Can Intensive Longitudinal Monitoring of Individuals Advance Cancer Research?

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The convergence of genomics, proteomics, systems biology, the Internet, and molecularly targeted drugs is transforming oncology. Striking advances in DNA sequencing have enabled the comprehensive assessment of a tumor's genome, epigenome, and transcriptome, whereas progress in mass spectrometry has allowed the broad characterization of proteins and metabolites [1, 2]. Sequencing normal tissue from a cancer patient allows germline polymorphisms characteristic of that individual to be distinguished from mutations specific to the tumor. This digital annotation of cancer has already taught us a fundamental lesson—that every cancer is different and that even individual cancers can be genetically heterogeneous [3]. The implications of this deceptively simple fact are profound. How do we reconcile cancer's uniqueness with clinical trials that must assume broad similarities among patients assigned to different treatment arms? An implication of cancer's vast diversity is that even the largest clinical trials cannot “even out” the differences between groups assigned to different treatments.

Molecular oncology's greatest clinical achievements thus far have come from studying single genetic lesions such as the Abelson–break point cluster (*BCR-ABL*) translocation or human epidermal growth factor receptor 2 (*HER2*) amplification [4]. The potent consequences of these alterations are sufficiently distinctive to be discernible clinically. However, most cancers arise from clinically indistinguishable combinations of aberrantly regulated genes. The limitations of most previous population studies can be seen from current studies. For example, the breakthrough melanoma drug vemurafenib, developed to target the mutant form of the BRAF protein, can stimulate tumor growth when the mutation is absent [5]. Like vemurafenib, most cancer drugs only work in specific molecular contexts; however, unlike vemurafenib, we generally don't

know what the right contexts are. How many drugs have failed clinical trials because they are effective in some molecular contexts but are ineffective or even cause harm in other contexts? In another example, bevacizumab is a monoclonal antibody that inhibits vascular endothelial growth factor A and inarguably benefits some patients with cancer; however, current methods have not allowed us to predict which patients will benefit, resulting in the recent withdrawal of U.S. Food and Drug Administration approval [6]. The utility of population-focused studies may be approaching an asymptote [2]. Cancer patients thus face an ever-widening gap between exponential advances in technology and the existing linear framework for identifying better treatments.

A central challenge in evaluating massive datasets is to discriminate biologically relevant signals from vast backgrounds of noise. The spotty success of genomewide association studies highlights the difficulty in detecting biologically relevant differences even in very large populations. In contrast, relatively little attention has been paid to maximizing signal-to-noise ratios in studies of small numbers of individuals. For cancer studies in the future, it will be critical to assure that both tumor and normal tissue are procured in a manner that maintains the integrity of the information they hold. Formalin fixation, the standard method for tissue processing, is detrimental for studies involving mRNA, metabolites, and many proteins. Even modest time intervals between tissue resection and processing can degrade RNA and confound immunohistochemistry assays [7]. Assuring high-quality tissue collection will be essential for future cancer trials.

Another vital area for development pertains to controls. Cancer patients provide opportunities for novel controls. One type is temporal: a patient's current tumor can be compared

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with tumor samples taken from the same patient at earlier time points of the disease. Such comparisons may cast light on mechanisms underlying disease recurrence or resistance to treatment. For example, sequential monitoring of patients receiving targeted therapies directed at *BCR-ABL* [8] or anaplastic lymphoma kinase–echinoderm microtubule-associated protein-like 4 (*EML4-ALK*) [9] revealed mechanisms of drug resistance and new opportunities for therapeutic intervention. In another example, sequential genome-scale monitoring recently provided insight into mechanisms by which acute myeloid leukemia can relapse [10] or evolve from antecedent myelodysplasia [11]. A second type of control is biological. A frequent finding during the treatment of patients with advanced cancer is that the disease progresses at some sites while remaining stable at other sites. A comparison of growing versus stable sites of disease may point to mechanisms underlying cancer progression and possibly opportunities for intervention. Although the heterogeneity of cancer within individual patients may promote the emergence of more than one mechanism of treatment resistance at a time, it is reasonable to hypothesize that favored mechanisms will predominate in the context of specific genetic backgrounds and exposures to past treatments.

It will be crucial to place patient-specific data into the context of an ever-expanding body of publicly available information. Open-access policies and computational methods for aggregating data across different platforms will provide vital tools. To determine the significance of differences detected between tumor tissue taken at different time points or between tumors from different anatomical sites, it will be necessary to estimate the variation that exists within a given tumor at a given time point. Clinical annotation and longitudinal monitoring will provide information about how cancer evolves within individuals over time. Given the enormous potential of transforming massive datasets from individual cancer patients into clinically effective treatments, it is sad that the fraudulent actions of a prominent investigator at Duke have cast a pall over the field [12]. In response, the National Academy of Sciences' Institute of Medicine recently issued a consensus report describing many of the obstacles associated with interpreting genome-scale ("omic") information and recommending that a "bright line" separate omic testing for research from omic testing to direct clinical care [13]. While these guidelines aim to protect cancer patients from poorly conducted science, it is imperative that they do not further distance cancer patients from scientific innovation. Interesting examples of exploiting omic information to inform the care of cancer patients have been reported [14–16] and constitute an important avenue for further exploration.

Attempts to use large-scale sequence data to inform clinical

decision making in cancer patients represent the earliest installments of what promises to be a multidecade effort. These early approaches attempt to find opportunities for intervention based on the direct identification of candidate targets [14–16]. Moving forward, we need to understand how genes collaborate to cause cancer. The resulting network models must ultimately explain how mutated and aberrantly regulated genes expropriate cell context-dependent signaling pathways to drive uncontrolled growth and must provide insights that can be exploited for therapy.

A fundamental question is whether or not medicine can advance based on the experiences of individuals. A recent landmark study from outside the cancer arena provides a glimpse into the future. Chen, Snyder, and colleagues longitudinally analyzed 20 blood samples taken from a single healthy individual (the study's senior author) over a 14-month period [17]. Meticulous construction of that individual's personal genome provided unprecedented resolution, allowing maternally and paternally inherited alleles to be distinguished and RNA editing events to be discerned. An assessment of genetic disease risk pointed to an unexpected predisposition to type 2 diabetes mellitus. Analysis of transcripts, proteins, and metabolites generated more than three billion data-points over a period of 400 days. Remarkably, during that period, the individual indeed developed clinically evident type 2 diabetes following a respiratory syncytial virus (RSV) infection. Longitudinal analysis of RNA sequencing data pointed to an association among allele-specific expression, RNA editing, and immune responses, and the integrated analysis of transcriptional, proteomic, and metabolomic profiles suggested a link between the individual's immune response to RSV and the emergence of type 2 diabetes. Although far from definitive, these results demonstrate that careful longitudinal monitoring of individuals can generate novel insights and hypotheses, and they suggest that combining the experiences of larger numbers of patients may eventually allow causal relationships to be discerned.

Longitudinal monitoring of individual cancer patients may similarly unveil new associations that have heretofore remained beyond the reach of population-based studies. Realizing the full potential of cancer research specifically, and personalized medicine generally, requires ways of using collections of intensively monitored individual patient experiences to assign confidence limits to our observations and, ultimately, new ways to think about causality.

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