

Preclinical activity of the type II RAF inhibitor tovorafenib in tumor models harboring either a *BRAF* fusion or an *NF1*-LOF mutation

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Abstract 1972

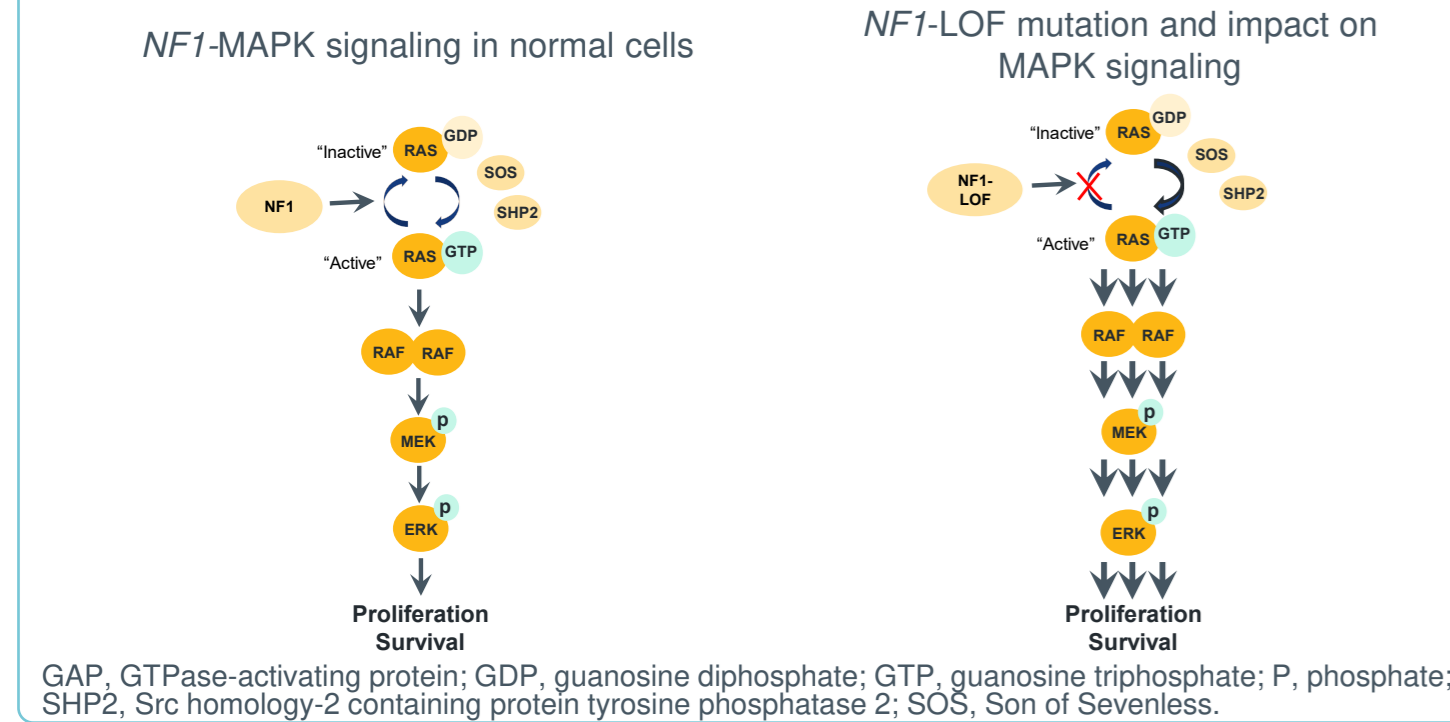
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Background

- Tovorafenib is an investigational, selective, CNS-penetrant, small molecule type II RAF inhibitor, which inhibits both RAF monomers and dimers and has been shown preclinically to impact the *KIAA1549::BRAF* fusion¹
- Tovorafenib is being evaluated as a monotherapy in relapsed/progressive pediatric low-grade glioma (pLGG) harboring *RAF* alterations (NCT04775485)^{2,3} and in combination with the MAPK/ERK kinase (MEK) inhibitor, pimasertib, in patients ≥ 12 years of age with recurrent, progressive, or refractory solid tumors with MAPK pathway alterations (NCT04985604)⁴
- Tovorafenib inhibits BRAF V600E, wild-type BRAF and CRAF, but does not result in paradoxical activation of MAPK signaling in tumors harboring *BRAF* fusions (e.g., *KIAA1549::BRAF* fusions)¹
- Tovorafenib has demonstrated significant anti-tumor activity in multiple tumor xenograft models harboring *BRAF* or *RAS* mutations¹
- This report describes the impact of tovorafenib alone or in combination with pimasertib in adult and pediatric tumor models harboring a *BRAF* fusion or a neurofibromin 1 loss of function (*NF1*-LOF) mutation

Fig 1. *NF1*-LOF upregulates MAPK signaling



Materials and methods

- Cell viability and phospho-ERK (pERK) assessment:** pERK levels were assessed by MSD-ELISA *in vitro*
 - Proliferation was assessed using CellTiter-Glo (CTG)
 - Daily repeated application of tovorafenib was applied to maintain drug concentration due to the tendency of tovorafenib to adhere to plastic and fetal bovine serum (FBS) over time, when in solution
 - Daily application of DMSO was included
- In vivo* efficacy studies:** Anti-tumor activity was assessed in tumor models
 - Randomization completed at 100–200 mm³
 - Tovorafenib dosed orally daily (QD) for up to 28 days at clinically relevant doses (i.e., 17.5 or 25 mg/kg)
 - Tumor volume and body weight were measured twice weekly
 - Endpoint plasma was collected to confirm drug concentration
- In vivo* PK-PD studies:** Single dose PK-PD studies were completed in tumor-bearing mice
 - Randomization was done at 300–500 mm³
 - A single oral dose of 17.5 or 25 mg/kg tovorafenib was administered
 - Tumors and plasma were collected for analysis
- Combination studies:** Synergy was assessed *in vitro* and *ex vivo* using 5x5 or 6x6 matrix in 2D or 3D, respectively
 - Synergy scores: calculated based on the Bliss Synergy model

Results: *In vivo* studies

Fig 2. Tumor regression in *AGK::BRAF* fusion melanoma PDX

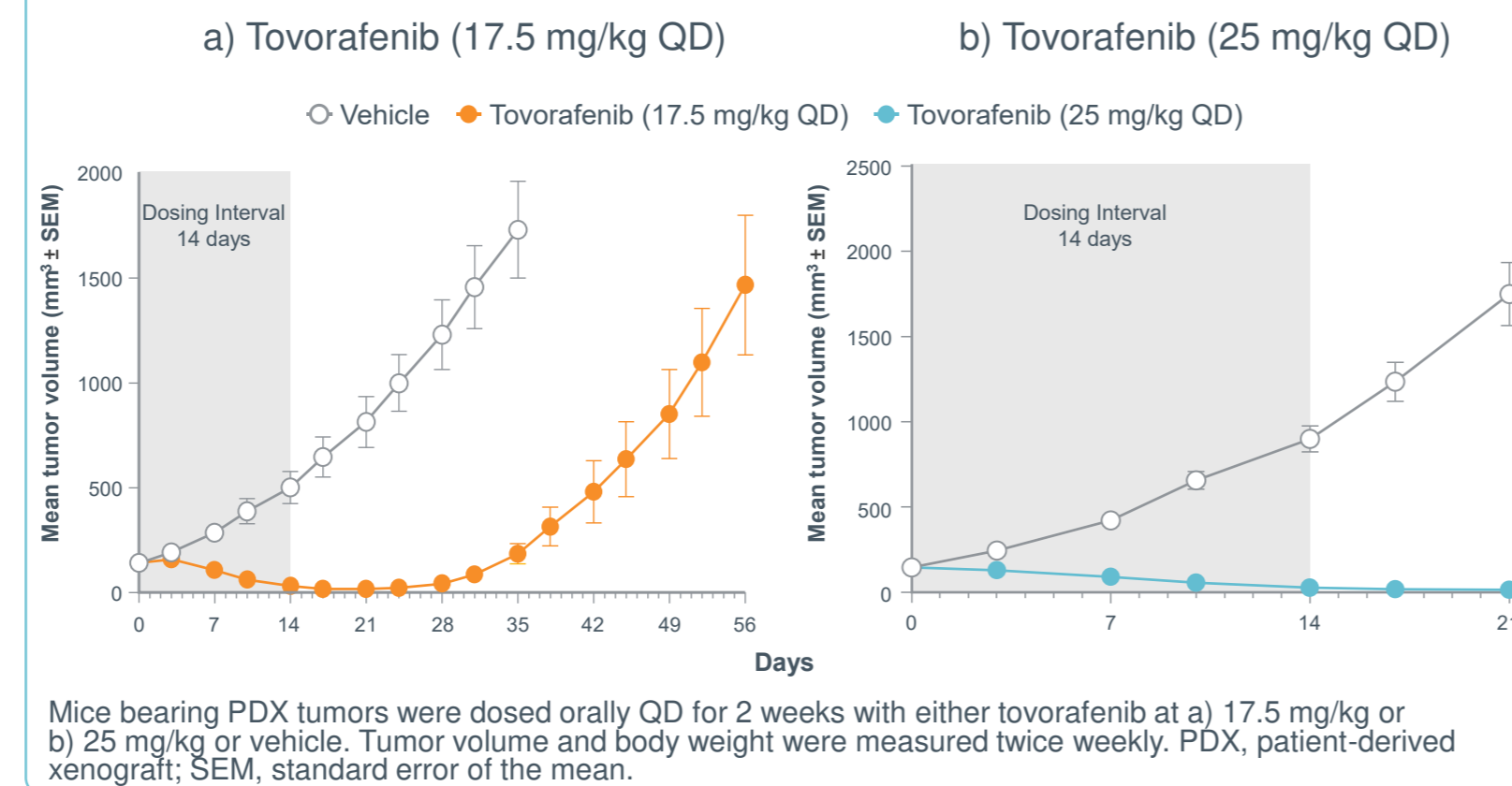


Fig 3. Lack of anti-tumor activity in *NF1*-LOF tumor models

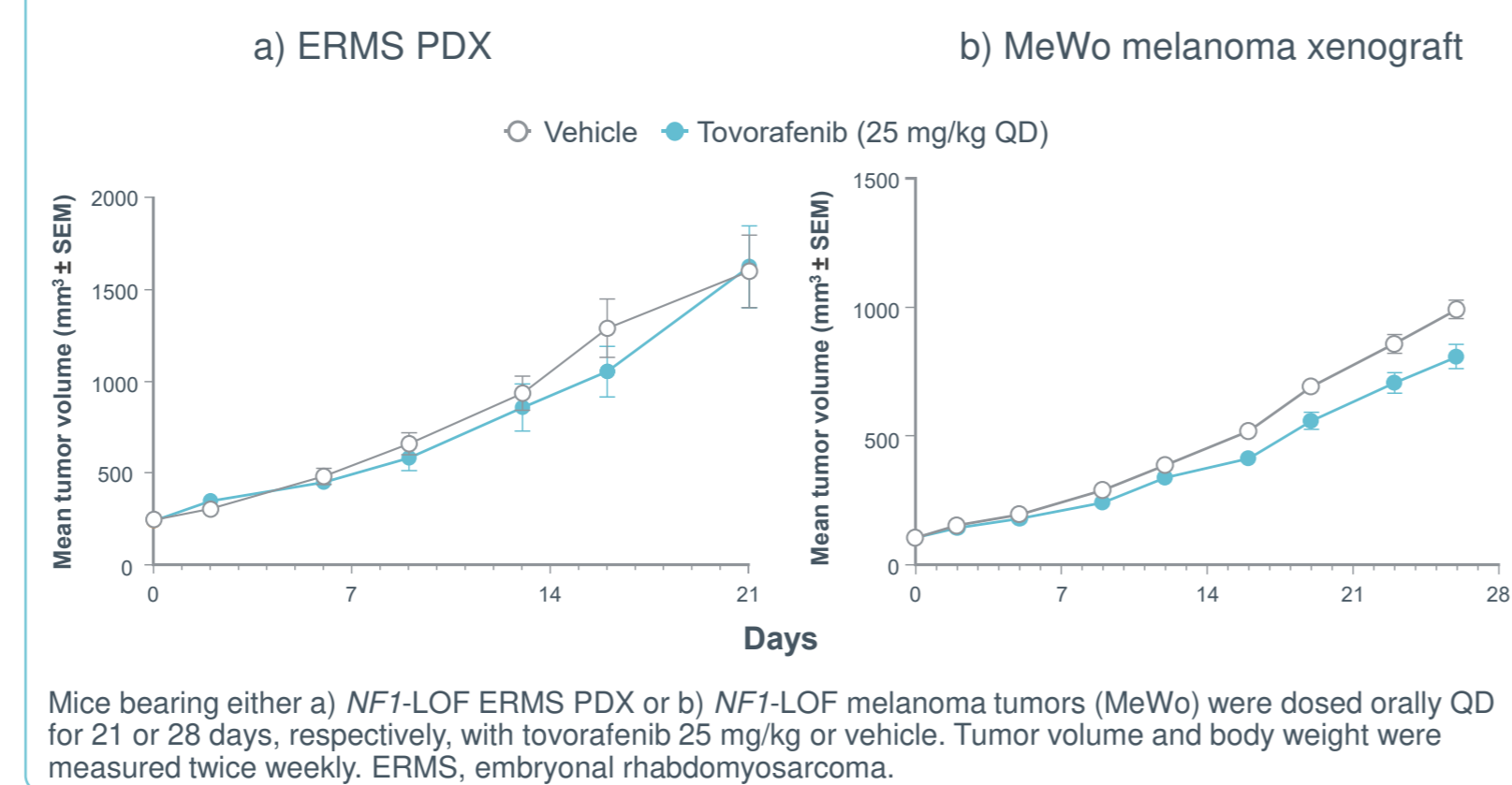
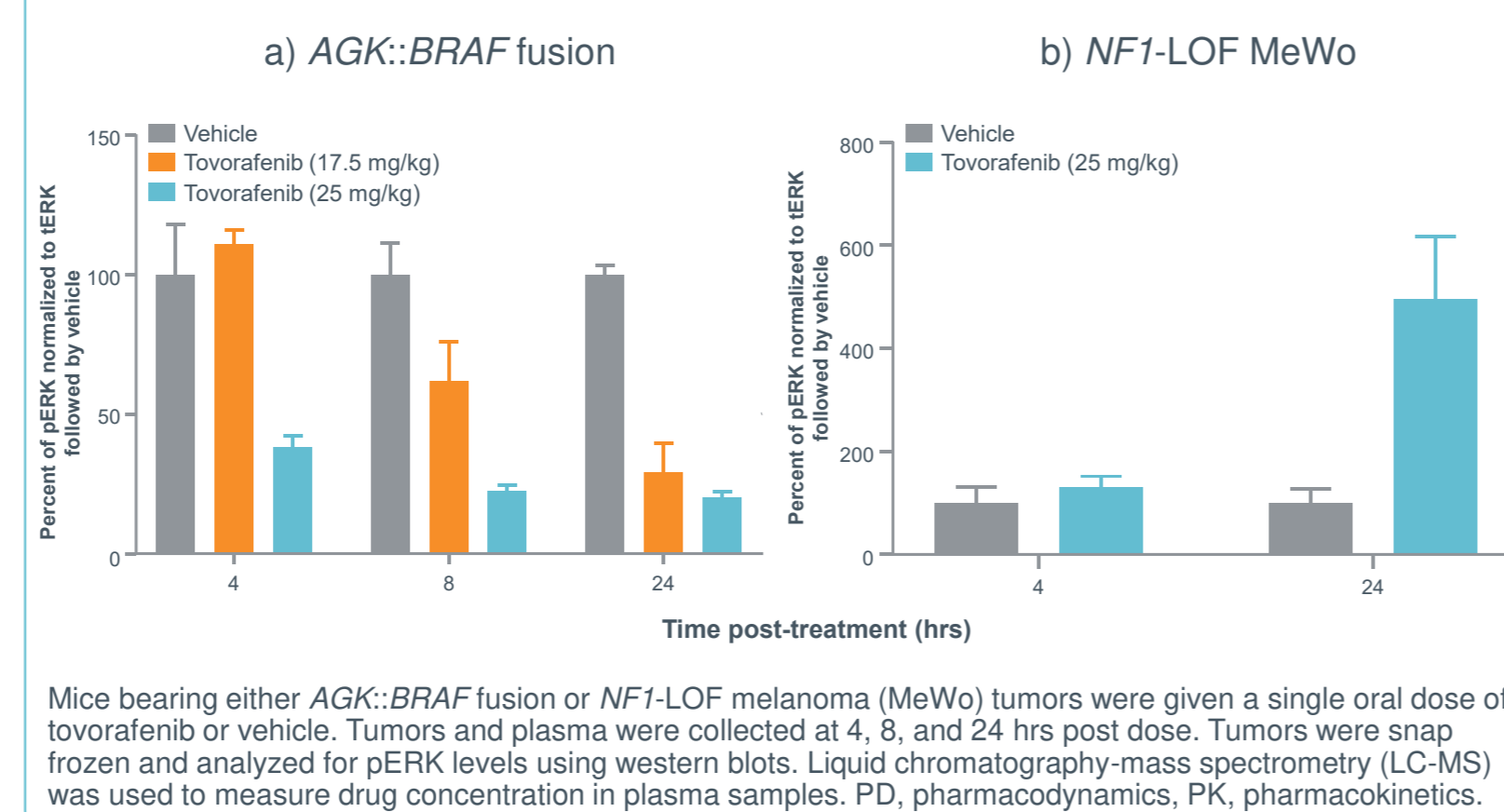


Fig 4. Differences in pERK modulation observed *in vivo*



Results: *in vitro* studies

Fig 5. pERK modulation in *NF1*-LOF and BRAF V600E tumor cell lines

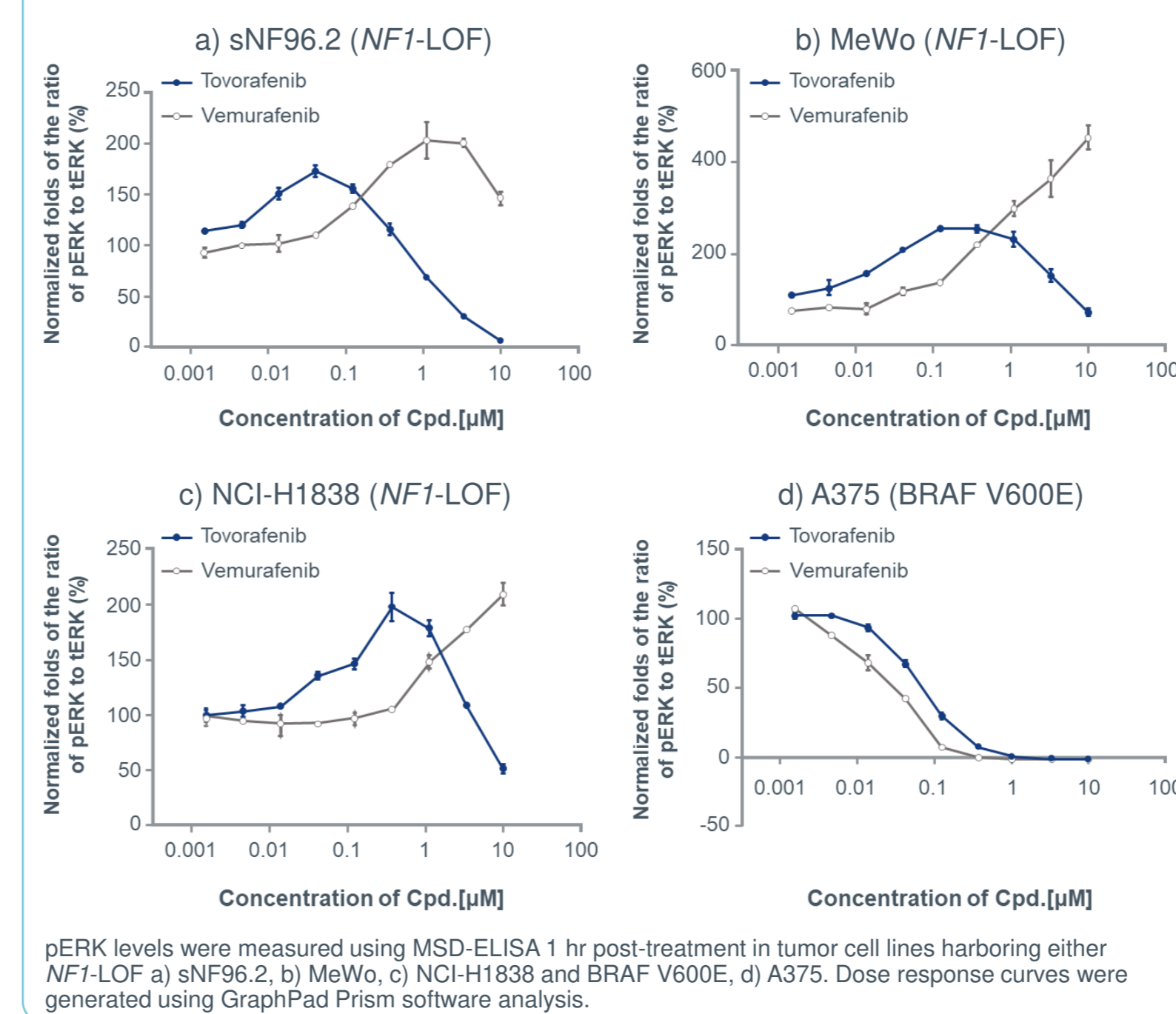


Table 1. EC₅₀ for pERK and proliferation across tumor cell lines

Tumor cell lines	Mutations	Tissue type	pERK EC ₅₀ μM		Proliferation EC ₅₀ μM	
			Tovorafenib	Vemurafenib	Tovorafenib	Vemurafenib
sNF96.2	<i>NF1</i> -LOF	MPNST	1.6	>10	0.99	6.0
MeWo	<i>NF1</i> -LOF	Melanoma	8.3	>10	7.5	4.5
NCI-H1838*	<i>NF1</i> -LOF	Lung	>10	>10	>10	>10
A375	BRAF V600E	Melanoma	0.07	0.02	0.62	0.09

Number of experiments for sNF96.2, MeWo and A375, n=2. *For NCI-H1838, n=1. Cell viability was assessed using CTG 72 hrs post-treatment. Repeated application of tovorafenib was done in CTG assay. pERK levels were measured using MSD-ELISA 1 hr post-treatment. EC₅₀ pERK and proliferation values were determined using GraphPad Prism software analysis. EC, effective concentration; MPNST, malignant peripheral nerve sheath tumor.

Results: *in vitro* or *ex vivo* combination studies

Fig 6. Synergy observed with tovorafenib + pimasertib in *NF1*-LOF MPNST cell line sNF96.2 *in vitro*

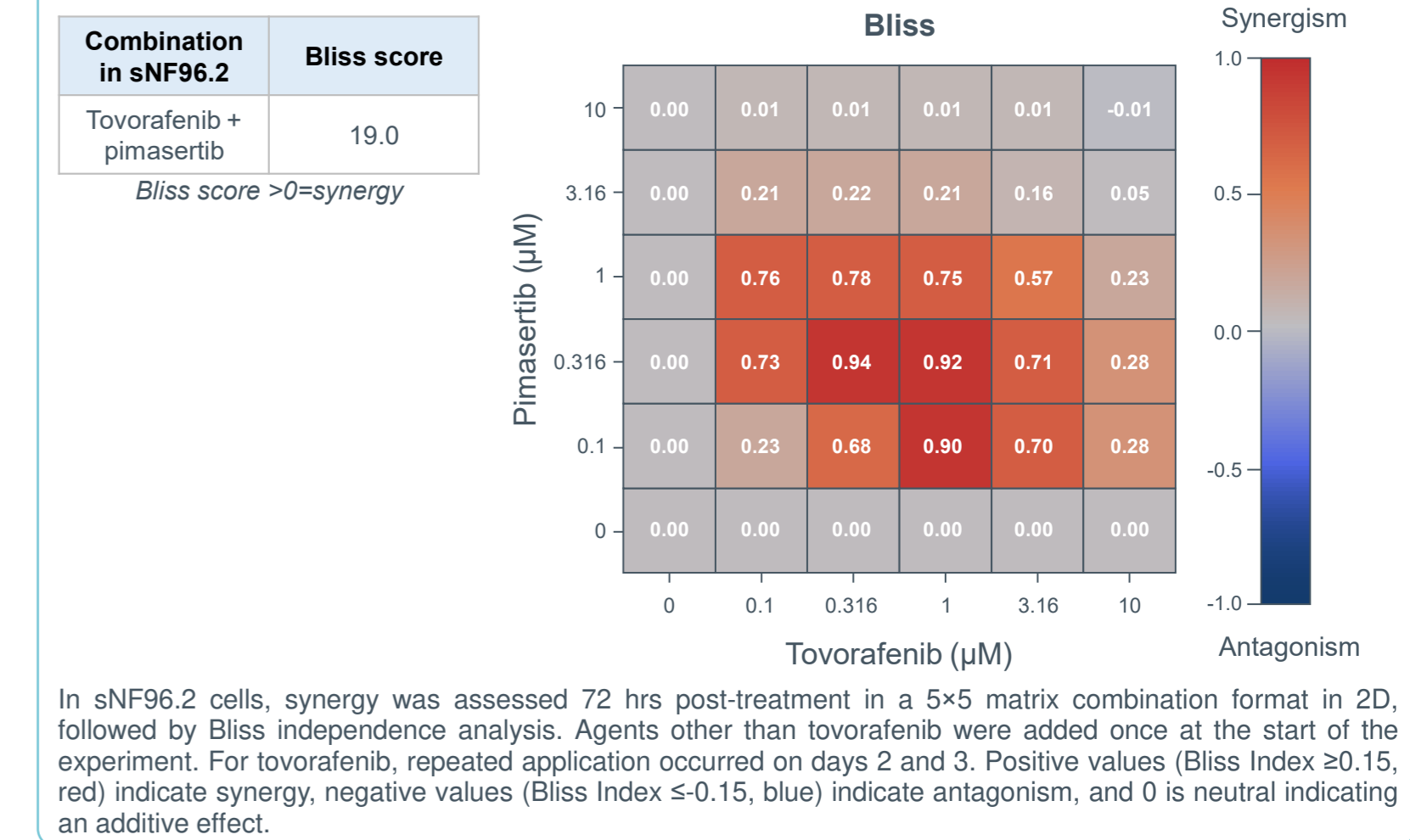
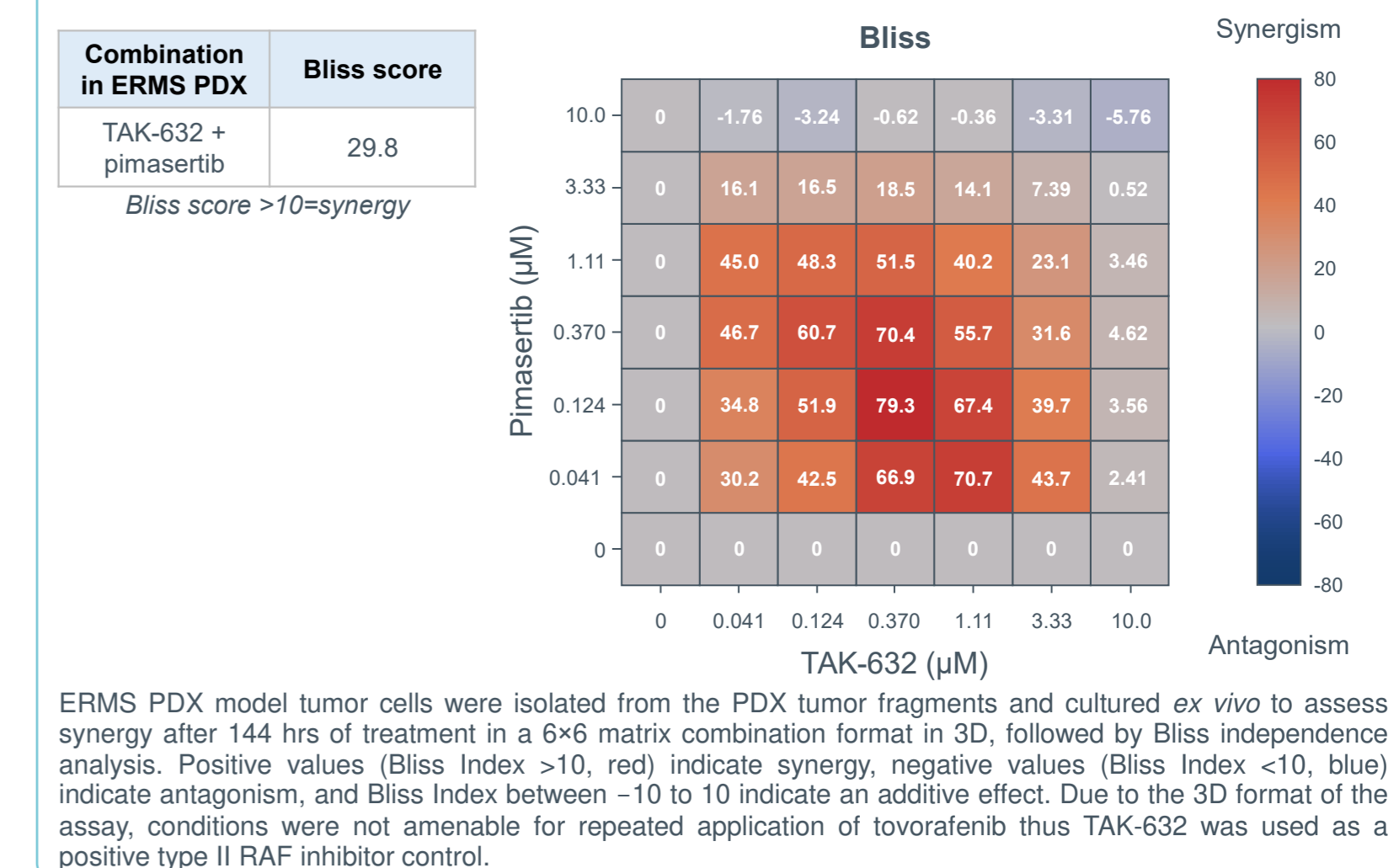


Fig 7. Synergy observed with TAK-632 + pimasertib in ERMS PDX model *ex vivo*



Conclusions

- In vivo***
 - AGK::BRAF* fusion PDX:** tovorafenib resulted in tumor regression and pERK inhibition at clinically relevant doses
 - NF1*-LOF models and tumor cell lines:** lack of anti-tumor activity and lack of pERK inhibition was observed in response to tovorafenib
- In vitro***
 - NF1*-LOF tumor cell lines:** increased pERK was observed at lower concentrations of tovorafenib; decreased pERK was observed at higher concentrations of tovorafenib
 - The effect on pERK may reflect a potential role for ARAF in this setting, as tovorafenib has been demonstrated to be ARAF-sparing
 - Tovorafenib monotherapy may be ineffective in tumors harboring an *NF1*-LOF mutation**
- In vitro* or *ex vivo* combination**
 - Combining type II RAF inhibitors with pimasertib resulted in synergy in *NF1*-LOF tumor models
 - Tovorafenib and pimasertib combination may impact growth of tumors harboring an *NF1*-LOF mutation**

References

- Sun Y, et al. *Neuro Oncol.* 2017;19(6):774–785.
- Kilburn LB, et al. *Nat Med.* 2024;30(1):207–217.
- ClinicalTrials.gov website. <https://clinicaltrials.gov/ct2/show/NCT04775485>. Accessed March 13, 2024.
- ClinicalTrials.gov website. <https://clinicaltrials.gov/ct2/show/NCT04985604>. Accessed March 13, 2024.