with shorter progression-free and overall survival with cetuximab treatment. Increased ERRB2 expression was detected in samples from two patients with acquired cetuximab resistance (PMID: 21900593) In multivariate analyses, ERBB2 amplification in 13 of 98 patients (cohort 1) and 16 of 70 patients (cohort 2) was associated with shorter progression-free survival with anti-EGFR antibodies (JCO Precision Oncology 2019 :3, 1-13).

For certain patients with ERBB2 overexpressed/amplified breast cancer, ado-trastuzumab emtansine, trastuzumab, pertuzumab in a combination regimen with trastuzumab (FDA, EMA, Health Canada, Swissmedic, NCCN, ESMO, and NICE), lapatinib (FDA, EMA, Health Canada, Swissmedic, NCCN, and ESMO), neratinib (FDA, EMA, NCCN), and trastuzumab in combination with hyaluronidase (FDA) are approved and recommended. Trastuzumab is approved and recommended for certain patients with ERBB2 overexpressed/amplified gastric cancer (FDA, EMA, Health Canada, Swissmedic, ESMO, and NICE).

Gene biological summary
ERBB2 (HER2) is an EGFR receptor tyrosine kinase, which activates PI3K/AKT/MTOR and RAS/RAF/MEK pathways to regulate cell proliferation, differentiation, growth, and apoptosis (PMID: 11350724). ERBB2 has no known ligand and exists in a constitutively "open" conformation, always able to form heteromeric dimers with ligand-bound HER1 (EGFR), HER3, or HER4 (PMID: 12605220). The most important domains in ERBB2 are the extracellular domain (residues 23-652) and the tyrosine kinase domain (residues 720-987), which contains an autoinhibitory loop (PMID: 18039657) (UniProt.org).

Variant group functional summary
ERBB2 (HER2) amplification indicates an increased number of copies of the ERBB2 gene. These mutations are highly correlated with overexpression of ERBB2 protein (PMID: 28445098) and have been demonstrated to be oncogenic (PMID: 23400474).

CCNE1 amplification

Tier II-C

Gene clinical summary
Alterations in CCNE1 are found in cancers, including ovarian, lung, breast, and hematological cancer. Amplifications result in cyclin E overexpression, driving cell cycle progression and cell proliferation (PMID: 27461360). There are currently no approved targeted therapies, but WEE1 and CHK1 inhibitors are under clinical investigation for CCNE1-amplified cancers (Clinicaltrials.gov).

Variant group clinical summary
Patients with solid tumors harboring CCNE1 amplifications match inclusion criteria for clinical trials with CHK1 inhibitors (NCT03253679), WEE1 inhibitors (NCT02873975), and ATR inhibitors (NCT03718091).

Gene biological summary
CCNE1 encodes cyclin E1. Cyclin E1 activates the cyclin-dependent kinases CDK1 which in turn phosphorylates and inactivates RB proteins, driving the transition from G1 to S phase during the cell cycle (PMID: 27461360). Important domains in CCNE1 include the nuclear localization signal (residues 12-17), the N-terminal cyclin box (residues 1260223), and the C-terminal cyclin box (residues 230-327) (PMID: 15660127).

Variant group functional summary
CCNE1 amplification indicates an increase in genomic copy number of the CCNE1 gene. CCNE1 amplifications are associated with cyclin E overexpression and drive cell cycle progression and cell proliferation (PMID: 27461360).

APC p.R876* stop gained variant

Tier II-C

Variant Group	Variant Oncogenicity	Position (GRCh38)	HGVS	Transcript
APC inactivating mutation APC truncating mutation	Loss of function (predicted)	Chr:5 Pos:112838220 Change:C>T	c.2626C>T p.Arg876*	ENST00000508376.6

Gene clinical summary
Alterations in APC, such as missense mutations, truncations, and loss of heterozygosity, are found in cancers, including colorectal cancer. Loss of APC function increases β-catenin levels and promotes tumorigenesis. Although APC is generally considered a tumor suppressor, truncation mutations cause both gain and loss of APC functions. Germline mutations in APC cause familial adenomatous polyposis, which confers increased cancer risk (PMID: 28423402). WNT/β-catenin pathway inhibitors are under clinical and preclinical investigation (PMID: 28731148). In preclinical studies, a small molecule inhibitor of cholesterol biosynthesis, TASIN-1, selectively kills cells with APC truncations (PMID: 27798265).

Variant group clinical summary
No therapies are indicated for colorectal cancer (CRC) based on germline or somatic APC mutation status. Patients with familial adenomatous polyposis harboring inactivating APC mutations match inclusion criteria for clinical trials, such as trials with drugs including an anti-IL-23 antibody (NCT03649971), a fatty acid synthase inhibitor (NCT02980029), tyrosine kinase inhibitors (NCT01282502)(NCT02961374) and an indirect MTOR inhibitor (NCT03095703).

Preclinical studies support sensitivity of CRC cells harboring APC inactivating mutations to inhibitors of truncated APC (PMID: 27798265), CTNNB1 (PMID: 23071338), tankyrase (PMID: 23539443), MTOR (PMID: 18768809)(PMID: 20080688), and EGFR (PMID: 17909047).

Gene biological summary
APC is a multi-functional protein that forms a complex to promote β-catenin degradation, antagonizing the WNT/β-catenin pathway, preventing activation of genes that regulate cell proliferation, migration, differentiation and survival (PMID: 28506769). APC also interacts with microtubules, playing a role in cell division and genome stability (PMID: 21859464). The important domains in APC include an N-terminal oligomerization domain, armadillo repeats (residues 453-767), an intrinsically disordered domain (residues 800-2843), including SAMP repeats, a basic region (residues 1866-1893), and a C-terminal domain (residues 2803-2843) (PMID: 21859464) (PMID: 28423402) (UniProt.org).

Variant functional summary
APC R876* results in a premature truncation of the APC protein at amino acid 876 of 2843 (UniProt.org). Due to the loss of several known functional domains (UniProt.org)(PMID: 17881494), this mutation is predicted to lead to a loss of function.

Variant group functional summary
APC inactivating mutations result in a loss of function of the APC protein, either through truncation or inactivating missense substitutions (PMID: 21859464)(PMID: 24086117). Germline mutations in APC cause familial adenomatous polyposis, which confers increased cancer risk (PMID: 28423402).

APC truncation indicates that a nonsense mutation or frameshift created a premature termination codon in the APC tumor suppressor (PMID: 24086117).

APC p.S1356* stop gained variant

Tier II-C

Variant Group	Variant Oncogenicity	Position (GRCh38)	HGVS	Transcript
APC inactivating mutation APC truncating mutation	Loss of function (predicted)	Chr:5 Pos:112839661 Change:C>G	c.4067C>G p.Ser1356*	ENST00000508376.6

Gene clinical summary
Alterations in APC, such as missense mutations, truncations, and loss of heterozygosity, are found in cancers, including colorectal cancer. Loss of APC function increases β-catenin levels and promotes tumorigenesis. Although APC is generally considered a tumor suppressor, truncation mutations cause both gain and loss of APC functions. Germline mutations in APC cause familial adenomatous polyposis, which confers increased cancer risk (PMID: 28423402). WNT/β-catenin pathway inhibitors are under clinical and preclinical investigation (PMID: 28731148). In preclinical studies, a small molecule inhibitor of cholesterol biosynthesis, TASIN-1, selectively kills cells with APC truncations (PMID: 27798265).

Variant group clinical summary
No therapies are indicated for colorectal cancer (CRC) based on germline or somatic APC mutation status. Patients with familial adenomatous polyposis harboring inactivating APC mutations match inclusion criteria for clinical trials, such as trials with drugs including an anti-IL-23 antibody (NCT03649971), a fatty acid synthase inhibitor (NCT02980029), tyrosine kinase inhibitors (NCT01282502)(NCT02961374) and an indirect MTOR inhibitor (NCT03095703).

Preclinical studies support sensitivity of CRC cells harboring APC inactivating mutations to inhibitors of truncated APC (PMID: 27798265), CTNNB1 (PMID: 23071338), tankyrase (PMID: 23539443), MTOR (PMID: 18768809)(PMID: 20080688), and EGFR (PMID: 17909047).

Gene biological summary
APC is a multi-functional protein that forms a complex to promote β-catenin degradation, antagonizing the WNT/β-catenin pathway, preventing activation of genes that regulate cell proliferation, migration, differentiation and survival (PMID: 28506769). APC also interacts with microtubules, playing a role in cell division and genome stability (PMID: 21859464). The important domains in APC include an N-terminal oligomerization domain, armadillo repeats (residues 453-767), an intrinsically disordered domain (residues 800-2843), including SAMP repeats, a basic region (residues 1866-1893), and a C-terminal domain (residues 2803-2843) (PMID: 21859464) (PMID: 28423402) (UniProt.org).

Variant functional summary
APC S1356* results in a premature truncation of the APC protein at amino acid 1356 of 2843 (UniProt.org). This mutation has not been characterized, however, due to the effects of an APC truncation at I1572 (PMID: 10346819), it is predicted to reduce the ability of APC to regulate beta-catenin.

Variant group functional summary
APC inactivating mutations result in a loss of function of the APC protein, either through truncation or inactivating missense substitutions (PMID: 21859464)(PMID: 24086117). Germline mutations in APC cause familial adenomatous polyposis, which confers increased cancer risk (PMID: 28423402).

APC truncation indicates that a nonsense mutation or frameshift created a premature termination codon in the APC tumor suppressor (PMID: 24086117).

MSH6 c.3646+4A>C splice region & intron variant

Tier II-D

Variant Group	Variant Oncogenicity	Position (GRCh38)	HGVS	Transcript
not available	Unknown	Chr:2 Pos:47805711 Change:A>C	c.3646+4A>C	ENST00000234420.9

Gene clinical summary
Alterations in MSH6 are found in cancers, including somatic mutations in colorectal and endometrial cancer (PMID: 25194673). Inactivating mutations in MSH6 cause MMR-deficiency (dMMR) and microsatellite instability (MSI). Germline mutations in MSH6 cause Lynch syndrome, which confers increased cancer risk (PMID: 27838401). In preclinical studies, MMR-deficiency conferred resistance to some chemotherapies (PMID: 15279797). MMR-deficient tumors of several cancer types have been shown to be sensitive to immune checkpoint inhibitors in clinical trials (PMID: 28770222).

Gene biological summary

MSH6 is an NTPase in the mismatch repair (MMR) pathway. Guided by PCNA, MSH2/MSH6 heterodimers recognize small mismatches, insertions or deletions (1-3 nucleotides) and recruit MLH1/PMS2 heterodimers to initiate repair. Important domains in MSH6 include the PCNA binding domain (residues 4-11), the MMR binding domain (residues 362-518), the connector domain (residues 519-717), the lever domain (residues 718-934 and 1009-1075), the clamp domain (residues 935-1008), and the ATPase domain (resdues 1076-1360) (PMID: 28765196).

CCND3 c.575-1G>C splice acceptor & intron variant

Tier II-D

Variant Group	Variant Oncogenicity	Position (GRCh38)	HGVS	Transcript
not available	Unknown	Chr:6 Pos:41936696 Change:C>G	c.575-1G>C	ENST00000372991.8

Variants of Unknown Significance

The variants listed here are not sufficiently characterized in the current literature and variant databases, and are therefore, currently, of uncertain or unknown clinical significance. They are reported here for future reference in the event they become clinically significant in light of additional supporting evidence.

CDC6 amplification	**GRB7** amplification	**SETDB1** amplification	**STARD3** amplification
RET p.A1020V			

✓ Electronically Signed by Samantha Perakis | *10/16/2019*

Appendix

NAVIFY™ Mutation Profiler disclaimer

Decisions on patient care and treatment must be based on the independent medical judgment of the treating physician, taking into consideration all applicable information concerning the patient's condition, including information not contained in this report such as other potentially clinically significant information. The information available in this report is obtained from third party sources (such as biomedical literature, medical guidelines, and publicly available data such as drug labels and clinical trials) and is subject to change over time based on future scientific and medical findings. The selection of any, all or none of the potential therapies identified in this report resides entirely within the discretion of the treating physician, and this report makes no promises or guarantees that such potential therapies will be effective in the treatment of any patient.

3rd party attributions

A portion of the somatic gene variant annotations and related content have been provided by The Jackson Laboratory Clinical Knowledgebase (JAX-CKB™)

Tier definitions (Clinical region: EU)

Tier I-A: Approved therapy. Included in professional guidelines.

Tier I-B: Well-powered studies with consensus from experts in the field.

Tier II-C: Approved therapies for different tumor types or investigational therapies. Multiple small published studies with some consensus. Inclusion criteria for clinical trials.

Tier II-D: Limited clinical or preclinical studies.

Tier III (VUS): Variants of Unknown Clinical Significance.

Tier IV: Benign or likely benign variants (not included in the report).

Software and content version numbers

NAVIFY(TM) Mutation Profiler Version 1.1.0.3d9a34b, Release date: 08/26/2019

NAVIFY(TM) Therapy Matcher Version 1.1.0.3d9a34b, Release date: 08/26/2019

Roche content Version 2.5.0, Release date: 07/24/2019

CIViC Version 01-september-2019, Release date: 09/01/2019

ClinVAR Version 20190730, Release date: 07/30/2019

COSMIC Version v89.r2, Release date: 05/15/2019

dbNSFP Version 3.5, Release date: 08/06/2017

ExAC Version 1.0.r3, Release date: 02/27/2017

TCGA Version 18.0, Release date: 07/08/2019

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Your Lab
1700 Lincoln Blvd, Suite 20, Redwood City, CA 94063
labx.com / (650) 484 4040
Additional Information

Test Performed: **Somatic Panel**

Report Date Oct 30, 2019
Status -

Patient		Client		Specimen	
Patient Name		Client		Accession ID	C129_6
Date of Birth		Client ID		Specimen	
Age		Physician		Collection	
Sex	Male	Pathologist		Accession	Oct 30, 2019
Ethnicity				Primary Tumor Site	Colon / Rectum
Diagnosis	Colorectal cancer				

Result: Omitted

5 Clinically Significant Variants

2 Therapies Associated with Resistance

6 Therapies with Potential Clinical Benefit

2 Clinical Trials

Actionable Variants With Associated Therapies

		Approved Therapies			
Gene / Variant	**Allelic Fraction**	**Colorectal Cancer**	**Other Indications**	**Associated With Resistance**	**Clinical Trials**
ERBB2 amplification **Tier 1A** Pathogenic	-	lapatinib /trastuzumab pertuzumab /trastuzumab	-	EGFR tyrosine kinase inhibitor cetuximab	1
EGFR amplification **Tier 2C** Pathogenic	-	5-fluorouracil /irinotecan /leucovorin 5-fluorouracil /leucovorin /oxaliplatin cetuximab cetuximab panitumumab panitumumab	-	-	2

Variants Without Associated Therapies

Gene / Variant	Allelic Fraction	Function	Classification	Assessment
CCNE1 amplification	-	gain	**Tier 2C**	Pathogenic
APC c.2626C>T p.R876* g.112838220C>T	-	loss	**Tier 2D**	Pathogenic

Gene / Variant	Allelic Fraction	Function	Classification	Assessment
APC c.4067C>G p.S1356* g.112839661C>G	-	loss	**Tier 2D**	Pathogenic

Prognostic Outcomes

Gene / Variant	Indication	Prognosis	Outcome / Endpoint
ERBB2 amplification **Tier 1A** Pathogenic	Colorectal adenocarcinoma	favorable	progression-free survival
ERBB2 amplification **Tier 1A** Pathogenic	Colorectal adenocarcinoma	favorable	objective response
EGFR amplification **Tier 2C** Pathogenic	Colorectal cancer	favorable	time to progression
EGFR amplification **Tier 2C** Pathogenic	Colorectal cancer	favorable	survival duration
EGFR amplification **Tier 2C** Pathogenic	Colorectal cancer	favorable	progression-free survival
EGFR amplification **Tier 2C** Pathogenic	Colorectal cancer	favorable	objective response
EGFR amplification **Tier 2C** Pathogenic	Colorectal cancer	unfavorable	objective response

Therapeutic Implications for Colorectal Cancer

Therapies	Gene / Variant	Response	Therapies Description
EGFR tyrosine kinase inhibitor	***ERBB2*** amplification	Resistance	FDA approved EGFR tyrosine kinase inhibitors include erlotinib, gefitinib, afatinib, and osimertinib. Lapatinib and vandetanib also inhibit the EGFR

Therapies	Gene / Variant	Response	Therapies Description
	Tier 1A Pathogenic		tyrosine kinase but are not approved as such.
cetuximab	***ERBB2*** amplification **Tier 1A** Pathogenic	Resistance	Cetuximab, an epidermal growth factor receptor antagonist, is FDA-approved for treating patients with locally or regionally advanced squamous cell carcinoma of the head and neck in combination with radiation therapy; recurrent locoregional disease or metastatic squamous cell carcinoma of the head and neck in combination with platinum-based therapy with 5-FU; recurrent or metastatic squamous cell carcinoma of the head and neck progressing after platinum-based therapy; KRAS wild-type, EGFR-expressing, metastatic colorectal cancer in combination with FOLFIRI for first-line treatment, or in combination with irinotecan in patients who are refractory to irinotecan-based chemotherapy, or as a single agent in patients who have failed oxaliplatin- and irinotecan-based chemotherapy or who are intolerant to irinotecan; cetuximab is EMA-approved for treating patients with EGFR-expressing, RAS wild-type metastatic colorectal cancer in combination with irinotecan-based chemotherapy, in first-line in combination with FOLFOX, as a single agent in patients who have failed oxaliplatin- and irinotecan-based therapy and who are intolerant to irinotecan; squamous cell cancer of the head and neck in combination with radiation therapy for locally advanced disease, and in combination with platinum-based chemotherapy for recurrent and/or metastatic disease.
lapatinib/ trastuzumab	***ERBB2*** amplification **Tier 1A** Pathogenic	Predictive	Lapatinib, a tyrosine kinase inhibitor, and trastuzumab, a HER2/neu receptor antagonist, are FDA- and EMA-approved for treating patients with HER2 overexpressing breast cancer.
pertuzumab/ trastuzumab	***ERBB2*** amplification **Tier 1A** Pathogenic	Predictive	-
5-fluorouracil/ irinotecan/ leucovorin	***EGFR*** amplification **Tier 2C** Pathogenic	Predictive	-
5-fluorouracil/ leucovorin/ oxaliplatin	***EGFR*** amplification **Tier 2C** Pathogenic	Predictive	Leucovorin, oxaliplatin and fluorouracil are chemotherapeutic drugs used in combination to treat colorectal cancer. This treatment regimen is known as FOLFOX.

Therapies	Gene / Variant	Response	Therapies Description
cetuximab	***EGFR*** amplification **Tier 2C** **Pathogenic**	Sensitive	Cetuximab, an epidermal growth factor receptor antagonist, is FDA-approved for treating patients with locally or regionally advanced squamous cell carcinoma of the head and neck in combination with radiation therapy; recurrent locoregional disease or metastatic squamous cell carcinoma of the head and neck in combination with platinum-based therapy with 5-FU; recurrent or metastatic squamous cell carcinoma of the head and neck progressing after platinum-based therapy; KRAS wild-type, EGFR-expressing, metastatic colorectal cancer in combination with FOLFIRI for first-line treatment, or in combination with irinotecan in patients who are refractory to irinotecan-based chemotherapy, or as a single agent in patients who have failed oxaliplatin- and irinotecan-based chemotherapy or who are intolerant to irinotecan; cetuximab is EMA-approved for treating patients with EGFR-expressing, RAS wild-type metastatic colorectal cancer in combination with irinotecan-based chemotherapy, in first-line in combination with FOLFOX, as a single agent in patients who have failed oxaliplatin- and irinotecan-based therapy and who are intolerant to irinotecan; squamous cell cancer of the head and neck in combination with radiation therapy for locally advanced disease, and in combination with platinum-based chemotherapy for recurrent and/or metastatic disease.
cetuximab	***EGFR*** amplification **Tier 2C** **Pathogenic**	Predictive	Cetuximab, an epidermal growth factor receptor antagonist, is FDA-approved for treating patients with locally or regionally advanced squamous cell carcinoma of the head and neck in combination with radiation therapy; recurrent locoregional disease or metastatic squamous cell carcinoma of the head and neck in combination with platinum-based therapy with 5-FU; recurrent or metastatic squamous cell carcinoma of the head and neck progressing after platinum-based therapy; KRAS wild-type, EGFR-expressing, metastatic colorectal cancer in combination with FOLFIRI for first-line treatment, or in combination with irinotecan in patients who are refractory to irinotecan-based chemotherapy, or as a single agent in patients who have failed oxaliplatin- and irinotecan-based chemotherapy or who are intolerant to irinotecan; cetuximab is EMA-approved for treating patients with EGFR-expressing, RAS wild-type metastatic colorectal cancer in combination with irinotecan-based chemotherapy, in first-line in combination with FOLFOX, as a single agent in patients who have failed oxaliplatin- and irinotecan-based therapy and

Therapies	Gene / Variant	Response	Therapies Description
			who are intolerant to irinotecan; squamous cell cancer of the head and neck in combination with radiation therapy for locally advanced disease, and in combination with platinum-based chemotherapy for recurrent and/or metastatic disease.
panitumumab	***EGFR*** amplification **Tier 2C** Pathogenic	Sensitive	Panitumumab, an epidermal growth factor receptor (EGFR) antagonist, is FDA- and EMA-approved for treating patients with wild-type RAS (defined as wild-type in both KRAS and NRAS) metastatic colorectal cancer in combination with FOLFOX for first-line treatment, as monotherapy following disease progression after prior treatment with fluoropyrimidine, oxaliplatin, and irinotecan-containing chemotherapy; and panitumumab is EMA-approved for treating patients with wild-type RAS metastatic colorectal cancer in second-line in combination with FOLFIRI who have received first-line fluoropyrimidine-based chemotherapy (excluding irinotecan).
panitumumab	***EGFR*** amplification **Tier 2C** Pathogenic	Predictive	Panitumumab, an epidermal growth factor receptor (EGFR) antagonist, is FDA- and EMA-approved for treating patients with wild-type RAS (defined as wild-type in both KRAS and NRAS) metastatic colorectal cancer in combination with FOLFOX for first-line treatment, as monotherapy following disease progression after prior treatment with fluoropyrimidine, oxaliplatin, and irinotecan-containing chemotherapy; and panitumumab is EMA-approved for treating patients with wild-type RAS metastatic colorectal cancer in second-line in combination with FOLFIRI who have received first-line fluoropyrimidine-based chemotherapy (excluding irinotecan).

Available Clinical Trials

Gene / Variant	Trial Title Trial ID	Treatments	Trial Phase	Location / Contact
ERBB2 amplification **Tier 1A** Pathogenic ***EGFR*** amplification **Tier 2C** Pathogenic	A Phase II, Randomized, Active-Controlled, Multi-Center Study Comparing the Efficacy and Safety of Targeted Therapy or Cancer Immunotherapy Guided by Genomic Profiling Versus Platinum-Based Chemotherapy in Patients With Cancer of Unknown Primary Site Who Have	trastuzumab erlotinib pertuzumab	Phase 2	LKH-UNIV. KLINIKUM GRAZ; Klinische Abteilung für Onkologie, Graz, 8036, Austria Reference Study ID Number: MX39795 www.roche.com/about_roche/roche_worldwide.htm;

Gene / Variant	Trial Title Trial ID	Treatments	Trial Phase	Location / Contact
	Received Three Cycles of Platinum Doublet Chemotherapy NCT03498521			global-roche-genentech-trials@gene.com; 888-662-6728 ;
EGFR amplification **Tier 2C** Pathogenic	Phase Ib Study of Gevokizumab in Combination With Standard of Care Anti-cancer Therapies in Patients With Metastatic Colorectal Cancer, Gastroesophageal Cancer and Renal Cell Carcinoma NCT03798626	cetuximab bevacizumab gevokizumab	Phase 1	Novartis Investigative Site, Salzburg, 5020, Austria Novartis Pharmaceuticals; novartis.email@novartis.com; 1-888-669-6682;

Individual Variant Interpretations

Gene	***ERBB2***
Variation	-
Exon	-
Nucleotide	-
	-
	-
Amino Acid	amplification
Function	gain
Allelic Fraction	-
Classification	**Tier 1A**
Assessment	Pathogenic

Interpretation
ERBB2 is an oncogene involved in cell proliferation and growth through activation of RAS/RAF/MAPK and PI3K/AKT/MTOR pathways [14, 18]. Amplification and gain-of-function mutations cause ERBB2 activation [44, 52]. ERBB2 alterations are reported to be mutually exclusive with EGFR and KRAS mutations in non-small cell lung cancer [7, 43].

Gene	***CCNE1***
Variation	-
Exon	-
Nucleotide	-
	-
	-
Amino Acid	amplification
Function	gain
Allelic Fraction	-
Classification	**Tier 2C**
Assessment	Pathogenic

Interpretation
CCNE1 is an oncogenic protein involved in cell cycle control [17]. Amplification and protein overexpression cause CCNE1 activation [28, 19, 47].

Gene	***EGFR***
Variation	-
Exon	-
Nucleotide	-
	-
	-
Amino Acid	amplification
Function	gain
Allelic Fraction	-
Classification	**Tier 2C**

Interpretation
EGFR is an oncogene involved in cell growth and differentiation through activation of the PI3K/AKT/MTOR and RAS/RAF/MAPK pathways [39]. Amplification, gain-of-function mutations, and protein overexpression cause EGFR activation [23, 57, 35, 41]. EGFR mutations are reported to be mutually exclusive with ALK rearrangements and KRAS mutations in non-small cell lung cancer [13, 42, 43].

Assessment	**Pathogenic**

Gene	***APC***
Variation	-
Exon	16
Nucleotide	NM_000038.6: g.112838220C>T c.2626C>T
Amino Acid	p.R876*
Function	loss
Allelic Fraction	-
Classification	**Tier 2D**
Assessment	**Pathogenic**

Interpretation
APC is a tumor suppressing WNT pathway antagonist involved in cell survival and adhesion [58, 26]. Loss-of-function mutations and promoter methylation cause APC inactivation [34, 21, 24].

Gene	***APC***
Variation	-
Exon	16
Nucleotide	NM_000038.6: g.112839661C>G c.4067C>G
Amino Acid	p.S1356*
Function	loss
Allelic Fraction	-
Classification	**Tier 2D**
Assessment	**Pathogenic**

Interpretation
APC is a tumor suppressing WNT pathway antagonist involved in cell survival and adhesion [58, 26]. Loss-of-function mutations and promoter methylation cause APC inactivation [34, 21, 24].

Genes Tested

Test information such as gene name and hot spot region can be included in this section.

Methods and Limitations

EXAMPLE Statement including sample type (FFPE, etc), method of extraction, amplification reactions, panel targeted regions, sequencing technology, etc. Additionally, a description of the data analysis software(s), genome of reference and the sensitivity of the methods should be described.

QIAGEN Clinical Insight (QCI™) is a variant analysis, interpretation and decision support tool for research and clinical labs analyzing human genetics data and is not intended to be used for diagnostic purposes. QCI Interpret software includes the following underlying databases, data reference sets and tools; QIAGEN Clinical Insight-Interpret (5.6.20191025), Ingenuity Knowledge Base (W-release 191004.002), CADD (v1.4), Allele Frequency Community (2019-09-25), EVS (ESP6500SI-V2), Refseq Gene Model (2019-02-05), JASPAR (2013-11), Ingenuity Knowledge Base Snapshot Timestamp (2019-10-04 15:56:49.0), Vista Enhancer hg18 (2012-07), Vista Enhancer hg19 (2012-07), Clinical Trials (W-release), PolyPhen-2 (v2.2.2), 1000 Genome Frequency (phase3v5b), ExAC (0.3.1), iva (Oct 4 10:42 iva-1.0.1200.jar), PhyloP hg18 (2009-11), PhyloP hg19 (2009-11), DbSNP (151), TargetScan (7.2), GENCODE (Release 29), CentoMD (5.3), OMIM (May 26, 2017), gnomAD (2.1.1), BSIFT (2016-02-23), TCGA (2013-09-05), Clinvar (2019-06-05), DGV (2016-05-15), COSMIC (v89), HGMD (2019.2), SIFT4G (2016-02-23)

Clinical Significance of Variants Based on AMP / ASCO / CAP Guidelines*

Strong Significance	**Tier 1A**	• Biomarker predicts response or resistance to an FDA or EMA approved therapy, according to drug label or professional guidelines for this diagnosis • Biomarker included in professional guidelines is prognostic or diagnostic for this diagnosis
	Tier 1B	• Biomarker predicts response or resistance to a therapy for this diagnosis based on well-powered studies • Biomarker is prognostic or diagnostic for this diagnosis based on well-powered studies
Potential Significance	**Tier 2C**	• Biomarker is associated with response or resistance to an FDA or EMA approved therapy, according to drug label or professional guidelines but only for different diagnosis • Biomarker is an inclusion criterion for an active clinical trial • Biomarker is prognostic or diagnostic based on multiple small studies
	Tier 2D	• Biomarker shows plausible response or resistance based on case or preclinical studies • Biomarker may assist in disease diagnosis or prognosis based on small studies
Uncertain Significance	**Tier 3**	• Biomarker has uncertain clinical significance and not known to be likely benign or benign

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