BIOENGINEERING

Drug testing in the patient: Toward personalized cancer treatment

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Two different devices show that delivery of cancer drugs directly into tumors in vivo can indicate cancer sensitivity; if implemented in clinical practice, these devices have the potential to reduce indiscriminate drug use, to improve survival, and to reduce unnecessary adverse effects (Jonas *et al*. and Klinghoffer *et al*., this issue).

Cancer now affects nearly one in two people in the developed world. Despite a plethora of new drugs, and the application of ever more complex analyses of molecular mechanisms, survival rates in many common cancers have been slow to improve (*1*). Several factors are responsible, among which are late diagnosis and the life-threatening nature of some cancer types owing to their anatomical location. In many cases, the reason for cancer's persistence is the suboptimal choice of therapeutic agent and, even if the correct drug or combination is given initially, drug resistance develops and tumor responsiveness becomes difficult to achieve.

In this issue of *Science Translational Medicine*, two different groups engineered devices to improve drug selection by means of directly injecting drugs into the patient and analyzing cancer response within the tumor microenvironment itself (*2*, *3*).

MINDING THE KNOWLEDGE GAP

In infectious diseases, the ease with which we can cultivate pathogens in vitro and evaluate sensitivity to agents has helped clinicians select treatments for individual patients. However, a patient's cancer cells frequently fail to survive in vitro, and biopsies of a tumor are often too small for multiple analyses and difficult to obtain throughout evolution of the disease.

Little is understood about the nature of the response of cancer to systemic therapy. In fact, even though upfront or "neoadjuvant" systemic therapy has been given to many thousands of patients with various cancers, the literature regarding the effects of treatment on the cancers themselves is primarily limited to radiological or histological analyses, often combined with immunohistochemical (IHC) and multi-omic analyses of tissue extracts. Traditionally, the descriptive nature of histopathology has been limited by the tendency of clinicians to put all cancers, once resected, into either formalin or a freezer, thus killing the very cells that need to be characterized to understand—and thus to eradicate—the disease.

In addition, we do not fully know the relationships among gene expression, treatment effects, and the cells (stromal, inflammatory, and immune) and vessels surrounding the tumor. A disproportionate effort has gone into developing methods for omic quantification in bulk tumor extracts versus developing the bioengineering, physics, and microanalytic tools that could be used to study the effects of therapies on different tumor compartments, cell types, and niche microenvironments.

Resistance to therapy is also poorly understood. In vitro studies in cancer cell lines have shown that resistance is due to many factors, especially expression of drug transporters, tumor-stroma cross-talk, clonal evolution of cancer cells, and mutations and amplification of genes responsible for repairing drug-induced lesions. But it is difficult to determine which mechanism dominates in individual cancer patients (*4*).

The importance of studying cancer "in the patient" is highlighted by the fact that cancers growing in patients differ from in vitro models in several respects. Preclinical models of cancer, including patient-derived xenografts (PDX), rarely mimic the complex microarchitecture of solid tumors. These models also do not have the critical environmental and physical characteristics of a tumor, including complex inflammatory cell infitrates; variable vascularization; defective, disorganized, and leaky blood vessels and lymphatics; increased stiffness of extracellular matrix; and high interstitial fluid pressure, although recent work has shown that driver mutations are indeed recapitulated in PDX models (*5*).

Tumor microarchitecture profoundly affects drug diffusion and distribution, owing to different binding affinities to extracellular components. High bulk tumor penetration by a drug does not necessarily mean high intracellular accumulation (*6*). Because of relative feasibility, most work is descriptive, focusing on the tumor vasculature. Transcytosis plays a minor role in drug transport in tumors as compared with transvascular drug transport occurring by diffusion and convection across an irregular endothelial cell lining. Unlike normal tissues in which the vessels are regularly spaced and allow even oxygen diffusion, tumors have avascular areas, limiting diffusion of oxygen, nutrients, and drugs.

INSIDER INFORMATION

In this issue of *Science Translational Medicine*, Jonas *et al*. (*2*) and Klinghoffer *et al*. (*3*) both propose a similar solution to studying tumors in the patient: to introduce the drugs directly into the tumor in situ, before resection, and assess the cytotoxic or proapoptotic effects on cancer cells.

Jonas and colleagues engineered a small, cylindrical device, 820 μm in diameter, designed to release different drugs from 16 discrete reservoirs. The device was implanted into tumors of several different types via a biopsy needle and left in situ for 24 hours (Fig. 1). After this time, the device and tissue could be removed with a coring needle for IHC analysis. Combinations of drugs could be released from a single reservoir, and there was the potential for temporal drug sequencing by changing the well size or embedding the drugs in a polymer for controlled diffusion.

As a first step to linking drugs to their effects, Jonas *et al*. demonstrated a correlation between apoptosis [caspase-3 cleavage (CC3)] and drug concentration and one between cell proliferation (Ki67) and tumor type. To combat heterogeneity in cancer response to drugs—which is a known challenge in diagnostics—the authors implanted several devices into different areas of one tumor and used replicate wells in one device. The authors were further able to assess local pharmacodynamics and diffusion rates and distance. To obtain an accurate readout, it was necessary to implant the device close to the non-necrotic periphery of the tumor. The authors further calculated that drugs released from the device diffused 200 to 300 μm into tumor tissue.

After characterizing their tiny drug

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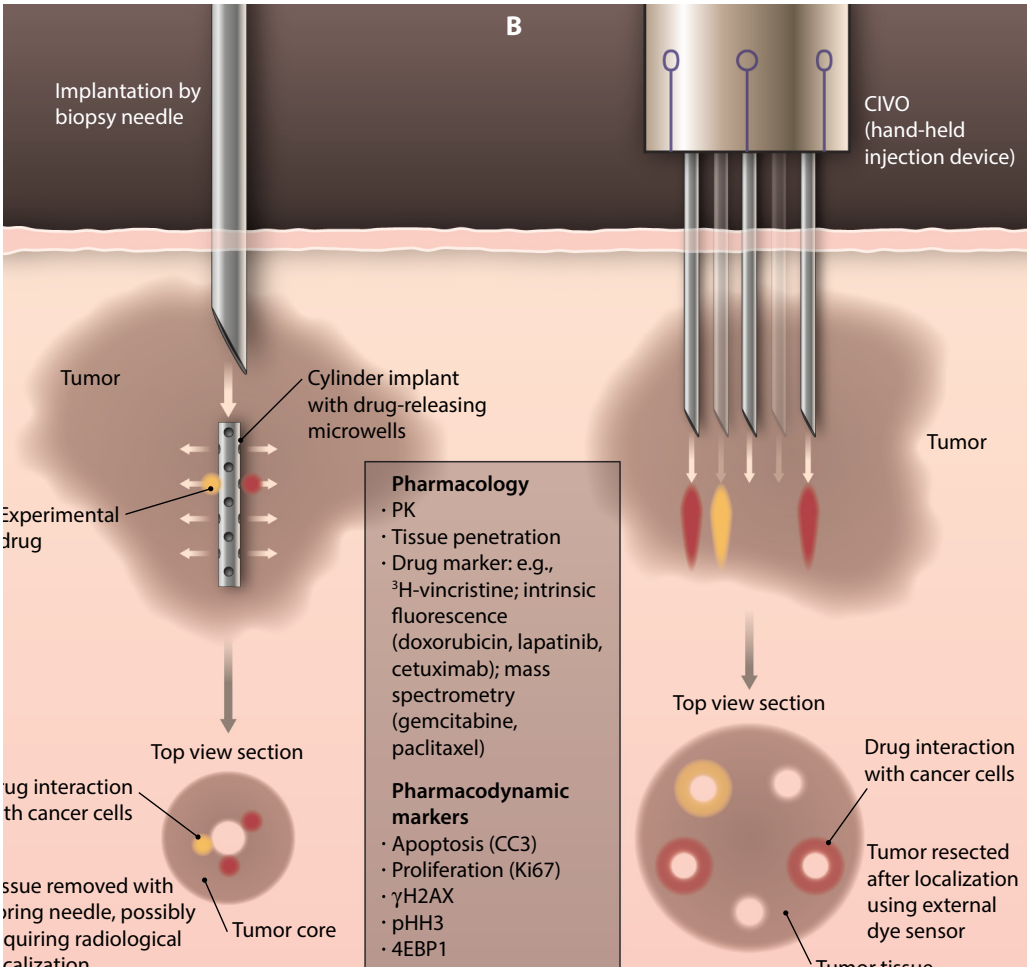

Fig. 1. Personalized drug sensitivity testing. Two different devices described in this issue of *Science Translational Medicine* (*2*, *3*) allow for in vivo drug sensitivity testing and biomarkers analysis in patient tumors. (**A**) The Jonas *et al*. device is implantable and can evaluate up to 16 different drugs simultaneously. (**B**) The Klinghoffer *et al*. device, called CIVO, microinjects up to six different drugs before tumor resection and has been tested in rodent and canine models and in human patients. Various pharmacologic and pharmacodynamic markers were evaluated in both studies to demonstrate that device outputs reflected in vivo, systemic response to therapy.

delivery device, Jonas *et al*. aimed to correlate localized tumor response to drugs with systemic treatment—which would ultimately be the utility of such a device in patients. The authors used doxorubicin in animal models of human melanoma, prostate, and breast cancers and found that the device mirrored the systemic response but with less variation. In a mouse model of human triple-negative breast cancer, the ranking of effective therapies as determined with the device was the same as the whole-animal response to systemically administered drugs (*2*).

The report from Klinghoffer *et al*. (*3*) describes a technology called "CIVO" that has a similar intent but differs from the Jonas device in important respects. CIVO is an array of up to six needles. The operator injects drugs through different needles as they are withdrawn, leaving a 6-mm "track" containing the drug and a co-injected inert tracking dye (Fig. 1). Similar to Jonas *et al*., after injection and incubation in situ, the tumor tissue was excised, and the effects of drug on cancer cells were measured. CIVO also comes with an automated analytical package that measures the readout and takes account of the cell phenotype infused, allowing for the construction of multiple readout curves for each tumor. The operator can choose which area to infuse; further, the visible track allows the cellular response to be correlated with biologically distinct microenvironments, as judged by a panel of biomarkers, offering the potential for combinatorial interrogation of drugs.

The principal preclinical focus of Klinghoffer *et al*. was a human lymphoma xenograft model treated with vincristine. The drug was imaged within a 2-mm limit of distribution beyond the injection epicenter. It was possible to monitor phamacodynamic effects of vincristine by using phosphohistone H3 and CC3 assays (Fig. 1). Other drugs and their mechanism-specific effects were also examined; for example, doxorubicin and the active metabolite of cyclophosphamide induced γH2AX (γ–H2A histone family, member X) phosphorylation in cells; prednisolone, a drug that is used in lymphoma treatment regimens, enhanced apoptosis induced by vincristine, while being inactive as a single agent.

A drug-resistant version of the lymphoma model was unexpectedly responsive to cyclophosphamide, as predicted with the CIVO device. Resistant tumor–bearing animals were treated with cyclophosphamide and were rendered tumor-free. Notably, standard in vitro culture of these resistant cells did not show responsiveness to cyclophosphamide, indicating the importance of in-tumor screening of drug efficacy. Klinghoffer *et al*. also checked the sensitivity of their lymphoma models to 97 approved cancer drugs and found that the resistant cells, but not the chemotherapy-naïve cells, were most sensitive to a previously unidentified mammalian target of rapamycin (mTOR) inhibitor; this finding, as with the example above, correlated with tumor response in vivo.

Importantly, Klinghoffer *et al*. moved CIVO forward into clinical testing (*3*). In dogs, the authors could deliver and track vincristine microinjections, and local cell responses to drug could be evaluated after tumor resection. The authors further tested CIVO in four human patients, demonstrating the feasibility of the approach to injecting superficial tumors (lymph nodes, in this case) and evaluating cancer cell death after 24 hours. This preliminary assessment in a small number of patients indicated that

the insertion of the device was well tolerated and safe within the limitations of this very early evaluation. A larger clinical trial is now planned for CIVO.

REALITY CHECK

Both devices in this issue of *Science Translational Medicine* have a lot in common (Fig. 1). The Jonas device has the advantages of interfacing with an existing clinical tool: the biopsy needle. Thus, the tumor tissue is accessed and imaged easily, enabling the drugs to be directly compared. The CIVO device is a microinjection platform that delivers drugs to a larger swath of tumor, thus offering the possibility of evaluating more biomarkers over a broader field. Although these are novel approaches to in vivo drug testing, there are disadvantages to these technologies. The issue of heterogeneity, although less of a problem than with other tests, may still present problems; drug treatment can select for dominant outgrowths of small populations of cells, including progenitor cells, that may ultimately be responsible for the regrowth and resistance to treatments. The authors measured tumor responsiveness within 200 μm to 2 mm of their devices, and resistant or responsive cells may not be in this vicinity.

Recent studies have shown that metastases evolve and survive in vivo by developing driver mutations, enabling cells to both overcome effects of treatments and survive in sometimes hostile environments in distant organs (*7*); thus, determining the effectiveness of treatment of the primary cancer by use of these technologies may not predict which therapies would be effective in metastatic disease. A solution to this problem could be the assessment of drug sensitivity of different metastases. To do this, the devices could be modified for implantation or injection under radiographic control in different organs—for example, combining positron emission tomography with probes to delineate heterogeneous areas of the metastasis.

Tumors are characterized by elevated interstitial fluid pressure (IFP) and high concentrations of proteins in the extracellular fluid, both of which impede drug diffusion. The implantation of these devices may disturb the tumor IFP and produce misleading results. Other confounding factors include tumor hydraulic pressure, elastic modulus, and the effect of collagen density (*8*), as well as tumor cell density and changes in intratumoral pH, which can vary widely within tumors, leading to differential effectiveness of therapeutics in different areas of the tumor (*9*). Thus, drug response measured with these devices may not fully reflect the in vivo situation, and more studies will be necessary to correlate local response with systemic effects.

A major advantage of these platforms is enabling testing sensitivity of cancer cells with the attendant stromal cells, immune cells, and vasculature—also known as the "microenvironment"; without these associated cell types, in vitro results can be misleading. A recent publication (*10*) attempted to answer this question using a machine-learning approach, in which various conventional parameters recapitulated patient-specific intratumoral heterogeneity, including the omic landscape. The system claimed to phenocopy the patient's tumor microenvironment by using tumor explants maintained in defined matrix support, matched to the individual's matrix system, in autologous patient serum. Such models, although enabling ex vivo evaluation of in vivo phenomena, may be too complex for clinical translation in the near future, unlike the methodologies described in *Science Translational Medicine* (*2*, *3*).

Although the methods of drug assessment described by Jonas *et al.* and Klinghoffer *et al.* do not address issues of absorption and metabolism, they are able to evaluate cell penetration, drug stability in the tumor environment in patients, and effectiveness in causing apoptosis and inhibiting cell proliferation. All cancer drugs have adverse effects, and one less obvious benefit of these systems is the ability to construct dose-response curves, thus informing clinicians about the degree of sensitivity; it is often the case that oncologists give large doses of chemotherapy when a smaller dose would suffice.

An additional advantage of these technologies includes the fact that they can potentially address the problem of drug discovery for cancer therapeutics. Many chemotherapeutic agents require activation in vivo, and for these, in vitro testing may yield misleading results, unless the activated drug is used in the test system. As pointed out in both papers (*2*, *3*), we are facing unprecedented challenges in drug discovery because of the high attrition rate. Indeed, the pharmaceutical industry has generated more potentially useful agents in the past 20 years for cancer treatment than in the entire history of cancer drug development, but the cumbersome and expensive journey needed for drug evaluation hinders the efficient translation of their work to benefit cancer patients.

PERSONALIZED DRUG TESTING

These techniques offer a possible alternative to the "hit and miss" way of using anticancer drugs in patients that has unfortunately become accepted practice; instead, these devices offer a personalized system for assessing drug sensitivity in vivo and tailoring therapy accordingly. Several hurdles need to be overcome before this can happen. The first is to validate more markers of drug action and effect. The relatively small number of these histological markers mentioned in the papers reflects the general paucity of adequate markers; Ki67, in particular, is infamous for being operator-dependent. Reliable biomarkers that reflect the activity of many targeted drugs are few and far between. Second, the two papers here focus on breast and prostate cancer and lymphoma, but others should be investigated because these approaches may be more useful in some cancers than others.

Further, one technology could be more suited to a particular cancer rather than others by virtue of location and size. Recurrent cancer presents different problems of access, size, and extent of organ invasion (lung, central nervous system, and bone, for example) compared with those of primary cancers; the technologies may have to undergo modification before being used for these indications. The devices then need to be evaluated in clinical trials in patients with metastases, to compare response rates with conventional approaches to treatment selection. In this context, it will be important to determine whether quality of life of patients can be improved by limiting the number of unnecessary and toxic therapies that so many patients have to endure. The other major question is whether the selection of more appropriate patient-specific adjuvant therapies, as determined with these devices, will improve survival in patients with potentially curable, localized cancers. Engineering the new technologies is the first important step. But only carefully designed clinical trials will answer these crucial questions.

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