SLAS Discovery
2020, Vol. 25(7) 672–683


DOI: 10.1177/2472555220915830
journals.sagepub.com/home/jbx


A Perspective on Expanding Our Understanding of Cancer Treatments by Integrating Approaches from the Biological and Physical Sciences

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Abstract

Multicellular systems such as cancer suffer from immense complexity. It is imperative to capture the heterogeneity of these systems across scales to achieve a deeper understanding of the underlying biology and develop effective treatment strategies. In this perspective article, we will discuss how recent technologies and approaches from the biological and physical sciences have transformed traditional ways of measuring, interpreting, and treating cancer. During the SLAS 2019 Annual Meeting, SBI[2] hosted a Special Interest Group (SIG) on this topic. Academic and industry leaders engaged in discussions surrounding what biological model systems are appropriate to study cancer complexity, what assays are necessary to interrogate this complexity, and how physical sciences approaches may be useful to detangle this complexity. In particular, we examined the utility of mathematical models in predicting cancer progression and treatment response when tightly integrated with reproducible, quantitative, and dynamic biological measurements achieved using high-content imaging and analysis. The dialogue centered around the impetus for convergent biosciences, bringing new perspectives to cancer research to further understand this complex adaptive system and successfully intervene therapeutically.

Keywords

cancer, organoids, organ-on-chip, imaging, cell viability, mathematical modeling

Introduction

Cancer is a highly complex adaptive system, which makes it challenging to study. Despite significant advances in drug development, many promising therapies fail to translate to the clinical setting. 2D drug screening assays have been the mainstay in drug development, yet they lack important aspects of the tumor microenvironment (TME), such as cancer-associated fibroblasts (CAFs), immune cells, blood and lymphatic vessels, and extracellular matrix (ECM), which are known to influence drug response,[1,2] as well as many other aspects of tumorigenesis. As a result, we are faced with the following question: Do we need a complex system to study such a complex disease? We believe that answers to this question lie at the intersection of the biological and physical sciences, also known as *quantitative biology*.[3] In this context, we are using the term *quantitative biology* to represent a growing field of researchers that apply quantitative approaches and technologies to analyze and model biological systems. Performing such interdisciplinary studies will lead to significant advances in our understanding and treatment of cancer.

In the cancer research community, there has been a surge in the development of biologically inspired 3D model systems (e.g., organoids or organ-on-chip) with the goal of incorporating physiologically relevant features of the tumor and the surrounding microenvironment. Engineering techniques, including micropatterning and microfluidics, are key advancements in this arena.[4,5] With progress being made in developing cutting-edge biological models, we are compelled to expand our assay repertoire to enable interrogation and interpretation of these more complex systems. There is a need to identify robust and reliable methods to

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Received Jan 10, 2020, and in revised form Feb 21, 2020. Accepted for publication March 4, 2020.

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Table 1. In Vitro Model Systems.

System Type		Disadvantages	Advantages
2D systems	Single cell types	Simple; do not translate to human biology	High-throughput; inexpensive; ease of use; amenable to drug screening
	Heterocellular cultures	Missing spatial information; difficult to study time dynamics; challenges defining optimal culture conditions	Amenable to high-throughput assays; cellular cross-talk
3D systems	Organoids	Expensive; heterogenous in size; assays are less developed; often lack stromal cells	Recapitulate aspects of tumor; patient-specific; amenable to high-throughput drug screening
	Organ-on-chip	Expensive; assays are less developed; technically challenging; platform variability	Tunable; mechanical forces can be studied; multiplexing capabilities

Table 2. Drug Screening Assays.

Experimental Assay	Readout	Timescale	Advantages	Disadvantages
CellTiter-Glo	ATP concentration; cell viability	Endpoint	Quick, high-throughput	Bulk measurement; single time point
Confocal/widefield imaging	Morphology; cell volume; growth/death rates;[7] metabolic signature (epifluorescence)[63]	Real-time (minutes to hours)	Distinguishes between cell types; subcellular information with fluorescently tagged proteins; accessible; multiple time points	Large data files; lower-throughput
Fluorescence lifetime imaging microscopy (FLIM)	Morphology; cell volume; metabolic signature[61,64,67]	Real-time (minutes to hours)	Distinguishes between cell types; subcellular information; multiple time points; early indication of drug effects; label-free; low phototoxicity; deep penetration	Highly dependent on signal-to-noise; less accessible; lower-throughput

quantify in vitro 3D samples and translate the findings for clinical applications. For example, several methods are currently being used to measure drug response based on metabolic or imaging-based readouts of cell viability.[6,7]

From these technological innovations, researchers are creating patient-derived mini-tumors in the laboratory for drug testing. However, it is impractical to experimentally test all the possible dosing regimen iterations (dose, timing, and scheduling of multiple drugs) that may be efficacious. Conventional dosing schedules are designed to give cytotoxic drugs at the maximum tolerated dose (MTD) until time of progression. The use of experimentally calibrated mathematical models can help explore an enormous treatment space available for optimal scheduling, rather than relying on MTD. Groundbreaking work by Anna Kane Laird showed that the growth kinetics of most untreated tumors can be described using a Gompertzian growth model.[8] Based on this observation, Larry Norton and Richard Simon developed a mathematical model that predicted a dose-dense chemotherapy schedule would achieve improved clinical outcomes, which was later validated in a randomized clinical trial of breast cancer patients.[9,10] This was the first example of a mathematical model optimizing treatment schedules in the clinic.

In this perspective, we discuss how the convergence of the physical and biological sciences provides us with a new perspective on cancer and the ability to more successfully intervene therapeutically. We highlight several examples of promising 3D biological model systems to study cancer complexity (**Table 1**), assay types that are relevant to interrogate this complexity (**Table 2**), and how computational modeling approaches may be useful to detangle this complexity with respect to optimal drug treatment strategies (**Fig. 1**). We summarize many of the discussion points debated during the SBI[2]-hosted Special Interest Group (SIG) at the SLAS Annual Meeting in 2019. The work described herein is not meant to be an exhaustive review of the cancer research landscape but rather to highlight key concepts that will be influential in a new era of precision cancer medicine.

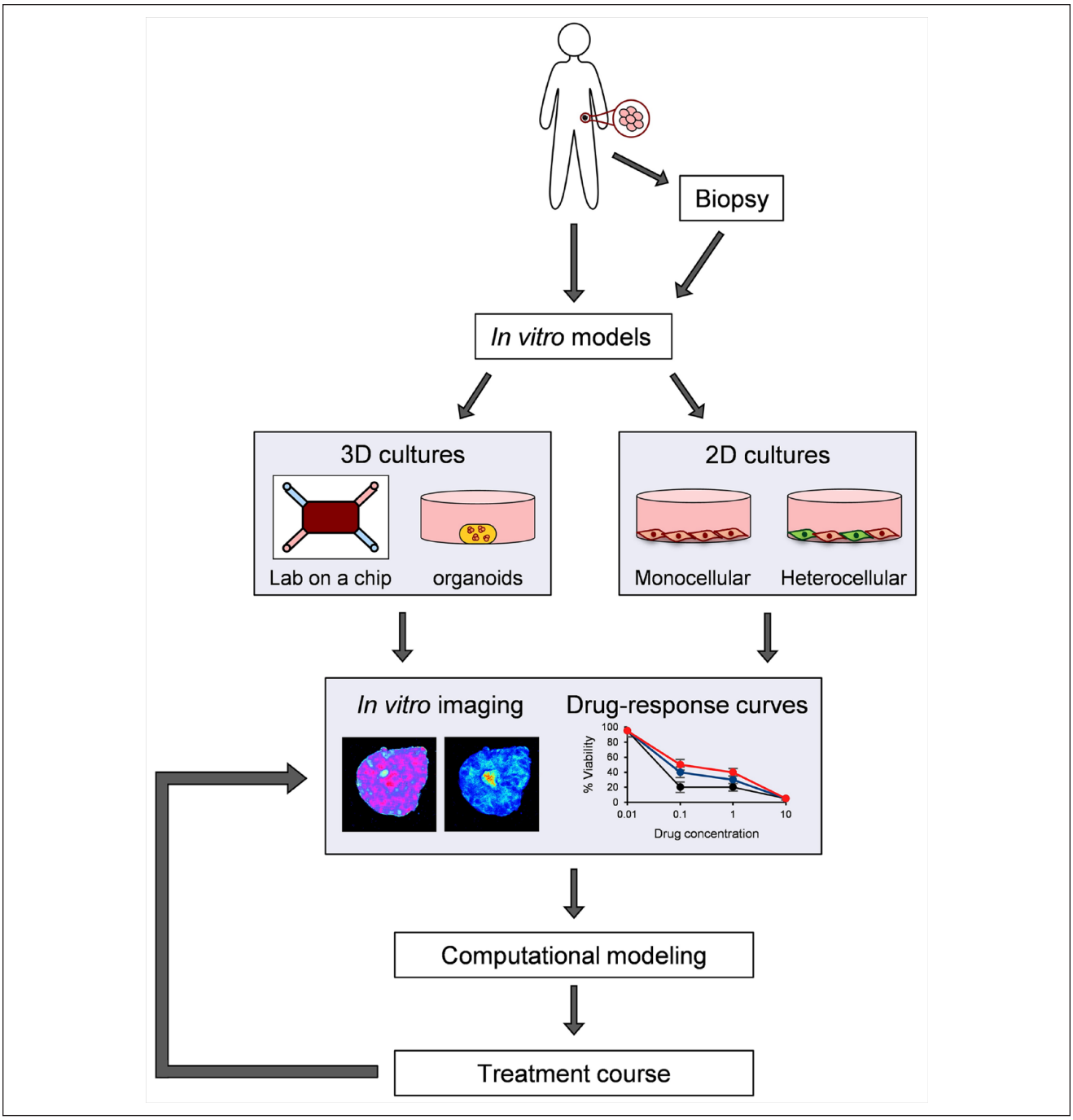

Figure 1. Workflow of integrating experimental data of patient-derived samples into mathematical models to make optimal treatment strategy predictions. The results generated from initial testing are iterated and refitted to increase model accuracy.

Main Text/Sections

What Biological Model Systems Are Appropriate to Study Cancer Complexity?

Current in vitro 2D models are highly simplistic and do not imitate human physiology. Furthermore, in vivo animal models often fail to recapitulate human biology due to mismatches in species-specific regulatory networks and host microenvironments.[11,12] Simply put, there is a need for more physiologically relevant systems to model the complexity of cancer. However, the level of complexity required to improve the success rate of drug development from bench to bedside is under debate. Several questions continue to surface: What aspects of biology should be

recapitulated in a model system and to what extent? Often, the answers come down to the context that is being studied. As George E. P. Box so famously quoted, "All models are wrong, but some are useful."[13] While this was in reference to statistical models, it can undoubtedly be applied to biological models (or mathematical models for that matter, as discussed later). Therefore, to improve the number of drugs that are clinically efficacious, we should aspire to develop biological models that are predictive of patient responses in the clinic.

There are many in vitro and in vivo models and techniques being developed to mimic tumor complexity (e.g., spheroids, bioprinting, patient-derived xenografts, and genetically engineered mouse models) to supplement current in vitro systems. In this perspective, we focus on two cutting-edge 3D culture systems, organoids and organs-on-chips, that capture patient heterogeneity and complex microenvironmental components that are invaluable for drug testing. Three-dimensional model systems better mimic the cell–cell and cell–ECM interactions found in vivo. Organoids are multicellular, self-assembled 3D structures that recapitulate aspects of the structure and function of the organ they were derived from.[14–16] They can be established from embryonic stem cells, adult stem cells, or induced pluripotent stem cells (iPSCs).[17] Recent studies have shown that organoids derived from patient tumors mimic and even predict patient drug response.[17–21] Jabs et al. compared primary cells isolated from the same patient grown in 2D monolayer versus 3D organoid cultures and detected differing drug effects between the two culture types.[22] They found that the drug response in organoid cultures was better linked to the genomic alterations present in the patient tissue. A study by Vlachogiannis et al. was one of the first to compare organoid drug screening results with clinical trial data. The authors measured the drug response of organoids derived from a small cohort of patients (n = 21) with metastatic gastrointestinal cancers and reported that the ex vivo drug screen had a positive predictive value of 88%.[18] Another recent study using organoids derived from pancreatic cancer patients detected gemcitabine sensitivity or resistance in the organoid model that matched the patient's stable or progressive disease, respectively.[20] The organoid field is still in its infancy, and while many of these studies suffer from small sample sizes, so true predictive power is difficult to assess, the organoid model holds great promise for use in precision medicine.

Researchers have shown that organoids are amenable to drug screens given their ability to be expanded and banked.[23] However, organoid-to-organoid variability is an important consideration when performing a drug screen. It remains to be seen how this intra- and interpatient heterogeneity will impact measuring and identifying treatment response.[14] In addition, while it is understood that organoids comprise stem and differentiated cell types,[16] more work needs to be done to further characterize these cell types and understand their spatial organization within the organoid. It is possible that further characterization (using CRISPR-CAS9 or lineage tracing techniques) will ultimately allow scientists to deal with the variability inherent in this model system. The lack of stromal cells (i.e., immune, endothelial, and CAF cells) present in the organoid model also limits their utility for certain drug types that are dependent on stromal presence (i.e., immunotherapy and anti-VEGF therapy). Co-culture systems are beginning to emerge,[24–26] but there is less control over spatial arrangements, and there are complications with culture conditions that are conducive to heterocellular growth. Engineering techniques such as 3D bioprinting are beginning to address some of these concerns.[27]

While organoids can recapitulate tissue-level functions, their architecture is much simpler than that of in vivo organs. Microfluidic organ-on-chip systems build on this complexity by incorporating cellular compartments to mimic tissue–tissue interfaces and functionality of an organ. These devices are made of glass or a polymer such as polydimethylsiloxane (PDMS) that, when coated with ECM, function as scaffolds for culturing different cell types in close proximity.[28] Furthermore, these systems incorporate physiological physical forces (e.g., fluid shear force and cyclic strain) that are lacking in other in vitro systems but have been shown to influence cancer cell growth and drug response.[29] Organ-on-chip technologies have been used to study a wide variety of cancer biological mechanisms, recently outlined by Sontheimer-Phelps et al.[30] Moreover, these devices have been used to understand drug toxicity (on- and off-target).[31] An important study using rodent, dog, and human liver-chips illustrated species-specific drug toxicities, suggesting that the human-derived organ-chips could better predict which drugs are safe for humans.[32] A multi-organ-on-chip system (integration of heart, liver, and several cancer types) was recently used to test for both anticancer efficacy and toxicity, a key preliminary example of how organ-on-chip technologies could be used in the drug development pipeline to measure on- and off-target drug response in one in vitro system.[33] While the increased complexity of this model system is promising and exciting, it can be more challenging to establish in the laboratory setting and less amendable to high-throughput experiments. Therefore, using the chip platform to study cancer drug treatments has been limited to demonstrating how various aspects of the TME influence drug response. To highlight a few examples, research groups have shown how CAFs,[34] endothelial cells,[35] mechanical forces,[29] and immune cell interactions[36,37] alter tumor cell response to drugs. Simpler versions of organs-on-chips have been used to screen drugs as a precision medicine approach.[38] To improve adoption by the cancer community and address some of the key challenges with this model system, several

organ-on-chip devices are now commercially available, providing reproducible, quality-controlled products with user-friendly microfluidic interfaces and engineered components in multiwell formats that are amenable to screening.[39]

Targeting the TME in addition to the tumor has gained momentum with the recent success of immunotherapies; however, there are mixed results and many unanswered questions, largely due to the heterogeneous nature of the TME. Additionally, the influence of cancer drugs is often focused on the tumor cells without considering the effects on the stromal bystanders and the feedback that may occur. For example, a recent landmark study showed that bacteria in the microbiome can metabolize gemcitabine to an inactive form and thus influence drug response.[40] Co-culture methods, specifically in 2D and basic 3D systems, have been instrumental in understanding the cross-talk between the tumor and single components of the TME;[41–43] however, the TME is a milieu of cell types and other factors that coexist and influence each other. This is an area of research where traditional in vitro culture systems can be very informative as they are easier to adapt to include additional cell types. When using the more complex model systems, such as organoids or organs-on-chips, to answer these research questions, it is imperative that they are tunable and scalable, allowing for microenvironmental factors to be added in a stepwise fashion, hopefully providing a more predictive model for clinical translation of drug compounds. Scientists have engineered remarkably complex model systems, but the assay development has lagged because of the additional spatiotemporal intricacies that have been introduced. In the following section, we shift focus toward assays that can integrate with these more complex model systems to measure drug response and some of the gaps that are still left to fill.

What Assays Are Necessary to Interrogate This Complexity?

When conducting drug screening assays to determine drug efficacy, it is important to note that some drugs cause cytostatic effects (i.e., inhibiting cell proliferation), while others result in cytotoxic outcomes (i.e., inducing cell death). One must consider these different drug-induced cellular effects when conducting in vitro drug testing and interpreting assay-specific data. The desired clinical outcome is often complete eradication of the tumor burden (although this treatment strategy is being challenged with a new approach termed *adaptive therapy* that is described in more detail in the next section).

A widely used assay to determine cell viability is the MTT assay, which quantifies the amount of tetrazolium cleaved by NAD(P)H-dependent cellular oxidoreductase enzymes by live cells.[44] However, when it comes to more complex 3D models, additional metabolic-based assays such as CellTiter-Glo 3D have been shown to be more effective to determine cell viability.[45–48] The advantages of this assay include its scalability, ease of use, and rapid readouts of ATP levels using a luminometer, which many laboratories are equipped with. Although it is an indirect measure of live cell percentages, CellTiter-Glo 3D has been used in numerous studies to assess drug sensitivity within biological model systems.[21,49–51] In particular, recent tumor organoid studies have used this method to generate drug response curves for large compound library screens.[21,49] These assays are also useful within the organ-on-chip systems to test liver cell viability as a measure of off-target drug toxicities[52] or to measure cancer drug effectiveness.[39] A caveat with the CellTiter-Glo assay is that it relies on the assumption that the ATP levels measured are proportional to the number of live cells present. This discrepancy between cell number and ATP proliferation assays can occur for specific drug classes as well as sample types,[53] including 3D organoids, where there are gradients of oxygen and nutrients resulting in cellular regions that are more metabolically active than others.[54–56] Quiescent cells within nutrient-deprived regions produce decreased ATP levels, yet these differences are averaged across organoids.[57,58] This bulk analysis overlooks potential metabolic heterogeneity resulting from organoid size, spatial location, or cell type differences. In addition, as the model systems become more complex with multiple cell types, these assays cannot distinguish between different populations of cells. Consequently, one should use caution in assessing overall viability and appreciate that this assay type is incapable of distinguishing between cytotoxic versus cytostatic drug effects, since percent cell death cannot be explicitly measured here.

A unique aspect of many of the microfluidics-based organ-on-chip systems is the presence of effluent cell culture media that can be continuously collected. This media can be used to interrogate liver enzyme activity by mass spectrometry,[33,59] measure secreted factors via a multiplexed cytokine array,[37] or quantify specific protein concentrations by enzyme-linked immunosorbent assay (ELISA).[35,59] While these methods are exciting because they offer a relatively easy way to track changes in cell byproducts over time without disturbing the cells within the device, they are indirect measurements of functionality and viability. In addition, as these systems become more complex with additional cell types (some current complex systems include up to four different cell types[59]), it is difficult to tease out signatures from the various cell populations.

As most benchtop assays used to assess cell viability are based on metabolic activity, advances in fluorescence microscopy techniques have also been used to conduct similar measurements. Fluorescence lifetime imaging microscopy (FLIM) has been used to measure the metabolic signature of live biological samples in real time.[60] This is done by the

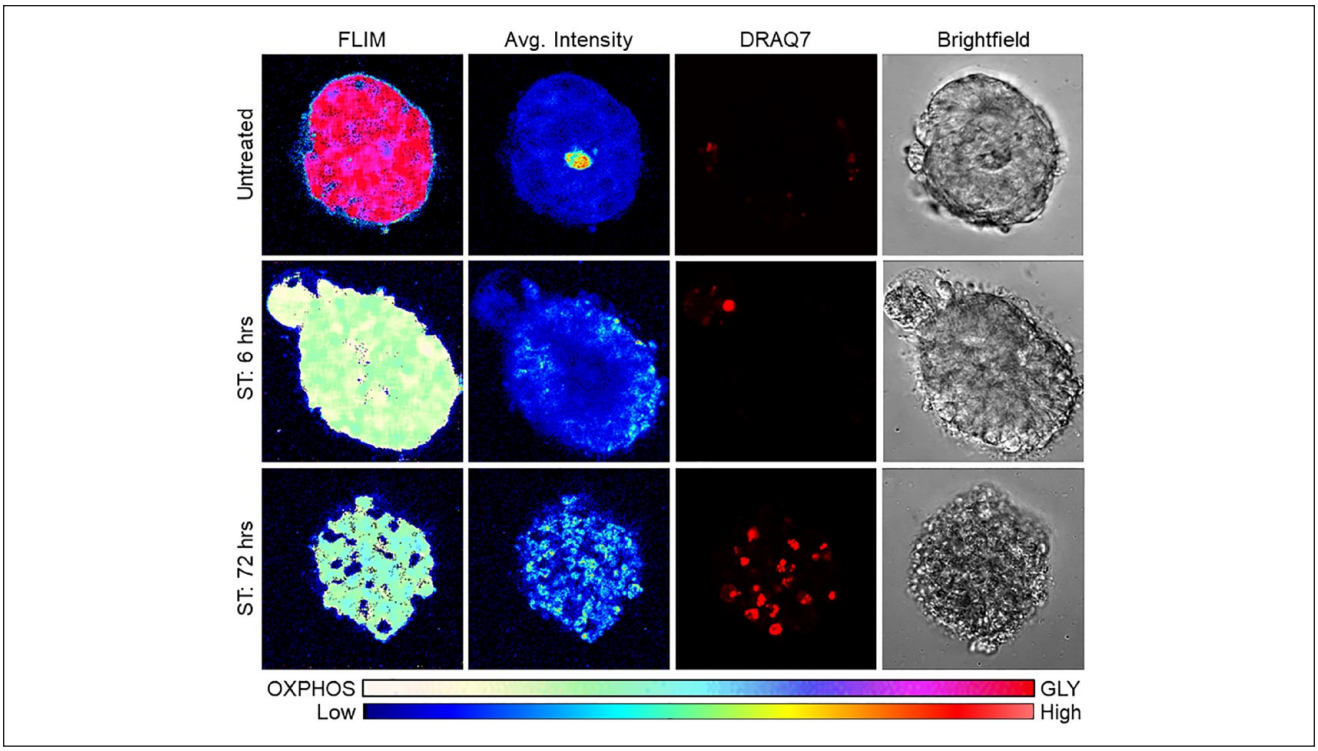

Figure 2. FLIM imaging of patient-derived colorectal cancer organoids. FLIM images of staurosporine (protein kinase inhibitor)-treated organoids show changes in FLIM metabolic signature (cyan/yellow coloring) after 6 h of treatment but low DRAQ7$^+$ (dead cell dye) cells. After 72 h, increased DRAQ7 signal is observed with a sustained shift in FLIM OXPHOS.

fluorescence lifetime of metabolites NADH or FAD and correlating these lifetimes to states of glycolysis (GLY) or oxidative phosphorylation (OXPHOS).[61,62] A shorter lifetime of cytosolic NADH is indicative of GLY, while the longer lifetime of protein-bound NADH signifies OXPHOS. The reverse is true for FAD. An advantage of this method is that NADH and FAD are autofluorescent species; thus, there is no fluorescent labeling necessary to obtain a readout. This method of imaging has been adapted for bulk measurements by utilizing a plate reader[63] or for distinct objects by coupling it with a microscope.[64,65] By expanding this setup to multiphoton excitation on a microscope, phototoxicity of the sample is decreased and deeper penetration is possible for large tissue samples of up to 3 mm.[66] This technique can also be coupled with confocal imaging methods to measure cell morphological parameters or fluorescent markers of cell death.

Walsh and Skala et al. have shown that by measuring the optical redox index (OMI), a parameter that depends on the lifetime of NADH and FAD, they were able to see heterogeneous drug response within their human patient- and mouse-derived tumor organoids.[6,54,67] The spatial information obtained using this method allows for further optimization of therapies to ensure that all subpopulations of tumor cells will be targeted for cell death. Further studies show that FLIM of NADH is a promising tool for drug screening using patient-derived samples and predicting clinical response.[68] However, it depends on expensive commercially available microscopy instrumentation that may be difficult to adopt widely within the cancer research community. A drawback of this imaging technique is that it relies on a high signal-to-noise ratio in order to accurately measure the lifetime, which can be difficult for tissue samples where there is often a large amount of scattering of photons. Nevertheless, our preliminary studies have shown that FLIM of NADH is able to determine the drug response of patient-derived colorectal cancer organoids at an earlier time point than dead cell stains (**Fig. 2**), therefore suggesting that FLIM may be a more sensitive and impactful technique to look at therapy effectiveness. FLIM, among other microscopy techniques, captures tumor cell heterogeneity (both spatially and temporally) that is not possible with CellTiter-Glo. These methods provide additional quantitative phenotypic data that may prove useful for mathematical modeling approaches.

The previously discussed assays are measurements of metabolic readouts and indirect correlates of cell death. Confocal and widefield imaging methods coupled with vital dyes (e.g., propidium iodide and trypan blue) have been widely used to characterize cell death directly.[7,69] Garvey et al. have applied these techniques using a high-content

imaging platform to screen drug responsive and resistant populations within co-culture systems.[7] They were able to distinguish each cell type and quantify multiple parameters such as birth and death rates, cell morphology, area, and percent viability over multiple time points and across large populations of cells. Dose–response curves generated from these measurements provide another method of assessing cell viability through imaging. These quantitative imaging techniques can be very informative in elucidating TME–tumor interactions when combined with more traditional in vitro model systems that integrate a co-culture approach.

Applying imaging-based approaches to 3D models, such as organoids and organ-on-chip platforms, delivers quantitative spatial-temporal information that can be collected quickly across multiple images and culturing conditions and is of great value to the quantitative biology community.[22,34,37,70–72] The use of fluorescently labeled proteins widens the possibility of observing multiple cell types or subcellular proteins and organelles. This becomes particularly useful when answering biological questions of how protein-targeted drugs promote cell death. In addition, genetic methods (e.g., CRISPR-CAS9 techniques[73]) can be used to track cellular changes that may occur during treatment, such as the outgrowth of drug-resistant subclones. However, as the assays become more complex with an increasing number of fluorescent labels and images collected, a bottleneck can occur when attempting to analyze such large image files. Significant energy has been invested in instrument and software development to overcome this bottleneck and improve throughput.[74,75] Machine learning techniques to help with object identification and segmentation are improving processing speed. Furthermore, label-free methods can relieve the analysis bottleneck by relying on the quantification of morphology and volume from brightfield images to assess cell death.[70,76,77] As discussed previously, the incorporation of stromal cells in 3D models is becoming more prevalent. However, one of the challenges with cell viability assays that rely on bulk metabolic measurements is the difficulty in distinguishing between different cell types in heterocellular cultures. Imaging-based approaches are advantageous in these settings given the ability to fluorescently label different cell types or apply machine learning algorithms to deconvolve cell populations.[7] Regardless of the assay of choice, proper controls are needed to conclude that a therapy is inducing cell death.

The goal of any drug screening assay is to be able to optimize treatment regimens to be the most effective for patients. IC50 values have become the accepted standard to quantify the potency of a drug at inhibiting a specific biological function, most commonly viability. It is defined to be the concentration in which the population of live cells is 50% of the control, or untreated, values.[78,79] Similar values such as EC50,[80] which is the drug concentration with 50% of maximum effect, are other attempts to summarize drug effects within a single value. One question is whether a single number is relevant to sum up the complexity of drug response and whether potency at the midpoint of a dose–response curve (i.e., IC50) is the most important difference to capture between drugs or cell types. Some argue that additional parameters other than potency are important measurements to consider, including the drug maximum effect or steepness of the dose–response curve.[81] As part of the NCI60 human tumor cell line drug screen, the Mean Graph and COMPARE algorithms were developed to rank and display drug response data that significantly inhibited tumor cell growth based on GI50 (50% growth inhibition), TGI (total growth inhibition), or LC50 (50% lethal concentration).[82,83] While a thorough investigation of these various parameters can be informative to depict systematic variation in drug response, it is easy to default to IC50 since it represents a single number that can be shared within the research community. With the recent introduction of complex models and analysis tools, including machine learning of high-content imaging data, scientists are generating a plethora of phenotypic descriptors that can describe unique biology. In order to replace the simplistic IC50 value, we must make these descriptors more interpretable, standardized, and available to the general cancer community.

Given the intricacies of the biological models and assay types previously discussed, mathematical modeling tools may help fill in some of the knowledge gaps in our understanding of cancer and have the potential to make more accurate predictions of drug response. In the following section, we shift focus toward the application of mathematical modeling tools to determine optimal treatment strategies.

How Can Physical Sciences Approaches Be Useful to Detangle This Complexity?

A pivotal time in cancer treatment occurred in the 1960s when H. Skipper, in collaboration with F. Schabel and W. Wilcox, developed a mathematical model of tumor cell growth and determined that each dose of drug kills a constant fraction of tumor cells.[84] This work changed the rationale for drug dosing and led to the use of drug combinations, which is still standard practice in cancer treatments today.[85] Several other approaches have been developed to mathematically model tumor growth kinetics, including exponential, power law, logistic, Mendelsohn, Gompertz, and von Bertalanffy models. Mathematical models can have great utility in investigating cancer progression (e.g., tumor growth, angiogenesis, and metastasis) and treatment response when tightly integrated with reproducible, quantitative, and dynamic biological measurements.[86] They are also advantageous for generating and testing new hypotheses. It is significantly cheaper and quicker to run thousands of in silico simulations versus thousands of in vitro or in vivo experiments. One could consider a basic outline of the

pipeline from mathematical model to the clinic to encompass the following steps: (1) develop and refine model; (2) analyze model and make predictions; (3) parameterize model with experimental data; (4) validate with experiments; repeat steps 1–4 as needed; (5) optimize treatment strategies (also consider pharmacokinetics and toxicity constraints); (6) test strategies in vitro and in vivo; and (7) test in clinic.

There are several mathematical modeling approaches that are being employed in the cancer research domain.[86] Two broad categories include mechanistic models and machine learning models.[87] Mechanistic models are useful in gaining a deeper understanding of the mechanisms driving cancer and how best to intervene. On the contrary, machine learning models are more phenomenological and are useful in exploiting large amounts of data to make predictions. One challenge with machine learning models is the availability of data—both quantity (volume) and quality (spatial and temporal)—needed to make reliable predictions. The field is somewhat divided between those who believe we must gain a deeper understanding of the mechanisms driving cancer progression and others who believe we do not need to understand them if we have the tools to predict the outcome. It is unclear which method will prevail or whether hybrid models will begin to emerge.

A promising use of mathematical modeling is to identify the most effective chemotherapy regimens to achieve desired endpoints, such as minimizing total tumor burden or reducing the drug-resistant cell fraction.[88,89] All too often during drug development, the main focus is on the drugs and corresponding mechanisms of action. However, as Norton stated, "Dose and schedule are the forgotten parts of the puzzle—and that's a tremendous shame."[90] Norton's work on dose-dense chemotherapy is a major success story for mathematical oncology. Several other mathematical models have guided prospective clinical trial protocols for cancer treatments[91,92] (NCT03557372 and NCT03768856). To continue to move this work into the clinic and increase adoption by the general cancer research community, a few challenges must be overcome. Similar to biological models, investments need to be made in defining mathematical model standards and sharing analysis tools.[93] Furthermore, we need to generate robust, quantitative data to calibrate these models. The predictive power of mathematical models relies on the quality of the preclinical models as well as the integrity of the data. Traditional viability assays, such as CellTiter-Glo, lack specific data qualities that are needed to parameterize mathematical models. Quantitative data from high-throughput multiplexed imaging data sets, as outlined in the previous section, are very informative in describing multicellular behaviors.[94] Additionally, one needs patient-specific clinical data to help translate mathematical model predictions to the clinical setting. Several national repositories host publicly available cancer patient data (e.g., The Cancer Genome Atlas [TCGA]) and patient-derived preclinical cancer models (e.g., Patient-Derived Models Repository [PDMR]), which are useful resources to help achieve this goal.

A major challenge with any cancer treatment is the emergence of drug resistance. Developing biological models of drug resistance through genetic manipulation or in vitro selection is critical to the success of therapeutic scheduling. Combining mathematical modeling with the data generated from these preclinical models can be useful in designing strategies that minimize the outgrowth of the resistant population. The Goldie–Coldman hypothesis states that the presence of drug-resistant clones is dependent on mutation rate and tumor size.[95] Based on this assumption, Goldie and Coldman created a mathematical model suggesting that alternating cycles of two different chemotherapies would result in the best solution to target resistant cells and achieve remission. Researchers are now using evolutionary principles to guide treatment strategies—the intent is not to give the MTD but rather to provide the minimum effective dose over repeated cycles to minimize the likelihood of treatment resistance occurring.[96] Adaptive therapy is a mathematical modeling approach that relies on continuous variation of the drug treatment to keep tumor sizes static rather than complete shrinkage of the tumor burden.[97] This novel idea is to extend patient survival by maintaining a balance of chemosensitive cells that can suppress the growth of the chemoresistant population. Gatenby et al. have developed a clinical trial (NCT02415621) for metastatic castrate-resistant prostate cancer patients based on previously published modeling results on adaptive hormone therapy, and the pilot study outcomes are promising for patients.[97–99] Collectively, these studies are encouraging for a future of rationally designed cancer treatment strategies based on data-driven mathematical model predictions.

Summary/Conclusions

Cancer is a complex problem of many interconnected layers, some of which have not been discovered or fully appreciated yet. We need to bring new approaches and perspectives to transform the way we do drug development in cancer research.[100] During the SIG, we engaged in a lively discussion on the topics summarized in this perspective. Some of the key highlights include the following: (1) there is no perfect biological model system to emulate cancer; however, models across scales (2D and 3D) can have utility if they are capable of predicting the in vivo behavior one sets out to investigate; (2) results from traditional cell viability assays (e.g., MTS and CellTiter-Glo) can be misleading and should be replaced with more quantitative approaches, such as high-content imaging; (3) representing drug response by a single number (i.e., IC50) should not be the standard metric; however, there was no consensus on what should

replace it; and (4) data-driven mathematical modeling can help understand the complexities of cancer by providing opportunities to explore and test new scenarios that are impractical or overwhelming to test in the preclinical setting. It is important to note that mathematical models are only as useful as the data they are trained on. As our preclinical models become more predictive of clinical scenarios, we can integrate the resulting data into mathematical models combined with high-performance computing to enable a robust and scalable drug development pipeline. Multidisciplinary interactions among biologists, mathematicians, engineers, and oncologists are empowering new approaches and understandings of cancer biology that are critical for our quest toward precision medicine.

Acknowledgments

S.M.M. would like to thank Drs. Jasmine Foo, Paul Macklin, and Stacey Finley for countless thought-provoking discussions on mathematical modeling and fruitful collaborative efforts. The authors would also like to thank Dr. Colleen Garvey for her review and edits of this perspective article.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: S.M.M.'s work to develop and interrogate 3D patient-derived biological models and to parameterize mathematical models of cancer was funded by the National Cancer Institute (U01CA232137, MPIs: Finley, Macklin, and Mumenthaler; R01CA180149, MPIs: Agus, Atala, and Soker; U54CA143907, PI: Hillis).

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