DCC-3084, a RAF dimer inhibitor, broadly inhibits *BRAF* class I, II, III, *BRAF* fusions, and *RAS*-driven solid tumors leading to tumor regression in preclinical models

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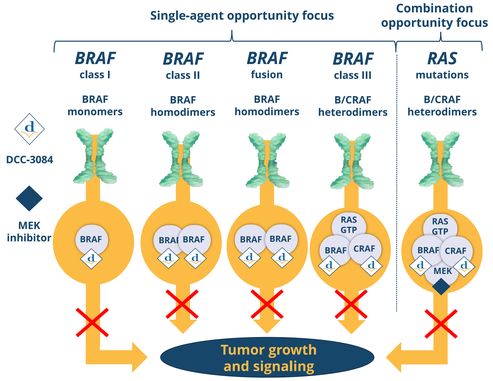
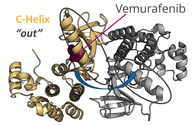
Introduction

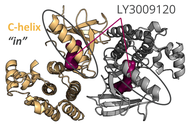
- DCC-3084 is a potent and selective switch-control inhibitor of BRAF and CRAF kinases shown preclinically to target all relevant aberrant signaling mechanisms (monomers, homodimers, heterodimers)[1-3]



Switch-control mechanism for engineering a RAF dimer inhibitor



Top: Vemurafenib binds the **C-helix switch** of all different species of BRAF (monomers, homodimers, and heterodimers) in the ***"C-helix out"*** state.[4] This binding mode limits inhibitor binding to only 1 monomer of the BRAF dimer, resulting in **transactivation** of the unbound monomer and enhanced pathway signaling



Bottom: LY3009120 (DP-4978) binds the BRAF **C-helix switch** in the ***"C-helix in"*** state and the activation switch in the ***"DFG out"*** state, enabling inhibitor binding to both monomers.[2] LY3009120 induces minimal paradoxical activation and fully inhibits RAF dimers

PDB 3OG7[4] (vemurafenib) and 5C9C[2] (LY3009120). Images rendered in PyMOL.

Methods

- Inhibition of RAF kinases, including off-rate analysis, was measured using recombinant enzymes
- X-ray crystallography was used for structure-based drug design
- Cellular proliferation was measured using resazurin to monitor cell viability
- Synergy in cells was measured using Bliss scores[5] and curve shift analysis
- Inhibition of ERK or RSK phosphorylation was measured by AlphaLISA or ELISA
- Pharmacokinetics in the plasma, brain, and CSF compartments were measured following oral dosing in Wistar rats
- *RAF*- and *RAS*-mutant mouse xenograft models were used to assess pharmacokinetics, pharmacodynamics, and efficacy

Results

DCC-3084 is a potent and selective inhibitor of BRAF and CRAF

Inhibitor	*BRAF* IC_{50} (nM)	*CRAF* IC_{50} (nM)	*ARAF* IC_{50} (nM)	$BRAF^{V600E}$ IC_{50} (nM)	*BRAF*/*CRAF* $t_{1/2}$ (hrs)
DCC-3084	**71**	**34**	**903**	**2**	**>20**
Tovorafenib	654	338	2300	6	2
Naporafenib	38	29	720	28	10
Belvarafenib	31	51	276	3	>20
Exarafenib	182	87	2600	31	>20
JZP815	48	18	261	46	>20

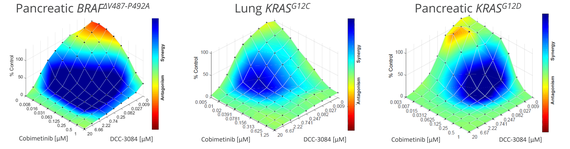
Twelve-point dose with 3-fold dilutions starting at 10 mM. [ATP] = 1 mM.
Recombinant ARAF assay designed to more accurately reflect inhibition of ARAF in cells.
$t_{1/2}$ determined in the presence of BRAF and CRAF. [ATP] = 1 mM.

DCC-3084 exhibits overall best-in-class inhibition of cellular proliferation in *BRAF* class I, II, and III mutant and *RAF* fusion human cancer cell lines

Inhibitor	A375 IC_{50} (nM)	Colo-205 IC_{50} (nM)	HT-29 IC_{50} (nM)	BxPC-3[a] IC_{50} (nM)	H2405 IC_{50} (nM)	WM3928 IC_{50} (nM)	WM3629 IC_{50} (nM)
Mutation class	**I**	**I**	**I**	**II**	**II**	**Fusion**	**III + *NRAS***
DCC-3084	**54**	**174**	**13**	**61**	**74**	**42**	**3**
Tovorafenib	3000	6880	5270	1100	603	669	305
Naporafenib	438	2142	228	19	465	90	3
Belvarafenib	144	486	128	59	149	14	2
Exarafenib	170	624	101	254	549	98	17
JZP815	141	290	47	200	47	133	2

Inhibition of cell proliferation of 12-point dose with 3-fold dilutions starting at 10 mM.
[a]BxPC-3 data are IC_{50} (nM) for inhibition of pERK measured by AlphaLISA after 4-hour treatment.

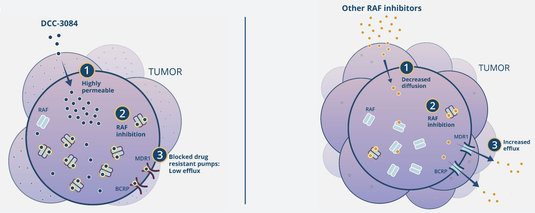
DCC-3084 synergizes with the MEK inhibitor cobimetinib in *BRAF* class II and *KRAS*-mutant cell lines



Cell line	Cobimetinib IC_{50} (nM)	DCC-3084 IC_{50} (nM)	DCC-3084 + cobimetinib IC_{50} (nM)	Fold change
BxPC-3	526	2300	14	**164**
H358	80	1260	42	**30**
HPAF-II	177	309	12	**26**

Nine-point dose with 3-fold dilutions starting at 20 mM for DCC-3084. Nine-point dose response with 2-fold dilutions for cobimetinib. Single agent IC_{50} for cobimetinib and DCC-3084 are the absolute IC_{50} corresponding to 50% of the DMSO control. IC_{50} for DCC-3084 + cobimetinib calculated for DCC-3084 at IC_{50} for cobimetinib. Fold change is DCC-3084 + cobimetinib relative to the IC_{50} of DCC-3084 alone.

DCC-3084 inhibits both the MDR1 and BCRP drug-resistance efflux transporters

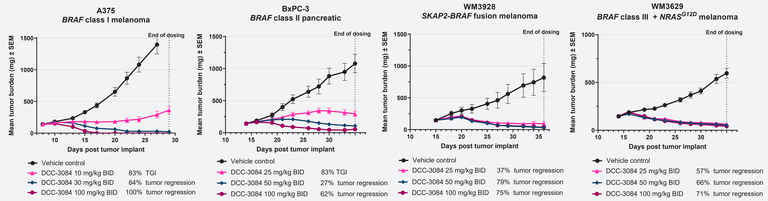


DCC-3084 exhibits excellent permeability and low efflux, and is a strong inhibitor of the MDR1 and BCRP drug transporters

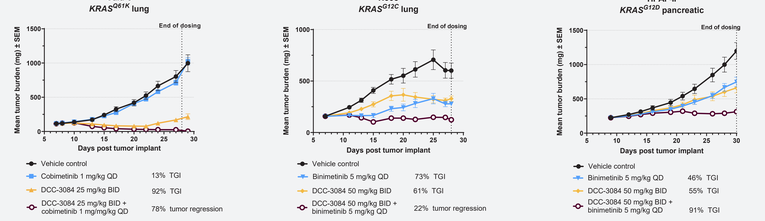
Inhibitor	T. sol pH 1.6 (μM)	MDR1 P_{app} A-B	MDR1 efflux ratio	MDR1 IC_{50} (nM)	BCRP P_{app} A-B	BCRP efflux ratio	BCRP IC_{50} (nM)
DCC-3084	**408**	**21**	**1.1**	**79**	**33**	**0.8**	**74**
Tovorafenib	<1.6	16.9	1.2	>10,000	6.1	13.2	706
Naporafenib	21	3.9	6.2	2300	4.9	16.6	2400
Belvarafenib	25	0.5	0.2	>100	0.9	3.2	17
Exarafenib	1518	2.8	13.9	>10,000	1.1	58	>10,000
JZP815	213	5.0	2.1	1900	26.1	1.5	1130

P_{app} A-B has units of 10^{-6} cm/s. Efflux ratio = P_{app} B-A (apparent permeability coefficient from basolateral to apical direction)/P_{app} A-B (apparent permeability coefficient from apical to basolateral direction)

Single-agent DCC-3084 produces tumor regression in *BRAF* monomer–, *BRAF* homodimer–, and *B/CRAF* heterodimer–mutant cancer models



DCC-3084 exhibits single-agent activity in *BRAF*/*CRAF*-driven, *KRAS*-mutant cancer models and further combines with MEK inhibitors



Data represent mean ± SEM. Tumor models were established to 100–300 mm^3 prior to dosing.

DCC-3084 exhibits good CNS penetration properties *in vivo*

Inhibitor	AUC [brain]/AUC [plasma]	Kp_{uu}	Classification[6]
DCC-3084[a]	**0.49**	**0.30**	**Moderate**
Tovorafenib	0.33	0.05	Low
Naporafenib	0.11	0.05	Low
Belvarafenib	1.74	0.87	High
Exarafenib	0.02	0.01	Low

Free fraction was determined based on percent rat brain and plasma binding.
[a]DCC-3084 brain and plasma concentrations were measured in Wistar rats after 5 days of oral BID dosing at 30-mg/kg. Brain and plasma concentrations for other inhibitors were measured in Wistar rats after a single oral 30-mg/kg dose.

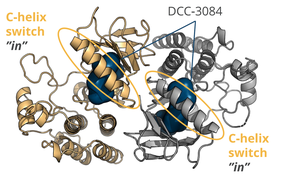
DCC-3084 exhibits strong accumulation in tumors

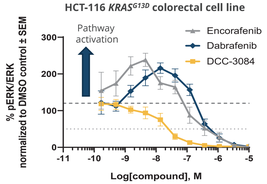
Model	Time (hrs)	pRSK % inhibition	Plasma AUC_{0-last} (ng*h/mL)	Tumor AUC_{0-last} (ng*h/mL)	Tumor/ plasma
A375 30 mg/kg BID	2	**92**	13,800	26,500	**1.9**
	6	**83**			
	10	**79**			
BxPC-3 50 mg/kg BID	2	**92**	16,800	28,500	**1.7**
	6	**75**			
	10	**23**			

PK/PD determined at steady state after 5 or 7 days of repeat oral dosing.

DCC-3084 switch-control binding mechanism limits paradoxical stimulation

DCC-3084 was designed to force the main activation DFG switch into an ***"out"*** state and the **C-helix switch** to an ***"in"*** state. This allows DCC-3084 to bind both monomers of a RAF dimer (left), enabling pathway inhibition in *RAS*-mutant cancers and limiting pERK activity due to paradoxical pathway activation observed with first-generation BRAF inhibitors (right).





CONCLUSIONS

- DCC-3084 is a potential best-in-class pan-RAF inhibitor engineered using Deciphera's proprietary switch-control platform
- DCC-3084 is a potent and selective inhibitor of BRAF and CRAF kinases shown preclinically to target all relevant aberrant signaling mechanisms (monomers, homodimers, heterodimers)
- DCC-3084 exhibits high permeability, good CNS penetration, and tumor tissue accumulation
- DCC-3084 exhibits long residency time, low efflux, and potent inhibition of efflux transporters linked to drug resistance to enable durable efficacy
- Strong preclinical data in cancers driven by *RAF* or *RAS* mutations supports exploration of single-agent and combination opportunities
- Submission of Investigational New Drug Application to FDA is planned for second half of 2023

PRESENTED AT THE AMERICAN ASSOCIATION FOR CANCER RESEARCH (AACR) ANNUAL MEETING
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CORRESPONDING AUTHOR/DISCLOSURES

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All authors are/were full time employees of Deciphera Pharmaceuticals, LLC and own/owned Deciphera Pharmaceuticals, LLC stock or options.

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ABBREVIATIONS

ARAF, serine/threonine protein kinase A–rapidly accelerated fibrosarcoma; ATP, adenosine triphosphate; AUC, area under the concentration time curve; AUC_{0-last}, AUC from time 0 to last measured concentration; BCRP, breast cancer resistance protein transporter; BID, twice daily; BRAF, v-Raf murine sarcoma viral oncogene homolog B1; CNS, central nervous system; CRAF, serine/threonine-protein kinase C-Raf; CSF, cerebrospinal fluid; DFG, aspartic acid–phenylalanine–glycine; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; ERK, extracellular signal–regulated kinase; GTP, guanosine triphosphate; hrs, hours; IC_{50}, half maximal inhibitory concentration; Kp_{uu}, unbound partition coefficient (free brain concentration/free plasma concentration); KRAS, Kirsten RAS; M, molar; MDR1, multidrug resistance mutation transporter; MEK, mitogen-activated protein kinase kinase; NRAS, neuroblastoma RAS; PERK, protein kinase R-like endoplasmic reticulum kinase; PK, pharmacokinetics; p.o., orally; pRSK, phosphorylated RSK; QD, once daily; RAF, rapidly accelerated fibrosarcoma; RAS, rat sarcoma small GTPase protein; RSK, ribosomal s6 kinase; SEM, standard error of the mean; $t_{1/2}$, half-life; TGI, tumor growth inhibition; T. sol, thermodynamic solubility; WT, wild-type.

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