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Precision oncology based on omics data: The NCT Heidelberg experience

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Precision oncology implies the ability to predict which patients will likely respond to specific cancer therapies based on increasingly accurate, high-resolution molecular diagnostics as well as the functional and mechanistic understanding of individual tumors. While molecular stratification of patients can be achieved through different means, a promising approach is next-generation sequencing of tumor DNA and RNA, which can reveal genomic alterations that have immediate clinical implications. Furthermore, certain genetic alterations are shared across multiple histologic entities, raising the fundamental question of whether tumors should be treated by molecular profile and not tissue of origin. We here describe MASTER (Molecularly Aided Stratification for Tumor Eradication Research), a clinically applicable platform for prospective, biology-driven stratification of younger adults with advanced-stage cancer across all histologies and patients with rare tumors. We illustrate how a

Key words: next-generation sequencing, precision oncology, whole-exome sequencing, clinical trial design, personalized medicine

Abbreviations: CME: continuing medical education; CRAFT: Continuous ReAssessment with Flexible exTension; CUP: carcinoma of unknown primary; DKTK: Deutsches Konsortium für Translationale Krebsforschung, German Cancer Consortium; DTH: DataThere-House; FFPE: formalin-fixed paraffin-embedded; ICGC: International Cancer Genome Consortium; MASTER: Molecularly Aided Stratification for Tumor Eradication Research; MTB: molecular tumor board; NCT: National Center for Tumor Diseases; NSCLC: non-small cell lung cancer; PDO: patient-derived organoids; PDX: patient-derived xenografts; NGS: next-generation sequencing; RPPA: reverse phase protein array; STS: soft-tissue sarcoma; TCGA: The Cancer Genome Atlas; WES: whole-exome sequencing; WGS: whole-genome sequencing

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standardized workflow for selection and consenting of patients, sample processing, whole-exome/genome and RNA sequencing, bioinformatic analysis, rigorous validation of potentially actionable findings, and data evaluation by a dedicated molecular tumor board enables categorization of patients into different intervention baskets and formulation of evidence-based recommendations for clinical management. Critical next steps will be to increase the number of patients that can be offered comprehensive molecular analysis through collaborations and partnering, to explore ways in which additional technologies can aid in patient stratification and individualization of treatment, to stimulate clinically guided exploratory research projects, and to gradually move away from assessing the therapeutic activity of targeted interventions on a case-by-case basis toward controlled clinical trials of genomics-guided treatments.

Challenges and Opportunities of Precision Oncology

Cancer research in the last decade was teeming with discoveries of promising targeted therapies and associated predictive biomarkers that enable their selective application. In particular, the cancer community has been thrilled and enticed by the results of large genome sequencing efforts, such as those undertaken by The Cancer Genome Atlas[1] and the International Cancer Genome Consortium,[2] which raise hopes that better outcomes for patients can be achieved by thorough molecular characterization of their tumors. From more detailed molecular characterization of distinct histologic entities and subsequent individualization of therapy[3] to the identification and efficient targeting of individual genetic alterations across different cancers,[4] clinical translation has always been on the heels of genomic discovery. Next-generation sequencing (NGS) was rightly hailed as the key methodological breakthrough to realize the successful clinical application of many aspects of precision oncology. Consequently, NGS technologies have dominated the landscape of precision cancer medicine, although they are known to be difficult to standardize and implement in routine clinical laboratories. It became apparent early on that the more we know about the molecular differences within and among tumor entities, the more difficulties in clinical translation we may encounter. All NGS methods based on limited amounts of tumor tissue have an inherent error of underestimating tumor heterogeneity, possibly resulting in therapies that only partially target widely heterogeneous tumors. In addition to the limitations in spatial resolution of tissue-based NGS, the missing temporal resolution of sequencing continuously evolving tumors at single time points is a rising concern. There is also a continuous debate as to which NGS-based approach (multi-gene panel sequencing, whole-exome sequencing [WES], or whole-genome sequencing [WGS]) is best suited for molecular characterization and stratification of tumors in a clinical setting.[5] Due to the lack of standardization of bioinfomatic and data curation pipelines, ensuing clinical interpretations are not easily comparable between institutions. Moreover, in times of ever smaller, molecularly defined patient cohorts, we only begin to grapple with the incompatibility of our current clinical trial designs, which rely on statistically robust information from several hundred to thousands of patients. Finally, we are far from understanding context-specific variations among tumors harboring the same molecular alteration, stressing the need for comprehensive, multidimensional characterization of human tumors and leading to increased interest in epigenomics, proteomics, metabolomics and other technologies as molecular stratification tools.

Omics-Driven Precision Oncology in Heidelberg

The development of genomics-driven precision oncology was spearheaded by a number of institutions, each of them taking a somewhat different approach to analyze and interpret NGS data from solid tumors in a clinically relevant manner. Three basic models of academic precision oncology programs using NGS were created. First, to rapidly screen large numbers of patients, cancer gene panels encompassing dozens to several hundred genes were developed and tested in an academic setting. This approach allows swift patient stratification and enrollment in basket trials, as exemplified by the MSK-IMPACT test developed at Memorial Sloan Kettering Cancer Center[6] and efforts at the Institute of Pathology at Heidelberg University Hospital.[7,8] Other institutions took advantage of the increasing availability of commercial alternatives for NGS testing in order to quickly validate the concept of precision oncology through matching patients with drugs according to genomic profile.[9] Third, an integrative approach including WES and transcriptome sequencing was shown to be practicable in a clinical setting.[10]

To leverage the broad expertise in cancer genomics at the German Cancer Research Center (DKFZ) for improved clinical care of cancer patients,[11] the DKFZ-Heidelberg Center for Personalized Oncology (DKFZ-HIPO)[12] was founded in 2011. Among the dozens of projects supported by HIPO is the MASTER (Molecularly Aided Stratification for Tumor Eradication Research) trial, a molecular stratification program for younger adults with advanced-stage cancer across all histologies and patients with rare tumors that was launched at the National Center for Tumor Diseases (NCT) Heidelberg. The initial goals of MASTER were to assess the feasibility of prospective WES and RNA sequencing in a clinical setting and to demonstrate that such an approach can provide relevant diagnostic information and create therapeutic opportunities, to prepare the ground for interventional clinical trials in patient cohorts that are stratified according to genetic profile. To this end, we have implemented a standardized workflow for selection and consenting of patients, evaluation of

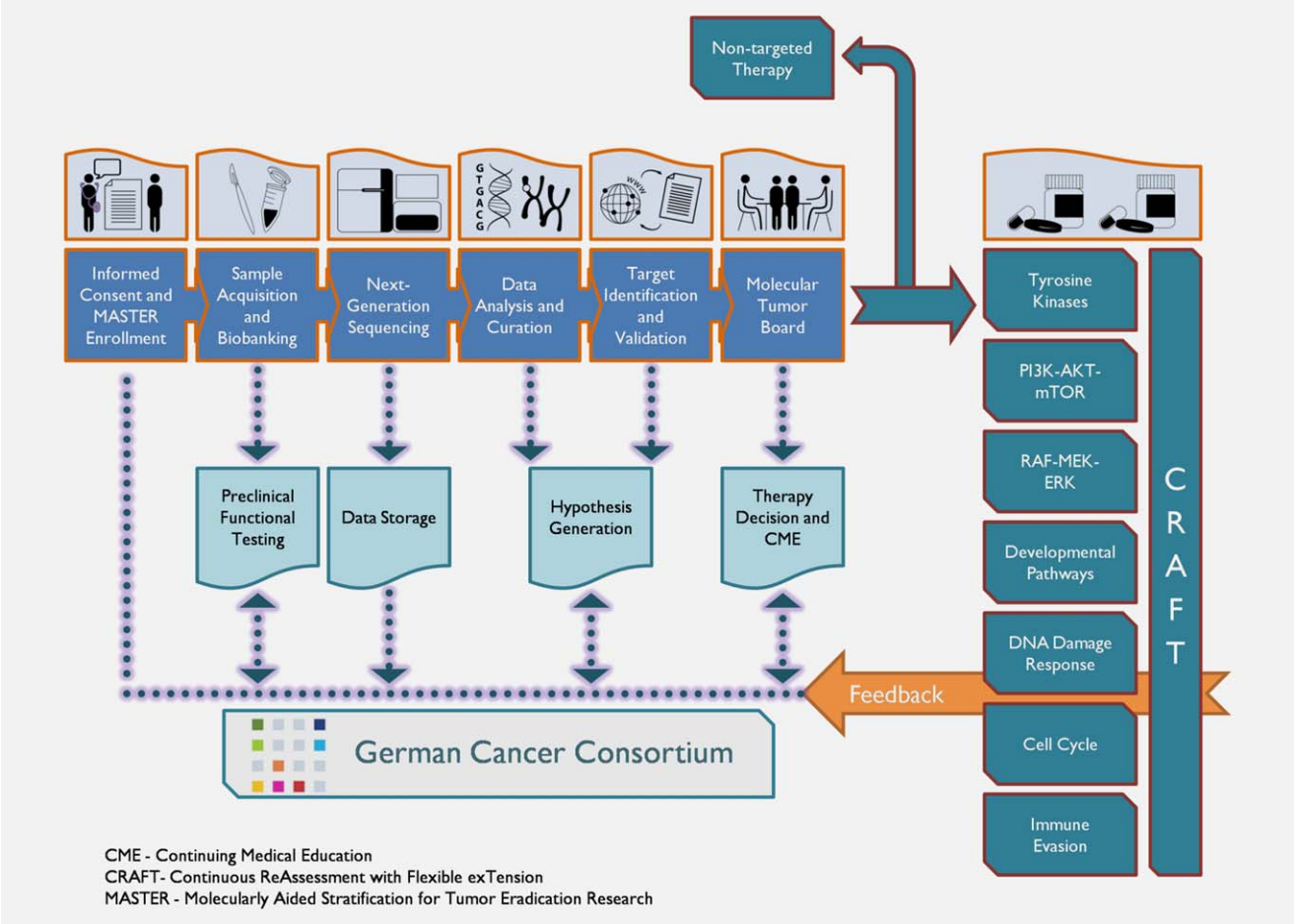

Figure 1. Workflow of the MASTER molecular stratification program.

tumor histology and cellularity, sample processing, molecular and bioinformatic analysis, validation of potentially actionable findings by orthogonal methods, and interdisciplinary evaluation and reporting of results through a dedicated molecular tumor board (MTB; Fig. 1). Systematic enrollment began in 2013, and as of March 2017, >550 patients representing a broad spectrum of entities have been analyzed (Fig. 2*a*). Our cohort includes adult patients below the age of 51 years with advanced-stage cancer across all histologies and patients with rare tumors, defined as an incidence of fewer than 1 case per 100,000 per year. We exclude patients who are unlikely to gain significant clinical benefit from NGS-based molecular analysis, such as individuals without measurable disease, patients with a life expectancy of <6 months or a Karnofsky Performance Status of <70%, and patients with a curative treatment option available at the time of enrollment. The current age limit of 50 years is based on several considerations. First, WES and RNA sequencing of all adult cancer patients is currently not feasible due to logistical challenges and financial constraints. Second, there is an unmet medical need in younger patients with advanced-stage cancers. Third, cancers arising in younger adults are underresearched and, as a consequence, less well understood. Fourth, there is a higher likelihood in younger adults of identifying potentially actionable driver mutations, that is, a more favorable driver/passenger mutation ratio, due to lower genetic complexity compared to advanced-stage cancers in older patients. Fifth, younger patients usually have fewer comorbidities and are therefore more likely eligible for genomics-guided experimental therapies.

One of the specific aims of MASTER was to determine the feasibility of WES/WGS and transcriptome sequencing in a clinically relevant time frame, and we have now achieved a turnaround time of <6 weeks from biopsy to a decision by the MTB, held on a weekly basis. Systematic evaluation of molecular alterations by the MTB has allowed stratification of patients into treatment baskets defined by specific molecular pathways and/or cellular processes (Fig. 1). We currently assign actionable genetic alterations, such as point mutations, changes in DNA copy number, aberrant gene expression, or gene fusions, to 7 different intervention baskets: tyrosine kinase signaling, PI3K-AKT-mTOR signaling, RAF-MEK-ERK signaling, developmental pathways (e.g., Hedgehog signaling), DNA damage response signaling, cell cycle regulation, and immune evasion (Figs. 1 and 2*b*). Due to the large number of druggable tyrosine kinases, this basket constitutes

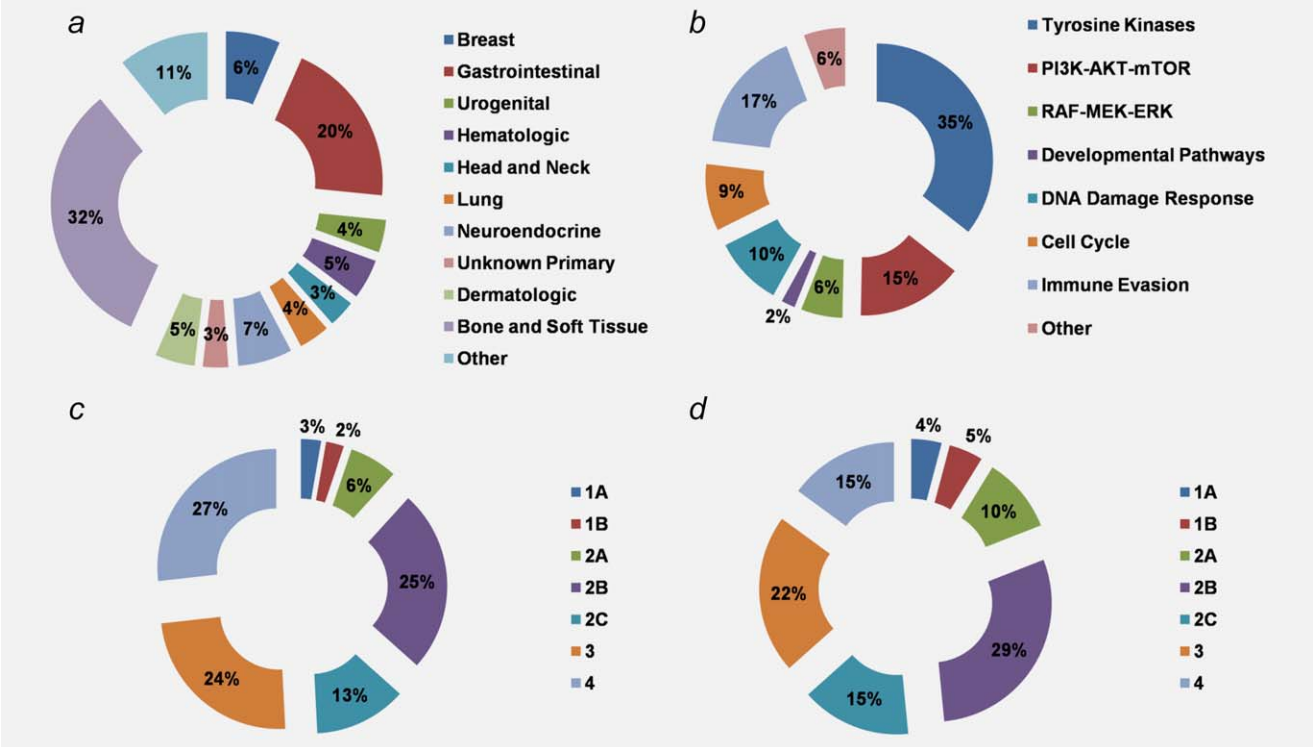

Figure 2. Distribution of cancer entities and recommendations for clinical management in the MASTER molecular stratification program. (a) Distribution of patients according to cancer entity. (*b*) Distribution of recommendations for clinical management according to intervention basket. (*c*) Distribution of recommendations for clinical management according to evidence level (all recommendations). (*d*) Distribution of recommendations for clinical management according to evidence level (highest-ranking recommendation).

the most prominent category, closely followed by the immune evasion basket (defined by high mutational load and/or *PDL1* amplification/overexpression as determinants of response to immune checkpoint inhibition[13]), and the PI3K-AKT-mTOR signaling basket. In the majority of cases, the molecular data provide a rationale for more than one drug or more than one basket. For that reason, actionable mutations are further categorized according to the level of evidence available for the association between a molecular alteration and response to a specific drug (Table 1). These evidence-based categories integrate clinical (levels 1 and 2) and preclinical (levels 3 and 4) evidence regarding the predictive strength of a specific molecular alteration across different tumor entities and provide a standardized and practical system for rapid clinical orientation. In a number of cases, candidate actionable targets are dropped due to a low probability of success based on a documented lack of exceptional responders within a specific histologic entity, a medical history suggesting resistance, or the concomitant presence of known resistance factors. These limitations notwithstanding, we currently identify at least one targetable lesion and provide a potential rationale for experimental therapy in approximately 75% of patients. In nearly two-thirds of these cases, the decision is supported by clinical evidence and in large parts based on clinical observations in other tumor entities (level 2B). Interestingly, depending on whether we take into account the best recommendation by the MTB or consider all possible therapeutic targets, our treatment decisions are based on preclinical evidence in up to 50% of cases (Figs. 2*c* and 2*d*). Although MASTER does not yet include an interventional clinical trial, the implementation rate of recommended targeted therapies (in-label, off-label, on-study, compassionate use) has been steadily increasing to >35%.

The decision to implement WES and transcriptome sequencing as stratification tools was taken deliberately in light of the fact that our patient cohort encompasses a high proportion of rare cancer entities with few, if any, well-defined driver genes. We are aware of the limitations of this approach, as WES does not provide the same depth of coverage as targeted sequencing using subgenomic gene panels.[21] Other biases may arise from differences in sequencing methodologies and bioinformatic strategies. Nevertheless, this approach has been found to be highly reproducible across experienced sequencing facilities and thus amenable for clinical use.[22] We believe that the true advantages of precision oncology will manifest themselves only through comprehensive molecular characterization of large patient cohorts and successful integration of genetic data with clinical information from individual patients. Even without complete understanding of the molecular complexity of individual cancers and without immediate clinical application of the genomic data, we are confident that the prospective collection of

Table 1. Levels of evidence

		Example
1A	Drug is approved for the same tumor type harboring the specific biomarker.	Crizotinib in NSCLC with EML4-ALK fusion[14]
1B	Predictive value of the biomarker or clinical effectiveness of the corresponding drug in a molecularly stratified cohort was demonstrated in an adequately powered prospective study or a meta-analysis.	Erlotinib in NSCLC with EGFR amplification[15,16]
2A	Predictive value of the biomarker or clinical effectiveness of the drug in a molecularly stratified cohort was demonstrated in a prospective trial with biomarkers as a secondary objective or an adequately powered retrospective cohort or case-control study in the same tumor type.	Vemurafenib in NSCLC with BRAF V600E mutation[4]
2B	Predictive value of the biomarker or clinical effectiveness of the drug in a molecularly stratified cohort was demonstrated by clinical data in a different tumor type.	Everolimus in NSCLC with PTEN loss-of-function mutation, loss, or deletion[17]
2C	Case study or single unusual responder indicates the biomarker is associated with response to the drug, supported by scientific rationale.	Sorafenib in NSCLC with BRAF G469V/R mutation[18,19]
3	Preclinical data (*in vitro* or *in vivo* models and functional genomics) demonstrate that the biomarker predicts response of cells to drug treatment.	Dasatinib in NSCLC with DDR2 mutation[20]
4	Biological rationale exists that links the drug to the altered signaling pathway or relevant basket. No reported clinical or preclinical data on the response to the drug.	Panobinostat in NSCLC with SMARCA4 loss-of-function mutation

comprehensive genomic data is as crucial for future medical research as the long-term storage of formalin-fixed paraffin-embedded material has repeatedly proven to be in the past. With this goal in mind, we took the next logical step and have recently implemented WGS within MASTER. This endeavor will not only prospectively collect sequencing data from a large, clinically annotated patient cohort, but possibly also offer unique insight into rare structural variants and alterations in non-coding regions of the genome, such as enhancers and other regulatory elements, that are not assessable by other methods but may prove clinically relevant in future.

One of the major logistic challenges of MASTER is the acquisition and shipment of fresh frozen samples from >60 participating facilities to the central sample processing laboratory at the DKFZ in Heidelberg. Upon arrival, frozen sections are obtained from individual tumors for evaluation of histology and tumor cell content, which is required to be at least 20%, by a board-certified pathologist. In the sample processing laboratory, protocols have been developed for standardized extraction of DNA, RNA and protein from tumor samples of various origin. DNA from normal tissue, consisting of peripheral blood mononuclear cells or saliva, is also extracted. Following quality control, DNA and RNA are submitted to the DKFZ Genomics and Proteomics Core Facility for exome capture, library preparation, and NGS at a mean coverage of ~150-fold for WES and ~60-fold for WGS. Alignment files (for DNA) and raw sequencing data (for RNA) are provided by the sequencing core facility. Thereafter, dedicated bioinformaticians at the DKFZ execute pipelines for the detection of single-nucleotide variants, small insertions or deletions, structural variations and copy number alterations in the DNA data as well as alignment, expression quantification and fusion gene detection in the RNA data. The results are automatically aggregated into a spreadsheet and then manually inspected for annotated druggable lesions, which subsequently undergo visual control. Inspection of germline variants is only performed for a subset of predefined cancer predisposition genes. A team of translational oncologists analyze the resulting variants for actionable targets. Prioritized targets are validated by the Center for Molecular Pathology at the Institute of Pathology using orthogonal techniques such as Sanger sequencing, multi-gene panel sequencing, *in situ* hybridization and immunohistochemistry. A detailed report is generated after the weekly MTB and distributed to the treating physician. The turnaround time for this workflow is about 6 weeks.

The realization that we need to address the varied challenges of precision oncology in long-term, multi-insitutional efforts has spurred the extension of MASTER to 9 additional comprehensive cancer centers within the German Cancer Consortium (DKTK).[23] Specifically, we aim to implement the MASTER workflow across all DKTK Partner Sites to develop a DKTK-wide clinical cancer genome sequencing program. By leveraging the consortium's combined expertise in genomics, molecular mechanism-based therapy, and clinical trial design, this initiative provides a unique opportunity to systematically expand the cohort of genomically characterized

patients, evaluate the utility of precision oncology on a national scale, and enable therapeutic trials in larger, molecularly stratified cohorts. A joint DKTK MTB will convene weekly and will be responsible for identifying therapeutic choices in individual patients, stratifying cases into intervention baskets and determining clinical trial eligibility based on a comprehensive molecular report. This cross-institutional forum will also serve as a valuable educational and training resource for practicing clinicians, who as a result will be optimally prepared to formulate and implement genomically informed treatment decisions.[24] Thanks to the infrastructure for high-volume sequencing provided by DKFZ-HIPO, DKTK MASTER will be in a position to systematically test the clinical application of WES/WGS and RNA sequencing in a concerted, interdisciplinary and cross-institutional effort, and prepare the ground for addressing the elementary question of whether matching treatments with individual molecular profiles will lead to improved patient outcomes.

Beyond Case Reports

Personalized or precision medicine invariably leads to steadily decreasing numbers of patients who will actually benefit from a given treatment. In addition, due to the rising number of treatment options, there is an exponential increase in possible drug-combination and drug-sequencing permutations. Clinical trial design and statistical analysis of precision medicine have to adapt to these developments and create tools that allow extraction of scientifically sound and clinically relevant information from basket, umbrella or "N of 1" approaches.[25–29] While we are awaiting the results of such novel precision oncology trials, we can try to bridge the gap by focusing on broad evaluation of precision medicine programs[9] or study exceptional responders to targeted therapies[30] to better understand the molecular mechanisms at play. Within MASTER, we were able to take the second approach and illustrate that a personalized strategy can have wide-ranging diagnostic as well as therapeutic consequences for individual patients. Due to our diverse patient cohort encompassing many rare cancer entities, we were able to detect several unanticipated genetic associations, some of which might have been missed using targeted NGS approaches. For instance, we detected a *PDGFRA* mutation in a case of soft-tissue sarcoma (STS) not otherwise specified, suggesting a diagnosis of gastrointestinal stromal tumor, and an *EWSR1-WT1* fusion, typically associated with desmoplastic small round cell tumor, in a neuroendocrine tumor. Overall WES and transcriptome sequencing has led to reconsideration of some aspects of the clinical diagnosis in approximately 5% of our patients. Subsequent morphomolecular reassessment confirmed the molecular disease categorization in some cases, but rejected the respective suggestion in others, highlighting the need for a truly integrative approach to correctly classify such difficult cases. We were also able to narrow down the origin of several carcinomas of unknown primary (CUP) based on specific genetic alterations. Furthermore, we experienced that comprehensive molecular profiling has not only the potential to contribute to diagnostic categorization, which invariably includes thorough histopathologic reevaluation, but also identifies actionable targets and thus guides targeted therapy in CUP patients. Recently, we reported a case of an advanced-stage malignancy classified as poorly differentiated STS that was refractory to chemotherapy, where molecular profiling within MASTER and additional histopathologic analyses established the diagnosis of a poorly differentiated adenocarcinoma. We also identified a high mutational load and a focal high-level amplification on chromosome 9p, harboring *PDL1*, in this tumor. These findings provided a rationale for immune checkpoint inhibition with pembrolizumab and resulted in a long-lasting near-complete remission in this patient.[31] In a patient with metastatic sinonasal carcinoma, we identified a *KIT* exon 11 mutation that prompted imatinib treatment, which resulted in partial remission of all tumor manifestations lasting >10 months. Upon sequencing of the recurrent tumor, a secondary imatinib-resistant *KIT* exon 17 mutation was detected and provided a molecular rationale for treatment with regorafenib or sorafenib, which are known to be effective in this setting.[32] In another report, we performed WES and transcriptome sequencing to uncover *ERBB2* amplification as promising target in a patient with metastatic gallbladder cancer. This patient subsequently received targeted therapy with pertuzumab and trastuzumab in combination with nanoparticle albumin-bound paclitaxel and achieved a durable complete response.[33] In addition to the detection of common molecular alterations in rare tumors, in MASTER we also have the opportunity to study the "long tail" of less frequent or private genetic alterations.[34] Such data are invaluable in jump-starting individual laboratory research projects into the functional and mechanistic consequences of these molecular lesions. In a case of a histiocytic sarcoma harboring an atypical *BRAF* mutation, for instance, we determined the functional interaction of *BRAF* with mutant *HRAS* to promote oncogenic signaling.[35] In some cases, we are able to gain insight into tumor evolution, such as in a patient with acute promyelocytic leukemia who developed meningeal relapse. Here, we found an activating mutation in the catalytic domain of the FLT3 receptor tyrosine kinase at relapse, which allowed us to trace back this mutation to a minuscule subclone that was present in the bone marrow at original diagnosis and defined a subset of cells able to escape standard therapy.[36] The observation of amplified *FGFR1* in a patient with metastatic leiomyosarcoma led to the preclinical investigation of FGFR1 as a putative therapeutic target in 3 independent cohorts of STS patients. This study revealed that FGFR inhibitor sensitivity in STS models is primarily determined by FGFR1 expression levels and supports further evaluation of FGFR inhibitor therapy in this group of diseases.[37] These first individual reports about successful personalized treatments and translational research projects originating from the MASTER program not only underscore the clinical

need for broad testing of common genetic variants in rare and unclassifiable tumors, but also demonstrate the multitude of opportunities for translational research opened up by comprehensive genomic profiling in a clinical context.

Incidental Germline Findings

When applying WES to cancer genomes, workflows usually include sequencing of matched blood samples to exclude private germline variants and only include somatic variants in downstream bioinformatics pipelines to select for cancer-relevant mutations. However, especially in our cohort of younger cancer patients and/or patients with rare tumors, we anticipated an enrichment of patients with a possible hereditary background. If germline variants would be excluded in such cases *a priori*, relevant pathogenic mutations responsible for cancer development might be missed.[38] Importantly, such mutations could also be therapeutically relevant. This applies in particular to genes linked to DNA damage response signaling, where germline mutations are known to be responsible for hereditary breast and ovarian cancer, and patients with mutations in these genes might respond to PARP inhibition or platinum-based chemotherapy.[39] Therefore, the MASTER program includes calling of potentially pathogenic germline mutations using dedicated bioinformatics pipelines and clinical evaluation by a medical geneticist since 2015. Indeed, since implementing germline variant evaluation, we have identified several patients with pathogenic germline variants in known tumor susceptibility genes, for example, *BRCA1/2*, *PALB2* or *NF1*, that not only led to treatment recommendations but also had important implications for further surveillance of the patients and their families. On the other hand, the majority of rare or private germline variants identified are variants of uncertain significance (class 3 variants) according to the standards and guidelines of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology.[40] Further evaluation of such variants is an important issue that needs to be addressed in future studies, particularly if such variants are identified in potentially targetable genes and/or genes known to be associated with hereditary cancer matching the patients' cancer type and family history. Besides challenges in data interpretation, ethical and legal issues need to be taken into account, and structured evaluation and reporting of incidental germline findings need to be implemented for all clinical germline WES- and WGS-based data.[41] Therefore, a consensus on the discovery, validation by orthogonal methods, interpretation, and reporting of germline findings within MASTER has been developed. Specifically, a board-certified medical geneticist participates in the MTB and is responsible for interpretation of germline variants. In case a relevant germline mutation is identified, recommendation for referral to a medical geneticist and genetic counseling is provided during the interdisciplinary discussions.

Current and Future Developments

Assessment of circulating biomarkers

An integral part of MASTER is the prospective identification of biomarkers to predict the therapeutic activity of targeted therapies in genetically defined patient cohorts, which will help us evaluate their efficacy across different cancer entities during upcoming clinical trials. We are hopeful that we can achieve our goals more rapidly by multiplex evaluation of multiple circulating biomarkers, such as cell-free tumor DNA, exosomes or tumor cells.[42] These may allow for a more precise and dynamic measurement of cancer evolution during therapy and may be better suited for assessing its spatial and temporal heterogeneity, as well as monitoring the emergence of molecular resistance mechanisms.[43,44] "Liquid biopsies" based on detection of circulating, cell-free DNA are one of such promising avenues, able to assess the mutation status and the occurrence of resistance mutations, possibly reducing the need for repeated tumor biopsies.[45] Specifically, in a subset of MASTER patients receiving small-molecule kinase inhibitors, we will perform sequential liquid biopsies to non-invasively monitor mutations in a panel of cancer genes. These mutations can be evaluated as indicators of response or, in the case of rising allele frequencies or newly emerging mutations, acquired resistance to targeted therapy associated with tumor progression and poor outcome. Whole-exome sequencing has also been successfully used for detecting novel driver mutations in addition to secondary resistance mutations in circulating tumor DNA.[46] This method may provide complementary information to the genomic analysis of the tumor and address its clonal heterogeneity.[47] Integration of circulating biomarkers and pharmacogenomic data with clinical information may help us modify our decision algorithms, assess the evolution of resistance mechanisms, move forward the design of adaptive clinical trials, and generate further hypotheses for translational research projects.

Functional testing

A wide range of technologies for *ex vivo* analysis of tumor cells for translational applications have been established within the DKTK. Two of them, expansion and study of tumors using patient-derived organoids (PDOs) or patient-derived xenografts (PDXs), are actively pursued for clinical application.[48,49] These models may offer a faithful representation of the human tumor that is accessible to empirical testing of drugs prior to their use in the patient. Patient-derived organoid- and PDX-based investigations will be guided and accompanied by WES and WGS.[50] Proof of principle for clinical application of sensitivity testing using PDOs has already been successfully accomplished within the DKTK. For example, *in vitro* characterization of organoids derived from a patient with pancreatic adenocarcinoma confirmed sensitivity to PARP inhibition, which was predicted based on a germline *PALB2* variant identified in the MASTER program

(M. Reichert, personal communication). Others have described successful identification of actionable targets and drug combinations in PDX representing STS[51] and melanoma.[52] Within DKTK MASTER, we plan to investigate the feasibility of personalized tumor models derived from patients with high-risk STS or chordoma, as these represent a large subset of our study population and at the same time display a highly unfavorable prognosis due to the lack of efficient systemic therapies. This makes them ideal candidates for the development of patient-derived *in vitro* and *in vivo* models. These models will be invaluable for identification of potentially actionable targets and pathways using pharmacologic and radiotherapeutic approaches. They can also be used to systematically investigate functional dependencies by focusing on individual genes or by performing unbiased functional genomic screens. These efforts will help realize the promise of interdisciplinary personalized oncology in STS and chordoma patients through multidimensional tumor characterization and clinical implementation of treatment recommendations guided by assessment of potentially actionable functional dependencies in patient-derived tumor models. Yet unsolved challenges of these methods are rooted in the heterogeneity or subclonal evolution of PDXs.[53] We are convinced that effective treatments can be identified through rapid establishment of *ex vivo* tumor models in addition to a comprehensive genomic characterization.

Targeted proteomics

Cancer can be characterized by its genomic alterations, but it also needs the translation of genetic information from the driving mutation into oncogenic function of the corresponding protein.[47] The complexities of molecular interactions and signaling dynamics of a cellular system rise exponentially when taking into consideration not only DNA and mRNA, but also protein expression and posttranslational protein modifications. By now, while NGS of tumor DNA and mRNA allows better stratification and tailored therapy of patients, we are well aware of the limitations of these methods, including a blind spot to any translational and posttranslational alterations. Using highly quantitative proteomics technologies such as reverse phase protein array (RPPA), we address these shortcomings and aim to validate protein expression and protein phosphorylation on the way to comprehensive profiling of relevant pathways and druggable alterations. RPPA technology allows simultaneous, quantitative analysis of total, cleaved, or posttranslationally modified (e.g., phosphorylated, glycosylated, acetylated) cellular proteins in a large number of samples. Because of its high sensitivity and accurate quantification, we hope that RPPA technology will provide invaluable information about the expression and functional status of cancer-associated proteins.[54] Ongoing collaborations help us develop and optimize RPPA to allow for more precise charting of active molecules in individual patients. Although we have not yet integrated RPPA into the MASTER workflow, we have retrospectively analyzed protein samples from patients to confirm the feasibility of this method in a clinical setting. Clinical implementation of RPPA requires exact and reliable determination of a given expression level in order to allow selection of a specific therapy based on the presence or absence of a signal. To this means, and in addition to pre-analytical sample quality and the presence of high-affinity and specific antibodies, normalization and quantitation of samples as well as standardized bioinformatic and statistical analysis are crucial.[55] RPPA technology has been successfully used for the identification of activated molecular pathways associated with prognostic and therapeutic relevance in lung and breast cancers.[56,57] Furthermore, as RPPA technology is increasingly incorporated into precision oncology trials,[58] we can advance the development of novel, investigator-initiated clinical trials to prospectively validate newly identified molecular targets as well as biomarkers of therapeutic response. Validation of response and resistance markers will also help us define rational drug combinations for further clinical testing.

MASTERing big data

The aforementioned examples add to our current understanding of cancer, but represent only a selection of translational opportunities possible within the DKTK network and rooted in MASTER. While other high-throughput technologies may still be in their infancy, the innovative and relentless pursuit of research questions raised by NGS-generated "big data" drives our current and future cancer research efforts. To meet the increasing need of clinicians to rapidly access their patients' genetic profiles in order to make genomically informed therapeutic decisions, we aim to develop and optimize a software-based approach to NGS data interpretation. We plan to integrate publicly available open-source databases[59] that assess the clinical relevance of genetic variants and evaluate evidence for individual molecular targets into the routine workflow of DKTK MASTER. We further collaborate with software developers on projects to link patient-level data obtained from electronic health records and corresponding NGS data in an integrative database, named DataThereHouse (DTH). The DTH will be able to process data from several sources, including the clinical information system of Heidelberg University Hospital, the clinical cancer registry, a radiotherapy database, biobank records, local and external clinical trial databases as well as the NGS data from individual patients. In its final form, the DTH will provide clinicians and researchers with comprehensive, real-time access to patient-related records and perform fast and efficient in-depth descriptive and explorative analysis of these data. Furthermore, this system will be an accessible gateway to NGS-generated data and facilitate their visualization, exploration, analysis and quality assessment. We expect that these Information Technology solutions will substantially increase the throughput of clinical interpretation and provide the possibility of standardized evaluation and treatment recommendations based on NGS data. We are confident that

most of the aforementioned preclinical and translational efforts will have a direct impact on the design and realization of planned clinical trials, which will be able to benefit from standardized evaluation of NGS data.

Art and CRAFT of Clinical Trials in Precision Oncology

The art to translate latest-generation molecular diagnostics into solid and reliable, evidence-based clinical treatment has just started and necessitates the craft of systematic exploitation of this knowledge for therapeutic benefit in prospective, controlled clinical trials. However, the conventional strategy to generate clinical evidence by moving stepwise from phase 1 to randomized phase 3 trials is not suited for molecularly diverse patient populations. Challenges in performing clinical trials in precision oncology were underlined by the results of SHIVA, the first randomized phase 2 trial using molecular profiling (including NGS) and molecularly matched treatments in patients with advanced solid tumors.[60] Due to its discouraging conclusions, this study has drawn significant attention but also raised questions about its statistical design, biological rationale and therapeutic decision algorithms.[61] In light of these facts, SHIVA should be regarded as a transformer rather than a destroyer of the paradigm of precision oncology.[62] Therefore, umbrella and basket concepts with parallel assessment of toxicity and efficacy as well as seamless transitions from early to later stages of drug and treatment strategy development are necessary additional components of trial design to meet the challenges of precision oncology.[63] Currently, treatment recommendations resulting from molecular profiling in MASTER can be translated into clinical action in about one-third of cases, mandating further development of robust clinical data to identify the benefits of targeted approaches to otherwise intractable cancers. The newly established NCT Precision Medicine in Oncology (PMO) program will provide a unique opportunity to evaluate in a reliable and structured manner the clinical efficacy of targeted therapies based on comprehensive molecular profiling, and will give patients access to targeted, molecular mechanism-based treatment approaches that are otherwise not or only rarely available. The NCT PMO program as logical continuation and extension of MASTER has already spurred the development of 2 molecularly guided clinical trials, NCT PMO-1601 (evaluation of the CDK4/6 inhibitor palbociclib in CDKN2A/B-deleted tumors) and NCT PMO-1603 (evaluation of PARP inhibition in combination with trabectedin in tumors with defective DNA repair). In addition, the evaluation of multiple different targeted treatment approaches in parallel study arms within the CRAFT (Continuous ReAssessment with Flexible exTension) concept (NCT PMO-1602; Fig. 1) exemplifies one of our current approaches. However, flexibility in adaptive study designs will be essential to account for diverse stages of drug and treatment strategy development from single-arm to multi-arm randomized approaches. Thus, MASTER will serve as versatile platform for rapid clinical translation of biology-driven precision oncology strategies.

Concluding Remarks

In summary, we are convinced that the implementation and continuous refinement of a program for cross-entity, multidimensional tumor characterization in a clinical setting will improve our understanding of cancer through stimulation of fundamental research and technology development, and will help realize the promise of informed, personalized oncology through biology-guided patient stratification and individualization of therapy. These goals are best achieved through intense, interdisciplinary collaboration on the local, national and international level. Last but not least, such a framework provides an ideal environment for the education and training of scientists working in the field of applied cancer research as well as physicians who need to be increasingly aware of the clinical impact that new insights into cancer biology will have in the immediate future.

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