IN THE SPOTLIGHT

A Critical Need for Better Cancer Immunotherapy Models: Are Organotypic Tumor Spheroid Cultures the Answer?  
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Summary: Immunotherapy has transformed the therapeutic landscape of cancer, but the preclinical evaluation of combination approaches that will deepen and broaden its clinical benefit has lagged far behind due to the lack of expedient and easily accessible ex vivo human systems. In this issue, Jenkins and colleagues and Deng and colleagues report the use of organotypic cultures of tumors derived from mice and humans containing both tumor cells and cells from their local immune microenvironment to recapitulate the in vivo use of immune checkpoint inhibitors and extend the application of this system to therapeutic combinations of immune checkpoint blockade and molecularly targeted agents. Cancer Discov; 8(2): 143-5. © 2018 AACR.

See related article by Jenkins et al., p. 196 (4).
See related article by Deng et al., p. 216 (5).

The introduction of effective off-the-shelf immunotherapies, particularly those agents targeting the PD-1/PD-L1 axis, has revolutionized the cancer therapy landscape. These agents are capable of producing durable responses, translating to prolonged clinical benefit, even after therapy cessation, and can induce rare cures, even in metastatic disease (1). However, despite clinical activity of these agents across a broad array of tumors, only a minority of patients respond in most tumor types. Therefore, the research community must actively seek novel, rationally designed combinations of immunotherapies with other immune approaches, targeted agents, chemotherapy, or other modalities to enhance response rates. However, the number of potential combinations to be clinically assessed vastly exceeds the number of patients available to effectively test even a fraction of them all (2). Furthermore, the level of preclinical evidence needed to advance combinations to the clinic requires extensive time and resources. Therefore, many trials are being initiated with weak rationale and are simply empiric in nature. Thus, effective means to identify, prioritize, and develop combinations are sorely needed.

Because immunotherapies harness both local and systemic immunity and require dynamic interplay between multiple cell types present in the tumor microenvironment, traditional approaches to modeling the efficacy of immunotherapies in the laboratory often fail to accurately recapitulate in vivo models. Thus, murine models are often employed to overcome these challenges, but are time- and resource-intensive. Inconsistencies between the murine and human immune systems are well known (3), and few, if any, effective models for acquired resistance to immunotherapies have been established in the literature. Thus, overcoming these challenges in the preclinical evaluation of immunotherapy will require the introduction of novel model systems that could be performed quickly and easily ex vivo, lending themselves to screening strategies. Furthermore, model systems adaptable to mouse cancer models and human cancer that can dynamically assess the interplay between cell types in the tumor-immune microenvironment critical to clinical responses within the treated patient with cancer could help rapidly advance the field. Such a model system could not only help prioritize combination strategies to translate to the clinic, but also identify biomarkers of immunotherapy response (baseline or early on-therapy).

In this issue of Cancer Discovery, two reports of the use of organotypic spheroid cultures derived from both human [patient-derived organotypic tumor spheroids (PDOTS)] and mouse [mouse-derived organotypic tumor spheroids (MDOTS)] tumors were employed to model short-term responses to PD-1/PD-L1-targeted therapies ex vivo using a novel microfluidic culture system (4, 5). In the first of the two articles, Jenkins and colleagues validated that spheroids (40–100 μm) derived from tumors contained relatively similar fractions of T lymphocytes (CD3+, CD4+, and CD8+), myeloid (CD11b+ and/or CD11c+) populations (Fig. 1A; ref. 4). Using cytokine expression profiling as a functional readout of activity, along with fluorescence cell imaging, they could demonstrate anti–PD-1–mediated T-cell activation in culture, along with subsequent tumor cell death. Organoids derived from heterogeneously responding/nonresponding isogenic CT26 tumors demonstrated that some features of resistance to immunotherapy could also be captured in these culture systems. Subsequently, the authors provided a proof-of-principle demonstration of the use of organotypic tumor cultures to predict efficacy of immunotherapeutic combinations with a novel TBK1/IKKe inhibitor, which was similarly effective in both the CT26-derived MDOTS and in the CT26 in vivo model. Finally, they performed PDOTS analysis across a wide

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variety of immunotherapy-responsive tumor types, identifying some unique patterns of cytokine expression in response to anti–PD-1 treatment (upregulation of CCL19 and CXCL13 in resistant patients) that may associate with poor outcomes. This remains to be validated functionally in a suitable experimental fashion and in far greater numbers of patients.

In the second report, by Deng and colleagues, the PDOT/MDOTS systems were also used in support of the beneficial effects of CDK4/6 inhibition on tumor immunity (5). Through application of multiple in vitro and in vivo model systems, Deng and colleagues demonstrate CDK4/6 inhibition can enhance T-cell activation and expression of TH1 cytokines in large part due to NFAT activation. The enhanced TH1 and CTL response is accompanied by improved homing of T cells to tumor sites and decreased presence of immunosuppressive myeloid cells. In addition, regulatory T cells (Treg) are preferentially impaired due the selective antiproliferative effects of CDK4/6 inhibition on these cells. The investigators study in vivo effects in both genetically engineered mouse models of lung cancer and tumor allograft (lung and colon cancer) models to demonstrate enhanced T-cell activation, decreased presence of Treg and myeloid cells, and enhancement of antitumor effects in combination with anti–PD-1 antibody.

So how do the PDOTS/MDOTS contribute to these studies? PDOTS can respond to CDK4/6 inhibition with increased release of TH1 cytokines. Using MDOTS with MC38 (allograft colon adenocarcinoma), the investigators were able to show that CDK4/6 inhibition in combination with anti–PD-1 can enhance the death of tumor cells, reduce chemokines such as CCL2, CXCL1, and CCL3 that are known to impair TH1 activation, and require the presence of T cells for these effects. The MDOTS findings are consistent with in vivo animal models, and the investigators are able to demonstrate similar findings in human PDOTS in seemingly more physiologic ex vivo conditions.

These findings in combination with two recent Nature articles by Goel and colleagues and Zhang and colleagues come to the same conclusion; small molecules targeting CDK4/6, already in the clinic, can enhance tumor immunity with improved antitumor effects when combined with PD-1/PD-L1 inhibition (6, 7). Interestingly, each of the publications support a different mechanism for this effect. In this article, it is NFAT activation, CD8+ and CD4+ T-cell trafficking, and the antiproliferative effects of CDK4/6 inhibition on subsets of immune cells including both myeloid and regulatory T cells (Fig. 1B). In contrast, the article by Goel and colleagues (7) focuses on the effect of CDK4/6 inhibition on an E2F target, DNMT1, which regulates the methylation of endogenous retroviral (ERV) sequences and, through upregulation of ERV expression, leads to type III IFN expression and enhanced antigen processing machinery gene expression and thereby enhanced antigen presentation. Additionally, through DNMT1, antiproliferative effects of CDK4/6 inhibitors decrease the presence of Tregs in tumors. Finally, Zhang and colleagues demonstrate enhanced cancer immunity with CDK4/6 inhibition through the effect of cyclin D/CDK4 on SPOP and E-cadherin, which in turn effect degradation of the PD-L1 molecule (6). In this manner, CDK4/6 inhibitors increase PD-L1 expression and, in combination with PD-1 inhibitors, enhance the antitumor effects. Therefore, although these complex preclinical findings all are in support of the combination of CDK4/6 inhibitors with anti–PD-1, PDOTS
and MDOTS provide additional validation. At this time, MDOTS/PDOTS findings are only supportive and on their own are insufficient to make the case for this combination of agents. As this simple and rapid model is developed further, there is hope that it will independently provide more mechanistic and definitive answers. Ultimately, further development of PDOTS/MDOTS could lead to a primary approach to select and optimize the best combinations of small molecules, chemotherapy, or other immune agents in combination with the immune checkpoint inhibitors anti-PD-1/PD-L1.

**LIMITATIONS**

Of course, these *ex vivo* systems are not the ultimate solution for discovering and testing effective immunotherapy combinations, but rather a new instrument in the toolbox. PDOTS/MDOTS lack the complete immunologic picture of systemic immunity and cannot recapitulate the dynamics of lymphoid structure T-cell priming, which is thought to be one major target for anti-CTLA4 responses (8). Other factors like the influence of the microbiome (outside of what is already established in the immune contexture of the tumor) also cannot be dynamically explored (9). Although the work focused on recapitulation of the tumor-immune compartment with spheroid establishment, it is unclear whether other stromal features, such as fibroblasts and endothelial cells, are stable in these cultures, which could influence experimental results.

Organotypic spheroid cultures have been used for some time, particularly in the cancer stem cell (CSC)–like field of research. It has been well established that such culture conditions are especially permissive to the proliferation of CSC-like cells and may induce a change in the representative tumor cell populations, even over a short culture period (10). Finally, one difference in the PDOTS/MDOTS system is the unique microfluidic culture conditions. An unanswered question is whether this culture system is required to effectively recapitulate tumor–immune interactions. If unnecessary, then the experimental model system would certainly be more widely utilizable by the general research community, but an assessment was not apparent in the data presented.

In summary, these articles together define some early development of *in vitro* models of the tumor microenvironment that is believed to be critical to sort through and prioritize the overwhelming potential combinations of immune-based therapies. Nevertheless, the findings presented here provide support for an exciting combination of a small-molecule CDK4/6 inhibitor with anti-PD-1, which is rapidly moving into the clinic based on this work and the work of others in preclinical models.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**REFERENCES**

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