Of Mice and Melanoma: PDX System for Modeling Personalized Medicine
Edward J. Hartsough and Andrew E. Aplin

Targeted therapies have advanced the treatment options for cutaneous melanoma, but many patients will progress on drug. Patient-derived xenografts (PDX) can be used to recapitulate therapy-resistant tumors. Furthermore, PDX modeling can be utilized in combination with targeted sequencing and phosphoproteomic platforms, providing preclinical basis for second-line targeted inhibitor strategies. Clin Cancer Res; 22(7) April 1, 2016/C211 doi:10.1158/1078-0432.CCR-15-3054

In this issue of Clinical Cancer Research, Krepler and colleagues describe the generation of patient-derived xenograft (PDX) models created from twelve BRAF V600E–harboring melanomas from individuals who have progressed on treatment with the BRAF inhibitors, vemurafenib or dabrafenib (1). Krepler and colleagues utilized these models to mimic progression on drug by maintaining mouse-bearing PDXs on BRAF inhibitor. They demonstrate that serial passaging in the PDX system faithfully recapitulates histologic features of the original tumor. By employing targeted sequencing panels and reverse-phase proteomic analysis (RPPA), the group categorizes mechanisms of resistance and tests strategies for second-line combination therapies. They identified cMET amplification and phosphorylation in a subset of progressing tumors that are sensitive to cMET targeting in combination with BRAF and MEK inhibitors.

Even before President Obama’s initiative on Precision Medicine, there have been significant efforts to develop more optimal tools that will help design personalized treatment strategies. One tool at the forefront of such efforts is the development of PDX models, which reflect the heterogeneity within tumors and maintain, at least in initial passages, components of the original tumor microenvironment. Despite some limitations, PDX models have the potential to improve cancer therapies and promote precision medicine efforts. As evidenced in this study, high-throughput screening modalities identified additional mutations/alterations in BRAF inhibitor–resistant PDX samples that, when targeted in combination, result in tumor shrinkage.

A likely advantage of the PDX model compared with standard cell line xenograft models is the ability of the former to better predict therapeutic response to targeted therapies and inform drug treatment scheduling to stave off the onset of resistance. One discussion within the melanoma field is the benefit of maintaining patients on continuous drug after the onset of disease progression. Whereas one study concluded that maintaining patients on BRAF inhibitors with progressive disease increases overall survival (2), there is at least one case report detailing patient benefit from discontinuing targeted therapy (3). Furthermore, multiple preclinical studies, as well as the current study, demonstrate that a “drug holiday” delays the onset of resistance and that drug cessation following the development of resistance is detrimental to optimal growth (4, 5). Given these differences and the notion that durable resistance develops from the outgrowth of minor populations within tumors, the PDX system is ideally suited to test scheduling options to determine effects on the duration of response and whether drug-tolerant cells, which are able to expand following drug removal, persist in the tumor. These represent increasingly important questions as the field moves toward combinatorial therapies and in the future may be coupled with in vivo signaling monitoring systems, such as quantitative ERK1/2 reporters, to assess the efficacy of scheduling across the whole tumor in a temporal and quantitative manner (6, 7).

A powerful application in this study is the utilization of multiple platforms to highlight the example of MET amplification associated with BRAF inhibitor resistance. Notably, the authors conclude that MET amplification, as determined by next-generation sequencing technologies, is not a sufficient indicator of its potential role in acquired resistance (1). In addition, they employ RPPA, a high-throughput technique to identify alterations in signaling pathways and growth regulatory proteins. RPPA revealed elevated phospho-cMET in two of three MET-amplified resistant tumors, and only the tumors displaying high phospho-cMET were found to be exquisitely susceptible to cMET inhibition. In the TCGA melanoma dataset, MET is mutated or amplified in approximately 14% of melanoma samples (65/478), and as noted in the study by Krepler and colleagues (1), only two of nine MET-amplified tumors with available RPPA data demonstrated an increase in phospho-cMET. This approach supports the notion to cross-reference next-generation sequencing data with phosphoproteomic pathway profiling to properly assess potential drug targets. In addition, these findings highlight a role for aberrant cMET activation in drug-resistant melanoma. Currently, there are multiple small-molecule inhibitors to cMET in clinical trials.

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for various cancer treatments, as well as mAbs designed to inhibit cMET signaling either by neutralizing cMET itself or sequestering its ligand, hepatocyte growth factor (HGF). One limitation of the NSG mouse model in this regard is that mouse HGF does not activate the human c-MET; thus, the authors are likely analyzing the effects of tumor-derived HGF or ligand-independent signaling. Utilization of human HGF knockin mice would permit analysis of the effects of stromal-derived growth factor.

In reality, the PDX system described by Krepler and colleagues can take several weeks to months to generate sufficient numbers of mouse “avatars” for “co-clinical trials” or drug assessments. Progressing patients may not be afforded this time frame; however, the knowledge gleaned from this system will most likely inform treatment options in other patients. An adaptation may be the \textit{ex vivo} treatment of patient-derived explants, similar to experiments carried out with prostate tissue (8). These \textit{ex vivo} model systems could provide rapid results more likely to guide patient treatment options in a real-time manner. Also important is the generation of models with selective tropisms. In particular, PDX models that give rise to brain metastases should be a focus, given the clinical unmet need in this area. Mouse models have demonstrated spontaneous metastasis of patient-derived breast cancer tissue to relevant sites of human breast cancer tropism (9). Spontaneous brain metastasis of primary melanoma has been demonstrated in mouse model (10), although this system does not utilize patient samples. Further work to establish brain metastatic model in a PDX platform needs to be explored.

Finally, the major obstacle to be overcome with PDX modeling is the use of a mouse with a severely compromised immune system. Immunodeficient mice are not suitable to investigate immunotherapy efficacy or the effect of targeted therapies on immune function. This hurdle is particularly relevant for cutaneous melanoma, in which the advances made with targeted small-molecule inhibitors have been paralleled with the remarkable clinical effects of immune checkpoint blockade agents, such the anti-CTLA4 antibody, ipilimumab, and the anti-PD1 agents, pembrolizumab and nivolumab. The number and type of immune cells infiltrating tumors will be important to predict the efficacy of immune checkpoint agents (11). Targeted and immunotherapy combination treatments in preclinical genetically engineered mouse models have demonstrated great promise (12). Furthermore, humanized mice that will serve to test immune checkpoint inhibitor therapies alone and in combination with targeted inhibitors on patient-derived samples representing the ultimate mouse avatar for personalized medicine are being established (13).

In summary, the study by Krepler and colleagues in this issue (1) validates the power of the PDX system to define drug targets of resistant patient tumors. As outlined in Fig. 1, their work suggests that proper identification of second-line

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**Figure 1.** Schematic workflow depicting the identification of second-line targets from drug-resistant PDXs through next-generation sequencing and RPPA platforms. Resistant PDXs are propagated to be utilized in assays testing the efficacy of therapeutics against potential resistance mechanisms to ultimately inform second-line treatments in resistant melanoma patients.
therapy targets requires cross-referencing of next-generation sequencing data with phosphoproteomic pathway analysis. This study will hopefully lead the charge to add high-throughput phosphoproteomic analysis to standard clinical diagnostic practices and further expand the use of mouse avatars to bolster our understanding of drug resistance.

Disclosure of Potential Conflicts of Interest

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References

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