Dimerization-induced activation of the integrated stress response kinase PERK by an investigational small molecule modulator, DP-9024

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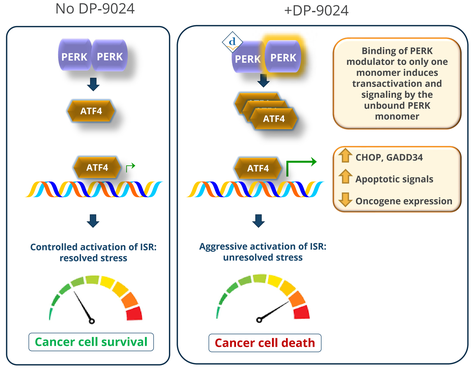
Introduction

- The Integrated Stress Response (ISR) is a major adaptive stress response pathway in cancers[1-4]
- The ISR kinase family member PERK plays an important role in the Unfolded Protein Response (UPR)[1-4]
- The UPR is considered an Achilles' heel in B-cell cancers, as myelomas and B-cell lymphomas are dependent on a well-balanced UPR pathway to cope with the high demand for protein folding and their secretory nature
- Given the double-edged–sword nature of the UPR, the activation of PERK and its downstream pathway can have cytoprotective or cytotoxic effects[5]
- In B-cell cancers, the UPR is at close-to-maximum cytoprotective capacity, such that further pharmacological stimulation of PERK drives a cytotoxic outcome leveraged to induce antitumoral effects



Methods

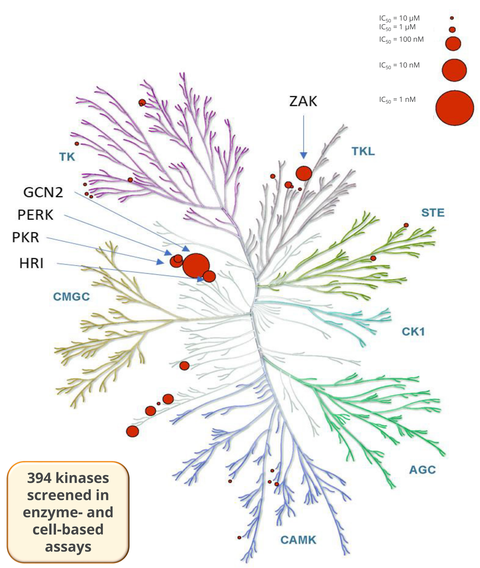
- Recombinant WT and mutant PERK constructs were assayed in the presence of DP-9024
- Structures of compound-bound PERK were determined by X-ray crystallography
- Kinome profiling was determined using enzymatic and cellular assays
- Cellular modulation of the ISR/UPR pathway (PERK, ATF4, and CHOP) or the apoptosis pathway (c-PARP, c-Caspase 3/7) was measured by Western blot, qRT-PCR, or ELISA
- The level of DP-9024–induced PERK activation was determined using a cellular nanoBRET dimerization assay using WT and mutant PERK constructs

Results

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

	Assay	DP-9024
Cellular assays	PERK dimerization assay (BRET; EC_{50}, nM)	274
	H929 ATF4 stimulation (fold increase versus control)	12
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

DP-9024 exhibits a selective kinome profile



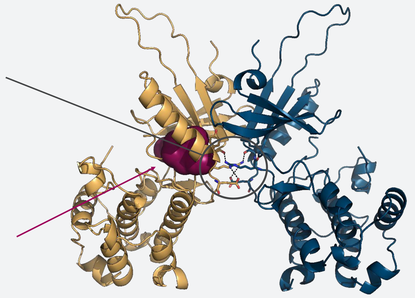
394 kinases screened in enzyme- and cell-based assays

Illustration reproduced courtesy of Cell Signaling Technology, Inc.

Structure of a close analog of DP-9024 bound to PERK monomer

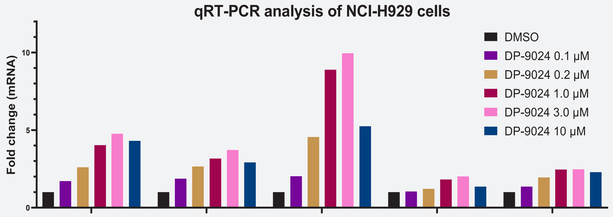
PERK forms a head-to-head dimer nucleated by trans-monomer hydrogen bond interactions with key arginine residues in the C-helix switch

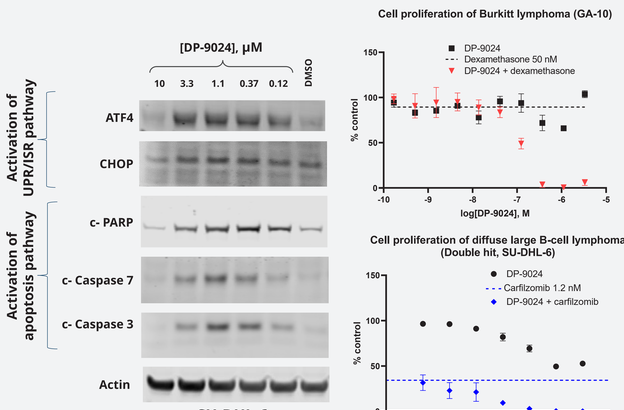
Binding the C-helix switch in the ***"C-helix out"*** state limits inhibitor binding to half of the dimer, **transactivating** the unbound PERK half of the dimer in the catalytically active, ***"C-helix in"*** state



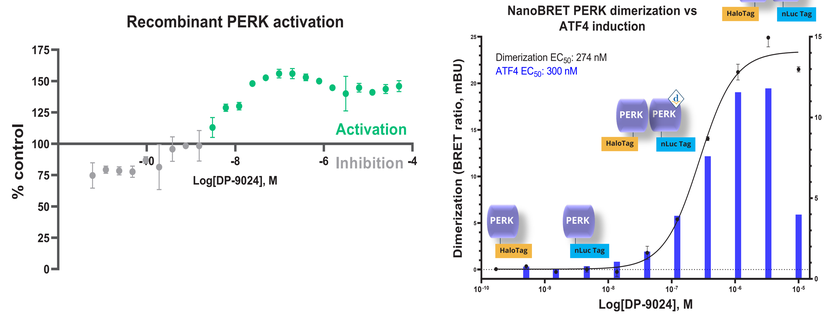
Both monomers were bound to compound under the crystal structure saturating conditions. In this figure, only 1 bound monomer is shown to represent the model of activation at or below IC_{50} of DP-9024 binding.

DP-9024 upregulates ISR pathway genes in B-cells, induces apoptosis, and synergizes with antimyeloma therapies





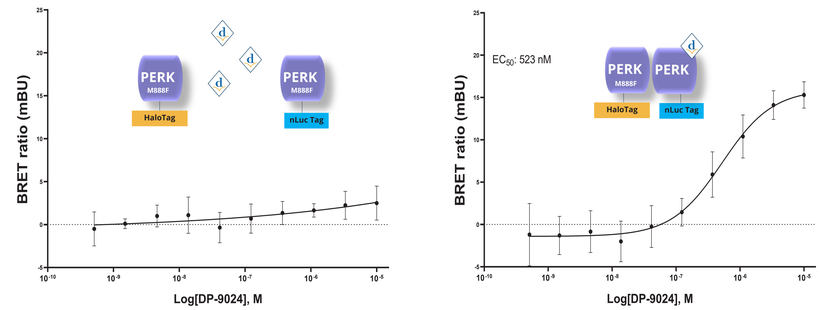
DP-9024 induces PERK dimer–mediated transactivation



Left panel: Recombinant PERK activity monitored in an *in vitro* TR-FRET assay.
Right panel: PERK dimer formation (left y-axis, black curve) induced by DP-9024 monitored by NanoBRET Protein-Protein Interaction system (Promega). ATF4 induction by DP-9024 monitored by ELISA (right y-axis, blue bars). ATF4 induction levels correlate with PERK monomer occupancy by DP-9024 and induced PERK dimerization.

Gatekeeper mutant M888F-PERK is unable to bind compound, therefore no dimer formation (BRET) is observed

Binding of DP-9024 to WT-PERK monomer is sufficient to restore heterodimer formation with binding-incompetent PERK-M888F



Left panel: PERK gatekeeper mutation M888F blocks DP-9204 binding and prevents PERK dimerization.
Right panel: WT-PERK binding to DP-9024 rescues dimerization with gatekeeper mutant M888F-PERK.

CONCLUSIONS

- DP-9024 is a potent and selective activator of PERK activity
- DP-9024 induces stimulation of the ISR family member kinase PERK through direct monomer binding and dimerization by DP-9024
- PERK activation leads to unresolved UPR stress that can potentially be leveraged as a novel mechanism to induce growth arrest in UPR-vulnerable cancers, including multiple myeloma and B-cell lymphoma

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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

ADME, absorption, distribution, metabolism, and excretion; ADP, adenosine diphosphate; AGC, protein kinase A, G, and C families; ASNS, asparagine synthetase; ATF4, activating transcription factor 4; BRET, bioluminescence resonance energy transfer; c-, cleaved; CAMK, Ca2+/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; EC_{50}, half maximal effective concentration; ELISA, enzyme-linked immunosorbent assay; ER, endoplasmic reticulum; 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; ISR, integrated stress response; mRNA, messenger RNA; nLuc, NanoLuc®; PARP, poly ADP-ribose polymerase; PERK, protein kinase R–like endoplasmic reticulum kinase; PKR, protein kinase R; qRT-PCR, real-time quantitative reverse-transcription polymerase chain reaction; STE, homologs of yeast sterile 7, sterile 11, and sterile 20 kinase family; TK, tyrosine kinase family; TKL, tyrosine kinase–like family; TR-FRET, time-resolved fluorescence energy transfer; UPR, unfolded protein response; WT, wild type; ZAK, sterile alpha motif and leucine zipper containing kinase.

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