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COMMENTARY

The future of cancer treatment using precision oncogenomics

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Abstract Clinicians should soon have the opportunity to use precision oncogenomics to tailor the optimal cancer treatment to a specific patient. Precision oncogenomics will incorporate different sequencing platforms depending on the goal of the sequencing result. For example, the sequencing strategy used in immuno-oncology for the design of a tumor-specific vaccine may be different than that used by oncologists following a patient for clearance of mutations from circulating tumor DNA in the peripheral blood. I will provide a broad overview of several of the ways that precision oncogenomics is likely to influence the field of oncology over the next several years building off the experience at the Genomics Tumor Board at Washington University in St. Louis and a case of small-cell neuroendocrine carcinoma of the endometrium as examples.

Cancer researchers are using the results from comprehensive tumor sequencing studies in an expanding number of ways in an effort to define the most appropriate choice of individualized therapy for cancer patients. When used clinically, this approach broadly defines the field of precision oncogenomics. In a relatively straightforward process, physicians can perform exome- or whole-genome sequencing (WGS) of tumor DNA to identify mutations that are specific targets of small molecule inhibitors or other therapeutics. Similarly, whole transcriptome profiling of tumors by RNA-seq can be used to identify alterations in gene expression or gene splicing that may be amenable to targeted therapies. Researchers can use sequencing results to infer tumor cellularity of their samples and use this information to target putative initiating mutations, which occur in the founding clone of a tumor. They must also consider subclonal architecture and tumor heterogeneity when using sequencing data for the selection of a treatment option with curative intent (McGranahan and Swanton 2017). Both DNA and RNA sequencing of tumors will become more clinically relevant as more and more drugs, which are selective against particular abnormalities found in cancer, become available in the clinic. However, developing specific agents that target loss-of-function mutations remains a pharmacologic hurdle and will continue to be a significant barrier to the application of this approach to all cancer-causing mutations clinically. Also, comprehensive sequencing to identify a matched targeted therapy will not be necessary for some types of cancer or for all cancers of a specific type. For example, the utility of sequencing cases of Philadelphia chromosome-positive chronic myeloid leukemia (CML) in its chronic phase would be extremely low given the efficacy of tyrosine kinase inhibitors with activity against the BCR-ABL fusion oncoprotein. Treatment with one of these tyrosine kinase inhibitors puts almost all patients with CML into long-lasting complete remission (Shah 2018). Other cancers, such as acute myeloid leukemia (AML), have a relatively small number of somatic mutations that drive the disease. Moreover, in AML, these mutations occur in a relatively small number of unique genes (Ley et al. 2013). Therefore, in this type of

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cancer, clinicians may be able to continue to use gene panel testing to identify the actionable drivers present in almost all cases.

Distinct from using sequencing results to identify targeted therapies, researchers can follow the mutational burden of disease in cancer patients over time in an attempt to tailor treatment based on the clearance of tumor-specific mutations. This approach may be agnostic to the prognostic or therapeutic relevance of particular mutations. For example, the clearance of mutations in circulating tumor DNA (ctDNA) in peripheral blood may identify patients who will likely enter into a complete remission and have a favorable outcome after chemotherapy treatment. Alternatively, patients who do not clear mutations in ctDNA may benefit from additional (or alternative) treatment (Oellerich et al. 2017). This approach is of particular pertinence in AML. Whereas comprehensive genomic sequencing of AML may currently have limited use for matched targeted therapy, researchers can follow for mutation clearance of multiple somatic variants detected via exome sequencing or WGS as a measure of residual disease that is predictive of relapse and long-term outcome (Klco et al. 2015).

Finally, precision oncogenomics will play a role in the rapidly evolving field of immunooncology. For example, researchers can identify predicted neo-epitopes, or tumor-specific antigens, which may occur because of the expression of genes that harbor nonsynonymous mutations. The predicted neo-epitopes can then be used to design an antitumor vaccine. In addition, sequencing can delineate the mutational profile of a tumor, such as a microsatellite unstable tumor that may be a suitable candidate for treatment with a checkpoint blockade inhibitor. Lastly, sequencing of tumors can be used to define the human leukocyte antigen alleles (and their expression profile) and detect the presence (or absence) of tumor-infiltrating lymphocytes. The results of these types of sequencing analyses may have implications for the optimal use of several different types of cancer immunotherapy (Palucka and Coussens 2016).

The Division of Oncology and the McDonnell Genome Institute started a Genomics Tumor Board (GTB) at Washington University School of Medicine in 2014 as our initial organized attempt at implementing precision oncogenomics. At the monthly conference, faculty and trainees were invited to present cancer cases as candidates for comprehensive sequencing and analysis. Advisory panels made up of genomic analysts, pathologists, and medical oncologists guided the discussion of the meetings, selected qualified cases, and determined the appropriate sequencing technology to be applied for each case. We also discussed the pertinent findings from the previously sequenced cases. Genetic counselors were key participants in the GTB. Funding for all sequencing and analysis work was provided by the McDonnell Genome Institute and the Division of Oncology. The intended result of the GTB was to increase knowledge, competence, and performance in the use and application of genetic and genomic sequencing tests by medical professionals in the cancer care field. We hoped that by reaching this objective we would improve the treatment of patients with cancer at our institution.

We envisioned two broad types of appropriate cases for the GTB. For the first type, the results of sequencing the case would have had a strong likelihood of guiding therapeutic decisions. For example, those patients with refractory or relapsed cancer who had exhausted standard-of-care therapy options for their disease but remained candidates for additional treatment would be selected and their tumors sequenced to identify other therapeutic options. For the second type, the results would have had a strong likelihood of shedding significant insight into cancer biology. This could lead to novel treatment options for cancers in which little was known of the genomic alterations driving the disease. However, even if the sequencing results did not influence or change a patient's treatment, the results could form the foundation for further research and treatment studies. Of course, the advisory panel considered if the timing of the case was appropriate for sequencing. Could the proposed

sequencing take place in a clinically actionable time frame? If not, was the sequencing worthwhile based on potential benefit of the discovery research alone? Next, the panel weighed whether an appropriate tumor sample (or samples) was available from which to isolate nucleic acid for sequencing. Additionally, the advisory panel had to confirm that the patient had provided informed consent on an appropriate sequencing protocol that would allow for return of results to the treating physician and patient. Finally, the cost of sequencing remains a factor even when the cancer cases are highly selected. The budget necessary for comprehensive sequencing studies such as exome sequencing or WGS, especially when including sequencing analysis costs, can become prohibitive as the number of cases increases. These types of studies are generally not covered for reimbursement under either federal or private health insurance plans (for more information, see CAG-00450N: Proposed Decision Memo for Next Generation Sequencing (NGS) for Medicare Beneficiaries with Advanced Cancer on CMS.gov).

When we started the GTB, the potential for cancer genomics to provide clinically meaningful results seemed to be strong. We believed (and still believe) that well-designed clinical trials that stratify cancer patient treatment based on tumor sequencing results are necessary to validate the use of any sequencing platform for routine patient care. However, we also understood that sequencing select "*N* of 1" cancer cases could yield important, clinically meaningful results. We expected most of our cases to be of the first type described above: cases that we sequenced in an attempt to identify novel therapeutic targets. Instead, after more than 2 years of conferences, almost all the candidate cases and those that moved forward to sequencing were of the second type: cases that we sequenced to primarily answer research questions. We recognize that we ran our GTB differently than other cancer centers run their molecular tumor boards, in which genetic results (often from gene panel testing and not more comprehensive sequencing) of many more cancer patients are discussed at each conference. These molecular tumor boards are explicitly clinically oriented and provide a much-needed service to clinicians in an effort to clarify and interpret genetic testing results. Nonetheless, most of our faculty and trainees wanted to use cancer sequencing through our GTB as a means to understand cancer biology and not as an effort to tailor a potentially untested treatment for a patient. Does our experience with the GTB portend resistance to the implementation of precision oncogenomics in the future? In general agreement with a pessimistic outlook, there have been a number of studies and editorial pieces published over the past few years that have been critical of the use of gene panel testing or other genomic sequencing platforms for use in tailoring treatment for precision medicine or precision oncology. Conversely, there have been several well-designed studies and editorial pieces that support the utility of cancer sequencing. Rather than discuss the merits and shortcomings of each of these manuscripts, my assertion is that we are still working to establish a role for precision oncogenomics in the clinic, and I strongly believe that the challenge is not insurmountable.

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As I have recounted previously, Dr. Tim Ley and other colleagues here at Washington University sequenced my leukemia genome in 2011 when I relapsed with acute lymphoblastic leukemia (ALL) for the second time. I had a hematopoietic cell transplant in 2008 after my leukemia relapsed for the first time. After the second relapse, I was treated with a standard salvage chemotherapy regimen. However, after the first cycle of chemotherapy, which had

life-threatening side effects, my leukemia persisted. There were no other available treatment options that would likely put the relapsed, refractory cancer into a lasting remission. Thankfully, the results of the sequencing changed everything. Analysts did not identify actionable targets from the exome or WGS of my relapsed leukemia sample (using DNA obtained from a skin biopsy at my original diagnosis as a normal or "germline" comparator). Yet, from the results of the RNA-seq data, a team of analysts led by Dr. Malachi Griffith identified that the *FLT3* gene was being overexpressed in my leukemia cells (when compared to *FLT3* expression in other ALL samples) (Griffith et al. 2016). I started taking the multikinase inhibitor sunitinib, which was FDA-approved for other types of cancer and was an antagonist of the wild-type FLT3 tyrosine kinase being expressed by my leukemic blasts. After starting sunitinib, the leukemia went into a prompt remission, and I went on to have a second hematopoietic cell transplant in 2011. I detail my own story again not to belabor it but instead to illustrate how I wish cancer genomics could play out on a large scale. In hindsight, and with much more experience in genomics and oncology, I realize how incredibly fortunate I was that sunitinib was effective in my case (Wartman 2015).

To conclude, I will summarize a case that we sequenced for our GTB last year. It illustrates that there may not always be a clear separation between the two types of prototypical cases that I have described above. Instead, the use of precision oncogenomics may not only inform the selection of treatment but may shed insight into cancer biology as well. To begin, a 53-yr-old woman presented to her local gynecologist with the complaint of postmenopausal bleeding for ~4 mo. Her gynecologist performed an ultrasound which revealed a uterine mass that was biopsied. The preliminary pathology from the outside hospital showed a poorly differentiated neoplasm, favoring an undifferentiated carcinoma. Subsequently, the patient underwent a total hysterectomy and bilateral salpingo-oophorectomy with pelvic and aortic lymph dissections. This revealed a T1bN0M0/FIGO stage IB, grade 3, small-cell neuroendocrine carcinoma of the endometrium. It was a 4.5-cm tumor with myometrial invasion and extensive lymphovascular space invasion. Zero of 21 pelvic lymph nodes were positive and 0 of 14 periaortic lymph nodes were positive for metastatic disease. The patient presented to an outside emergency department ~1 mo postoperatively with nausea and was found to have multiple brain metastases. There was significant mass effect of the right cerebellum resulting in compression of the fourth ventricle. She was taken to the operating room and underwent a right frontal ventriculostomy and right-sided posterior fossa craniotomy with tumor resection. Pathology confirmed a metastatic small-cell neuroendocrine tumor. The patient then underwent whole-brain irradiation and went on to complete four cycles of adjuvant carboplatin and etoposide systemic chemotherapy. She entered into a complete remission. Given that small-cell neuroendocrine carcinoma of the endometrium is a rare tumor with a poor prognosis, the patient's case was presented to the GTB as a candidate for comprehensive sequencing that would be used to identify actionable target alterations in the event of her relapse.

We performed exome, WGS, and RNA-seq on DNA/RNA isolated from the patient's initial tumor sample. The results were notable for two findings: (1) 901 SNVs or indels were called by three or more variant callers from exome/WGS data (805 of these passed manual review), and 305 of the called variants were indels near homopolymer stretches. This mutational profile was consistent with microsatellite instability (MSI-high). (2) We detected two mutations, which are likely biallelic, in *SMARCA4* (A314fs and e15-1 splice site mutation). Interestingly, inactivating biallelic mutations in *SMARCA4* were identified in 12/12 cases of small-cell carcinoma of the ovary, hypercalcemic type (Jelinic et al. 2014). The overlap of these mutations in these distinct types of tumors suggests the possibility of some relationship between the loss of *SMARCA4* and a small-cell carcinoma phenotype, which merits sequencing additional cases of small-cell carcinoma of the endometrium and further investigation. In our case, we also identified mutations in *SMARCA2* (N1227fs), *PTEN* (R14fs and

N32fs), *MSH3* (K383fs), and *ERCC2* (R378H). In concordance with the MSI-H mutational profile, routine immunohistochemistry performed on the tumor sample by pathology showed loss of nuclear expression of *MLH1* and *PMS2*. Germline genetic panel testing by a CLIA-approved laboratory revealed a pathogenic variant in *MLH1* (1559-2A>C splice acceptor variant) consistent with the diagnosis of Lynch syndrome. Apart from the implications of the *MLH1* germline variant on further cancer screening and management for the patient and her family, the patient's mismatch repair-deficient tumor may be preferentially susceptible to treatment with a checkpoint blockade inhibitor (Le et al. 2017). Given mutations in both *SMARCA2* and *SMARCA4*, drugs that alter the function of the SWI/SNF complexes, of which SMARCA2 or SMARCA4 are subunits, could be a therapeutic option for the patient in the future. Although speculative at this point, the patient's tumor might be sensitive to EZH2 inhibition as well (Wang et al. 2017). Although the sequencing results of this GTB case have not been as clearly "translatable" to the clinic as my own, they were clearly significant. The case provides an example of both the potential of precision oncogenomics by identifying several targets for therapy and also the pitfalls of the field as there is not strong evidence directly linking the therapeutic targets in the rare cancer to antitumor response or favorable outcome. Overall, advances in genomics and genomic analysis are making it possible to study cancer cases in a wide variety of different contexts with the goal of putting precision oncogenomics into practice. We should remain optimistic that several different platforms utilizing precision oncogenomics will be validated in prospective, randomized clinical trials over the next several years and the field will advance from the level of "*N* of 1" case studies to evidence-based standard-of-care for many cancer patients.

ADDITIONAL INFORMATION

Ethics Statement

The patient was enrolled in a single-institution, tissue-banking protocol approved by the human studies committee at Washington University. She provided written informed consent for comprehensive sequencing studies, including exome sequencing. The Washington University Institutional Review Board (IRB) approved this protocol.

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Competing Interest Statement

The author has declared no competing interest.

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