DP-9024, an investigational small molecule modulator of the integrated stress response kinase GCN2, synergizes with asparaginase therapy in leukemic tumors

Gada Al-Ani, Kristin M Elliott, Qi Groer, Aaron J Rudeen, Patrick C Kearney, Jeffery D Zwicker, Yu Mi Ahn, Stacie L Bulfer, Cale L Heiniger, Molly M Hood, Salim Javed, Joshua W Large, Max D Petty, Kristen L Stoltz, Bertrand Le Bourdonnec, Bryan D Smith, and Daniel L Flynn

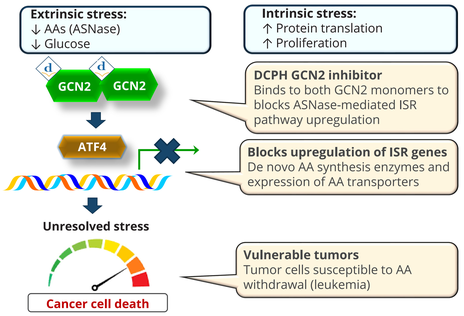
Deciphera Pharmaceuticals, LLC, Waltham, MA, USA

decīphera®

Poster: 4938

Introduction

- The Integrated Stress Response (ISR) is a major adaptive stress response pathway and plays an important role in cell fate determination in response to stress[1-4]
- Oncogene-addicted tumors are under high levels of extrinsic and intrinsic stress and are dependent on a well-balanced ISR to cope with the high metabolic demands for accelerated growth[1-4]
- The ISR is a double-edged sword of survival and cell death, and depending on context, modulation of the ISR kinase GCN2 can have either cytoprotective or cytotoxic effects[1-4]
- Activation of GCN2 was identified as a resistance mechanism to ASNase therapy in ASNS-low leukemic cells and MAPK-driven solid tumors[5-7]
- The inhibition of GCN2 in the context of ASNase-resistant leukemic cells can be pharmacologically leveraged to induce antitumoral effects



Methods

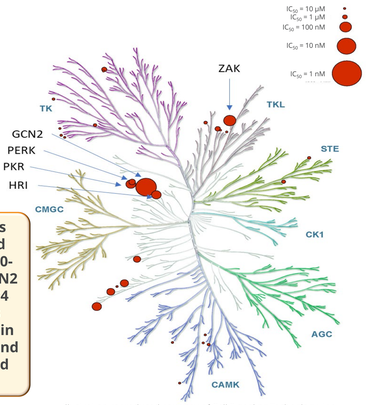
- Modulation of ISR kinases was characterized using enzymatic assays
- Kinome selectivity profiling was determined using enzymatic and cellular assays
- ISR pathway modulation was assessed using cellular assays of phospho-GCN2 and ATF4 by Western blot or ELISA under basal, ASNase-treated, or amino acid–starved conditions
- Sensitization of leukemic cells to ASNase was tested in cell proliferation assays *in vitro*
- *In vivo* compound-mediated reversal of ASNase-induced upregulation of tumoral ATF4 was determined in a leukemia PK/PD xenograft model
- Inhibition of tumor growth was determined in leukemia xenograft models *in vivo*

Results

DP-9024 was designed as a selective and potent modulator of ISR kinases that inhibits GCN2, with optimized pharmaceutical and selectivity profiles

	Assay	DP-9024
Cellular assays	HCT-116 –AA phospho-GCN2 inhibition (IC_{50}, nM)[a]	11
	HCT-116 –AA ATF4 (GCN2 inhibition; IC_{50}, nM)[b]	51
	CCRF-CEM ASNase cell proliferation (GCN2 inhibition; IC_{50}, nM)[c]	39
Off-target profile	Kinome and safety	Highly selective
	hERG (Predictor™ fluorescence polarization; IC_{20}, μM)	>20
ADME	Microsomal stability (human, mouse) % remaining at 60 min	64%, 70%
	Caco-2 (A-B, efflux ratio)	41, 1.6

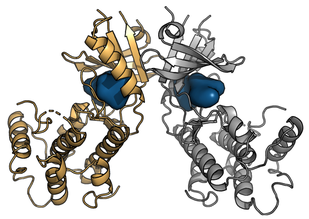
[a]Inhibition of GCN2 activation induced by AA starvation in HCT-116 colorectal cancer cells.
[b]Inhibition of GCN2-mediated activation of ATF4 induced by AA starvation in HCT-116 cells.
[c]Inhibition of cell proliferation by blocking ASNase-mediated GCN2 activation CCRF-CEM cells.



6 kinases inhibited within 100-fold of GCN2 out of 394 kinases screened in enzyme- and cell-based assays

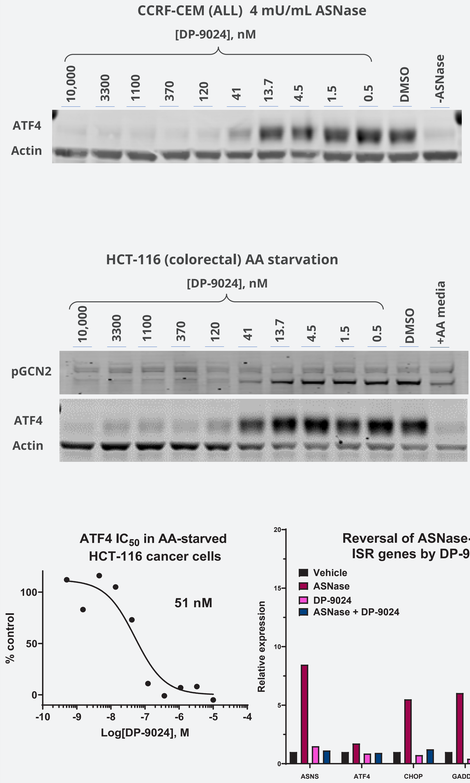
Illustration reproduced courtesy of Cell Signaling Technology, Inc.

X-ray crystal structure of DP-9024 bound to GCN2 dimer

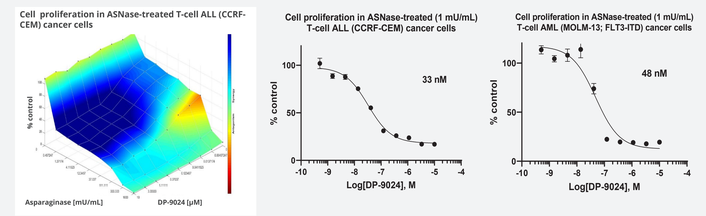


DP-9024 binds to both monomers of GCN2 and induces a "C-helix out" switch to inhibit GCN2

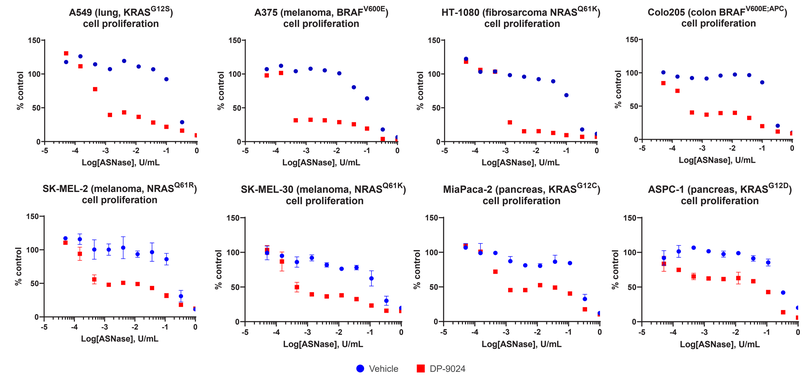
DP-9024 reverses ASNase- or AA starvation–mediated upregulation of the ISR pathway



GCN2 inhibition synergizes with ASNase to sensitize leukemic cells to asparagine depletion *in vitro*

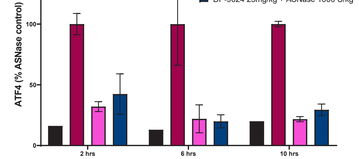


GCN2 inhibition synergizes with ASNase to sensitize solid tumor cells to asparagine depletion *in vitro*

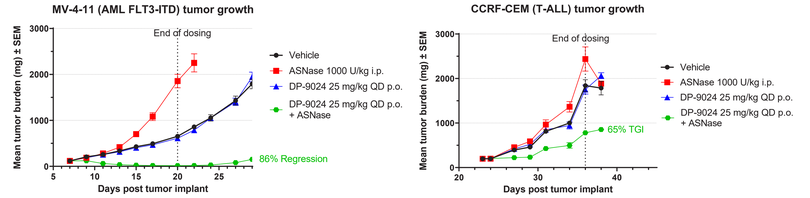


Note: A concentration of 1 μM DP-9024 was used in all the assessments above.

DP-9024 reverses ASNase-mediated upregulation of tumoral ATF4 in a PK/PD model and synergizes with ASNase to sensitize leukemic tumors to asparagine depletion in xenograft models *in vivo*



Oral dose (mg/kg)	Timepoint (hr)	DP-9024 plasma free drug level (ng/mL)	% inhibition of ATF4
50	2	85	81
	6	79	90
	10	74	98
25	2	69	69
	6	65	92
	10	45	88



CONCLUSIONS

- The ISR kinase GCN2 was identified as a resistance mechanism to ASNase in ASNS-low leukemic cells[7]
- Inhibition of the ISR pathway with DP-9024, the potent and selective small molecule modulator of GCN2, synergized with ASNase and sensitized leukemic cells to amino acid withdrawal *in vitro* as well as in leukemic xenograft models *in vivo*

PRESENTED AT THE AMERICAN ASSOCIATION FOR CANCER RESEARCH (AACR) ANNUAL MEETING
ORLANDO, FL, APRIL 14–19, 2023

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.

ACKNOWLEDGMENTS

Greg Plowman, Fred Reu, Cheryl Gradziel, Carla Marashio, Alex Thibonnier, Sihyung Yang, Dan Tanner, Forrest Stanley.
Editorial support was provided by AlphaBioCom, a Red Nucleus company, and was funded by Deciphera Pharmaceuticals, LLC.

ABBREVIATIONS

AA, amino acid; ADME, absorption, distribution, metabolism, and excretion; AGC, protein kinase A, G, and C families; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; APC, antigen presenting cell; ASNase, asparaginase; ASNS, asparagine synthetase; ATF4, activating transcription factor 4; BRAF, v-Raf murine sarcoma viral oncogene homolog B1; 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; DMSO, dimethyl sulfoxide; EIF4EBP1, eukaryotic translation initiation factor 4E binding protein 1; ELISA, enzyme-linked immunosorbent assay; FLT3-ITD, FMS-like tyrosine kinase 3 internal tandem duplication; GADD34, growth arrest and DNA damage–inducible protein 34; GCN2, general control nonderepressible 2; GPT2, glutamic-pyruvic transaminase 2; hERG, human ether-a-go-go–related gene; HRI, heme-regulated inhibitor; IC_{50}, concentration inducing 20% inhibition; IC_{50}, half maximal inhibitory concentration; i.p., intraperitoneally; ISR, integrated stress response; KRAS, Kirsten RAS; MAPK, mitogen-activated protein kinase; NRAS, neuroblastoma RAS; PD, pharmacodynamic; PERK, protein kinase R–like endoplasmic reticulum kinase; pGCN2, phospho-general control nonderepressible 2; PK, pharmacokinetic; PKR, protein kinase R; p.o., orally; QD, once daily; RAS, rat sarcoma small GTPase protein; SEM, standard error of the mean; SLC1A5, solute carrier family 1 member 5; STE, homologs of yeast sterile 7, sterile 11, and sterile 20 kinase family; T-ALL, T-cell acute lymphoblastic leukemia; TK, tyrosine kinase family; TKL, tyrosine kinase–like family; VEGFA, vascular endothelial growth factor A; ZAK, sterile alpha motif and leucine zipper containing kinase.

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 and Masson GR. *Biochem Soc Trans*. 2022;50(2):737-45.
4. Staschke KA and Wek RC. *Nat Cell Biol*. 2019;21(7):799-800.
5. Apfel V, et al. *ACS Pharmacol Transl Sci*. 2021;4(1):327-37.
6. Gwinn DM, et al. *Cancer Cell*. 2018;33(1):91-107.e6.
7. Nakamura A, et al. *Proc Natl Acad Sci USA*. 2018;115(33):E7776-85.