Mechanism of Tumor-selective Inhibition of Dimeric RAF by a Type 1 RAF inhibitor

Poster Number : LB294

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INTRODUCTION

Clinical efficacy of dimeric RAF-pathway-targeting inhibitors requires selective MAPK inhibition in RAS (MUT) tumors over RAS (WT) normal cells

1- Type 1.5 (*αC-OUT/DFG-IN*) – FDA-approved BRAF inhibitors

- Exploit **monomeric** signaling properties of **BRAF (V600E) tumors**[1]
- **Paradoxically activate MAPK pathway** in contexts where RAF signals as a **dimer**[2] (normal tissues, secondary mutations that convert BRAF (V600X) monomeric to dimeric, RAS-mutant driven tumors)

  ➔ **Toxicity as single agents in BRAF (V600E) tumors** and **ineffective in RAS-driven tumors**
- **BRAFi + MEKi combination leverages paradoxical activation** in normal tissues – **standard-of-care for treatment of BRAF (V600E) mutant melanoma**

2- Type 2 (*αC-IN/DFG-OUT*) - Pan-RAF inhibitors

- Effectively inhibit monomeric and dimeric RAF with minimal MAPK activation ➔ **Address a broad range of BRAF and RAS mutant tumors**[1,3]
- **Limited efficacy as single agents**
- Pan-RAFi + MEKi effectively inhibits RAF-dimer driven tumors but strongly **suppress MAPK signaling in normal tissues ➔ toxicities**

3- Type 1 RAF inhibitors (*αC-IN/DFG-IN*)

- Show **poor efficacy** in inhibiting both dimeric or monomeric RAF

ELV-3111, a **first-in-class Type 1 pan-RAFi**, exhibits the **broad utility of a Type 2 inhibitor** while safely combining **with MEK inhibitors,** similar to Type 1.5 inhibitor

HYPOTHESIS

Mechanistic insight into MAPK hyperactivation by Type 1 RAF inhibitors enables safe, tumor-selective combinations for dimeric RAF-driven tumors

CONCLUSION

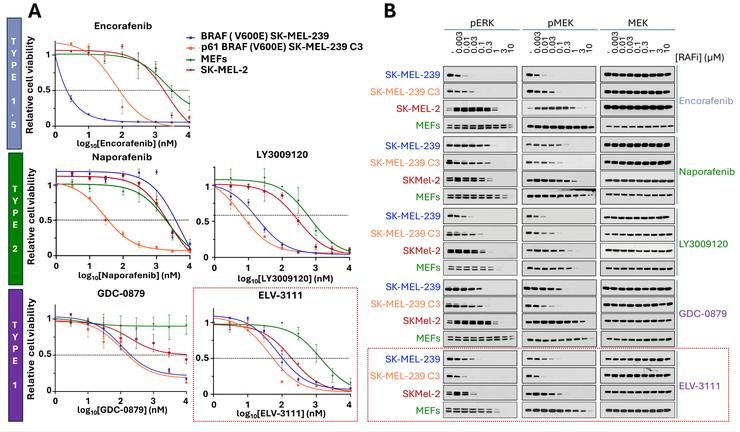
First-in-class Type 1 RAFi ELV-3111 (*αC-IN/DFG-IN*)

- **Highly potent**, inhibiting MAPK signaling in BRAF class I/II/III and RAS (MUT) cells *in vitro* and *in vivo*
- Induces **MAPK hyperactivation in normal cells** via a **RAS-dependent allosteric mechanism**
- **Leveraging Type 1 RAFi-Induced MAPK Hyperactivation in vertical MAPK combinations is expected to provide potent and safe treatment options for patients with dimeric RAF-driven cancers, compared to current treatments**

References:
1- Karoulia et al. Nat Rev Cancer (2017)
2-Poulikakos P. I. et al. Nature (2010)
3- Adamopoulos C et al. Cancer Discov (2021)
4- Machleidt, T. et al. ACS Chem. Biol (2015)
5- Dixon A.S et al. ACS Chem. Biol. (2016).

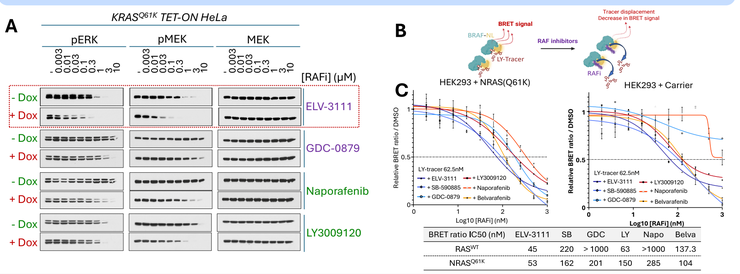
RESULTS

Figure 1: ELV-3111 Is a Type 1 RAF Inhibitor with Broad Activity and Potent Suppression of Monomeric and Dimeric RAF



BRAF (V600E) monomeric SK-MEL-239, BRAF (V600E) dimeric resistant SK-MEL-239 C3, NRAS (Q61K) SK-MEL-2 and RAS (WT) MEFs were treated with increasing concentrations of different RAFis (0–10 μM). **(A)** Cell viability was measured after 6 days. **(B)** MAPK signaling (pERK, pMEK) was assessed after 1 hour.

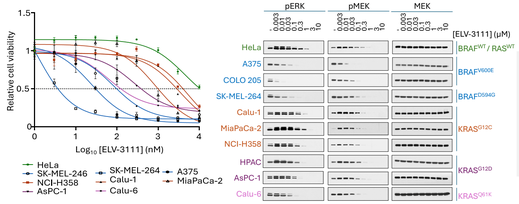
Figure 2: ELV-3111 Exhibits Enhanced Selectivity for RAS-Mutant over RAS-WT Cells compared to Type 2 RAF Inhibitors, Due to Superior BRAF Binding in RAS-MUT Context



BRET ratio IC50 (nM)	ELV-3111	SB	GDC	LY	Napo	Belva
RASWT	45	220	> 1000	63	>1000	137.3
NRASQ61K	53	162	201	150	285	104

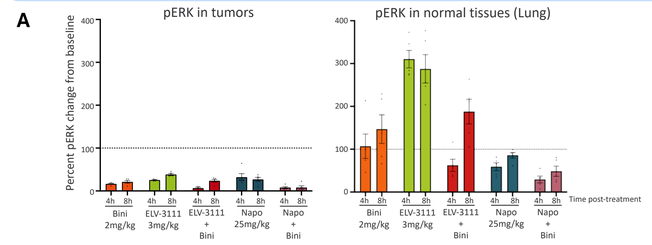
(A) KRAS (Q61K) was induced or not in doxycylin-inducible HeLa cells model, followed by treatment with different Type 1 (purple) or Type 2 (green) RAFis for 1 hour. MAPK signaling levels were evaluated. **(B)** HEK293 cells were co-transfected with BRAF-NanoLuc with or without NRAS(Q61K) for 24 hours, then treated with 62.5 nM RAFi-tracer in combination with increasing concentrations of different type 1 or type 2 RAF inhibitors, for 2 hours. **(C)** BRET signals, enabled real-time measurement of drug-target interactions[4], were measured.

Figure 3: ELV-3111, as a Single Agent, Demonstrates High Potency in Cells Driven by Class 1, Class 2, and Class 3 BRAF Mutants, as well as RAS(MUT) Cell lines



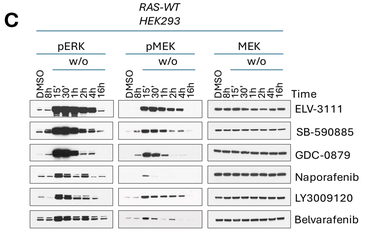
A panel of cancer cell lines, harboring different classes of BRAF or RAS mutations, was treated with ELV-3111 **(A)** Relative cell viability was assessed after 6 days and **(B)** MAPK signaling levels were evaluated after 1 hour.

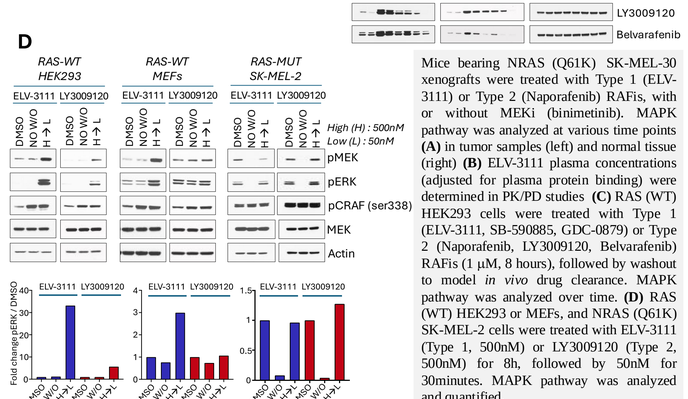
Figure 4: ELV-3111 Induces Robust MAPK Hyperactivation In Normal Tissue *In Vivo* as a Result of Declining Drug Concentrations – A Characteristic of Type 1 RAFis



B

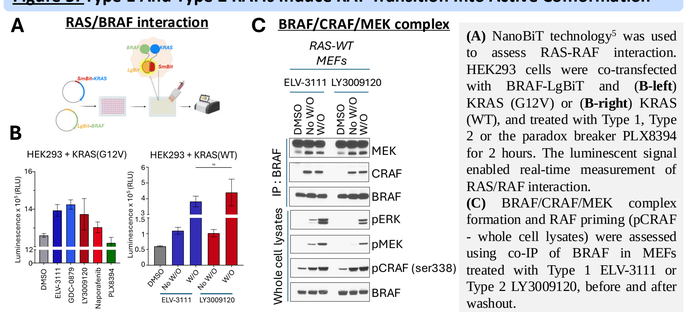
PK/PD	2h	4h	6h	8h
Free 3111	464	204	188	117





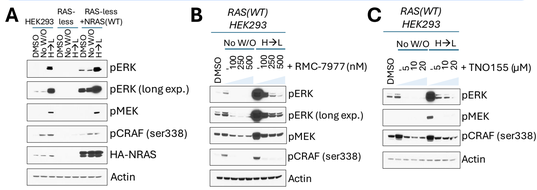
Mice bearing NRAS (Q61K) SK-MEL-30 xenografts were treated with Type 1 (ELV-3111) or Type 2 (Naporafenib) RAFis, with or without MEKi (binimetinib). MAPK pathway was analyzed at various time points **(A)** in tumor samples (left) and normal tissue (right) **(B)** ELV-3111 plasma concentrations (adjusted for plasma protein binding) were determined in PK/PD studies **(C)** RAS (WT) HEK293 cells were treated with Type 1 (ELV-3111, SB-590885, GDC-0879) or Type 2 (Naporafenib, LY3009120, Belvarafenib) RAFis (1 μM, 8 hours), followed by washout to model *in vivo* drug clearance. MAPK pathway was analyzed over time. **(D)** RAS (WT) HEK293 or MEFs, and NRAS (Q61K) SK-MEL-2 cells were treated with ELV-3111 (Type 1, 500nM) or LY3009120 (Type 2, 500nM) for 8h, followed by 50nM for 30minutes. MAPK pathway was analyzed and quantified.

Figure 5: Type 1 And Type 2 RAFis Induce RAF Transition Into Active Conformation



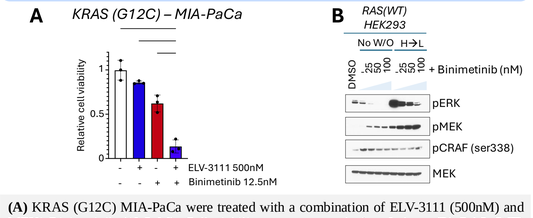
(A) NanoBiT technology[5] was used to assess RAS-RAF interaction. HEK293 cells were co-transfected with BRAF-LgBiT and (**B-left**) KRAS (G12V) or (**B-right**) KRAS (WT), and treated with Type 1, Type 2 or the paradox breaker PLX8394 for 2 hours. The luminescent signal enabled real-time measurement of RAS/RAF interaction. **(C)** BRAF/CRAF/MEK complex formation and RAF priming (pCRAF - whole cell lysates) were assessed using co-IP of BRAF in MEFs treated with Type 1 ELV-3111 or Type 2 LY3009120, before and after washout.

Figure 6: Type 1 RAFi-induced MAPK Hyperactivation in Normal Cells is RAS-dependent



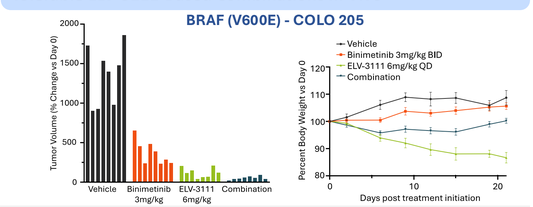
(A) Parental or RAS-less HEK293, and RAS-less HEK293 expressing NRAS (WT) were treated with ELV-3111 for 8 hours, followed by 50nM for 30 minutes. HEK293 cells were treated with ELV-3111 in combination with **(B)** panRAS-ONi (RMC-7977) or (C) SHP2i (TNO155) for 8 hours, followed by 50nM of ELV-3111 in combination. MAPK pathway was analyzed.

Figure 7: Type 1 RAFi-induced MAPK Hyperactivation Enables Potent ELV-3111-Based Combinations – *In-Cell Proof of Concept*



(A) KRAS (G12C) MIA-PaCa were treated with a combination of ELV-3111 (500nM) and MEKi Binimetinib (12.5nM). Cell viability was measured after 6 days. **(B)** RAS (WT) HEK293 cells were treated with a combination of ELV-3111 (500nM) and MEKi Binimetinib for 8 hours, followed by 50nM ELV-3111 for 30minutes. MAPK signaling was assessed.

Figure 8: Exploiting MAPK Hyperactivation for Effective and Tolerable ELV-3111-Based Combinations *In Vivo*



(C) Mice bearing BRAF (V600E) COLO-205 xenografts were treated with ELV-3111 as a single agent or in combination with Binimetinib (MEKi). Tumor growth and tolerability (body weight changes) were assessed overtime.

Figure 9: Rational Type 1 Combinations for Improved Therapeutic Index in Dimeric RAF-Driven Cancers