Pan-RAF inhibitor DAY101 exhibits preclinical activity in preclinical tumor models harboring BRAF alterations beyond BRAF V600E mutation

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DAY101 is a pan-RAF inhibitor

- DAY101 (TAK-580, MLN2480, BIIB-024) has demonstrated to be an oral, CNS-penetrant, selective, type II pan-RAF kinase inhibitor that exhibits equipotency against the BRAF V600E mutation and wild-type BRAF and CRAF
- DAY101 has been shown to inhibit both RAF monomers and dimers without resulting in paradoxical activation of the MAPK pathway in wild-type BRAF as observed with type I BRAF inhibitors
- Preclinical *in vitro* studies have demonstrated that DAY101 does not induce paradoxical activation of MAPK signaling in models that express *KIAA1549-BRAF* fusion[1]
- Ability of DAY101 to inhibit both RAF monomers and dimers may enable broader clinical application beyond BRAF V600 mutations, including RAF fusions, non V600 BRAF mutations and KRAS mutations
- The combination of DAY101 and MEK inhibitors was assessed preclinically in models harboring various genomic alterations

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1. Sun Y et al., Neuro Oncol 2017; 19:774-85.

DAY101 and TAK-632 exhibited comparable biochemical and cellular characteristics

Assay	DAY101	TAK-632
BRAF wild-type IC_{50} nM	10.1	8.3
CRAF wild-type IC_{50} nM	0.7	1.4
BRAF V600E IC_{50} nM	7.1	2.4
EC_{50} nM A375 (BRAF V600E)	240	160

Potency of DAY101 against BRAF, CRAF and BRAF V600E was assessed by a TR-FRET biochemical assay. Cellular viability was measured using CellTitre-Blue and EC_{50} values were determined using GraphPad Prism software analysis.[2] Cell viability for TAK-632 was measured using CellTitre-Glo[3].

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2. Elenbass et al., *EJC Suppl* 2010;8(7):40-1 (abstr 105). 3. Okinwa et al., J Med Chem 2013; 56 (16): 6478-91.

Single agent activity of DAY101 in tumor cell lines

Tumor cell line	DAY101 IC_{50} µM (repeated application)	DAY101 IC_{50} µM (single application)	MEKi-2 IC_{50} µM	DAY101 IC_{50} µM (previously reported)[1]
A375 (BRAF V600E)	0.14	1.071	0.04	0.24
HCT116 (KRAS G13D/PIK3CA H1047R)	1.66	ND	2.10	3-10

Cell viability was assessed using CellTiter-Glo after 72 h incubation. For DAY101, repeated application was done on days 2 and 3 to maintain drug concentration due to the tendency to adhere to plastic over time when in solution. This is not an issue for suspensions or short-term mechanistic assays. As a control, DSMO was also added on days 2 and 3.

1. Sun Y et al., Neuro Oncol 2017; 19:774-85.

Synergy was observed in response to DAY101 + MEKi in non V600 BRAF mutant cell lines *in vitro* or PDX models *ex vivo*

Assay format	Cell line	BRAF mutation	BRAF mutation class	Tumor type	Synergy score	
					DAY101 + MEKi-1	DAY101 + MEKi-2
Cell lines (2D)	A375	V600E	1	Melanoma	5	2
	NCI-H1755	G469A	2	Lung	11	12
	MDA-MB-231	G464V	2	Breast	20	14
	NCI-H1666	G466V	3	Lung	1	2
PDX models (3D)	MEXF 2104	V600E	1	Melanoma	3	3
	MEXF 1870	K601E	2	Melanoma	12	8
	MEXF 622	G469R	2	Melanoma	5	4
	MEXF 1876	G469V	2	Pancreatic	1/-1	-4

BI: >0 = synergy; <0 = antagonism

Synergy was assessed in a 5x5 matrix combination format in a 2D monolayer assay run for 72 h, followed by Bliss independence analysis. All compounds were added once at the start of the experiment. PDX models were assessed in a 5x5 matrix combination format in a 3D clonogenic assay followed by Bliss independence analysis. In the 3D clonogenic assay, repeated application of DAY101 was done every 2–3 days whereas combination partners were only added once at the start of the experiment. At maximum colony formation, between 8–13 days, colony counts were performed. A positive number indicated synergy as the number of combination pairs which achieved a Bliss Index ≥0.15. A O score indicated additive effects with Bliss Index between -0.15 and +0.15 for all combination pairs. A negative number indicated antagonism as the number of combination pairs which achieved a Bliss Index of ≤-0.15.

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MEKi: MEK inhibitor

Single agent activity in PDX models harboring BRAF fusions treated with pan-RAFi TAK-632 and MEKi *ex vivo*

Compound	Melanoma PDX *AGK-BRAF*		Mixed Mullerian PDX *KIAA1549-BRAF*		Colon PDX *AGAP-BRAF; BRAF-AGAP3*		Pancreatic PDX *TNS3-BRAF*	
	IC_{50} µM	Max % inh	IC_{50} µM	Max % inh	IC_{50} µM	Max % inh	IC_{50} µM	Max % inh
TAK-632	0.55	95.7%	2.29	88.2%	3.23	77.6%	2.88	74.9%
MEKi-1	0.21	86.9%	5.09	69.1%	3.22	70.4%	4.60	62.9%
MEKi-2	0.29	86.5%	7.42	60.8%	4.87	63.3%	7.12	56.9%

Single tumor cells isolated from the PDX tumor tissues were seeded into 96 well plates. Compounds were added the following day. Cells were treated for 7 days. CellTitre-Glo was used the readout. GraphPad Prism was used to calculate the IC50s.

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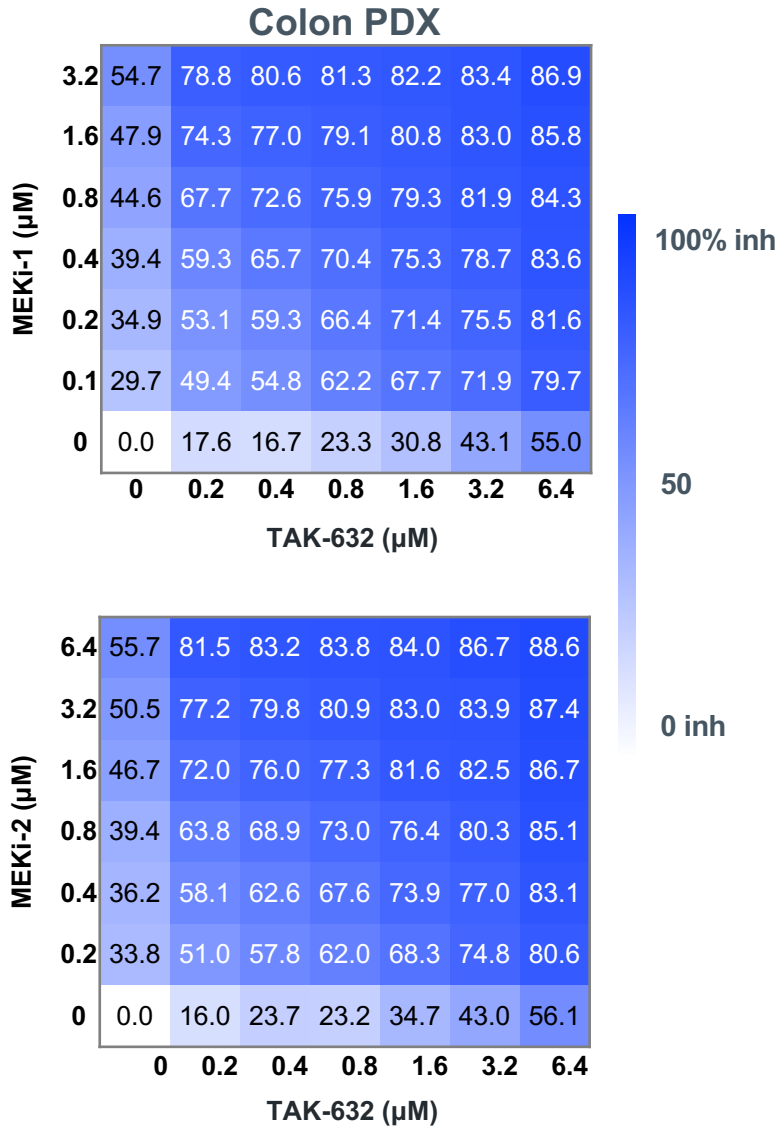
Inh: inhibition; MEKi: MEK inhibitor

Synergy was observed in response to TAK-632 + MEKi in BRAF fusion PDX models *ex vivo*

Synergy score of combination	Melanoma PDX *AGK-BRAF*	Mixed Mullerian PDX *KIAA1549-BRAF*	Colon PDX *AGAP3-BRAF; BRAF-AGAP3*	Pancreatic PDX *TNS3-BRAF*
TAK-632 + MEKi-1	12.2	35.3	26.7	35.5
TAK-632 + MEKi-2	10.7	33.5	27.7	36.9

CI: >5 = synergy; <-5 = antagonism



Single tumor cells isolated from PDX tumor tissue were seeded into 96-well plates. Compounds were added the following day with a 6x6 combination matrix for 7 days. Combination Index synergy scoring was determined, and Loewe score presented. A score higher than 5 indicated synergy and a score less than -5 indicated antagonism.

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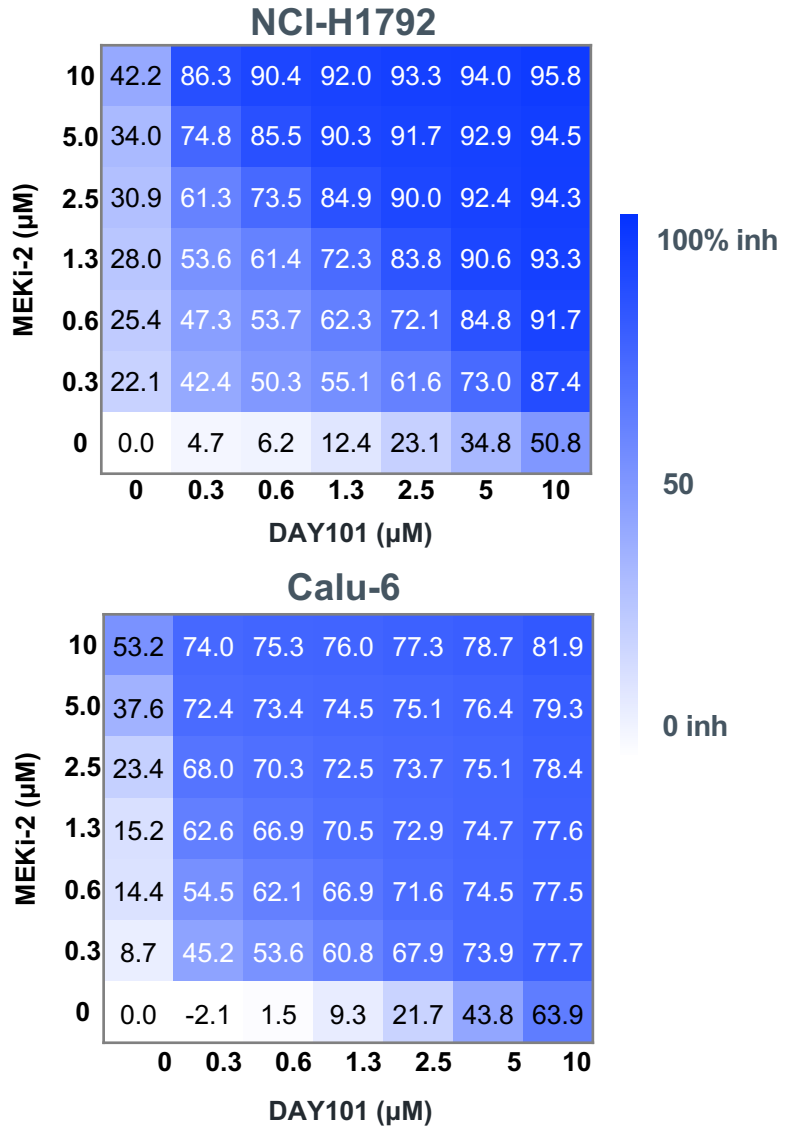
Inh: inhibition; MEKi: MEK inhibitor

Synergy was observed with DAY101 + MEKi-2 in KRAS mutant tumor cell lines *in vitro*

Mutation	Cell line	Tumor type	DAY101 + MEKi-2
KRAS G12C	NCI-H358	Lung	5.6
	NCI-H1792	Lung	40.9
	Calu-1	Lung	7.6
	NCI-H23	Lung	15.7
	NCI-H2122	Lung	15.0
	SW756	Cervix	17.1
KRAS G12V	SW620	Colon	5.4
	SW480	Colon	13.6
	Capan-2	Pancreas	9.8
	NCI-H727	Lung	6.9
KRAS G12D	LS513	CRC	22.3
	HPAC-1	Pancreas	8.8
KRAS Q61	NCI-H460	Lung	11.1
	Calu-6	Lung	30.2
KRAS G13D/PIK3CA H1047R	HCT116	Colon	18.9
KRAS G12S	A549	Lung	9.2
BRAF V600E	A375	Melanoma	6.2

CI: >5 = synergy; <-5 = antagonism



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Cells were seeded in 6x6 combination matrix in 2D format. For DAY101, repeated application was done on days 2 and 3. All other compounds were added only once at the start of the experiment. Combination Index synergy scoring was determined, and Loewe score presented. A score higher than 5 indicated synergy and a score less than -5 indicated antagonism. Inh, inhibition

Conclusions

- Single agent activity was observed in a melanoma AGK-BRAF fusion PDX model in response to a pan-RAF inhibitor or a MEK inhibitor *ex vivo*

- Combination of a pan-RAF inhibitor and MEK inhibitor impacted tumor models harboring RAS/RAF alterations. Synergy was observed in:
  - Non V600 BRAF tumor cell lines or PDX models in response to DAY101 + MEKi combination *in vitro* or *ex vivo*, respectively
  - BRAF fusion PDX models in response to pan-RAFi + MEKi combination *ex vivo*
  - KRAS mutant tumor cell lines in response to DAY101 + MEKi *in vitro*

- DAY101 is currently being investigated in a phase 2 trial for the treatment of BRAF-altered, relapsed or progressive LGG in patients 6 months to 25 years of age (FIREFLY-1, NCT04775485)

- A phase I/II trial to evaluate DAY101 in combination with MEKi pimasertib to treat solid tumors with MAPK alterations is planned