COMMENTARY

Fishing for answers in precision cancer medicine

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Zebrafish Xenotransplants Enter Precision Oncology

"Which drug should I prescribe to the cancer patient in front of me?" This question is an inevitable riddle for many oncologists. While the list of US Food and Drug Administration-approved biomarker-driven targeted therapies in oncology grows every year, and clinical trials for new immunotherapy agents soar, chemotherapy is still widely used in the treatment of many cancer types. Even with the advent of new therapies, response rates that are often close to or below 50% still make it a coin toss to guess which drug a specific patient will respond to (1). The field of precision medicine has emerged to address this question and to arm oncologists with tools to make better predictions in prescribing treatment, based not on group averages but on a patient's unique tumor genotype or phenotype. In this vibrant and growing field, genomic sequencing technologies and functional in vitro and in vivo drug testing using patient-derived tumor xenotransplantation into immunocompromised mice have been the dominant research directions.

In PNAS, Fior et al. (2) bring zebrafish xenografts into the fray of precision medicine with a proof-of-concept study that illustrates the use of xenotransplantation of patient-derived colorectal carcinomas in zebrafish larvae as a fast in vivo drug testing platform. They optimized xenotransplantation of human colorectal cancer cells into zebrafish larvae that have not yet developed an adaptive immune response. Transplantation into transparent larvae allows for microscopic assessment of some phenotypic properties of the cancer cells (e.g., angiogenesis, migration); however, foremost, it is amenable for rapid parallel drug testing with high statistical power. To show the model's value for precision medicine, Fior et al. (2) tested drugs that are the current standard of care for metastatic colorectal cancer, moving from a first-line treatment with cytotoxic chemotherapy [5-FU+oxaliplatin+folinic acid (FOLFOX) and 5-fluorouracil(FU)+irinotecan+folinic acid (FOLFIRI)] to second- and third-line–targeted agents (Cetuximab and Regorafenib, respectively) following the National Comprehensive Cancer Network/European Society for Medical Oncology guidelines. They performed a head-to-head comparison of drug treatment response of colorectal cancer cell lines in zebrafish and mouse xenotransplant and successfully showed the ability to identify different responses to chemotherapy agents in fish in 4 days. Finally, they moved on to a proof-of-concept study illustrating the applicability of the method for precision medicine by testing drug sensitivities on five zebrafish patient-derived xenografts obtained from resected colorectal tumors.

Of Fish, Mice, and Men

Knowing a patient's chemosensitivity profile offers a powerful tool to guide therapeutic decisions that can have a profound impact on health outcomes, as is exemplified by the impact of antimicrobial susceptibility testing on the management of infectious diseases (3). The trial and error approach that oncologists are currently forced to adopt due to lack of predictive tools results in significant consequences for both the individual patient and the healthcare system. A patient being treated with an ineffective drug does not have a neutral cost, as many treatments used in oncology come with several side effects. Some of these can be severe and life-threatening, requiring hospitalization for acute management, while others might have lasting consequences, like therapy-related secondary cancers or long-term toxicity, which are particularly burdening for pediatric and young adult cancer survivors (4–7). Delaying access to an effective drug allows the disease to progress or develop resistance. Additionally, as suggested by recent clonal evolution studies, previous treatment with chemotherapy might cause the tumor to evolve and even reduce the efficacy of immunotherapy approaches (8). These negative effects on the health outcome of patients also correspond to an increased economic cost for patients, their families, and the healthcare system as a whole, particularly if we consider the rising cost of drugs in oncology and hospital care for patients who have cancer (9).

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To address this unmet need, chemoprofiling of the patient's own tumor has been proposed by drug testing short-term cultures and tumor organoids in vitro and mouse xenotransplants in vivo. With the addition of a zebrafish larvae xenotransplantation platform (2), a few considerations are in order:

i) Both in vitro and zebrafish larvae xenotransplant assays require little starting material, and therefore allow testing of many conditions in parallel, with limited cost and high statistical power. Mice xenotransplants, on the other end, require a significantly higher number of cells, and are a more expensive and work-intensive model, limiting the possibility to scale up for testing multiple conditions in parallel.

ii) This difference in cost and scalability is partially due to the difference in infrastructure footprint, facilities, and reagents required for the different models. Each approach presents different barriers to adoption as a routine practice in cancer centers and hospitals. For example, animal facilities housing immunocompromised mice would require special conditions and larger spaces and would have a higher operational cost per patient tested than a tissue culture room, or a fish facility dedicated only to generating larvae thanks to the high fecundity of zebrafish and low maintenance costs. In vitro systems and zebrafish larvae xenotransplants have a much higher potential for automation to increase throughput, standardization, and reproducibility of the process.

iii) Unlike mice xenotransplants, which require weeks to months, in vitro assays and zebrafish larvae xenotransplants have a very short readout time, with a chemosensitivity profile available in less than a week from the tumor biopsy. This time window is short enough to impact and inform the therapeutic decision-making process for picking a first-line treatment. The potential translational impact of personalized mouse xenografts, in fact, has been limited to correcting the course of treatment and picking a second- or third-line treatment.

iv) An ever-growing body of genomic data from many tumor types is illustrating the importance of clonal dynamics and cancer evolution as major reasons behind primary and secondary drug resistance (10). As Fior et al. (2) discuss in their paper, the longer time and higher cell number used in the mouse assay will likely allow a better analysis of clonal dynamics and evolution of drug resistance from minor clones compared with the fast xenotransplant in zebrafish assay or other in vitro assays.

v) The assay's performance might also depend on the drug tested, where drugs with a non–cancer cell-autonomous mechanism of action and/or resistance would be better assessed in an in vivo system. Fior et al. (2) show successful testing of cytotoxic chemotherapy agents (FOLFOX and FOLFIRI) and targeted treatments (Cetuximab and Regorafenib). Conservation of the target or interspecies differences between humans and fish might hinder the testing of some drugs that target the tumor microenvironment.

Ultimately, which approach will be better suited for a certain tumor type will significantly depend on its specific predictive power and its cost-effectiveness for translation in a clinical setting. Years of unsuccessful clinical translation of findings based on drug testing in mouse xenotransplants have called into question to what extent transplantation models at large can recapitulate actual human patients. For drug testing purposes, the lack of coevolution with the tumor microenvironment and immune system, as well as differences in physiology, could render xenotransplant models into somewhat glorified cell incubators.

The preliminary patient sample-derived zebrafish xenotransplantation results reported by Fior et al. open the exciting opportunity to rapidly test the platform's validity as a prognostic tool in colorectal cancer and other tumor types in larger prospective clinical studies.

Thanks to advances in genome editing, the parallel rise of genetically engineered mouse and zebrafish cancer models might become competing or complementary approaches, but both lack the speed and clonal complexity of zebrafish and mouse xenotransplants, respectively, and present greater barriers to upscaling for clinical application (11–13).

Similarly, while the explosion of genome sequencing is already reaching clinical applications (14), our still limited knowledge of the functional impact of many mutations and evidence for epigenetic and transcriptional mechanisms of drug resistance make functional chemosensitivity profiling assays a necessary and powerful complement to sequencing-based predictions (15).

The preliminary patient sample-derived zebrafish xenotransplantation results reported by Fior et al. (2) open the exciting opportunity to rapidly test the platform's validity as a prognostic tool in colorectal cancer and other tumor types in larger prospective clinical studies. Additionally, they offer an opportunity to test the combination and possible integration of multiple approaches (e.g., zebrafish xenografts and DNA sequencing and/or mouse xenografts). In the ever-growing repertoire of -omics technologies and functional tools available for precision medicine, one plus one might very well equal three in prediction power. Cost-effectiveness studies will become of paramount importance to sort through the many options on the table, and move the most useful ones toward real clinical translation.

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