Genomic oncology: moving beyond the tip of the iceberg

Jane de Lartigue, PhD

Historically, cancer has been diagnosed and treated on the basis of the tissue of origin. The promise of personalized therapy, matched more precisely to an individual’s tumor, was ushered in with the development of molecularly targeted therapies, based on a greater understanding of cancer as a genomic-driven disease. Here, we discuss some of the evolution of genomic oncology, the inherent complexities and challenges, and how novel clinical trial designs are among the strategies being developed to address them and shape the future of personalized medicine in cancer.

The evolution of genomic oncology

In the 15 years since the first map of the human genome emerged, genetics has become an integral part of medical practice worldwide. Oncology is no exception; the genetic origins of cancer were suspected more than a century ago and it is now well understood that most cancers are driven by genetic alterations that disrupt key cellular pathways involved in tumor survival and progression.

Drugs targeting these alterations have been developed, giving rise to a novel treatment paradigm. Genomic oncology uses the genetic aberrations within a tumor to direct “personalized” cancer care with targeted therapies that include biologics, small molecules, and immunotherapies. The small molecule tyrosine kinase inhibitor imatinib was the prototypical targeted cancer drug and demonstrated significant success in patients with chronic myeloid leukemia (CML) whose disease is driven by a chromosomal aberration that results in a fusion between the breakpoint cluster region (BCR) and Abelson murine leukemia viral oncogene homolog 1 (ABL) genes.

Targeted therapies such as imatinib can be safer and more effective than broad-acting, indiscriminate cytotoxic therapies like chemotherapy, but it has become increasingly clear that greater effect is derived from these drugs when they are used in molecularly-selected patient sub-populations. Indeed, as exemplified by the use of epidermal growth factor receptor (EGFR)-targeted therapy in patients with lung cancer, even the most effective targeted therapies can fail if used in the wrong patient population.

In recognition of this issue, the oncology field has developed molecular biomarkers that can predict response, or lack thereof, to targeted therapy. Drugs are now commonly being evaluated in trials that select eligible patients on the basis of biomarker positivity, and a number of companion diagnostics have been codeveloped to assist in these efforts (Table 1). Notable successes include the development of the monoclonal antibody trastuzumab for patients with breast cancers that have human epidermal growth factor receptor 2 (HER2) gene amplification or HER2 protein overexpression, and the small molecule BRAF kinase inhibitor vemurafenib in melanoma patients with mutations in the BRAF gene.

The rapid clinical development of anaplastic lymphoma kinase (ALK) inhibitors like crizotinib for the treatment of ALK-driven non-small-cell lung cancer (NSCLC), demonstrates the potential for more streamlined development of drugs when targeted patient populations are evaluated from an earlier stage.

The identification of molecular drivers in various cancer types and the development of biomarker-based diagnostics, to identify patients with these specific alterations, have helped to select patients most likely to benefit from targeted therapies, resulting in the approval of more than 30 targeted therapies for oncologic indications. However, in the decades following the development of imatinib, researchers have come to realize that it is an exception rather than the rule. CML is driven predominantly by a single genetic driver – BCR-ABL – identified in more than 90% of patients, but in the majority of cancer types, there may be multiple drivers in play, which are all causally implicated in the development and progression of a given tumor. In addition to these drivers, studies have unveiled a host of molecular alterations that occur as a result of the genomic disarray of cancer and don’t necessarily confer a selective advantage to the tumor, so-called passenger mutations. Distinguishing between the...
two can be a daunting task10 and, adding to the challenge, researchers have recently identified another type of alteration – latent drivers, which are mutations that behave like passengers but, coupled with other mutations, can drive cancer development and drug resistance.11

In spite of many laudable successes, there have been a greater number of failures in the development of targeted therapies. Furthermore, they have not proven to be the cure many hoped for; responses are often short-lived and tumor recurrence all but inevitable. Many lessons have been learnt from these failures and limitations and it is becoming increasingly clear that we have merely touched the tip of the iceberg in genomic oncology.

**Cataloguing cancer genes**

Since the early days of the Human Genome Project, the development of next-generation sequencing (NGS) technologies have made it substantially cheaper and faster to...
sequence an entire genome. We have entered the much-anticipated era of the “$1,000 genome” from a cost of several hundred million dollars just a decade ago. Oncologists are taking advantage of the ready availability of this technology to sequence tumor genomes using biopsied samples in an effort to unravel the molecular underpinnings of different cancers and facilitate broader, more effective implementation of personalized cancer therapy.

The National Cancer Institute (NCI) and National Human Genome Research Initiative (NHGRI) funded The Cancer Genome Atlas (TCGA) in 2006 in a comprehensive and coordinated effort to catalog all cancer genes, starting with ovarian cancer and glioblastoma multiforme and expanding to include samples from 11,000 patients across 33 tumor types. TCGA sample collection was completed in 2013, but data are still being analyzed. Most recently, the mutational landscape of human skin cutaneous melanoma was characterized, using sequencing data from 303 malignant melanoma patients collected through the TCGA. The study illustrated the high mutational burden of melanoma, confirmed the dominant drivers of this cancer type, and identified new genes that may be involved in its development.

Many cancer research institutes and pharmaceutical companies are now also conducting tumor profiling studies on patient biopsy samples. Since 2011, researchers at Dana-Farber – Brigham and Women’s Cancer Center and Dana-Farber – Boston Children’s Cancer and Blood Disorders Center have been carrying out a large-scale cohort study called Profile, which has enrolled more than 35,000 participants and generated more than 12,000 tumor profiles.

There have also been considerable efforts to provide more accurate gene expression profiles using improvements in RNA-based sequencing technologies and to profile the tumor proteome (evaluating differences in protein expression), epigenome (assessing epigenetic alterations such as DNA methylation and histone modification, which can also impact gene expression), and metabolome (to assess changes in tumor cell metabolism, which have also been linked to the development of cancer). The ultimate goal is to combine these “-omics” technologies to gather a complete picture of a tumor and how it differs from normal cells and to determine which of these changes is responsible for the development of cancer.

Currently, about 10% of the known genes in the human genome are known or suspected to play a role in cancer, and around 140 of these have been shown to be driver genes. A study published in the journal Nature in 2014 attempted to determine how close we were to a complete catalog of all cancer genes. The study, led by Michael S Lawrence, a computational biologist at the Broad Institute in Boston, involved a largescale genomic analysis across more than 5,000 human cancers and their matched normal tissue pairs in 21 tumor types. Using a technique called down-sampling, the researchers examined how the number of cancer genes identified changes with sample size. They found that there is still an upward trend in the cancer gene discovery curve, which suggests that many novel genes are yet to be discovered; indeed, this study itself identified 33 new cancer genes.

Challenges and complexity
The identification of novel cancer genes and the widespread clinical application of this vast array of genomic data to guide personalized medicine is not without challenges. Pan-cancer analyses, such as the Lawrence et al study, have highlighted one of the most significant issues that limit the promise of targeted therapy — there is substantial mutational heterogeneity in cancer within any given tumor type from patient to patient and even within the same patient. There is a “long-tail effect”, wherein a minority of cancer genes are mutated at a high frequency (>20%) across tumor types, whereas the majority of genomic alterations occur infrequently from tumor to tumor.

There is additional complexity in the fact that the mutational profile of the tumor can evolve over time and in response to treatment. This may explain why different tumors with the same driver mutation have different responses to therapies targeting that aberration. It also means that, even if therapies are effectively matched at the beginning of treatment, molecular evolution may mean that this changes over time.

The TCGA has also performed a pan-cancer analysis, which was published in 2014; it demonstrated that 2 cancer subtypes (eg, lung and breast) may actually be more similar to each other than to others from the same tissue–of-origin, which reinforces the idea that molecular profile is potentially more important than histology in guiding treatment strategies.

Rising to the challenge: novel clinical trial designs
One of the principal limiting factors in the development of effective targeted therapies is that they continue to be evaluated in clinical trials that are driven by tumor histology and, when biomarkers are used to select a patient subpopulation, typically only a single driver mutation is evaluated. Many experts have called for an overhaul of the clinical development of molecularly targeted therapies and, in recent years, this call has begun to be answered.

A number of next-generation clinical trials have been developed. Basket trials are independent of tumor histology and recruit patients with a variety of different cancer types, based on the presence of a specific molecular alteration(s). Umbrella trials (Table 2) focus on one kind of cancer, but patients are assigned to different treatment arms by match-
<table>
<thead>
<tr>
<th>Trial name</th>
<th>Cancer type</th>
<th>Description</th>
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| BATTLE-2          | Advanced lung cancer that has progressed on first-line chemotherapy | Open-label, multicenter, biopsy-driven, adaptively randomized phase 2 trial  
  4 arms: Erlotinib  
MK-2206 (AKT inhibitor)  
MK-2206 + selumetinib (MEK inhibitor)  
Sorafenib                                                                                                                   |
| I-SPY 2           | Newly diagnosed, locally advanced breast cancer | Neoadjuvant phase 2 clinical trial designed to test investigational agents in combination with standard chemotherapy in women with newly diagnosed, locally advanced breast cancer.  
Active clinical investigations: Trebananib (Angiopoietin 1/2 neutralizing peptibody) ± trastuzumab  
Ganitumab (IGF-1R mAb) + metformin  
MK-2206 ± trastuzumab  
Pertuzumab + trastuzumab  
Pertuzumab + T-DM1  
Graduated agents: Veliparib + carboplatin  
Neratinib                                                                                                                      |
| NCI-MATCH         | Advanced solid tumors and lymphomas that are refractory to or have progressed on standard therapy | Multiple, single-arm phase 2 trials in which patients are biopsied before enrollment and subsequently matched to a therapeutic arm that targets their specific molecular abnormalities.  
Will begin with 10 arms: Crizotinib – ALK rearrangement  
Crizotinib – ROS1 translocations  
Dabrafenib + trametinib – BRAF V600E/K mutations  
Trametinib – BRAF fusions/non-V600E/K mutations  
Afatinib – EGFR activating mutations  
Afatinib – HER2 activating mutations  
AZD9291 – EGFR T790M mutations and rare EGFR activating mutations  
T-DM1 – HER2 amplification  
VS6063 – NF2 loss  
Sunitinib – cKIT mutations                                                                                                                        |
| LungMAP           | Squamous-cell lung                        | Multidrug, multi-substudy, biomarker-driven trial in which patients are matched to an investigational treatment that may target their specific molecular alterations.  
4 substudies: Sub-Study A – tumors with none of the studied genetic alterations assigned to MEDI4736 (PD-L1 mAb)  
Sub-Study B – tumors with PIK3CA mutations assigned to chemotherapy (Arm B1; 50%) or GDC-0032 (PI3K inhibitor; Arm B2; 50%)  
Sub-Study C – tumors with CCND1, CCND2, CCND3, or CDK4 gene amplification assigned to chemotherapy (Arm C1; 50%) or palbociclib (Arm C2; 50%)  
Sub-Study D – tumors with FGFR gene amplification, mutation or fusion assigned to chemotherapy (Arm D1; 50%) or AZD4547 (FGFR inhibitor; Arm D2; 50%)                                                                 |
| ALCHEMIST         | Early-stage resected non-squamous NSCLC   | Tumor specimens removed during surgery and tested for specific gene mutations then matched to adjuvant treatment with drugs that target those mutations but have only been approved for use in advanced lung cancer. The ALCHEMIST-Screening study will examine tumor specimens for ALK and EGFR alterations and patients with these alterations will subsequently be referred to either or two treatment trials testing adjuvant treatment with crizotinib [ALCHEMIST-ALK] or erlotinib [ALCHEMIST-EGFR]. |
| NCI-IMPACT        | Advanced solid tumors                     | Patients’ tumors biopsied and those with specified mutations of interest randomized 2:1 to Arm A (to receive treatment prospectively identified to target that mutation/pathway) or Arm B (to receive treatment not identified to target that pathway)  
4 treatment regimens, 3 pathways, and 20 targeted genes will be evaluated:  
Patients with BRAF, KRAS, NRAS, HRA5 mutations, or NF1 loss of function – GSK1120212 (MEK inhibitor)  
Patients with AKT1, PIK3CA, MTOR gain of function or PTEN, FBXW7 loss of function – everolimus (mTOR inhibitor)  
Patients with ATM, ATR, ERCC1, MLH1, MSH2, NBN, or RAD51 loss of function – veliparib (PARP inhibitor) + temozolomide  
Patients with PARP1, PARP2, TP53 loss of function – MK1775 (Wee1 inhibitor) + carboplatin                                                                 |

Table 2 continued on page 304
ing the molecular makeup of their tumor to a specific drug. These trials can be thought of as parallel phase 2 trials being conducted in a single entity, exploring different treatments based on the molecular makeup of a cancer (umbrella) or looking across cancer types to find responses to a given targeted therapy (basket). They have an adaptive design, allowing the right drugs to be delivered quickly to the right patients. 

The NCI has developed its own initiative that combines aspects of both umbrella and basket trials; NCI-MATCH (Molecular Analysis of Therapeutic Choice) began in July and will enroll around 3,000 patients with multiple cancer subtypes whose disease has progressed on at least one line of standard therapy. NGS will be used to search for 143 mutations targeted by drugs already approved for other indications or that have shown efficacy in late-stage trials. Up to 25% of patients will have rare cancers. The study is taking place at 2,400 sites and will start with 10 treatment arms.

The effectiveness of these trials is contingent on the strength of the evidence linking a molecular alteration to cancer development and the efficacy of drugs targeting that pathway. Success is not always guaranteed. The recently completed CUSTOM (Molecular Profiling and Targeted Therapies in Advanced Thoracic Malignancies) trial highlighted both the strengths and weaknesses of the basket trial design. Five targeted therapies were evaluated in patients with advanced NSCLC, small-cell lung carcinoma, and thymic malignancies grouped by molecular markers. On the one hand, it showed that a large number of patients are not required to identify therapeutic efficacy in some cases (15 patients with NSCLC and an EGFR mutation were enrolled in the erlotinib arm and were sufficient to achieve an overall response rate of 60%), on the other hand, a sufficient number of evaluable patients could not be enrolled in most treatment groups owing to a low frequency of the target mutations involved.

Indeed, study of these less frequent alterations (which Lawrence et al showed likely make up the majority of unidentified mutations in cancer) can prove particularly difficult. Some are so rare that they have only been identified in the...
context of a negative clinical trial, in the single patient who responds to a particular therapy. It is estimated that between 1% and 10% of patients are exceptional responders and, in the past they would typically have been ignored. Now, researchers are beginning to mine these responses to gain insight into the molecular mechanisms driving them.26,27

The idea is to reverse the “genotype-to-phenotype” drug development paradigm by retrospectively analyzing data from clinical trials in which an exceptional response was observed and using NGS technologies to identify the underlying molecular events. This strategy has seen significant successes in recent years and a range of different molecular mechanisms have been reported in numerous different tumor types (Table 3).

<table>
<thead>
<tr>
<th>Targeted strategy/therapeutics</th>
<th>Details of exceptional response</th>
<th>Proposed molecular mechanism</th>
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<tbody>
<tr>
<td><strong>mTOR inhibitors</strong></td>
<td></td>
<td></td>
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<tr>
<td>Everolimus (Afinitor)</td>
<td><strong>Patient 74-year-old female</strong></td>
<td>Mutations in TSC1 and NF2 genes, which activate mTORC1, increase sensitivity to everolimus$^a$</td>
</tr>
<tr>
<td>Malignancy</td>
<td>Advanced bladder cancer</td>
<td></td>
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<tr>
<td>Response</td>
<td>Experienced disappearance of cancer in 3 months and continued to do well for 4 years of follow-up</td>
<td></td>
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<tr>
<td>Everolimus + pazopanib (Votrient)</td>
<td><strong>Patient 70-year-old male</strong></td>
<td>E2419K and E2014K mutations in mTOR gene, which activate the mTOR pathway$^b$</td>
</tr>
<tr>
<td>Malignancy</td>
<td>Metastatic urothelial carcinoma</td>
<td></td>
</tr>
<tr>
<td>Response</td>
<td>Complete radiographic response lasting 14 months</td>
<td></td>
</tr>
<tr>
<td><strong>Multikinase inhibitors</strong></td>
<td></td>
<td></td>
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<tr>
<td>Sorafenib (Nexavar)</td>
<td><strong>Patient 66-year-old female</strong></td>
<td>S214C mutations in the ARAF gene$^b$</td>
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<tr>
<td>Malignancy</td>
<td>Stage IV lung adenocarcinoma</td>
<td></td>
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<tr>
<td>Response</td>
<td>Near-complete response with clinical and radiographic remission for 5 years</td>
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<tr>
<td>Sorafenib + temsirolimus (Torisel) + bevacizumab (Avastin)</td>
<td><strong>Patient 55-year-old female</strong></td>
<td>BRAF gene fusion (KIAA1549-BRAF) PTEN loss$^b$</td>
</tr>
<tr>
<td>Malignancy</td>
<td>Spindle cell neoplasm that progressed after conventional chemotherapy</td>
<td></td>
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<tr>
<td>Response</td>
<td>Durable disease control and symptomatic benefit</td>
<td></td>
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<tr>
<td><strong>Sunitinib (Sutent)</strong></td>
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<td></td>
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<tr>
<td>IGF-1R monoclonal antibodies + mTOR inhibitors</td>
<td><strong>Patient 52-year-old male</strong></td>
<td>Amplification of RET gene$^c$</td>
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<tr>
<td>Malignancy</td>
<td>Platinum-refractory right-sided testicular germ-cell tumor</td>
<td></td>
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<tr>
<td>Response</td>
<td>Clinical and biochemical response</td>
<td></td>
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<tr>
<td>IMC-A12 + temsirolimus</td>
<td><strong>Patients 24-year-old female and 21-year-old male</strong></td>
<td>Activation of mTOR pathway for initial response and subsequent resistance Mutations in PTPRD and GRB10 genes for response to combination therapy$^d$</td>
</tr>
<tr>
<td>Malignancy</td>
<td>Advanced Ewing sarcoma</td>
<td></td>
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<tr>
<td>Response</td>
<td>Near-complete tumor regression with IGF-1R antibody monotherapy, followed by relapse. Female patient then achieved sustained complete response, and male patient achieved complete response for 4 months following combination therapy</td>
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<tr>
<td>HDAC inhibitors</td>
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<td>Vorinostat (Zolinza) + temsirolimus</td>
<td><strong>Patient 26-year-old female</strong></td>
<td>Immune dysregulation, involving an imbalance between effector and functional T-regulatory cells Activation of mTOR pathway$^e$</td>
</tr>
<tr>
<td>Malignancy</td>
<td>Hodgkin lymphoma refractory to standard therapy</td>
<td></td>
</tr>
<tr>
<td>Response</td>
<td>Near-complete response allowing second allogeneic stem-cell transplant</td>
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