Clinical trials for precision oncology using next-generation sequencing

The demonstrated genomic heterogeneity of human cancers is having major impacts on the development and evaluation of cancer therapeutics and molecular diagnostics. Many new cancer drugs target somatic alterations in tumors and are being developed with companion diagnostics. Oncology drug development and practice are likely to become increasingly stratified and utilize the enrichment Phase III trial paradigm. Although this paradigm includes an increasing number of successes, single-agent molecularly targeted treatment of metastatic disease will generally provide limited patient benefit. More substantial gains will require better understanding of crosstalk among signaling pathways, ability to combine drugs and use of drugs at initial diagnosis. Early phase discovery clinical trials in which patients will have genome-wide tumor characterization at diagnosis and at critical retreatment points will provide data sets for learning how to effectively match therapeutics to genomic alterations. However, moving tumor genomics to clinical oncology entails many practical challenges. We review some of these challenges and the clinical studies that are being undertaken to translate genomics to clinical oncology.

KEYWORDS: cancer biomarker · cancer clinical trial · next-generation sequencing · personalized medicine · precision medicine

Large tumor sequencing studies have demonstrated that human cancers of a given histologic type are often heterogeneous with regard to the mutations that drive their invasion and that the mutations present in individual tumors have major influences on the natural history of the tumor, and its responsiveness to therapy. These findings are having a major impact on the development and evaluation of cancer therapeutics and molecular diagnostics. The paradigms for development, evaluation and administration of cancer treatments have been organized around the primary site of the disease and the new findings will have major influence on all levels of oncology research and practice. With this perspective, we will review some new types of clinical studies that are utilizing high-throughput DNA sequencing.

Phase III clinical trials for molecularly targeted drugs & companion diagnostics in oncology

Most recent success in oncology therapeutics development has been based on the paradigm illustrated in Figure 1. The development begins with the discovery of a recurrent somatic mutation in tumors of a given primary site in tumor DNA sequencing studies. If the mutation occurs in a gene that is considered drugable (e.g., a kinase), then the second step is to develop a drug that inhibits the pathway dysregulated by the constitutively activated oncogene. The third step is to evaluate the drug in clinical trials in which patients are selected based on harboring the mutation. Vemurafenib [1] and crizotinib [2,101] are two recent examples. This paradigm has worked best in cases where the target protein is not widely expressed or essential for normal tissues.

The Phase III trial generally used for the recent successes of this paradigm is the randomized enrichment design shown in Figure 2 [3,4]. With this approach, patients are screened using a clinical-grade test for identifying patients with tumors predicted to be responsive to the drug. Those who are ‘test-positive’ are randomized to receive either the test drug or a standard of care control; those ‘test-negative’ are not included in the clinical trial. The US FDA has voiced some concerns about the enrichment design on the grounds that the drug can only be approved for a restricted population if a companion diagnostic test is also approved, and how the test can be approved if the Phase III trial does not demonstrate that the new drug is ineffective in the ‘test-negative’ patients. This creates an ethical problem, however, since one cannot place patients on a clinical trial of a drug if one does not think it will benefit the patient. The FDA has accepted the enrichment design in recent cases where there was a strong biological rationale or Phase II data for believing that the...
drug is not likely to be effective in test-negative patients. They have also described a regulatory pathway for approval of the companion diagnostic test on the basis of the Phase III randomized enrichment trial – the intended use of the test is to identify patients for whom an effective drug exists, not to distinguish patients who will benefit from the drug from those who will not.

In some cases there may be a subsequent Phase III trial for test-negative patients. There may be some effectiveness of the drug in test-negative patients because no test is perfect and some of the negatives may be false negatives. The drug may also have important off-target effects. In many cases, however, it is appropriate to withhold exposure of test-negative patients to the drug until it is shown effective in the patients whom it is expected to benefit. Unfortunately, most molecularly targeted cancer drugs have normal tissue toxicity since they affect key signaling pathways and are not specific for mutated forms of the targeted protein.

Since cancer biology is complex, it is, in many cases, not possible to define the most appropriate predictive biomarker based solely on preclinical data. For example, the drug target can be mutated or amplified, as in the case of the EGFR receptor, and the impact of the alteration may be mitigated by the presence of another genomic alteration. Consequently, it can be useful to not be overly restrictive in eligibility for the Phase II clinical trials of drugs developed with this paradigm. Studying a broader set of patients enables one to confirm or refine the biological basis for the approach, and also facilitates determination of the best criterion to use for test positivity in the subsequent Phase III trial. There may also be several related candidate tests and the Phase II data on an unselected population may help resolve which best identifies responsive patients.

When there is not compelling biology or Phase II data supporting the use of a predictive biomarker with a defined cut-point, eligibility for the Phase III trial should not be restricted by a candidate marker of uncertain relevance. This may indicate, however, that the mechanism of action of the drug is not sufficiently well understood to go forward with clinical development. As Schilsky points out: “Failures in the development of targeted therapy have not, in most cases, been failures of the drug, but failures of our understanding of that drug target. Thus, the successful development of new targeted anticancer drugs will be driven by a deeper understanding of the biology of human cancer, accompanied by the development of specific and reproducible assays to assess the target in human biospecimens.” The ‘all-comers’ approach is, unfortunately, often used in the manner traditional for post-hoc subset analysis. This is characterized by no prospective planning for evaluating the treatments in subsets determined by candidate biomarkers, no adequate sizing of the study for such analyses or adjustment for multiple testing, and no requirement that tumor tissue of all patients be submitted for biological analysis. Simon and others have described, however, a variety of prospectively planned all-comers designs for developing and evaluating a drug and predictive biomarkers that avoid these problems. These include designs in which the criterion for positivity is not prespecified and designs that select the best predictive classifier from among several candidates in an adaptive manner, while preserving the type I error of the clinical trial. This latter approach is being used in pivotal oncology clinical trials.

**Phase II trials**

A variety of new study designs are being used for biomarker-based Phase II clinical trials. These are mostly clinical trials for patients with...
tumors of a single primary site in which one or more candidate genomic biomarkers are being evaluated in parallel with the new drug. Pusztai and Hess [9], and Jones and Holmgren [10] have described extensions of Simon’s two-stage, single-arm, Phase II design for accommodating a single binary candidate marker. These designs are primarily focused on ensuring that promising activity of the drug is not missed in cases where its activity is restricted to ‘test-positive’ patients and yet excessive numbers of patients are not required in cases where its activity is sufficiently broad that the marker is not needed. Freidlin et al. have described a design for use with a single binary biomarker in a randomized Phase II design that enables one to determine whether the drug should be developed in a Phase III enrichment trial, an all-comers trial or dropped from further development [11].

There are many more complicated Phase II settings, where no natural cut-point of the biomarker is known in advance or where there are multiple candidate biomarkers. Such settings generally require much larger sample sizes than is traditional for Phase II clinical trials and Phase II biomarker trials also require pretreatment tumor tissue from the patients. Consequently, such clinical trials can be expensive and time-consuming. In many cases, the biological characterization of the patients’ tumors can be performed for analysis after patient entry using research-grade laboratory tests. If the tests are going to be used to select patients for the trial, however, then the tests must be clinical grade and performed in Clinical Laboratory Improvement Amendments (CLIA)-certified laboratories in real-time, and an investigational device exemption may be required from the FDA.

The BATTLE I trial in non-small-cell lung cancer (NSCLC) is an example of a Phase II clinical trial in which four different tests were evaluated in the context of four different drug regimens [12]. Treatment assignment among the four regimens was randomized, but the randomization weights varied as the trial progressed according to which treatment had the best performance within each of the four biomarker strata, using freedom from progressive disease at week 8 as the end point. Fresh tumor biopsy was required as an entry requirement. There were two main objectives of the adaptive randomization. One was to efficiently screen four treatments in four predetermined strata of NSCLC patients. The second objective was to provide patients with a trial in which they could feel that the design was adapting to assign them the drug regimen that was best for their form of the disease. Korn and Freidlin have raised questions about the effectiveness of such response adaptive randomization designs for reducing the

![Figure 2](image-url)

**Figure 2. Targeted-enrichment Phase III clinical trial design.** Patients are tested using a proposed companion diagnostic for measuring a biomarker predictive of benefit from new treatment. The test is analytically validated with prespecified criteria of positivity. Patients whose tumors are ‘test-positive’ are included in the randomized clinical trial comparing the new regimen to an appropriate control. ‘Test-negative’ patients are not included in the clinical evaluation of the new regimen. Rx: Treatment.
number of patients receiving what turns out to be a less active regimen [13]. Consequently, it is not clear whether this approach was more efficient relative to the use of optimal two-stage designs for each drug–biomarker combination. The I-SPY 2 Phase II design being conducted in breast cancer also uses an adaptive design with prespecified biomarker strata and multiple treatments [14]. A key challenge for the development of multiarm adaptive trials and trials that prospectively match tumors to drugs is obtaining access to a broad menu of drugs that represent potent inhibitors of their target pathways.

Analytical validation of next-generation sequencing & moving tumor genomics to clinical oncology

For clinical trials in which patients are selected based on a companion diagnostic test, the test should be analytically validated. This generally means that the test is reproducible and has low false-positive and -negative error rates. For next-generation sequencing (NGS)-based tests, the gold standard might be based on Sanger sequencing or estimating the error rates using DNA constructs created with known genomic alterations. A good discussion of analytical validation for NGS is given by Gargis et al. [15]. For tests in which there is no established gold standard, analytical validation means that the test is reproducible over time and is robust to real-world laboratory variation. The vast majority of tumor-sequencing studies have not used analytically validated sequencing and such research sequencing would not be adequate for clinical studies. Developing analytically validated NGS protocols is an important challenge for the new generation of clinical trials.

Analytic validation of NGS-based tests represents a new setting for validation where new guidelines need to be created. While Sanger sequencing only characterizes a small number of targeted regions in the genome, NGS can quickly examine hundreds of targeted regions or even the whole exome or genome. With NGS data, a wide variety of bioinformatics tools are available to move the raw sequence data to sample variant identification and the exact bioinformatics pipeline needs to be included in the analytic validation testing. Any software updates will require revalidation of the test. Given the broad reportable range of NGS tests, a laboratory cannot validate every possible variant observed during the trial. Variants must be categorized into classes within which the assumed performance with the assay will be equivalent, for example, simple nucleotide variants and, insertions and deletions might represent different categories and analytic performance metric estimates reported for each. With the inability to establish false-positive and -negative rates for every possible variant that might be observed during the course of the clinical trial, some studies require a secondary confirmation for each novel positive finding on a complementary platform.

In addition to the complexity of the bioinformatics pipeline, when a NGS test is used in a clinical trial, an informatics system needs to be in place to handle the quantity and complexity of the data and communications among the parties involved. The results of the NGS test need to be returned to the clinic in real-time and in a manner that is easy for oncologists to interpret and to act upon.

Large tumor-sequencing studies such as, the Cancer Genome Project in the UK [102] and The Cancer Genome Atlas in the USA have identified recurrent genomic changes in a variety of primary tumor sites. These data provide a scientific basis for treatment of individual patients based on the biological characterization of their tumors. Moving tumor genomics to clinical oncology entails many challenges, however. Some of these are listed in Box 1 and will be discussed below. The challenges involve logistics, ethics, bioinformatics, study design, regulation, analytical assay validation and interdisciplinary collaboration. Moving genomics to therapeutics involves using drugs for new indications and dealing with uncertainties regarding which mutations in a given gene affect the function of the protein product, which are important for the invasive properties of the tumor and which should be considered ‘actionable’ for administration of a drug that was developed for somewhat different mutations in a different primary site. There is much yet to learn about effective matching of drugs to genomically characterized tumors [17]. Treating patients with drugs selected based on current knowledge to block the dysregulation caused by genomic alterations can, however, provide a database for improving our knowledge of how to combine tumor genomics with therapeutics. It may be much less informative to treat patients without prospective biological characterization and hope to correlate responses to post hoc-assessed genomic tumor alterations; the latter approach may be useful for trying to understand unusually good responses to standard treatments.
Box 1. Questions to address in using high-throughput sequencing for oncology.

- Which patients could benefit?
- How will informed consent be obtained?
- How will incidental findings be dealt with?
- What type of sequencing should be performed?
- How will a computational pipeline for analyzing raw sequencing data be established?
- How will the tissue preparation, sequencing and computational pipeline for clinical use be analytically validated?
- How will sequencing be completed within a clinically relevant time frame?
- How will the sequencing results be interpreted and related to treatment options?
- How will the results be presented to the treating physician?
- How will sequencing results be collected and stored?

Discovery clinical trials using test panels of genomic alterations

A variety of cancer organizations have introduced early-phase clinical trial programs in which a patient’s tumor is biologically characterized and then a drug selected from a menu of available agents based on the characterization (Table 1). Von Hoff et al. used immunohistochemistry, FISH and transcript microarray profiling [18]. Of 106 patients who consented, 86 were biologically characterized and 66 patients having a wide range of tumor types were treated based on the biological characterization. The results of the biological analyses were reviewed by two study physicians. The results were considered in the context of the patient’s prior treatment history and comorbidities, and the identified targets were ranked. For each target, there was a list of FDA-approved drugs that were considered relevant for inhibiting the pathway activated by dysregulation of the target. Based on this information, a specific therapy was suggested to the treating physician and the patient was treated according to the package insert recommendations. There were six responses by the RECIST criteria in the 66 patients, three in breast cancer and one each in ovarian cancer, rectal cancer and NSCLC, with a response rate of 10%. The primary end point of the trial was whether the progression-free survival (PFS) of the study was more than 30% longer than that of the most recent treatment of the patient prior to entering the study. A PFS ratio of greater than 1.3 was seen in 18 of the 66 patients. This achieved the statistical objective of the trial, but is difficult to interpret in terms of whether the matching of drugs to biological characterization achieved better outcomes for the patients. The PFS of a patient is variable, both with regard to the pace of the disease, as well as the limited accuracy of measurement. The use of this end point is based upon the assumption that responses get shorter and shorter as the disease progresses, but whether a PFS ratio of 1.3 or more adequately accounts for sources of variability and potential biases is unclear.

Tsimberidou et al. reported on the results of a Phase I clinical trials program conducted at the MD Anderson Cancer Center (TX, USA) over a 4-year period [19]. The treating physicians requested all available molecular tests that were CLIA-certified at MD Anderson at the time a patient presented to the Phase I clinic. The pathology laboratory prioritized the panel of molecular aberrations for development on the basis of their known frequency in cancer and/or whether they were perceived as actionable, or as having other clinical relevance to patients. The report indicated that “patients whose tumors had a molecular aberration were preferably treated on a clinical trial with a matched targeted agent, when available;” however, it appears that treatment was decided upon by individual physicians.

<table>
<thead>
<tr>
<th>Study name</th>
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<tr>
<td>BRIM3 (vemurafenib)</td>
<td>Randomized, enrichment Phase III</td>
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<td>PROFILE 1007 (crizotinib)</td>
<td>Randomized, enrichment Phase III</td>
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<td>BATTLE I</td>
<td>Adaptive, randomized, Phase II, multiarm, four biomarkers</td>
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<td>ISPY II</td>
<td>Adaptive, randomized, Phase II, multiarm, multiple biomarkers</td>
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<td>IMPACT</td>
<td>Randomized, Phase Ib, rules-based matching design, 22 gene-targeted sequencing panel, four drugs</td>
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<td>SU2C melanoma BRAF wild-type</td>
<td>Randomized, Phase Ib, tumor board-based matching design, whole-exome and RNA-seq, multiple drugs</td>
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<td>SHIVA</td>
<td>Randomized, Phase Ib, tumor board-based matching design, 46 gene-targeted sequencing panel</td>
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<tr>
<td>MOSCATO</td>
<td>Nonrandomized, array CGH and targeted sequencing. No treatment on study</td>
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<tr>
<td>WINTHER</td>
<td>Nonrandomized, Phase Ib rules-based matching design</td>
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CGH: Comparative genomic hybridization.
and whether patients were matched or not was retrospectively determined. Over the 4-year period, approximately 2350 patients were seen in the Phase I clinic and 1283 had molecular analysis ordered. Of the 1144 who had adequate tissue available, 684 had no aberrations detected, 379 (33.1%) had one and 81 (7.1%) had two or more. Of the 379 patients with one aberration detected, 175 were treated with ‘matched’ therapy, 116 with ‘nonmatched’ therapy and 88 were excluded from the analysis for various reasons. Approximately 50% of both the matched and unmatched groups were treated in the escalation phase of Phase I studies. The overall response rate was 27% for the matched group (2% complete response; 25% partial response) versus 5% for the unmatched group. The 70 matched patients with \textit{BRAF} mutations had a 37% response rate, compared with 0% for the 14 nonmatched patients with \textit{BRAF} mutations. The response rate for matched patients without \textit{BRAF} mutations was 20%, compared with the response rate of 6% for unmatched patients without \textit{BRAF} mutations. However, the matched and nonmatched patients differed with regard to their primary site of disease, with the matched group dominated by patients with melanoma and thyroid, lung and breast tumors, and the nonmatched groups were more heavily represented by patients with colorectal and a wide range of other malignancies.

Sequist \textit{et al.} reported on the use of multiplexed genotyping of NSCLCs as routine clinical practice at the Massachusetts General Hospital (MA, USA) [20]. They used a \textit{SNaPshot}\textsuperscript{®} (Life Technologies, CA, USA) assay to simultaneously screen for mutations in hotspots of 16 genes using formalin-fixed paraffin-embedded tumor tissue. They also tested for an \textit{ALK} translocation using FISH. Among 552 patients with sufficient tissue for testing, 51% had one or more mutations detected, most commonly in \textit{KRAS} (24%), \textit{EGFR} (13%) and \textit{PIK3CA} (4%), and translocations involving \textit{ALK} (5%). Of the 353 patients with advanced disease, 22% were steered toward a genotype-directed therapy.

Several organizations are conducting or planning feasibility studies, clinical trials or national programs based on prospectively characterizing a broad range of the molecular alterations in patients’ tumors. The Stratified Medicine Programme led by Cancer Research UK (London, UK), the UK Technology Strategy Board (Swindon, UK), AstraZeneca (London, UK) and Pfizer (NY, USA) aims to collect samples from 9000 patients treated at seven Experimental Cancer Medicine Centers to determine the best way of running a genetic testing service, which could then potentially be implemented over the whole National Health Service [103]. The French National Cancer Institute (Boulogne-Billancourt, France) and French Ministry of Health (Paris, France) have set up a national network of 28 regional molecular genetics centers to provide, free of charge, molecular tests for all patients in their regions, regardless of the institution where they are treated [21].

In the MPACT trial being conducted at the National Cancer Institute (MD, USA), patients with metastatic disease of many primary sites who have exhausted standard treatments undergo biopsy and have their tumors characterized based on amplicon sequencing of 400 actionable variants in 22 genes [104]. Clinical-grade DNA sequencing conducted in a CLIA-certified laboratory with a minimum depth of coverage of 450x per targeted variant is performed. The genes sequenced fall into three pathways, RAS–RAF, PI3K and DNA repair, with one drug available for each of the first two pathways and two for the DNA repair genes. ‘Actionability’ of specific variants in the target genes is predefined based on literature documentation, previous reporting in the Catalogue of Somatic Mutations in Cancer database [105] and evidence of functional effect for loss-of-function genes. Patients whose tumors contain an actionable variant are randomized 2:1 to receive the drug targeting the dysregulated pathway or physicians’ choice among the other available drugs. Control patients will be eligible for treatment with the targeted drug after progression. The rules also cover how drugs are selected in cases with multiple actionable variants. Although a senior physician and the director of the sequencing laboratory oversee the matching of drugs to actionable genomic alterations, the system is rule-based rather than ‘tumor board’-based in which decisions are made for individual cases in a nonalgorithmic manner. Research-grade whole-exome sequencing will be subsequently performed for all patients on the MPACT clinical trial, but only the clinical grade targeted amplicon sequencing will be used for determining the targeted drug.

A 2:1 randomized design is also being used in a Stand Up To Cancer Foundation (CA, USA) study of the treatment of metastatic melanoma in patients whose tumors do not contain a mutation in the \textit{BRAF} gene. The melanoma protocol includes many drugs and an extensive clinical-grade genomic characterization, which will
include whole-exome sequencing and RNA-seq. The melanoma trial will utilize a tumor board for overseeing the matching of drugs to tumors.

The French SHIVA clinical trial is also a randomized Phase II proof-of-concept study comparing therapy based on tumor molecular profiling to conventional therapy in patients with refractory cancer of a variety of primary sites [22]. The SHIVA trial will identify hotspot mutations in 46 genes by targeted sequencing using the AmpliSeq™ (Life Technologies, CA, USA) cancer panel. Amplifications of interest will be assessed using Cytoscan HD™ (Affymetrix, CA, USA). One hundred patients will be accrued to each arm of the randomized trial. No more than 20% of the patients will have the same histologic type of cancer. PFS is the primary end point and control patients will be eligible for crossover after 8 weeks. The trial will be limited to drugs that are approved for clinical use in France because it was deemed too difficult to coordinate different pharmaceutical companies providing drugs in clinical development for the same trial.

The randomized designs have two distinct objectives. One is the testing of the null hypothesis that the policy of trying to match the drug to the genomics of the tumor is no more effective than a physicians’ choice strategy without using any tumor characterization beyond that used for standard of care. Whereas most clinical trials evaluate a single drug or regimen, the null hypothesis here relates to a matching policy for a given set of drugs and biomarkers available for the study. This makes it particularly important to obtain a broad enough menu of potent inhibitors of their targets. The policy is also determined by the type of genomic characterization performed and by the ‘rules’ for matching drug to tumor. If the matching is done by a tumor board and is not rule-based, or if the rules change frequently, then it will be difficult for others to utilize that policy, and the pragmatic value of the clinical trial will be limited. It may also be difficult for regulatory bodies to approve use of investigational drugs for use as decided by a tumor board rather than in a more rule-based manner. Consequently, it is important that the policy be transparent and that the duration of the trial be short, so that the rules do not change frequently. The use of a randomized control group ensures that comparisons of PFS between the matched and control groups are not biased by differences in patient characteristics or biases in assessment of progression. The proof-of-principle embodied by the null hypothesis may be more meaningful, however, in the melanoma trial of a single histologic category than in the other two cases where a wide range of primary sites of disease are included. Although, for advanced metastatic cancer, the primary site may not be important prognostically, the effectiveness of matching drugs to molecular alterations may vary with regard to the type of alteration and the drugs available for treating them, and this may in turn vary by primary site.

A second objective of the randomized studies is the screening for antitumor activity of individual drugs used in specific tumor contexts. For some primary sites, a gene may be mutated sufficiently frequently for the study to provide an adequate Phase II evaluation of the drug for that new indication. In many cases, however, the numbers will not be adequate for a proper Phase II evaluation. Nevertheless, the trial may serve to screen for drug–mutation matches for which there is a substantial degree of activity. This might be viewed as a Phase IB or Phase 1.5 screening trial in which leads must be confirmed in an expanded cohort of a follow-up trial. In this discovery mode, assessment of activity of a drug against tumors with a given mutated gene must take into account the possibility that the primary site may indicate a genomic context that may modulate activity of the drug against the alteration.

Several centers and organizations are conducting or planning nonrandomized studies or molecular characterization programs. These include the MOSCATO program at the Institut Gustave Roussy (Villejuif, France) [23] and the Worldwide International Networking Consortium international WIN Thér nonrandomized clinical trial [106]. MOSCATO profiles patients with refractory cancer using array comparative genomic hybridization and a panel of hotspot mutations in 96 amplicons from a biopsy performed in a metastatic site [23]. The mutations are identified using Sanger sequencing. Patients are triaged to specific Phase I trials according to the presence of a molecular abnormality. The primary end point is the PFS ratio. WINThér will be based on extensive biological analysis for patients with advanced cancer resistant to standard-of-care treatment. Patients with actionable mutations will receive a matching molecularly targeted agent. The trial will include 200 patients and the primary end point is the PFS ratio. Roy Chowdhury et al. [24] described the pilot MI-OncoSeq study they are conducting at the University of Michigan (MI, USA) using extensive integrative high-throughput sequencing [20]. They utilize shallow depth of coverage whole-genome sequencing to identify
copy number alterations and structural rearrangements, and deeper coverage whole-exome sequencing of the tumor and matched germline samples, as well as RNA-seq transcriptome sequencing to identify dysregulated expression and evaluate the functional products of genomic alterations. This evaluation is performed in a research laboratory and potentially actionable findings are verified in a CLIA-certified laboratory. They organized a Sequencing Tumor Board that incorporated expertise in clinical oncology, pathology, cancer biology, bioethics, bioinformatics and clinical genetics, and established that they could accomplish the characterization within 4 weeks at a cost in reagents of approximately US$5400 per patient.

The nonrandomized trials are sometimes called ‘N of 1’ trials in the sense that each patient is different and the outcome of treatment must be evaluated individually in terms of the individual characterization of his or her tumor. This nomenclature can be misleading, however. The ‘N of 1’ approach traditionally referred to a design in which individual patients were sequentially treated for multiple courses with either a test drug or control, with the sequence of treatment or control determined by randomization. This is clearly not possible for cancer studies, however. The only end point clearly interpretable for the nonrandomized studies is objective tumor response. Tumors generally do not shrink spontaneously and so an objective tumor response can usually be attributed to the effect of the drug. Objective responses for patients with far advanced metastatic disease are generally rare and can be used for discovering promising ways to target molecularly characterized tumors. PFS is much less interpretable in these nonrandomized studies. The pace of disease can vary substantially even in advanced cases and so comparing PFS between different subsets of patients is hazardous. Use of the ‘PFS ratio’ is also problematic. PFS is subject to measurement error and ascertainment bias depending on the frequency of surveillance. For a patient who has a PFS prior to entry on a study of 8 weeks, a PFS ratio in excess of 1.3 may only mean that progression was not declared at the first 8-week follow-up of the genomic-based study. This is not strong evidence of an effective treatment effect. In the future, it will be important to define more randomization questions to be addressed in the genomic characterization studies.

One approach for inferring associations between genomic characterizations and treatment response is to retrospectively identify so-called ‘exceptional cases’, patients with an exceptional response to a treatment, who had tumor samples collected at the beginning of the clinical trial. For example, Iyer et al. describe a single metastatic bladder cancer case of a durable complete response to everolimus [25]. The Phase II trial the patient was participating in did not achieve its primary end point. The authors performed whole-genome sequencing of the tumor to identify the cause of the exceptional response. The whole-genome sequencing identified 17,136 somatic missense mutations and insertions and deletions. From this list and sequencing results from additional cases, the authors identified a frameshift variant in TSC1 that may be predictive for everolimus sensitivity. The results of exceptional case studies can be used to guide genomic alteration-targeted treatment matching in prospective clinical trials.

There are two broad strategies for structuring prospective precision medicine programs. The first is a design similar to the MPACT study where the genomic profiling, actionable rules and treatment arms are all under one protocol [104]. Adaptive rules for adding or dropping treatments can be built in, but the entire process is within the study. The second design is to separate the genomic profiling and treatment studies. Here, a screening program with standardized CLIA laboratory-run tests would be performed and an individual genomic report returned. Treatment clinical trials can specify eligibility criteria based on the results of the report of the screening program, but are independent protocols. Since NGS tests evaluate many genes at the same time, they can be used to screen patients for a multitude of treatment studies. The advantage of having separate, but coordinating protocols for the screening and treatment is that this will allow more flexibility in the system.

Princess Margaret Hospital–Ontario Institute for Cancer Research currently has an open feasibility study where patients have their tumors genomically profiled and an expert panel prepares a molecular profile report identifying actionable, or potentially actionable, alterations, which is then placed in the electronic medical record, and given to the clinician for treatment decisions [26]. One possible action is to place the patient on a clinical trial open to patients containing one of the patients’ molecular alterations. A similar study is underway at Vanderbilt University (TN, USA) [27]. They have also developed the My Cancer Genome website, which...
contains curated information about specific variants found in a growing number of primary tumor sites [107]. This website provides current information to help physicians make appropriate treatment decisions based on the genomic alteration report provided based on the tumor profiling. The website also directly links into Clinicaltrials.gov and lists clinical trials that an individual with the given variant would be eligible for [108].

**Conclusion**

New paradigms are being developed for confirmatory, translational and discovery clinical trials in oncology. These changes result from the evidence that tumors of a given primary site or histologic type are genomically heterogeneous and that these differences have major influences on natural history and responsiveness to treatment. Success has been achieved in developing kinase inhibitors using an enrichment design with a companion diagnostic for selecting patients most likely to benefit (or deselecting those least likely to benefit). In most cases, however, degree of effectiveness for patients with metastatic disease has been limited and more substantial benefits are limited by lack of understanding of signaling pathways, difficulties in performing combination therapy studies and lack of potent drugs selective for mutated oncoproteins. Progress in the identification of recurrent somatic alterations in tumors has outstripped the ability of using this information therapeutically. Commonly mutated targets such as, transcription factors, tumor suppressor genes and RAS proteins are often not ‘drugable’. Many clinical trials are conducted without adequate characterization of genomic alterations in the tumors of the patients and many of the alterations are of low prevalence, making their study difficult. Many cancer organizations are putting in place mechanisms for bringing tumor genomic characterization to broader populations and are organizing early-phase clinical trials that will facilitate discovery of genomic alterations exploitable by available drugs. There are many challenges involved in implementing such discovery clinical trials and these include establishing the protocols and computational pipelines necessary to make NGS analytically validated for clinical use, obtaining availability of a menu of investigational drugs from pharmaceutical sponsors for broad discovery studies and developing resources and methods for evaluating the potential actionability of genomic alterations with regard to available drugs.

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<th>Executive summary</th>
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<td><strong>Background</strong></td>
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<td>• Large tumor-sequencing studies have established that human cancers of a given histologic type are often heterogeneous with regard to the mutations that drive their growth and invasion. These findings are having a major impact on the development and evaluation of cancer therapeutics and molecular diagnostics.</td>
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<td><strong>Phase III trials</strong></td>
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<td>• Most recent success in oncology therapeutics development has been based on the paradigm illustrated in Figure 1:</td>
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<td>– Discovery of a recurrent somatic mutation in tumors of a given primary site;</td>
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<td>– Development of a drug that inhibits the pathway dysregulated by the constitutively activated oncogene;</td>
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<td>– Development of a test that identifies the patients whose tumors harbor the target mutation;</td>
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<td>– Conduct an enrichment clinical trial in which patients are selected based on the test and randomized to a regimen containing the new drug, or to a standard of care control.</td>
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<td><strong>Analytical validating next-generation sequencing &amp; moving tumor genomics to clinical oncology</strong></td>
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<td>• For clinical trials in which patients are selected based on a companion diagnostic test, the test should be analytically validated.</td>
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<td>• Developing analytically validated next-generation sequencing protocols and computational pipelines are important challenges for the new generation of clinical trials.</td>
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<tr>
<td>• Moving tumor genomics to clinical oncology entails many challenges, involving logistics, ethics, bioinformatics, study design, regulatory affairs, analytical assay validation and interdisciplinary collaboration.</td>
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<td><strong>Discovery clinical trials using test panels of genomic alterations</strong></td>
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<tr>
<td>• Moving genomics to therapeutics research involves specifying hypotheses on which genomic alterations are to be considered actionable for which drugs. Tumor boards may assist in prioritization of hypotheses for individual patients, but only the clinical trials will provide evaluation of the hypotheses.</td>
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<td><strong>Conclusion</strong></td>
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| • There is much yet to learn about effective matching of drugs to genomically characterized tumors. Treating patients with drugs selected based on current knowledge to block the dysregulation caused by genomic alterations can, however, provide a database for improving our knowledge of how to combine tumor genomics with therapeutics. It may be much less informative to treat patients without prospective biological characterization and hope to correlate responses to post hoc-assessed genomic tumor alterations.
Future perspective
Oncology drug development will become increasingly stratified. Most successful drugs will be developed for targeting genomic alterations in conjunction with companion diagnostics. Reimbursement for drugs without companion diagnostics that only provide marginal average patient benefit will become increasingly difficult to sustain economically. Effective therapeutic development will become increasingly science driven and require substantial resources. New partnerships between academic research and industry are essential. Many recurrently altered genes are not drugable with current approaches and that constitutes a barrier to improving patient benefit. Surmounting this barrier is too high risk for investigator-initiated research or industry, and will require major new government-sponsored, focused initiatives. Single-agent molecularly targeted treatment of metastatic disease will generally provide limited patient benefit. More substantial gains will require better understanding of crosstalk among signaling pathways, ability to combine drugs and use of targeted drugs at initial diagnosis. Early-phase discovery clinical trials in which patients will have genome-wide tumor characterization at diagnosis and at critical retreatment points will provide data sets for learning how to effectively match therapeutics to genomic alterations. Such studies can also provide a setting for identifying drug combinations that are highly active against tumors bearing specific genomic alterations.

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**Websites**


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