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Tumor regression in novel triple therapy BRAF mutant metastatic melanoma patient-derived preclinical models

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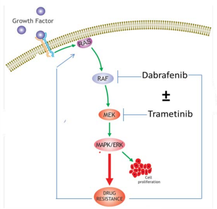
Abstract

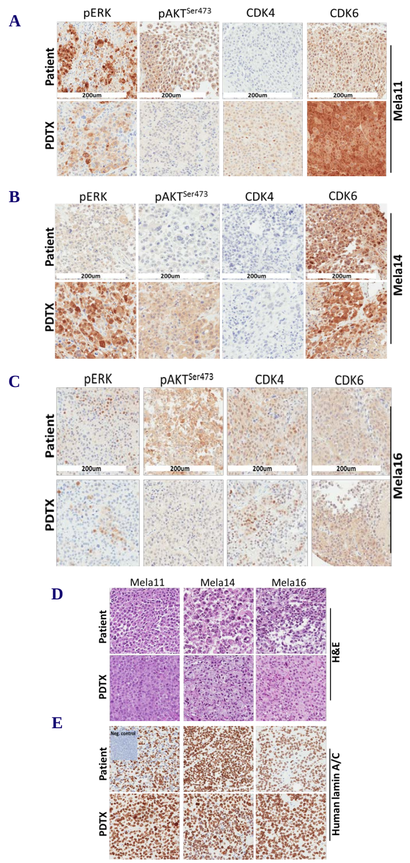
Metastatic melanoma (MM) is the most aggressive type of skin cancer contributing to approximately 80% of all skin cancer related deaths. In 2016, it was estimated that more 76,000 cases would be diagnosed in the United States. The high metastatic potential and aggressive clinical behavior of this disease makes it a major health problem.. As a consequence there has been notable development in novel targeted (i.e. BRAF inhibitors) and immune therapies (i.e. anti-PD1 and CTLA4 inhibitors) leading to enhanced overall survival. However, despite improvements in patient outcomes, most patients develop resistance within 6-11 months on dual BRAF/MEK inhibition, and 5 months on immune therapy, highlighting the need to identify new therapies that improve disease management and patient survival. Increased expression of CyclinD1 has been reported to occur as a mechanism of resistance to BRAF inhibition which also plays a role in reactivation of the canonical activating pathway in melanoma, MAPK. Thus, we hypothesized that targeting cyclin D1 using a CDK4/6 inhibitor (CDK4/6i) may enhance antitumor activity of standard of care when combined with a BRAF inhibitor (BRAFi) and MEK inhibitor (MEKi). We tested our hypothesis using patient-derived tumor xenograft (PDTX) and patient derived cell lines. The novel triple therapy drug combination (BRAFi/MEKi/CDK4/6i) was tested under two scenarios in PDTX models: 1) a naïve setting with established tumors and 2) a salvage setting where tumor growth had escaped standard of care BRAFi/MEKi treatment. In both scenarios, dramatic and significant tumor regression was observed in the PDTX models. The data are compelling with current plans for a phase 1/2 clinical trial.

Working hypothesis

Targeted therapies and immune therapies are two of the most effective therapies for patients with MM. However, most often patients develop resistance to either treatment modality. There are many mechanisms of drug-related resistance within tumor cells, including evasion of the immune system, activating mutations, activation of compensatory signaling pathways, and protein upregulation. Increased expression of cyclin D1 has been reported to occur in a portion of cases that are resistant to BRAF inhibition. Thus, we hypothesized that targeting cyclin D1 may enhance antitumor activity of current standard of care in patients with MM.



BRAF mutant PDTX mouse models are phenotypically and genetically similar to patient tumor source



Short tandem repeat analysis between patient and PDTX tumor tissues

	AMEL	D5S818	D13S317	D7S820	VWA	TH01	TPOX	CSF1PO	D18S51	D3S1358	D8S1179	FGA
pt. 11	XY	11,12	11,12	10	18,19	7,9.3	8	10,12	13,17	16	13,14	21
Mela11 PDTX	XY	11,12	11,12	10	18,19	7,9.3	8	10,12	13,17	16	13, *	21
pt. 14	XX	12	11,12	9,11	16,20	9, 9.3	8	10,12	10.1,14	15,16	12,14	24,26
Mela14 PDTX	XX	12	11,12	9,11	16,20	9, 9.3	8	10,12	10.1,14	*, 16	12,14	24,26
pt.16	XY	12,13	13,14	8	18,19	8,9.3	11,12	12	14	16,18	12,15	21,22
Mela16 PDTX	XY	12,13	13,14	8,11	18,19	8,9.3	11,12	12	14	16,18	12,15	21,22

Figure 1. Oncogenic, histologic and phenotypic comparison between tumor source and *BRAF*V600E MM PDTX mouse models. (**A-C**) Patient tumor tissues and corresponding PDTX tissues were stained for oncogenic proteins commonly upregulated or overexpressed in MM patients. (**D**) Mela11 and Mela16 PDTX tumors resemble a monomorphic architecture compared to patient tumor tissue, whereas Mela14 cells, in both models, are more pleomorphic. (**E**) Patient and PDTX tissue samples were FFPE and stained for human lamin A+C via IHC. The A/C components of lamins specifically support components of the human nuclear envelope. Nuclear staining is indicative of positive identification of human cells. The non-stained cells shown in the patient Mela11 model are lymphocytes. The negative control is represented by a pancreatic tumor harboring mouse cells. (**F**) STR analysis was used to determine origin validation by comparing genomic DNA between original patient tissue and PDTX tissues
pt, patient

Therapeutic treatment responses in PDTX mouse models match those observed in patients

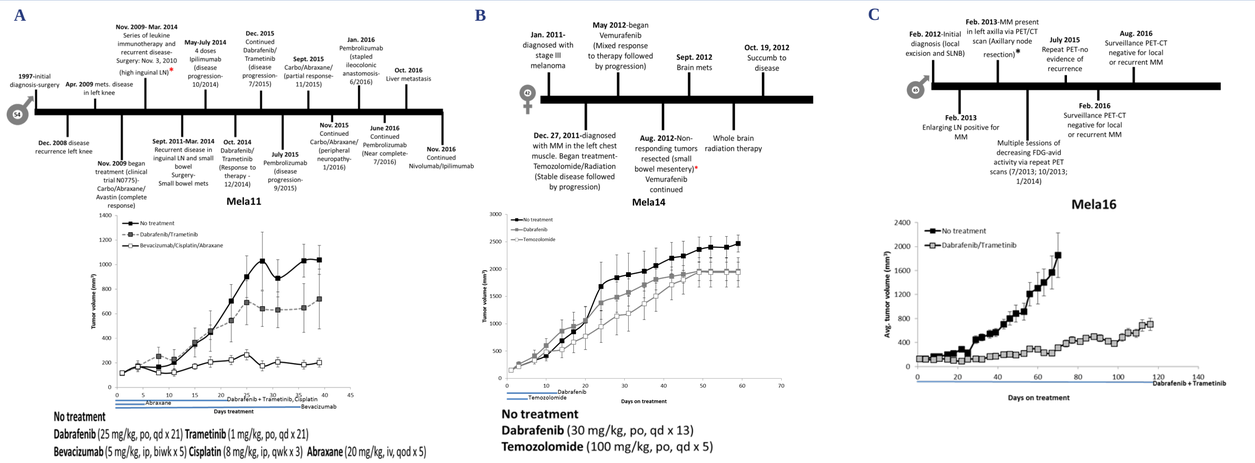
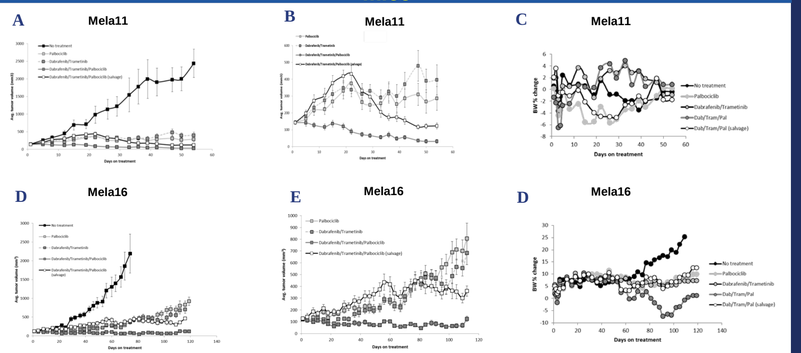


*__Figure 2.__ **Treatment responses in PDTX mouse models are similar to the matched patient.** (**A**, **B**)*BRAF* mutant PDTXs were treated with similar targeted therapy combinations as their respective patient and response to treatment between patient and matched PDTX were compared. Patient response to therapy was mimicked in the PDTX mouse models, suggesting the clinical relevance in utilizing these models to develop novel therapies for patient treatment. (**C**)The lack of a therapeutic regimen in patient Mela16 prevented direct comparisons between patient and PDTX response to therapy. Instead, the *in vivo* combination of dabrafenib/trametinib is shown to highlight the distinct differences between the 3 *BRAF* mutant patients: (**A**) patient with heavy treatment burden, (**B**) a patient with drug resistant tumors, and (**C**) a patient without systemic therapy. Asterisks indicate when tumors were collected for *in vivo* analyses

LN, lymph node; mets, metastatic; carbo, carboplatin; SLNB, sentinel lymph node biopsy; PET/CT, positron emission tomography-computerized tomography; FDG, fludeoxyglucose

Novel combination therapy causes partial and near complete response in tumor regression in naïve and BRAF/MEK inhibitor drug resistant MM PDTX mice



Treatment regimens: (1) No treatment **(2)** Palbociclib (100mg/kg) po; qd **(3)** Dabrafenib (25 mg/kg) po; qd, Trametinib (1 mg/kg) po; qd **(4)** Dabrafenib (25 mg/kg) po; qd, Trametinib (1 mg/kg) po; qd, Palbociclib (100mg/kg) po; qd **(5)** Dabrafenib (25 mg/kg) po; qd, Trametinib (1 mg/kg) po; qd, Palbociclib (100mg/kg) po; qd (**at time of tumor progression**)

__Figure 3.__ **Antitumor effects of dabrafenib, trametinib, and palbociclib in combination in *BRAF* mutant PDTX mouse models.** Athymic nude (*nu/nu*) mice bearing Mela11 (**A-B**) and Mela16 (**D-E**) patient tumors were treated with multiple therapeutic agent combinations and mean tumor volume (mm^3) + standard error was recorded. Figures (**B**) and (**E**) emphasize the significant effect triple therapy has on tumor growth, both as a combination therapy given together at once and as a salvage therapy when palbociclib is added to dabrafenib plus trametinib at tumor progression; p-values were measured at treatment endpoint. Compared to the current standard of care treatment, triple therapy combination ± salvage therapy was statistically significant (*p-value<0.05). Overall, the novel combination therapy was well tolerated, as indicated by body weight measurements (**C, F**). There was, however, a drop in weight loss in Mela16 while on triple therapy. A one week drug holiday helped recover loss in weight (**F**). po, by mouth; qd, everyday treatment; mg/kg, milligrams per kilograms

Conclusions

1. PDTX mouse models genetically match patient origin and maintain expression of human cells across multiple tumor passages *in vivo*
2. Treatment response to therapy between patient and matched PDTXs are similar
3. Palbociclib combined with dabrafenib and trametinib provided a durable treatment response compared to current standard of care combination in *BRAF* mutant PDTX mouse models

Future directions

1. Validate treatment efficacy of novel triple therapy combination in remaining PDTX mouse models
2. Determine mechanisms of antitumor activity
3. Examine predictive biomarkers for response and resistance to therapy following *in vivo* treatment