Characterization of novel patient derived melanoma xenografts and cell lines in response to targeted therapies

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Abstract

Pre-clinical models exhibit melanoma-specific patient characteristics

Purpose: Develop pre-clinical models that are applicable to clinical practice with more efficacious bench-to-bedside translation. One avenue proposed in this study is the use of patient derived xenograft (PDX) models. These models have previously been reported to have the ability to predict clinical activity as they are biologically stable in terms of gene expression pattern, tumor architecture, and mutation status (1). Additionally, as PDX models serve as individualized experimental models, there have been many reports of success stories in patient treatment and outcomes (2-4). The detailed characterization of our pre-clinical melanoma models will represent a resource rich analysis for predictive biomarker discovery and drug activity, especially the design of novel combination therapeutic agents for metastatic melanoma.

Experimental Design: A panel of STR validated pre-clinical metastatic melanoma models were developed from human tumor tissue, including established cell lines, cell-line derived xenograft models and PDX models. Each model was molecularly characterized to confirm a match to that of the originating patient tumor via protein immunohistochemistry (IHC), miRNA quantitative polymerase chain reaction (QPCR), DNA short tandem repeat (STR) analysis, and Sanger sequencing.

Results: All pre-clinical models were positive for the melanoma-specific marker Melan-A (among others not shown) confirming a melanoma phenotype. BRAF, NRAS and KIT mutations were identified in our models matching their respective patient tumor. Growth patterns among all models were established, resembling similar growth characteristics. Response to monotherapy treatments in vivo was demonstrated in the BRAF mutant model, Mela14.

Conclusions: Mutation status data (i.e. BRAF, RAS), patient therapeutic history and a future analysis of oncogene overexpression (pERK, pAKT, pPI3K, pPTOR, etc.) will provide the platform and rationale behind the use of specific therapeutic agents per model with the intent to design novel combination therapies that may reduce treatment related side effects, increase efficacy, and prolong survival in a patient setting.

Experimental Design

• Patient tumor biopsy tissues were harvested and collected for either cell culture (cancer tissues), in vivo subcutaneous implantation into nude mice (4-6mm3 sections), formalin fixed paraffin embedded (FFPE) (150 mm3 sections), and cryogenically frozen.
• Patient tumor tissues were subcutaneously implanted into the flank of nude mice to monitor tumor growth. Established tumors were then cryogenically frozen before further mouse serial passages.
• Established cell lines were subsequently passaged and used for in vitro drug proliferation assays and subcutaneous injections into nude mice to monitor tumor growth.
• All model types derived from the initial patient tumor biopsy are analyzed for molecular and histological characterization, and various drug screens.

Growth and therapeutic response characteristics of pre-clinical models

Molecular Target

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<th></th>
<th>Dabrafenib</th>
<th>IC50</th>
<th>MK-2206</th>
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<th>Trametinib</th>
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<td>Mela1</td>
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<td>N/A</td>
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<td>70.8μM</td>
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<td>Mela14</td>
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<td>2.5μM</td>
<td>8.5μM</td>
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<td>0.84μM</td>
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Monotherapy in vivo dose response: Mela14 PDX

Figure 6. Anomeric nude tumor bearing 14-day xenografts were initiated at established tumor weights (300-400mm3). Tumor xenografts were 20 days old when treatment was initiated. The data was demonstrated using three therapeutic agents dosing ranges from 0.08 to 0.25 μM (subcutaneous injections in 5 days’ intervals). Each xenograft was counted as 1 tumor to compare the growth inhibition in response to treatment. *p < 0.05

Future Directions

• Complete combination therapies in remaining pre-clinical models and assess sensitivity and response
• Validate antitumor synergy in PDX models
• Determine if PDX models mimic patient response to therapy
• Assess the potential for mutations in pre-clinical models
• Validate biomarkers for response to therapy in tumor tissues

References

6. These studies were supported by NCI Cancer Center Support Grant (CCSG) P30 CA008748, grant numbers CA112970, CA176651, and CA224539.
7. These studies were supported by NCI Cancer Center Support Grant

Acknowledgments

The authors thank Cancer Center Support Grant P30 CA008748 for providing core support and the Community Cancer Center Core Laboratory for tumor specimen processing and tissue management. The authors also thank the affiliated members of Mayo Clinic Jacksonville for their contributions to the development of the presented preclinical models.