DP-9149, an investigational small molecule modulator of the integrated stress response kinase GCN2, preclinically causes solid tumor growth inhibition as a single agent and regression in combination with standard-of-care agents

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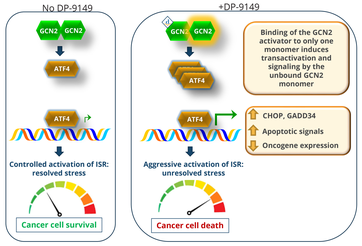
Poster: 1639

Introduction

- The Integrated Stress Response (ISR) pathway is a major adaptive stress response pathways in cancer and plays an important role in cell fate determination[1-4]
- Oncogene-addicted solid tumors are under high extrinsic and intrinsic stress, and they are dependent on well-balanced ISR pathway activity to cope with the high demand for accelerated growth[1-4]
- The ISR is a double-edged sword of survival and cell death, and depending on context, the activation of the ISR kinase GCN2 and downstream pathway can have cytoprotective or cytotoxic effects[1-4]
- Activation of the ISR through stimulation of GCN2 in solid tumors can be pharmacologically leveraged to induce antitumoral effects, especially for oncogene-addicted solid tumors[3]



Methods

- Activation of ISR kinases was characterized using enzymatic assays
- Kinome selectivity profiling was determined using enzymatic and cellular assays
- Cellular modulation of the ISR pathway (phospho-GCN2, ATF4, CHOP, or the apoptosis pathway [c-PARP and c-Caspase 3/7]) was assessed via Western blot or ELISA
- *In vivo* upregulation of tumoral ATF4 was determined in a fibrosarcoma PK/PD xenograft model
- *In vivo* inhibition of tumor growth was determined in solid tumor xenografts

Results

DP-9149 was designed as a selective and potent activator of GCN2

	Assay	DP-9149
Enzymatic	GCN2 recombinant enzyme activation versus control	2.5 fold
Cellular assays	ATF4 stimulation versus control HT-1080 (fibrosarcoma; $NRAS^{Q61K}$)	15 fold
	ATF4 stimulation versus control H358 (lung; $KRAS^{G12C}$)	8 fold
	ATF4 stimulation versus control LoVo (colorectal; $KRAS^{G13D}$, APC^{mut})	6 fold
Off-target profile	Kinome selectivity and safety (Cerep)	Highly selective
In vivo	PK/PD	Target engagement achieved

DP-9149 exhibits a selective kinome profile

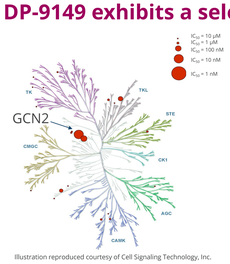
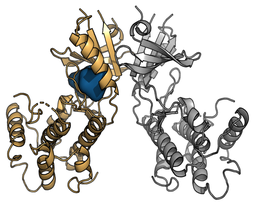


Illustration reproduced courtesy of Cell Signaling Technology, Inc.

394 kinases screened in enzyme- and cell-based assays

X-ray crystal structure of a close analog of DP-9149 bound to GCN2 monomer

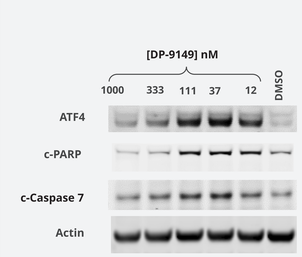


Both monomers were bound to compound under the crystal structure saturating conditions. In this figure, only 1 bound monomer is shown to represent model of activation at or below IC_{50} of DP-9149 analog binding.

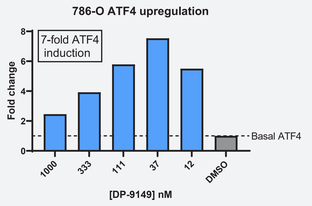
GCN2 forms a head-to-head dimer nucleated by trans-monomer hydrogen bond interactions with key arginine residues in the **C-helix switch**

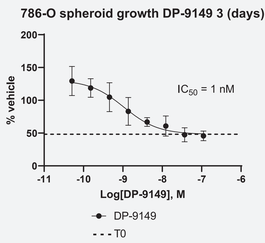
Binding **C-helix switch** in the ***"C-helix out"*** state limits inhibitor binding to half of the dimer, **transactivating** the unbound GCN2 half of the dimer in the catalytically active, ***"C-helix in"*** state

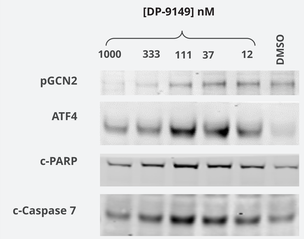
GCN2 activator DP-9149 upregulates the ISR/apoptosis pathway and inhibits spheroid growth as a single agent in oncogene-driven solid tumors *in vitro*



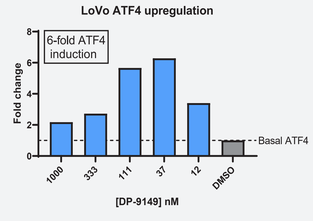
786-O (renal cell carcinoma; VHL^{mut})

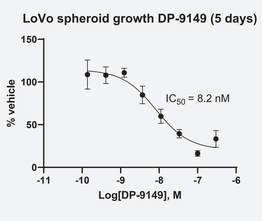




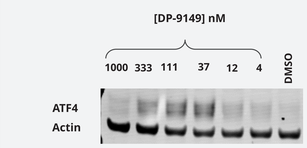


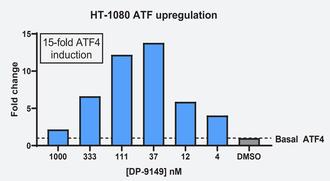
LoVo (colorectal; $KRAS^{G13D}$, APC^{mut})

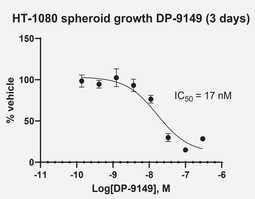




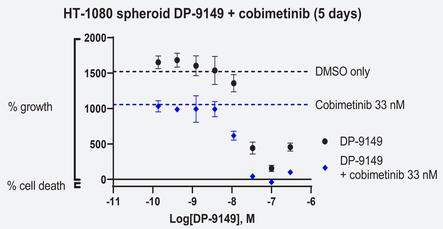
HT-1080 (fibrosarcoma; $NRAS^{Q61K}$)

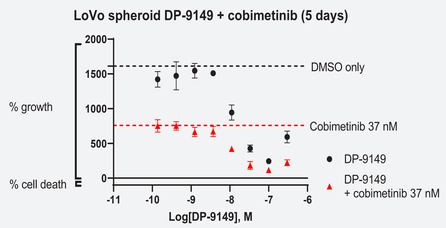






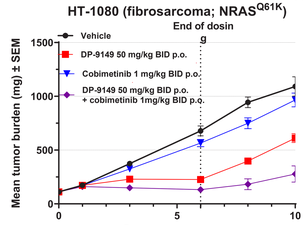
DP-9149 combines with MEK inhibition to promote regression in KRAS-driven solid tumors *in vitro*





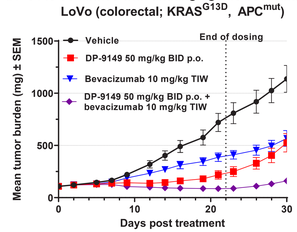
GCN2 activator DP-9149 upregulates the ISR, inhibits tumor growth as a single agent, and combines with standard-of-care therapy to induce tumor regression in solid tumor xenograft models *in vivo*

DP-9149 combines with MEK inhibitor cobimetinib in HT-1080 (fibrosarcoma; $NRAS^{Q61K}$)

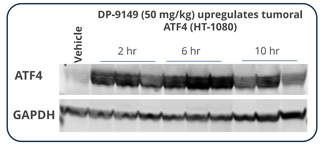


80% TGI (single agent)
3/10 Complete Regression in combo

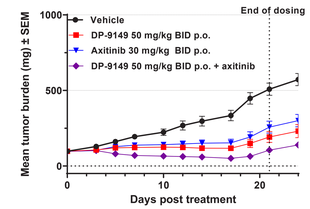
DP-9149 combines with antiangiogenic bevacizumab in LoVo (colorectal; $KRAS^{G13D}$, APC^{mut})



82% TGI (single agent)
17% Regression (combo)

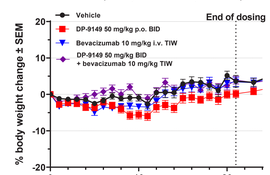


DP-9149 combines with antiangiogenic axitinib in 786-O (renal; VHL^{mut})

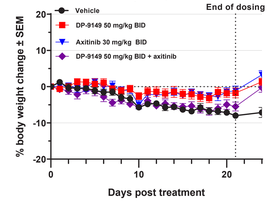


92% TGI (single agent)
56% Regression (combo)

LoVo (CRC; $KRAS^{G13D}$, APC^{mut})



786-O (renal; VHL^{mut})



CONCLUSIONS

- DP-9149 is a potent and selective activator of the ISR kinase GCN2
- DP-9149 binding to one protomer induces transactivation and signaling by the unbound GCN2 protomer
- GCN2 activator DP-9149 upregulated the expression of ATF4, an ISR pathway marker, and activated components of the apoptosis pathway, including c-Caspase 7 and c-PARP
- In solid tumors, *in vitro* and *in vivo*, DP-9149–mediated activation of the ISR pathway inhibited cell growth to induce tumor regression in oncogene-addicted solid tumors, including RAS-mutant cancers and VHL-mutant renal cancers, as a single agent and in combination with approved agents for the treatment of renal cell carcinoma

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

Gada Al-Ani (Galani@Deciphera.com). 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

ADP, adenosine diphosphate; AGC, protein kinase A, G, and C families; ATF4, activating transcription factor 4; BID, twice daily; c-, cleaved; CAMK, ca^{2+}/calmodulin-dependent protein kinase family; CHOP, C/EBP homologous protein; CK1, casein kinase 1 family; CMGC, family of kinases including cyclin-dependent kinases, mitogen-activated protein kinases, glycogen synthase kinases, and cyclin-dependent kinases; CRC, colorectal cancer; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; GADD34, growth arrest and DNA damage-inducible Gene 34; GCN2, general control nonderepressible 2; IC_{50}, half maximal inhibitory concentration; ISR, integrated stress response; i.v., intravenous; KRAS, Kirsten rat sarcoma virus; M, molar; MEK, mitogen-activated protein kinase kinase; NRAS, neuroblastoma RAS; PARP, poly-ADP ribose polymerase; PD, pharmacodynamic; pGCN2, phosphorylated GCN2; PK, pharmacokinetic; p.o., orally; RAS, rat sarcoma small GTPase protein; SEM, standard error of the mean; STE, homologs of yeast sterile 7, sterile 11, and sterile 20 kinase family; TGI, tumor growth inhibition; TIW, 3 times a week; TK, tyrosine kinase family; TKL, tyrosine kinase–like family; VHL, Von Hippel-Lindau.

REFERENCES

1. Pakos-Zebrucka K, et al. *EMBO Rep.* 2016;17(10):1374-95.
2. Licari E, et al. *Int J Biochem Cell Biol.* 2021;139:106059.
3. Gold LT, et al. *Biochem Soc Trans.* 2022;50(2):737-45.
4. Tang CP, et al. *Nat Chem Biol.* 2022;18(2):207-15.