Discovery and development of orally available sub-nanomolar potent checkpoint kinase 1 inhibitors as potential anti-cancer therapies

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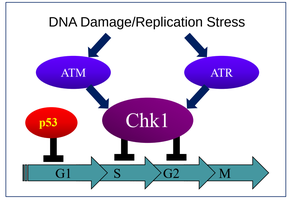
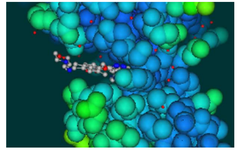
Checkpoint kinase 1 Inhibition: Cancer Therapeutic Rationale



- Chk1 kinase regulates S and G2/M cell cycle checkpoints
- Checkpoint activation in response to DNA damage or replication stress results in Chk1-mediated cell cycle arrest
- Inhibition of Chk1 results in checkpoint override, premature entry into mitosis and tumor cell death

Discovery & Optimization of Chk1 Small Molecule Inhibitors



ONT-2409 docked in Chk1 kinase ATP-binding pocket

- A pyrazole-amino-pyrazine chemical scaffold yielded a sub-nanomolar biochemical lead (ONT-2409)
- Chemical optimization for
  - Cellular potency
  - Selectivity
  - ADME/PK
  - hERG
- Several promising potential development candidates were identified with ideal drug properties
  - Balanced PK, selectivity, potency and in vivo efficacy
  - Potent single agent and synergistic combination efficacy in vitro and in vivo

ONT-2409 Overrides Cell Cycle Checkpoint Control in Gemcitabine-Treated MIA-Paca2 Cells

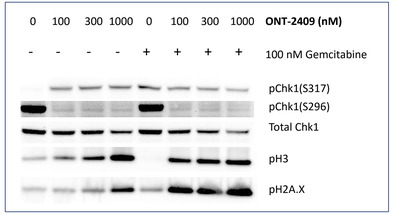


Figure 1. MIA-PaCa2 cells were treated with ONT-2409 alone and in combination with 100nM gemcitabine for 18h followed by immunoblot analysis of P-S317 Chk1, P-S296 Chk1, total Chk1, P-S10 Histone H3 and P-S139 Histone H2A.X

ONT-2409 Drives a Dose-Dependent Reduction in Phosphorylation of Chk1 S296 in MIA-Paca2 Cells

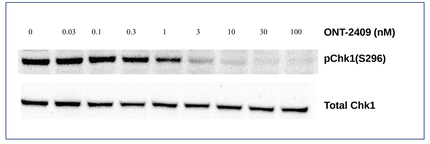


Figure 2. MIA-PaCa2 cells were treated with varying concentrations of ONT-2409 for 18h followed by cell lysis and immunoblot analysis of total and P-S296 Chk1. ONT-2409 inhibits CHK1 S296 phosphorylation with an IC50 of ~1nM.

ONT-2409 Selectively Inhibits a Subset of Tumor Cell Lines as a Single Agent

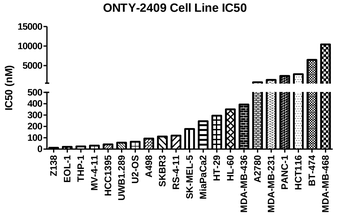


Figure 3. Tumor cell proliferation assays were run to evaluate the potency of ONT-2409 as a single agent. Cells were plated in a 96-well format and drug was added in serial half-log dilutions after cell attachment/plating. Assays were 72h in length post drug addition and quantitated using Cell-Titer Glo (Promega). The bar graph shows the absolute IC50 in nM on the Y-axis.

ONT-2409 Synergizes with Gemcitabine and SN-38

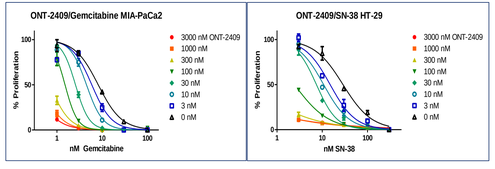


Figure 4. Tumor cell proliferation assays of ONT-2409 in combination with chemotherapeutics. Assays were 72h in length post drug addition and cell proliferation was measured using Cell-Titer Glo (Promega). Each line in the graph represents the chemotherapeutic dose response at a single concentration of ONT-2409. The left shift of each line with increasing ONT-2409 represents combinatorial drug activity.

ONT-2409 + Gemcitabine Demonstrate Strong Anti-Tumor Efficacy in an HT-29 Tumor Xenograft Model

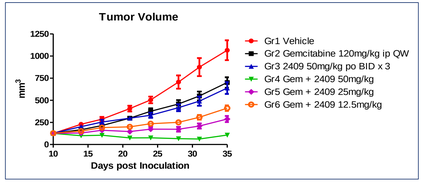


Figure 5. Nude mice bearing subcutaneous HT-29 tumor cell xenografts were treated with (hydroxypropyl)methyl cellulose vehicle (po BID x 3d/weekly cycle), gemcitabine (120mg/kg ip QW/cycle), ONT-2409 alone (50mg/kg po BID x 3d/cycle) or in combination with gemcitabine (120mg/kg gem dosed ip on d1 of cycle followed by 50, 25 or 12.5mg/kg ONT-2409 dosed po BID on d2-4/cycle, followed by 3d off both drugs). 3 dosing cycles were completed. Graphs show mean tumor volume +/- SEM (n=10/group)

Chk1i SAR Optimization: Potency & Clearance

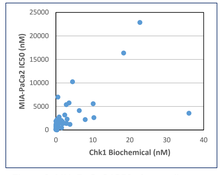


Figure 6. MIA-PaCa2 IC50s from cell proliferation correlate with biochemical IC50s

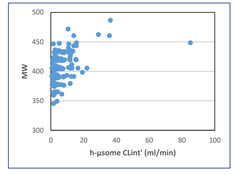


Figure 7. Lower intrinsic clearance in microsomes correlates with lower molecular weight

Biochemical and ADME Profiles of Chk1i Lead and Preclinical Development Candidate (PDC)

	PARAMETERS	ONT-2409	PDC-A
BIOLOGY	Chk1 biochemical IC50 (nM)	~ 0.1	~ 0.1
	MIA-PaCa2 IC50 (nM)	~ 250	~ 150
ADME	Permeability (A-B/B-A)	2/27	14/16
	Efflux	19	1
	$CL_{\mu,int}$ *(m/r/d/h)*	118/20/13/14	19/20/96/5
in vitro hERG	%inhibition @ 10 μM	58%	51%
Mouse PK	$T_{1/2}$ (h)	2	3
	Cmax (ng/ml)	331	294
	AUC (h.ng/ml)	1054	1769
	CL (ml/min/kg)	55	39
	F%	28	80

PDC-A is a Potent and Selective Chk1 Inhibitor

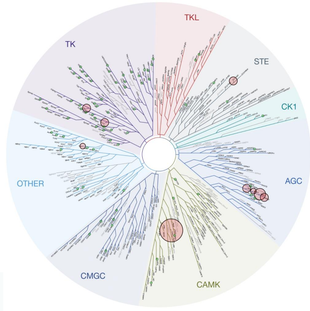


Table 1. PDC-A was screened against a panel of 120 kinases selected to represent kinases commonly involved in cancer. Percent inhibition was determined at 1μM and all kinases inhibited >80% and Chk2 are shown in the table. The IC50 for each of those kinases was determined and the selectivity relative to Chk1 was calculated. All assays were run at the ATP Km for each kinase. Cellular IC50s were derived from a signal transduction assays in relevant cell lines using phosphor-specific antibodies

Hit	Selectivity Kinase/Chk1 IC50	Cellular IC50 (nM)
Chk1	**1**	**1 (HT-29)**
Rsk3	36	nd
Flt3	32	> 5000 (MV-411)
Ret	69	nd
Rsk4	74	nd
Map4k4/Hgk	209	nd
Rsk2	72	nd
Rsk1	134	nd
Chk2	1405	nd

PDC-A Shows Potent Single Agent Activity on a Subset of Tumor Derived Cell Lines

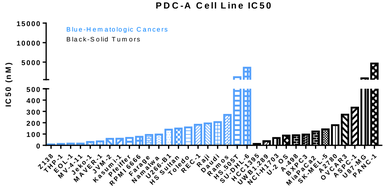


Figure 8. Tumor cell proliferation assays were run to evaluate the potency of PDC-A as a single agent. Cells were plated in a 96-well format and drug was added in serial half-log dilutions after cell attachment/plating. Assays were 72h in length post drug addition and quantitated using Cell-Titer Glo (Promega). The bar graph shows the absolute IC50 in nM on the Y-axis.

PDC-A Synergizes with Gemcitabine and Cytarabine

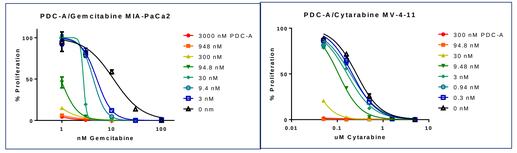
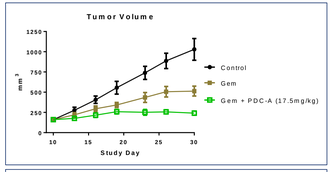


Figure 9. Tumor cell proliferation assays of PDC-A used in combination with chemotherapeutics. Assays were 72h in length post drug addition and cell proliferation was measured using Cell-Titer Glo (Promega). Each line in the line graph represents the chemotherapeutic dose response at a single concentration of PDC-A. Combinations of PDC-A with gemcitabine were conducted in MIA-Paca2 cells and combinations with cytarabine were conducted in MV-4-11 cells.

PDC-A in Combination with Gemcitabine Produces Enhanced Anti-Tumor Efficacy in an HT-29 Tumor Xenograft Model



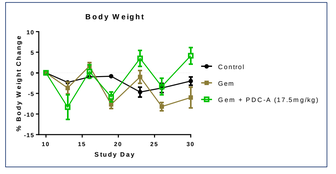


Figure 10. Nude mice bearing HT-29 tumor cell xenografts were treated with (hydroxypropyl)methyl cellulose vehicle (po BID x 3d/weekly cycle), gemcitabine (120mg/kg ip QW/cycle) or PDC-A in combination with gemcitabine (120mg/kg gem dosed ip on d1 of cycle followed by 17.5mg/kg PDC-A dosed po BID on d2-4/cycle, followed by 3d off both drugs). 3 dosing cycles were completed. Graphs show mean tumor volume (top and % body weight change (bottom) +/- SEM (n=6/group).

Summary and Conclusions

- Oncothyreon's novel pyrazole-amino-pyrazine Chk1 inhibitors are highly potent and selective for Chk1
  - Sub-nanomolar potency vs Chk1 with limited off-target kinase activity
  - Single digit nanomolar cellular potency against Chk1
- Attractive pharmaceutical properties
  - Oral bioavailability and low efflux ratio allows for flexible dose schedule, potential for treating MDR resistant and CNS metastasized cancers
  - Good metabolic stability, no CYP inhibition liability and excellent hERG inhibition index
- Compelling single agent and combinatorial anti-tumor activity
  - Potent single agent activity in select tumor cell lines and synergistic potentiation of gemcitabine or cytarabine *in vitro*
  - Well tolerated with enhanced tumor control in combination with gemcitabine *in vivo*

ONCOTHYREON