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Dishing out cancer treatment

Despite their limitations, *in vitro* assays are a simple means for assessing the drug sensitivity of a patient's cancer. After consulting experts in the community, we think such assays deserve a second look.

Selecting the right course of chemotherapy and kinase inhibitor is a daunting task. Although we now have decades of collective experience describing the average best course of action for a given tumor type, in too many cases in a given cancer patient, the selected drug regimen proves ineffective. In recent years, the search for biomarkers that can predict sensitivity or resistance to specific drugs has intensified, but so far, useful molecular signatures predictive of treatment outcomes for patients are few and far between. As a patient's cancer cells are usually readily available from biopsies or surgically resected tumors, shouldn't it be possible to test therapies on the cancer cells isolated from patients and identify the most effective drug and regimens?

Clinicians and scientists have asked this question from the very beginning of the modern era of cancer medicine. Starting in the mid-1950s researchers began to develop methods to isolate and culture cancer cells for assessing their sensitivity to an ever-growing collection of anti-cancer agents. Early on, the assays measured the drugs' effect on cell proliferation or cell viability in short-term culture. Subsequently, with the first wave of the cancer stem cell theory in the 1970s, so-called clonogenic assays, which measured the number of cells able to grow into single cell–derived colonies *in vitro,* were pioneered by Salmon, Hamburger and others. However, enthusiasm about the application of these assays in the clinic was severely dampened in 1983 by two influential commentaries, which voiced skepticism about the likelihood of these assays improving the outcome of cancer treatment (*N. Engl. J. Med.* **308**, 129–134; 154–155, 1983).

Thirty years later, though, the central conclusions from the original clonogenic assay articles still mainly hold. First, the correlation between drug resistance *in vitro* and *in vivo* is relatively high (up to 90% or more). Second, the correlation is lower for initial response of a tumor to a drug (40–70%) and many of the observed responses are transient and partial. Third, most clinical studies conducted to assess the utility of *in vitro* drug testing have been unsatisfactory because they were small, uncontrolled, nonrandomized and/or retrospective, or had other methodological problems. And fourth, there is enough evidence—albeit imperfect—that hints at the potential utility if the assays could be improved.

Unfortunately, the lack of immediate success led most of mainstream oncology to turn its back on *in vitro* drug sensitivity testing, and only incremental advances have been made in the development of the methods since the 1980s. This seems surprising as the technological advances of the past three decades offer the possibility to address many of the real or perceived weaknesses of existing cellular assays.

A key critique of the cultured tumor cell approach is that the cells are not representative of those in the patient. In the 1980s and 1990s, only relatively crude tests of cellular physiology and morphology were available to optimize protocols. Today, however, a whole gamut of genomic, transcriptomic, proteomic and metabolomic profiling technologies are available to systematically optimize the culture conditions and track cells *in vitro* so they resemble those *in vivo*.

Of course, we now know tumor cells are just part of the picture; a realistic assessment of drug sensitivity must provide a snapshot of the tumor microenvironment and its complex vasculature, stroma and immune cells. Although tumor cells have been co-cultured with various nonmalignant cells, and small fragments of cancer tissue have been cultured in collagen gels to approximate their native environment, much more effort is needed to cross-fertilize approaches in tissue engineering with those in cancer tissue preservation.

A place to start is likely to be hematological malignancies. Harvesting and culturing bloods cells is much easier than for solid tumors. In fact, blood cancers are the only types of tumors in which primary patient isolates are routinely used to validate lead compounds in the drug development process.

New methods for single-cell analysis should also enable assessment of the degree to which heterogeneity of the original tumor is retained in the *in vitro* model and of how different subpopulations of cancer cells are affected by the treatment regimen. This seems especially important as it might offer ways to find drug combinations that affect all cell populations and might result in more durable tumor responses than therapies that have large average effects but leave certain cancer cell populations unharmed (e.g., cancer stem cells). That said, in the clinic, obtaining samples that reflect the whole spectrum of tumor heterogeneity might be difficult, especially in cases where only miniscule amounts of tissue can be obtained or when much of the primary tumor is instead used for routine diagnostics.

A more intricate problem is determining the appropriate concentrations of drugs to test *in vitro*. Most cancer drugs have a very small therapeutic window, and the relationship between maximal serum concentrations and intra-tumor concentrations for any given tumor is not known. Here, advances in analytical and spatial computer simulation technology might be able to provide better estimates.

So will investing research funding in optimizing *in vitro* drug sensitivity assays provide more tangible benefits to patients than spending on existing approaches currently dominating cancer research, which focus on personal cancer genomes or biomarker discovery? At the moment, the jury is out on whether we can understand the biology underlying drug sensitivity or the likelihood that a complex phenotype can be boiled down to a limited number of molecular markers. In the meantime, optimizing *in vitro* screening methods might help us exploit our growing battery of drugs more efficiently until more rational ways of selecting drugs become available. After years spent on the sidelines, perhaps *in vitro* screening methods deserve another look.