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EDITORIAL – TRANSLATIONAL RESEARCH

Solving for Chemotherapeutic Sensitivity: Adapting "Black Box" Methods to Study Patient-Derived Tumor Organoids

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At their core, modern approaches to solve some of the world's biggest problems have a common limitation. The real world and the data produced in studying it are messy and complex. The myriad factors influencing the behavior of people, systems, and machines often are innumerable and obscured. Whether due to a lack of understanding in the context of limited factors or due to a process driven by infinitely complex influences, the short-term result is the same: problem-solving relies on the adaptation of heuristic methods in the context of hypothesis-driven research.

Science and engineering rely heavily on a method termed the "black box" problem to study the behavioral characteristics of a model or system. In any black box problem, the internal factors that drive the behavior of the system are obscured, and instead, the model is studied strictly by characterizing the external influences (the input) exerted on the system and observing the response (the output). With serial experiments, the behavior of the system can be predicted in response to a variety of controlled stimuli. The general approach is adaptable to many fields, with the metaphorical "black box" having contained computer systems, companies, biologic processes, and entire societies in the past. As the theory goes, if the black box model is good enough and inputs are well chosen and controlled, then the future behavior of the system represented by the black box can be reliably predicted.

Patient-derived organoids (PDOs) are the black box in clinically oriented cancer research. As contained systems, PDOs are enveloped quite literally in a cell membrane, with the capacity to precisely control perturbation (e.g., exposure to chemotherapeutics) and to allow observation of the resulting behavior (cellular fitness). In a black box approach, PDOs function well because although our understanding of the processes that control cellular behavior are continually improving, there remain challenges in scaling up the lessons from fundamental discoveries to have a direct impact on patient care. Notable exceptions abound, including tyrosine kinase inhibitors in stromal tumors and immunotherapeutics for tumors demonstrating microsatellite instability, but in the vast majority of difficult-to-treat gastrointestinal malignancies, we have yet to discover a silver bullet to address a solitary, dominant vulnerability-driving behavior.

In this issue of *Annals of Surgical Oncology*, Flood and colleagues[1] perform a systematic review to explore the data available in support of a strategy using PDOs to inform patient care. Focusing on colorectal cancer as a model disease, they accept the hypothesis that PDOs model the tumor from which they are cultured with high fidelity. With the PDOs accurately modeling each patient's disease, they then focus on data exploring the role of PDO pharmacotyping or drug-sensitivity testing against a panel of clinically relevant therapeutics to accurately predict clinical chemotherapeutic response. Akin to antibiotic testing in a clinical microbiology laboratory, a precision oncology

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approach would narrow clinical chemotherapeutic administration to those agents demonstrating maximal efficacy against PDO survival.

In evaluating the use of PDOs, the authors highlight three distinct biologic patterns of disease. In the first biologic pattern, studies exploring disease that lines the peritoneal surface are highlighted with a focus on pharmacotyping work intended to guide the debated therapeutic selection during hyperthermic intraperitoneal chemotherapy or hyperthermic intraperitoneal chemotherapy (HIPEC). This is an area that remains understudied in most high-volume PDO laboratories and is particularly intriguing because the clinical paradigm for care may be the most analogous to the translational methods (i.e., bathing the tumor directly in a chemotherapeutic solution). These data principally show that it is possible to perform the translational methodology in peritoneal surface malignancy, but to date, no clinical data are available to inform the capacity to serve as a prognostic biomarker in this setting.

The second biologic pattern investigated involves the systemic treatment of advanced colorectal cancers. Because some of the pioneering work performed to develop modern organoid technology was completed using LGR5+ intestinal stem cells,[2] a plethora of data are currently emerging to support the technical feasibility and good correlation between PDO pharmacotype and clinical response. Data in this space have further spawned at least two technology startups aimed at moving directly into the clinical care of patients. Investigations in this space are perhaps of the most immediate clinical significance. Even in metastatic disease, the combination of effective systemic chemotherapy and surgical resection can lead to cure. In this context, the future may be less about testing the biology of disease and more about testing for a chemotherapy to meet the biologic challenge in locally advanced, inoperable tumors or metastatic disease.

In the treatment of both synchronous and metachronous liver, lung, and peritoneal metastases, clinical response to therapeutics has a heterogeneity that may be captured by organoid profiling at different data points of the stepwise management, especially in understanding the tumor evolution and emerging resistance to therapy during the course of the treatment. Persister cells, the presumed seat of disease recurrence, also may be uniquely characterized by the establishment and *ex vivo* chemotherapeutic exposure of PDOs.

Finally, the third area of interest is the adaptation of PDOs to accurately characterize chemoradiotherapeutic response. The data remain quite exploratory, although further support for the use of PDOs to predict radiotherapeutic response would broaden the appeal of the technology to clinicians, researchers, and patients alike. For example, this translational technology has major potential implications for emerging clinical techniques such as the "watch-and-wait" strategy of localized rectal cancer and total neoadjuvant therapy. In a watch-and-wait approach, PDOs may serve as an optimal biologic reference for selecting patients for this strategy who have the best predicted and observed chemoradiotherapy response in order to support the decision for organ preservation in an attempt to distinguish those with a high likelihood of durable complete clinical response from those at highest risk of failure and tumor regrowth.

Integrating these findings in the performance of a meta-analysis brings into focus a few of the outstanding translational questions that remain to optimize the PDO technology and methods. The technology, by necessity, relies upon soluble growth factors in culture media to nurture the malignant epithelial component of the tumor at the expense of other cell types in the tumor microenvironment. Does the PDO culture, devoid of other cellular components in the tumor microenvironment, behave in a manner similar to that of *in vivo* tumor? Alternatively, is a more complex model incorporating other TME components necessary or warranted? As an example, adopting a similar pharmacotyping strategy to that described in most cases for emerging immunotherapeutic approaches would almost certainly require co-culture with the effectors of immunotherapeutic cell death.

Another outstanding issue calls into question the validity of early pharmacotyping altogether. The behavior of organoids in early culture, often called the establishment phase, can be greatly impacted by the waning cellular fitness and death of the cells not supported by specific growth factor supplementation.

Looking further forward, the question arises, what are the clinically relevant dose ranges for each agent proposed, and at what exact concentration *ex vivo* can we assume a good clinical response in the clinic? Many, including our group, have begun to assemble a large set of PDO pharmacotyping data from which the key clinical breakpoints (sensitive vs. resistant) can be correlated,[3] but do these data need to be acquired for all histopathologies and for all therapeutics? Extensive cross-validation of individual laboratories may be expected before clinicians are able to rely on pharmacotyping reference values derived from other cohorts.

Finally, how do we deal with the inevitable failures using this approach? Whether these failures arise due to limitations in methodology (immunotherapeutics in the absence of an immunocompentent model) or in biology (Mitogen Activated Protein Kinase inhibition demonstrating exquisite PDO cell-killing in *KRAS* mutant models with known clinical failure of the drug class), it is safe to assume that practical limitations will restrain clinical utility in some settings.

Despite these open questions, as highlighted by the study of Flood and colleagues, PDOs are a compelling emerging technology. Creating patient-specific tumor models offers many advantages when viewed from the perspective of a clinician interested in adaptation of emerging technologies in precision medicine initiatives. Unlike two-dimensional cell lines, the use of growth-factor supplementation and growth in a three-dimensional environment allows for rapid PDO establishment and maintenance of tumor heterogeneity with a high success rate. Mouse xenografts similarly preserve tumor heterogeneity and enable chemotherapeutic testing, but are limited by their high cost and lingering pace to development. Pharmacotyping is a direct and rational approach being studied across tumor types in an effort to provide evidence of predictive capacity. Importantly, however, a precision medicine approach that embraces PDOs but focuses on pharmacotyping alone may result in missed opportunities for patient care. Specifically, in certain settings, the sensitivity of emerging clinically relevant histopathologic techniques and molecular diagnostics is dramatically improved. Many of these tests, including next-generation sequencing technologies, often rely on the analysis of samples with high epithelial–stromal ratios. These ratios can be improved with a period of organoid expansion before molecular characterization.

Ultimately, the historical capacity to predict chemotherapeutic sensitivity in malignancy has been limited. Through population-based work, randomized controlled trials, and molecular study, we currently can provide reasonable guidance to patients with a new diagnosis. The era of precision medicine in difficult-to-treat gastrointestinal malignancy remains dependent upon the discovery of predictive biomarkers of clinical chemotherapeutic response that for many do not exist to date. Will PDO pharmacotyping follow the path of two-dimensional cell culture drug screens, in which high-throughput drug testing failed to live up to the promise of precision medicine? Can PDOs convert clinical chemotherapeutic selection into a black box problem with enough clinical fidelity to meet the need? Interest appears to be sufficient, from both basic science and clinical researchers, for time certainly to tell.

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