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EDITORIAL – TRANSLATIONAL RESEARCH

Patient-Derived Sarcoma Organoids Offer a Novel Platform for Personalized Precision Medicine

Adrian Sanchez-Fdez, PhD[1,2], Ashwyn K. Sharma, MD[1,2], Herve Tiriac, PhD[1,2], and Jason K. Sicklick, MD, FACS[1,2,3]

[1]Division of Surgical Oncology, Department of Surgery, University of California San Diego, San Diego, CA; [2]Moores Cancer Center, University of California San Diego, La Jolla, CA; [3]Department of Pharmacology, University of California San Diego, San Diego, CA

The promise of precision oncology has been to molecularly profile, stratify, and match patients' treatments with one or more drugs that are predicted to be effective for treating their cancers.[1–3] Numerous efforts have been made towards the development of methodologies to accomplish such a goal, including improvements in multi-omic analyses (i.e., DNA, RNA, and protein) with more rapid turnaround times of test results, novel bioinformatic tools, and drug screening approaches for use in real-time.[4] Altogether, these advances in the field are allowing for personalized precision cancer care to become more of a reality than ever before.

Oncology represents a particularly challenging field for precision medicine. Indeed, high inter-tumoral heterogeneity, as well as large spatial and temporal intra-tumoral heterogeneity is often observed in tumors with similar histology.[5] Sarcomas are a class of tumors that vividly highlight this complexity, because this catchall term encompasses more than 60 different sarcoma subtypes[6] and likely even more if we further subdivide individual histologies by anatomic location, molecular driver(s), and immune infiltrates (e.g., tertiary lymphoid structures).[7,8] These factors, taken together with the extremely low incidence of each sarcoma subtype, make it extremely difficult to perform clinical trials without pooling sarcoma patients together independent of histology. Despite the fact that sarcoma research has grown substantially over recent years, there still has been limited success in translating preclinical discoveries into therapeutic advances.[9] We are still using many of the same cytotoxic chemotherapeutic agents that we did one or two decades ago. This discrepancy between laboratory and clinical success is exacerbated by a general lack of preclinical models. Currently, two-dimensional cell line cultures are the primary and most widespread tools to study soft tissue and bone sarcomas. Although undeniably useful, these monolayer cell cultures fail to recapitulate the three-dimensional structure of tumors.[10,11] In addition, clonal cell lines are derived from only one cell type, and thus cannot capture the intra-tumoral heterogeneity found in clinical samples nor the tumor microenvironment (TME), which includes immune cells, cancer associated fibroblasts, blood vessels, and paracrine signaling networks. Therefore, developing novel ex vivo organoid models, which could potentially capture the three-dimensional structure, TME and cellular heterogeneity of these tumors, is of utmost importance. Ideally, these models would be translationally accurate, quick to develop, affordable, and easy to use.

In this current issue of the *Annals of Surgical Oncology*, Forsythe and colleagues report a novel approach for developing sarcoma patient-tumor organoid (PTO) models using a novel hydrogel-based method with a high success rate of establishment.[12] Organoid models are a powerful system to study patient-specific malignancies as they recapitulate an individual's unique tumor biology.[13,14] Furthermore, organoids provide the sarcoma field with a critical and necessary methodology to overcome the current dearth of research models. PTOs also allow researchers to investigate multiple therapies for specific sarcoma subtypes where rarity of the subtype may create

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J. K. Sicklick, MD, FACS
e-mail: jsicklick@health.ucsd.edu; jsicklick@ucsd.edu

challenges in obtaining an accurate representation of the tumor or performing a clinical trial. The authors not only described this application for the evaluation of chemotherapy efficacy in a variety of sarcoma subtypes, but also developed a more sophisticated immune-enhanced patient-derived sarcoma organoid (iPTO) system that incorporates patient-matched immune cells as a tool for testing immune checkpoint blockade. This platform could theoretically be leveraged for preclinical validation of *any* novel therapeutic agent or combination therapy. For instance, novel targeted therapy agents being studied in sarcomas, such as emactuzumab, SAR405838, MK-8242, and enzalutamide, could be investigated in specific histologies and molecular subtypes for histology-specific or biomarker-driven trial designs.[9,15]

This study highlights several advantages of utilizing fresh, non-expanded tumor specimens by demonstrating that passaging or immortalization of cells leads to an accumulation of changes in gene expression, which ultimately affect the reliability and accuracy of in vitro treatment responses. The rate at which these sarcoma PTOs were developed offers other advantages. According to the authors, the process of organoid biofabrication for drug testing can occur in an average of 7 days, allowing researchers to obtain treatment efficacy data in as low as 10 days after tumor biopsy or resection. This is theoretically rapid enough to allow for selecting a rational treatment regimen for patients without a significant delay in treatment initiation. With higher fidelity modeling of the original tumor and the ability to rapidly translate drug screening results back to the patient, there is potential for making PTO utilization a routine step in future personalized clinical care.

While promising overall, these PTOs have some limitations, and there remain several outstanding questions that need to be answered. Achieving precision personalized treatments requires us to unveil what specific molecular alterations are present in each tumor to identify therapeutic targets. But current commercially available, CLIA-approved next generation sequencing (NGS) assays generally have turnaround times in the order of several weeks to a month. Even circulating tumor DNA (ctDNA) blood-based assays generally take more than a week to return results. Thus, these crucial results cannot be utilized with these models to truly inform treatment decisions. For these reasons, we need to find a way to both speed up the turnaround time of NGS results, and/or lengthen the time that reliable PTOs can be kept in culture.

In this current study, the authors utilize a new hyaluronic acid and collagen-based extracellular matrix whose composition differs from other hydrogel formulations, such as Matrigel and fibrinogen,[16,17] as well as previously reported spheroid/organoid methodologies.[18] They report that this scaffold removes any interference from uncharacterized cytokines, mRNAs, and exosomes present in animal tumor-derived extracellular matrix (ECM) materials, although it is noteworthy that they utilized fetal bovine serum in the culture media. Presumably these sarcomas have cytokines or factors that may influence the activity and viability of the sarcoma cells in the presence of therapy. Using autologous materials and avoiding murine-derived components seems to be an obvious improvement for recapitulating tumor biology in a more refined manner,[19] but whether cells cultured with this method retain a closer gene expression profile as compared to those cultured with different approaches remains to be seen.

Perhaps the biggest limitation for PTOs is the potential scarcity of available biospecimens required to carry out experiments or, even worse, the impossibility of obtaining them in non-operative candidates. Forsythe et al. only performed the planned chemotherapy or immunotherapy screenings in 75% and 66.7% of PTO and iPTO sets, respectively. Some methodologies addressing these limitations have already been described[20] including use of smaller specimens after biopsy (e.g., fine needle aspirates). But these may lead to difficulties in generating enough organoids to test a wide enough array of drugs to tailor therapies effectively. This limitation certainly casts doubt on the current generalized applicability of this PTO platform. While cell expansion could solve this problem, fidelity to the source tissue may be compromised. Thus, there needs to be a reasonable balance between promptness of results and the broadness of their clinical applicability.

Another important question that remains to be answered is whether these PTOs can truly predict treatment responses in sarcoma patients. Organoid models have previously been shown to have 100% accuracy in predicting which chemotherapy agents will not be effective (i.e., negative predictive value). However, the same cannot be said about their positive predictive value.[12] As with most organoid models, cells from PTOs must undergo a multistep physicochemical procedure before they are established. This process may provoke gene expression and/or molecular alteration differences with respect to the native tumor that could therefore weaken their representativeness and potential for predicting treatment efficacy. The authors have also pointed out that intra-tumor heterogeneity or inadequate sampling are potential issues obstructing the translational power of organoid models. However, several additional factors affecting tumors within the human body (i.e., vascular oxygen gradients, drug pharmacokinetics and bioavailability, signaling molecules released by the TME, the loss of immune system complexity and capacity, etc.) cannot yet be satisfactorily accounted for in these ex vivo

models. Because of this, future investigations are still necessary to address the general weaknesses of PTOs and their predictive power for patient therapies.

Ultimately, Forsythe et al. demonstrate a novel and overall effective platform for establishing sarcoma organoids, which offers promise as a new method for studying this broad and complex compendium of tumors. Effective utilization of these PTOs will involve further demonstration of clinical predictivity and balancing their advantages and disadvantages depending on the patient, available biospecimens, or research plan; but this is certainly better than not having any models. By facilitating new modeling approaches in a rapid and reliable manner, these PTOs make it more feasible to expand our knowledge of heterogeneous sarcoma subtypes, as well as ultimately bridge the gap between translational research and therapeutic improvements with the hope that more personalized precision therapies can be employed for sarcoma patients.

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