Evaluation of antitumor activity of oral elraglusib in B16F10 melanoma model

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CONCLUSIONS

This is the first *in vivo* study to demonstrate that daily treatment with oral elraglusib is well tolerated, significantly inhibits tumor growth, and enhances antitumor immunity in the poorly immunogenic, highly aggressive B16F10 melanoma model.

- Repeated daily oral dosing was well tolerated in mice (7 days, QD and BID) and dogs (elraglusib tablet, 28 days).
- The Day 7 decrease in exposure was specific to mice (AUC0–24 0.4–0.5× Day 1) and was not seen in dogs (1.0–1.05× per mg/kg through Day 28), suggesting species differences in elraglusib metabolism.
- Efficacy of elraglusib SDD 100 mg/kg QD (TGI 50%) and BID (TGI 77%) predicts an elraglusib tablet HED of ~8 mg/kg (~500 mg) QD to BID. Human PK of daily elraglusib tablets remains to be determined.

These results support the clinical evaluation of elraglusib tablets in cancer patients.

BACKGROUND

Elraglusib (9-ING-41), a glycogen synthase kinase-3 inhibitor, exhibits a multimodal mechanism of action in human cancer. We previously reported that intravenous elraglusib combined with gemcitabine/nab-paclitaxel (GnP) met its primary endpoint of improved survival when compared to GnP alone in a randomized Phase 2 trial (the 1801 Part 3B trial, NCT03678883) in patients with metastatic pancreatic cancer. Here, we report, for the first time, the antitumor efficacy and immunomodulatory effects of oral elraglusib used as a single agent in a syngeneic mouse model of melanoma.

MATERIALS AND METHODS

Elraglusib formulations. Elraglusib IV solution (elraglusib 10 mg/ml solution in PEG400:EtOH:Tween80 75:17:8) was used for IV and IP injections in mice. Elraglusib oral solution (elraglusib 50 mg/ml solution in PEG400:EtOH:Tween80 94:5:1) or elraglusib spray-dried dispersion (SDD) (elraglusib SDD 20 mg/ml suspension in 10 mM sodium citrate buffer, 0.5% methylcellulose, 0.1% Tween 80, pH 4.0) were used for elraglusib oral administration in mice. The elraglusib SDD, composed of elraglusib and Eudragit L100 at a 1:1 weight ratio, is a component of the elraglusib tablet formulation, with each 1,000 mg tablet containing 500 mg of elraglusib SDD.

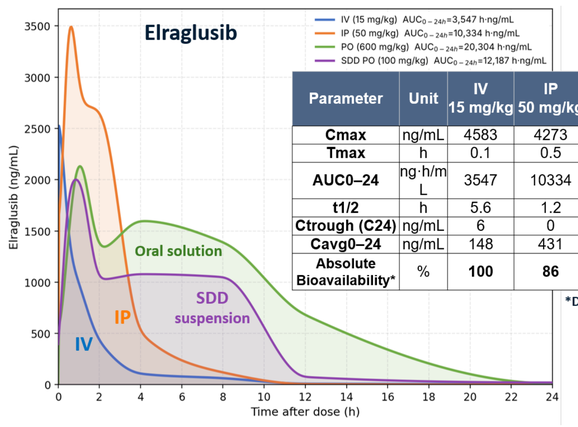
Mouse PK. Mice received elraglusib by intravenous injection at 15 mg/kg (single dose), by intraperitoneal injection at 50 mg/kg (single dose), and by oral gavage (7-day repeated dosing). Mice were administered either elraglusib oral solution at 600 mg/kg or an oral suspension of elraglusib SDD at 100 mg/kg (as elraglusib API), once daily by oral gavage for 7 days. Plasma was sampled on Day 1 and Day 7 at predose, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h after dosing.

Dog PK (elraglusib tablets): 28-day repeat-dose study. Dogs received elraglusib tablets (250 mg of elraglusib API per tablet) once daily with food. The 25 mg/kg group (1 tablet; actual dose 26.5–32.5 mg/kg) was dosed for 28 days. Plasma was sampled on Days 1, 15 and 28 at predose, 0.5, 1, 2, 4, 6, 8 and 24 h.

Plasma elraglusib was measured by a validated HPLC-MS/MS method (LLOQ 1.00 ng/mL) at BioAgilytix.

Elraglusib efficacy study. Oral elraglusib was evaluated in the subQ B16F10 syngeneic melanoma mouse model. Immunophenotyping of tumor samples was performed using a 42-parameter spectral flow cytometry assay.

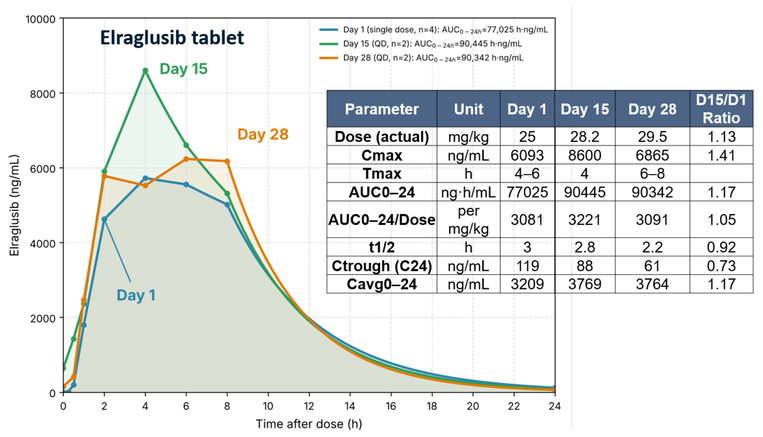
RESULTS



Parameter	Unit	IV 15 mg/kg	IP 50 mg/kg	Oral solution 600 mg/kg	Oral SDD 100 mg/kg
Cmax	ng/mL	4583	4273	2295	2028
Tmax	h	0.1	0.5	1.0	1.0
AUC0–24	ng·h/mL	3547	10334	20304	12187
t1/2	h	5.6	1.2	2.3	3.1
Ctrough (C24)	ng/mL	6	0	12	18
Cavg0–24	ng/mL	148	431	846	508
Absolute Bioavailability*	%	100	86	14	52

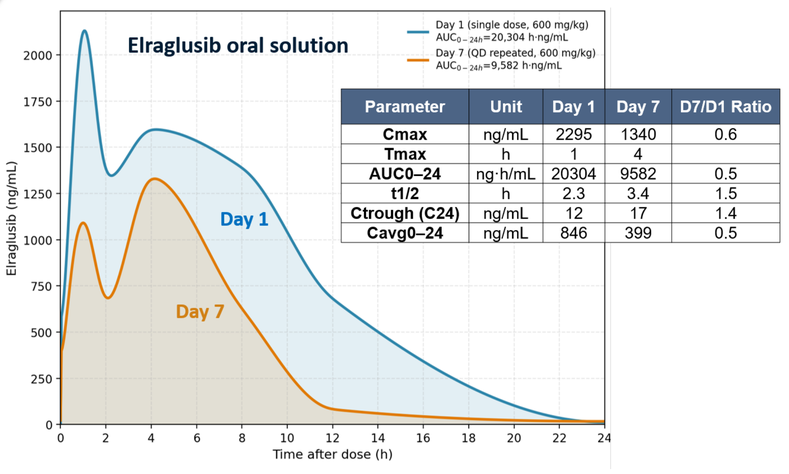
*Dose normalized

Elraglusib PK parameters in mice: single dose of elraglusib IV, IP or oral gavage. Adjusted for dose, oral elraglusib SDD suspension has about 3.6 times more exposure than the elraglusib oral solution. Oral bioavailability is about 52% (elraglusib SDD suspension) vs 14% (elraglusib oral solution).



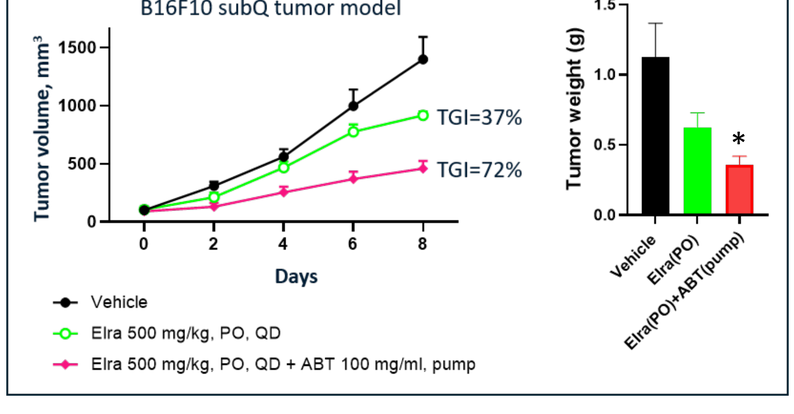
Parameter	Unit	Day 1	Day 15	Day 28	D15/D1 Ratio
Dose (actual)	mg/kg	25	28.2	29.5	1.13
Cmax	ng/mL	6093	8600	6865	1.41
Tmax	h	4–6	4	6–8	
AUC0–24	ng·h/mL	77025	90445	90342	1.17
AUC0–24/Dose	per mg/kg	3081	3221	3091	1.05
t1/2	h	3	2.8	2.2	0.92
Ctrough (C24)	ng/mL	119	88	61	0.73
Cavg0–24	ng/mL	3209	3769	3764	1.17

Elraglusib PK parameters in dogs: Day 1 vs Day 15 vs Day 28 (28-day repeated dosing, elraglusib tablet, 25 mg/kg, PO, QD). Unlike elraglusib SDD in mice (Day 7 AUC 0.4× Day 1), dog exposure to the elraglusib tablet does not fall with QD dosing: AUC0-24 is 1.17× Day 1 on Days 15 and 28 (1.05× and 1.00× per mg/kg dose). With t1/2 ≈ 2–3 h there is no meaningful accumulation; 25 mg/kg QD keeps exposure stable through Day 28. Elraglusib tablet BA (AUC-based) in dogs: 138% (fed).

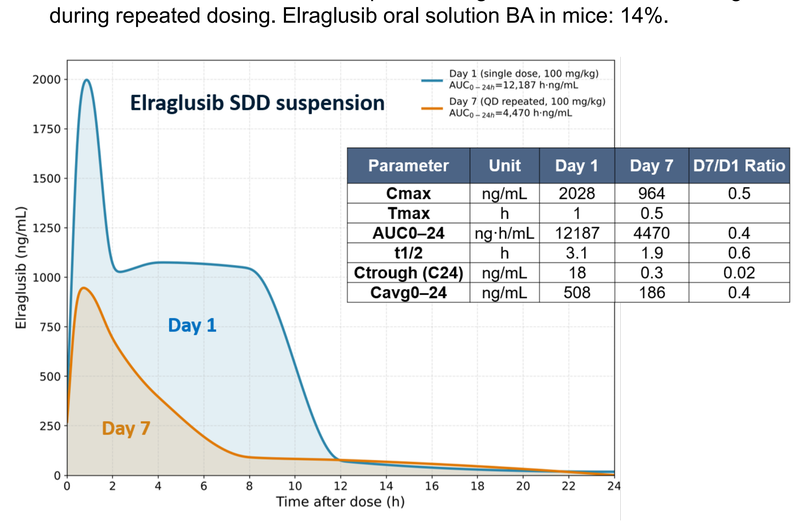


Parameter	Unit	Day 1	Day 7	D7/D1 Ratio
Cmax	ng/mL	2295	1340	0.6
Tmax	h	1	4	
AUC0–24	ng·h/mL	20304	9582	0.5
t1/2	h	2.3	3.4	1.5
Ctrough (C24)	ng/mL	12	17	1.4
Cavg0–24	ng/mL	846	399	0.5

Elraglusib PK parameters in mice: Day 1 vs Day 7 (7-day repeated dosing, elraglusib oral solution, 600 mg/kg, PO, QD). The experimentally observed reduction in AUC0-24 on Day 7 likely reflects nonlinear or time-dependent PK in mice, potentially including altered absorption, induction of metabolic clearance, transporter-mediated effects, or other adaptive biologic mechanisms occurring during repeated dosing. Elraglusib oral solution BA in mice: 14%.

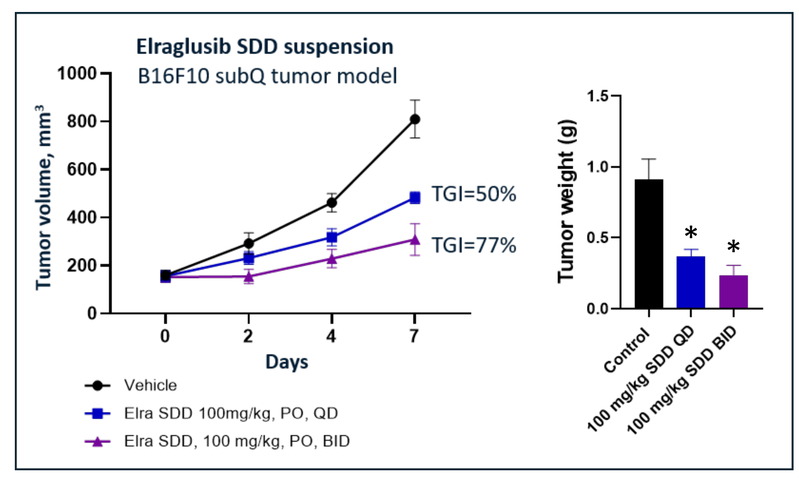


Daily treatment with elraglusib oral solution inhibits growth of B16F10 subQ tumors. Mice were inoculated subQ with B16F10 cells. Tumors were size matched and mice were randomized into 3 treatment groups: vehicle (N=5), elraglusib oral solution 500 mg/kg, PO, QD (N=5), and elraglusib + CYP inhibitor ABT (osmotic pump) (N=5). Points, mean tumor volume; bars, SE. Weight of resected tumors was measured. Columns, mean tumor weight; bars, SE.*P<0.05

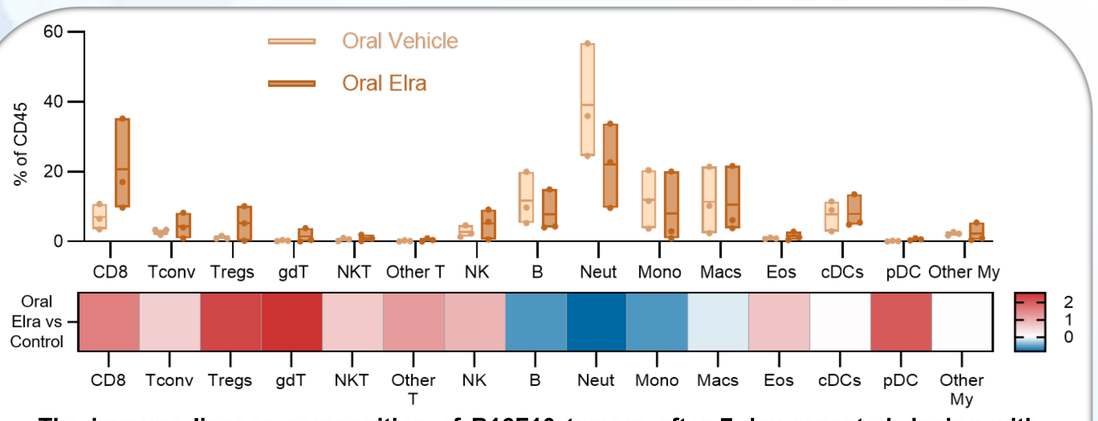


Parameter	Unit	Day 1	Day 7	D7/D1 Ratio
Cmax	ng/mL	2028	964	0.5
Tmax	h	1	0.5	
AUC0–24	ng·h/mL	12187	4470	0.4
t1/2	h	3.1	1.9	0.6
Ctrough (C24)	ng/mL	18	0.3	0.02
Cavg0–24	ng/mL	508	186	0.4

Elraglusib PK parameters in mice: Day 1 vs Day 7 (7-day repeated dosing, elraglusib SDD suspension, 100 mg/kg [elraglusib API], PO, QD). Elraglusib SDD QD exposure falls by Day 7, and more than with the elraglusib oral solution: AUC0-24 is 0.4× Day 1 for the SDD vs 0.5× for the oral solution. Elraglusib SDD suspension BA in mice: 52%.



Daily treatment with oral elraglusib SDD suspension inhibits growth of B16F10 subQ tumors. Mice were inoculated subQ with B16F10 cells. Tumors were size matched and mice were randomized into 3 treatment groups: vehicle (N=5), elraglusib SDD 100 mg/kg (as elra API), PO, QD (N=5), and elraglusib SDD 100 mg/kg (elraglusib API), PO, BID (N=5). Points, mean tumor volume; bars, SE. Weight of resected tumors was measured. Columns, mean tumor weight; bars, SE. *P<0.05. Elra, elraglusib.



The immune lineage composition of B16F10 tumors after 7-day repeated dosing with oral elraglusib. The heatmap illustrates relative changes in tumor-associated immune cell populations following treatment. Red and blue indicate higher and lower values, respectively, according to the accompanying color scale. Elra, elraglusib; CD8, $CD8^+$ T cells; Tconv, conventional $CD4^+$ T cells ($CD4^+$ $FoxP3^-$); Tregs, regulatory T cells ($CD4^+$ $FoxP3^+$); gdT, gamma delta (γδ) T cells; NKT, natural killer T cells; Other T, other T cells not assigned above; NK, natural killer cells; B, B cells; Neut, neutrophils; Mono, monocytes; Macs, macrophages; Eos, eosinophils; cDCs, conventional dendritic cells; pDC, plasmacytoid dendritic cells; Other My, other myeloid cells not assigned above.

In the B16F10 melanoma model, daily administration of oral elraglusib induced potent antitumor response. The activity of elraglusib was attributed to direct antitumor effect on cancer cells and to an increase and accumulation of CD8+ and NK cells together with decreased Ly6G+ neutrophil/PMN-MDSC-like cells in B16F10 tumors. These findings suggest that oral elraglusib shifts the tumor microenvironment toward a more immune-permissive and less myeloid-suppressive state, thereby enhancing antitumor immune responses.

Species-specific PK with repeated dosing. In mice, Day 7 exposure decreased with both the elraglusib SDD suspension (AUC0–24 0.4× Day 1) and the oral solution (0.5× Day 1), whereas dogs receiving elraglusib tablets maintained stable exposure through Day 28 (AUC0–24 1.2× Day 1; 1.00–1.05× per mg/kg dose). This suggests a mouse-specific, time-dependent increase in elraglusib clearance (e.g., metabolic induction), consistent with the enhanced antitumor activity of elraglusib oral solution combined with the CYP inhibitor ABT (TGI 37% → 72%). Repeated daily oral dosing was well tolerated in mice (7 days, QD and BID) and dogs (28 days).

HED prediction for the elraglusib tablet. The exposures associated with significant antitumor activity for elraglusib monotherapy in the B16F10 tumor model [TGI 50%; Week 1 Cavg ~350 ng/mL, AUC0–168 ~58,000 ng·h/mL) and BID (TGI 77%; projected Cavg ~680 ng/mL, AUC0–168 ~115,000 ng·h/mL)] are predicted to be achieved in patients at an HED in the range of 8 mg/kg (~500 mg for a 60 kg patient) QD or BID. While the HED is useful to inform phase 1 trial design, it is based on PK in mice where the metabolism of elraglusib, especially with repeat dosing, may be different than in other species including humans. Thus, human PK data obtained in phase 1 will be used to establish dose-exposure and exposure-effect relationships in humans that will support the identification of RP2D for elraglusib monotherapy.