Sequencing MEK inhibitor therapy after tovorafenib in *BRAF* fusion-driven cancers: Preclinical evidence of sustained tumor regression

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Background

- Pediatric low-grade gliomas (pLGGs) are the most prevalent pediatric brain tumor and make up 30% of all new childhood cancer diagnoses; 70% are driven primarily by *BRAF* alterations with *KIAA1549*::*BRAF* fusions the most common[1-6]
- ~60% of patients with pLGG are not cured by resection and have chronic, progressive disease often requiring multiple lines of treatment[7,8]
  - Chemotherapy is often selected as front-line therapy (BRAF V600E mutated tumors being an exception)[9]
  - Tovorafenib is a selective, CNS-penetrant, small molecule, type II RAF inhibitor,[6] and is the first and only FDA-approved treatment for patients ≥6 months of age with relapsed/refractory (r/r) pLGG with a *BRAF* fusion or a BRAF V600 mutation based on data from Arms 1 and 2 (pLGG arms) of the phase 2 FIREFLY-1 study (NCT04775485)[10-12]
    - MEK inhibitors (MEKi) are also options for treatment of r/r pLGG*.[9]
- The ideal sequence of tovorafenib and a MEKi in the r/r setting is an open question[9]
  - Clinical activity of tovorafenib after failure of prior systemic therapy, including MEKi, was demonstrated in FIREFLY-1;[10-12] however, the antitumor activity of MEKi following tovorafenib has not been established

*Dabrafenib/trametinib is FDA approved for patients 1 year of age and older with LGG with a BRAF V600E mutation requiring systemic therapy.[13]

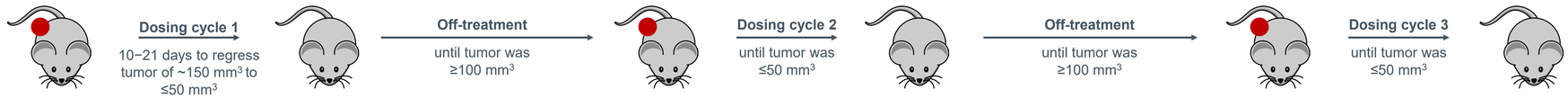
Study objectives

- Using 2 different *BRAF* fusion patient-derived xenograft (PDX) models, different treatment sequencing schedules were evaluated:
  - To assess the activity of MEKi in a post-tovorafenib treatment setting
    - Subsequent treatment with a MEKi (trametinib or selumetinib), including tumor regrowth post-MEKi followed by a second cycle of tovorafenib
  - To assess tovorafenib activity between MEKi treatments
    - Following initial treatment with a MEKi (trametinib) and regrowth off treatment, subsequent treatment with tovorafenib followed by tumor regrowth and retreatment with a MEKi treatment
  - To assess resistance to tovorafenib retreatment, including multiple courses and treating relatively larger tumors
- To explore the potential emergence of resistance with longer-term treatment with tovorafenib in a preclinical *BRAF* fusion tumor model

Materials and methods

- Tovorafenib demonstrated antitumor activity in an *AGK*::*BRAF* fusion PDX melanoma model;[14] this same model was used in the studies that follow, as *KIAA1549*::*BRAF* fusion PDX models are limited
- Studies were conducted using two different *BRAF* fusion PDX tumor models:
  - *AGK*::*BRAF* fusion melanoma: 6–8 adult female non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice/treatment group
  - Pediatric glioma model, anaplastic pleomorphic xanthoastrocytoma (APXA), bearing *CDC42SE2*::*BRAF* fusion: 15 adult male NOD rag gamma (NRG) mice/treatment group
- Mice were treated with tovorafenib or a MEKi during a series of on- and off- dosing cycles and off-treatment periods, on an individual basis (**Figure 1**)
  - Mean (±SEM, standard error of the mean [SEM]) tumor volume (mm^3) was evaluated throughout
    - Tumor volume was measured twice a week in individual mice
  - For additional dosing cycles, duration of treatment range was until the tumor size decreased to ≤50 mm^3

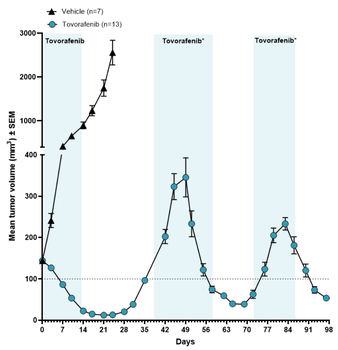
Figure 1. Overview of various *in vivo* study designs



Results

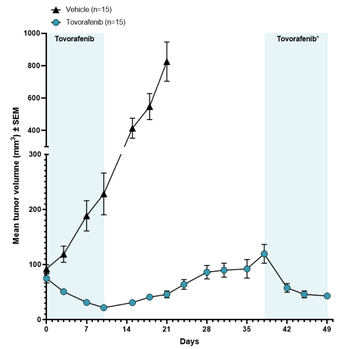
Figure 2. Tovorafenib retreatment induced responses in *BRAF* fusion tumors following initial treatment and regrowth off-treatment

A. *AGK*::*BRAF* fusion melanoma PDX



Mice were dosed with tovorafenib (25 mg/kg, oral, daily) or vehicle for 3 cycles of ~14 days (d).

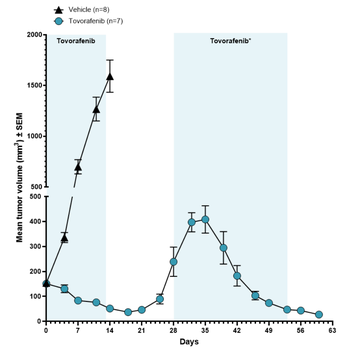
B. *CDC4SE2*::*BRAF* fusion APXA PDX



Mice were dosed with tovorafenib (25 mg/kg, oral, daily) or vehicle for 2 cycles (10 d and 11 d).

Figure 3. Relatively larger *BRAF* fusion tumors also responded to tovorafenib retreatment

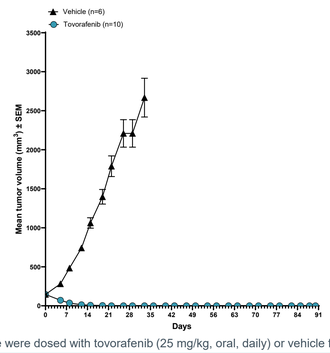
***AGK*::*BRAF* fusion melanoma PDX**



Mice were dosed with tovorafenib (25 mg/kg, oral, daily) or vehicle for 2 cycles of ~14 d/cycle. Tumor volume was ~150 mm^3 at start of initial treatment and ~400 mm^3 at start of retreatment.

Figure 4. Sustained regression with *in vivo* continuous tovorafenib dosing in *BRAF* fusion tumors

***AGK*::*BRAF* fusion melanoma PDX**

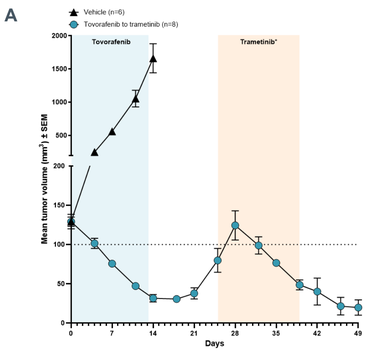


Mice were dosed with tovorafenib (25 mg/kg, oral, daily) or vehicle for 90 d.

Figure 5. MEKi treatment (A. trametinib and B. selumetinib) induced responses following initial tovorafenib treatment and tumor regrowth in *AGK*::*BRAF* fusion tumors

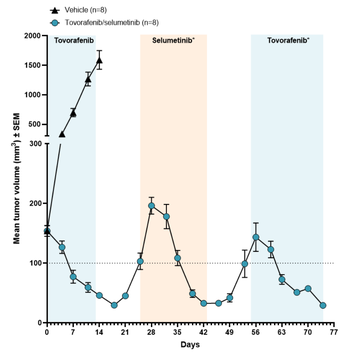
***AGK*::*BRAF* fusion melanoma PDX**

A



Mice were dosed with tovorafenib (25 mg/kg, oral, daily) or vehicle for 14 d. After a ~14 d off-treatment period, mice were treated with trametinib (1 mg/kg, oral, once daily) for ~14 d.

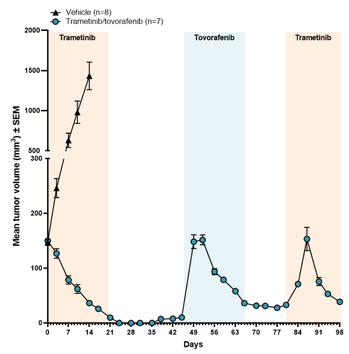
B



Mice were dosed with tovorafenib (25 mg/kg, oral, daily) or vehicle for 14 d. After a ~10 d off-treatment period, mice were treated with selumetinib (25 mg/kg, oral, twice daily) for ~18 d. After a second off-treatment period (16 d), mice received another cycle of tovorafenib (16 d).

Figure 6: Tumor regression was observed when tovorafenib was dosed in-between a MEKi

***AGK*::*BRAF* fusion melanoma PDX**



Mice were dosed with trametinib (1 mg/kg, oral, once daily) or vehicle for 20 d. After a 25 d off-treatment period, mice were treated with tovorafenib (25 mg/kg, oral, daily) for 21 d. After a second off-treatment period (15 d), mice received another cycle of trametinib (17 d).

Conclusions

- In an *AGK*::*BRAF* fusion PDX model, tovorafenib could be dosed first without impacting the ability of tumors to respond to a MEKi
  - Similar tumor regression was observed in *CDC4SE2*::*BRAF* fusion APXA PDX with tovorafenib treatment, demonstrating efficacy across different *BRAF* fusion tumor types
- Longer-term exposure was maintained with *in vivo* continuous tovorafenib dosing; significant tumor regression was maintained with no emergent tumors, indicating no evidence of emerging resistance
- These findings support the hypothesis that acquired resistance may be less common in *BRAF* fusion-driven tumors treated with tovorafenib

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