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Using avatars to win the fight over BRAF inhibitor resistance

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Patient-derived xenografts (PDXs) that are generated by transplanting fresh human tumor tissue into immunodeficient mice (avatars) have become a standard of translational cancer research. While much effort is currently being placed toward the development of patient-derived xenografts (PDXs) for pre-clinical studies of novel therapeutics, the appropriate setting for informing clinical management of patients has remained unclear (Hidalgo et al., 2014). The time required to develop the PDX and expand it for analysis of response to therapy precludes its utility for directing initial therapeutic regimes for patients. However, PDXs can be quite useful for the purpose of gathering information to inform second-line therapy after resistance to the first-line agent has developed. For example, patients with BRAF mutation are often treated with BRAF inhibitors or combined BRAF/MEK inhibitors. Characteristically, after only 9–10 months, resistance to therapy develops and alternative treatments are needed. At this time, it is wise to obtain genetic information on changes in the mutation/gene amplification status of key genes identified as drivers and then verify this with phosphoprotein analysis of the resistant tumor tissue. Based upon these data, appropriate second-line therapies might be implicated with greater success than is currently available. The proof of principal for this concept has been provided by the study recently published in *Clinical Cancer Research*. In this article, Krepler et al. observed the mean time to get PDX outgrowth of melanoma tumor implants was five weeks. They were able to analyze the genetic and phosphoprotein status of PDXs from 12 BRAF mutant melanoma patients who had developed resistance to BRAF inhibitor therapy. A number of common genomic alterations were detected: CDKN2A mutation in 9 of 12 samples; MAPK2 mutation in 2 of 12; BRAF amplification in 4 of 12; PTEN deletion or mutation in 7 of 12; and MET amplification in 3 of 12 (copy number 16, 9, and 93). One pair of matched samples acquired before and after BRAF inhibitor treatment showed that BRAF amplification was not present in the tumor prior to therapy. RPPA analysis was used to confirm that the genetically identified pathways were activated (pERK, pCycD, pAKT, pS6, pRSK, and a number of receptor tyrosine kinases (VEGFR, PDGFR, EGFR, cKIT, etc.). The authors then used this integrated genomic and proteomic information to design a rational second-line therapy utilizing PDX-bearing mice as subjects in their preclinical trials (Figure 1). For those PDX with biomarkers that indicated re-activation of the MAPK pathway and the PI3K pathway, treatment of PDX-bearing mice confirmed resistance to BRAF inhibition. In contrast, treatment with MEK/ERK/ PI3K inhibitors (encorafenib/ binimetinib/ BKM120) proved to be highly effective, while single agent treatment was not, likely based upon the activation of multiple pathways that resulted in resistance to the BRAF/MEK inhibitors initially. Surprisingly, there was no significant reduction in the levels

of phosphorylated AKT, S6, or ERK detected in response to the treatment with the combination of enco-rafenib/binimetinib/BKM120. Moreover, after the first 7 days of treatment, it appears that some resistance has developed, while a combination of ERK and PI3K inhibitors was more effective in inhibiting pRSK, pS6, and pMEK.

Another example of successful implementation of an integrated genetic and proteomic approach to inform second-line therapy was provided for BRAF inhibitor-resistant tumors with amplification of MET. The authors demonstrated that not all of the tumors with these genomic alterations are good candidates for cMET-targeted therapy. For instance, a PDX tumor with MET amplification but no detectable MET activity was intrinsically resistant to the cMET inhibitor capmatinib. In contrast, PDX tumors with MET amplifications and increased MET phosphorylation based upon RPPA analysis showed a remarkable response to the cMET inhibition combined with a MEK/ERK inhibitor, which caused regression of tumors in 10 of 10 mice over 21 days of treatment. Analysis of tumors treated with the combination of encorafenib, binimetinib, and capmatinib for the PDXs with high cMET activity showed evidence of regression with remarkable reduction in pERK, pMEK, cMET, and some reduction in pAKT. Therefore, analysis of genomic alterations in patient tumors alone may not be sufficient to predict effective therapy. A follow-up validation of signaling pathway activation associated with detected genetic changes will add precision to the identification of rational second-line therapies. The information required, however, is significant, including extensive exon sequence and intron sequence analysis as well as RPPA analysis of phosphoprotein status. Furthermore, such studies should consider intratumor heterogeneity which could be addressed by taking multiple samples from different areas of the tumor for PDX development.

The PDX-based personalized approach to development of appropriate second-line therapies for cancer patients could be highly valuable. Stability is among the key advantage of this model as the histology and genetic markup of the tumor is maintained throughout the several passages in mice as shown by Krepler et al. Further-more, a large study of 1000 PDXs reported remarkable reproducibility and the clinical translatability of this preclinical model, which makes it invaluable for evaluation of novel treatment modalities (Gao et al., 2015). We have extensively implemented PDXs to test promising therapeutic regimens, including combination of mitotic inhibitors with MDM2 antagonist, as well as to study the outcome of therapy-induced senescence (Vilgelm et al., 2015, 2016). However, due to the expense, timing, and complexity of the assays, one can envision a series of preclinical studies linked to clinical trials to demonstrate whether each individual PDX can consistently predict response to second-line therapy. Obviously, this will add to the complexity of the path report needed to inform the oncologist. Currently, commercial companies, such as Champion Oncology are doing this type of study and patients pay out of pocket for the analysis. The type of study proposed here by Krepler et al., if tested in a broader context of a multiple arm clinical trial, might lead to the establishment of new guidelines for therapy which could be covered by insurance companies once proven beneficial. As the authors point out, the PDX models described in this article do not take into account the effects of the targeted therapy combinations on the antitumor immune response. Moreover, it is not useful for examining the effects of immunotherapy. Currently, checkpoint inhibitors and other immunotherapeutic treatments are showing considerable promise for treatment of metastatic cancers, so viable

humanized mouse models will need to be developed to effectively utilize PDX models to predict response to immune therapies. Progress is being made in this area, but much more effort needs to be put forward to create a NSG mouse that produces the human cytokines needed for full differentiation of a human hematopoietic system from human CD34+ cells engrafted into the NSG mice.

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Figure 1.

Strategy for the selection of second line therapy proposed by Krepler et al. Tumor tissue from melanoma patients that relapsed on BRAF inhibitor therapy is implanted into immunodeficient mice for expansion. Resulting patient-derived xenograft tumors are subjected to genomic and proteomic analysis to gain information on the targetable mechanisms of BRAF inhibitor resistance that is then used to design personalized pre-clinical trial of potential second line therapies using PDX-bearing mice. Mut. - mutated, ampl. - amplified, activ. - activated.