# 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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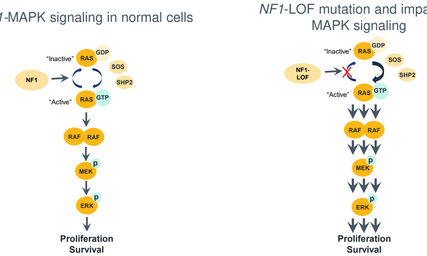
**Abstract 1972**

## 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* fusion[1]
- 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* mutations[1]
- 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



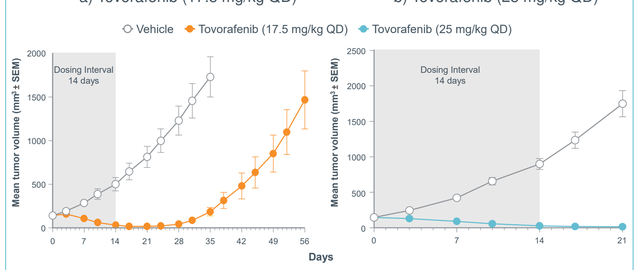

GAP, GTPase-activating protein; GDP, guanosine diphosphate; GTP, guanosine triphosphate; P, phosphate; SHP2, Src homology-2 containing protein tyrosine phosphatase 2; SOS, Son of Sevenless.

## 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 $mm^3$
  - 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 $mm^3$
  - 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 5×5 or 6×6 matrix in 2D or 3D, respectively
  - Synergy scores: calculated based on the Bliss Synergy model

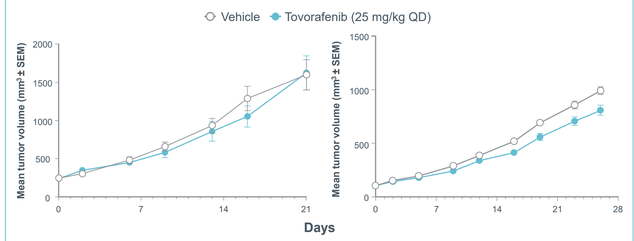
## Results: *In vivo* studies

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



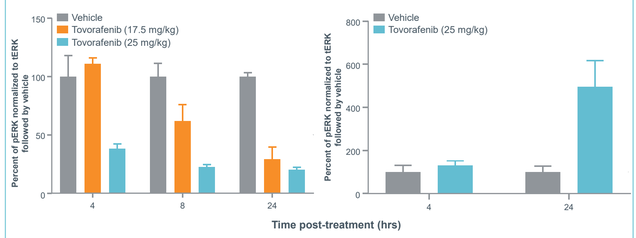

Mice bearing PDX tumors were dosed orally QD for 2 weeks with either tovorafenib at a) 17.5 mg/kg or b) 25 mg/kg or vehicle. Tumor volume and body weight were measured twice weekly. PDX, patient-derived xenograft; SEM, standard error of the mean.

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




Mice bearing either a) *NF1*-LOF ERMS PDX or b) *NF1*-LOF melanoma tumors (MeWo) were dosed orally QD for 21 or 28 days, respectively, with tovorafenib 25 mg/kg or vehicle. Tumor volume and body weight were measured twice weekly. ERMS, embryonal rhabdomyosarcoma.

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



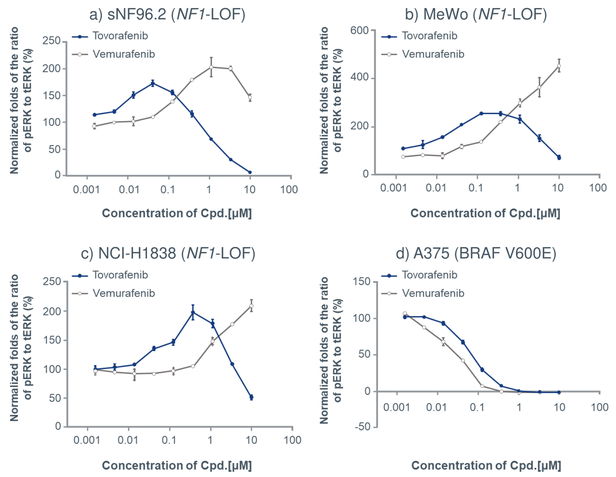

Mice bearing either *AGK*::*BRAF* fusion or *NF1*-LOF melanoma (MeWo) tumors were given a single oral dose of tovorafenib or vehicle. Tumors and plasma were collected at 4, 8, and 24 hrs post dose. Tumors were snap frozen and analyzed for pERK levels using western blots. Liquid chromatography-mass spectrometry (LC-MS) was used to measure drug concentration in plasma samples. PD, pharmacodynamics, PK, pharmacokinetics.

## References

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

## Results: *in vitro* studies

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




pERK levels were measured using MSD-ELISA 1 hr post-treatment in tumor cell lines harboring either *NF1*-LOF a) sNF96.2, b) MeWo, c) NCI-H1838 and BRAF V600E, d) A375. Dose response curves were generated using GraphPad Prism software analysis.

### Table 1. $EC_{50}$ 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 | *NF1*-LOF | MPNST | 1.6 | >10 | 0.99 | 6.0 |
| MeWo | *NF1*-LOF | Melanoma | 8.3 | >10 | 7.5 | 4.5 |
| NCI-H1838* | *NF1*-LOF | Lung | >10 | >10 | >10 | >10 |
| A375 | BRAF V600E | Melanoma | 0.07 | 0.02 | 0.62 | 0.09 |

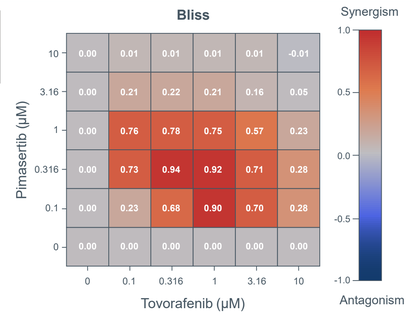
Number of experiments for sNF96.2, MeWo and A375, n=2. *For NCI-H1838, n=1. Cell viability was assessed using CTG 72 hrs post-treatment. Repeated application of tovorafenib was done in CTG assay. pERK levels were measured using MSD-ELISA 1 hr post-treatment. $EC_{50}$ pERK and proliferation values were 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*

| Combination in sNF96.2 | Bliss score |
|---|---|
| Tovorafenib + pimasertib | 19.0 |

*Bliss score >0=synergy*

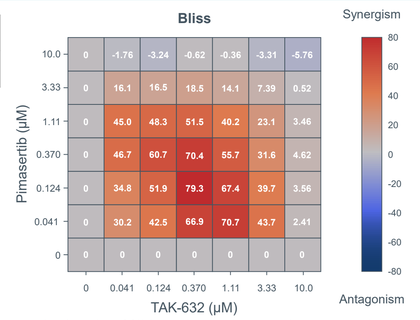



In sNF96.2 cells, synergy was assessed 72 hrs post-treatment in a 5×5 matrix combination format in 2D, followed by Bliss independence analysis. Agents other than tovorafenib were added once at the start of the experiment. For tovorafenib, repeated application occurred on days 2 and 3. Positive values (Bliss Index ≥0.15, red) indicate synergy, negative values (Bliss Index ≤-0.15, blue) indicate antagonism, and 0 is neutral indicating an additive effect.

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

| Combination in ERMS PDX | Bliss score |
|---|---|
| TAK-632 + pimasertib | 29.8 |

*Bliss score >10=synergy*




ERMS PDX model tumor cells were isolated from the PDX tumor fragments and cultured *ex vivo* to assess synergy after 144 hrs of treatment in a 6×6 matrix combination format in 3D, followed by Bliss independence analysis. Positive values (Bliss Index >10, red) indicate synergy, negative values (Bliss Index <10, blue) indicate antagonism, and Bliss Index between −10 to 10 indicate an additive effect. Due to the 3D format of the assay, conditions were not amenable for repeated application of tovorafenib thus TAK-632 was used as a positive type II RAF inhibitor control.

## 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**