

CRT Licensing Opportunity



Potent Inhibitors of Checkpoint Kinase 1 (Chk1)

- Lead series with low nM biochemical and sub-uM mechanism-based cellular activity
- Potentiation of genotoxic agent growth inhibition in colon cancer xenografts
- *In vitro* and *in vivo* biomarkers defined
- Chk1 crystallographic expertise - 59 X-ray structures determined for Lead Series

SMALL MOLECULES | Lead Optimisation - Proof of Principle

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Therapeutic Rationale

Chk1 is a serine/threonine kinase that is phosphorylated and activated in response to DNA damage, initiating a signalling cascade culminating in cell cycle arrest in the S and G2/M phases. Inhibition of Chk1 has been shown to abrogate cell cycle arrest leading to enhanced tumour cell death following DNA damage by a range of chemotherapeutics. Cells lacking intact G1 checkpoints through inactivation of p53 are particularly dependent on S and G2/M checkpoints and are therefore expected to be more sensitive to chemotherapeutic treatment in the presence of a Chk1 inhibitor, whereas normal cells with functional G1 checkpoints would be predicted to undergo less cell death [1].

Chk1 inhibitors may therefore provide a therapeutic strategy for enhancing the effectiveness of the genotoxic agents currently used in cancer treatment [2]. The most immediate target indications for Chk1 inhibitors are likely to be solid tumours where genotoxic therapy is currently a preferred clinical option (colon, ovarian and lung in particular).

Potent and Selective Chk1 Inhibitors with *In Vivo* Activity

Novel competitive inhibitors with low nM activity against Chk1 have been developed starting from a combined crystallographic bioassay template screen. The Lead Series is highly selective over the unrelated checkpoint kinase chk2 (100>10,000 fold), and against a wider panel of enzymes chosen to represent a spectrum of the kinome. Compounds from the Lead series abrogate the G2M checkpoint in cells

at sub-micromolar concentrations, and potentiate SN38 and Gemcitabine cytotoxicity by 3 to 29 fold in different colon tumour cell lines. The Lead Series has good *in vitro* ADME, good *in vivo* pharmacokinetic properties, and has demonstrated *in vivo* pharmacodynamic modulation of signalling through Chk1. Lead Compounds compare favourably with known clinical candidates in head-to-head *in vitro* and *in vivo* PD assays.

Compounds from the Lead Series have been investigated *in vivo* for efficacy in combination with irinotecan (Figure 1) and gemcitabine in human colon cancer xenografts in nude mice. Potentiation of approximately 2-fold, as measured by comparing tumour growth rates, was achieved by combining either genotoxic with a Lead Series Chk1 inhibitor. Additional cell based and *in vivo* models are being developed to evaluate the Lead Chk1 inhibitors, including *in vivo* models with gemcitabine in a pancreatic cell line.

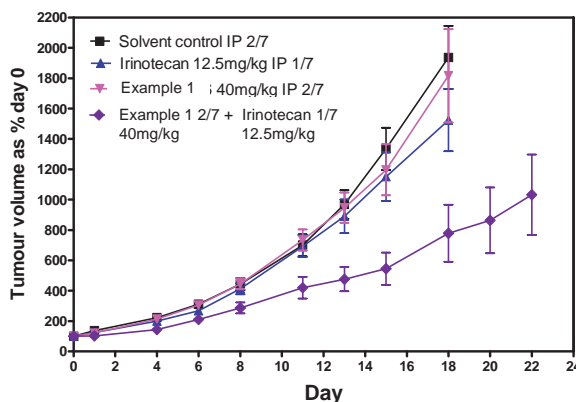


Figure 1: Example 1 potentiates the efficacy of irinotecan in SW620 colon cancer xenografts in mice

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Summary of Lead Series Data

	Lead Series
Chk1 IC ₅₀ (nM)	<0.5 - 50
Selectivity	100x - >100,000x
Checkpoint abrogation (μM)	0.01 - 1
Potentialiation of cytotoxicity <i>in vitro</i>	2x - 20x
Potentialiation of cytotoxicity <i>in vivo</i>	2x
MW	340 - 430
Ligand efficiency	0.31 - 0.48
CLogP	0.0 - 3.8

Biomarkers of Chk1 Inhibition

Lead compounds inhibit cytotoxic drug induced Chk1 autophosphorylation at Ser296 and block phosphorylation of CDK1 at Tyr15 *in vitro* and *in vivo* consistent with Chk1 inhibition and checkpoint abrogation. Compounds increase SN38 and Gemcitabine induced H2AX phosphorylation and PARP cleavage consistent with elevated DNA damage and tumour cell death.

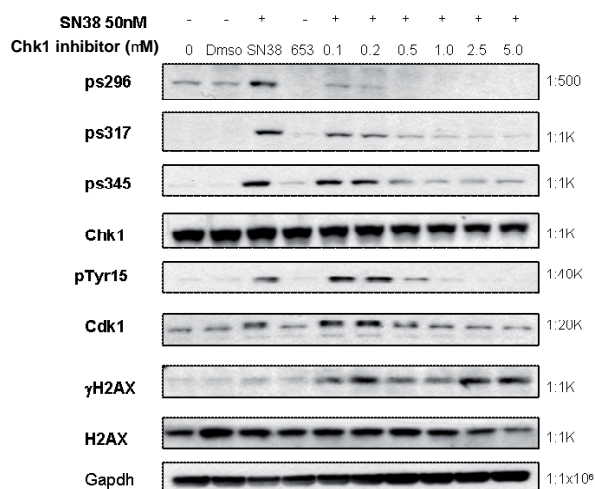


Figure 2 - Modulation of cellular biomarkers by a lead series Chk1 inhibitor in combination with SN38 in SW620 human tumour cells *in vitro*.

Crystallography

Multiple low MW hits were identified from a combined crystallographic-bioassay template screen [3], followed by iterative ligand-protein co-crystallography on multiple series to define SAR and guide improvements to potency. More than 90 co-crystal structures have been determined, 59 from the Lead Series.

Commercial Opportunity

CRT is offering prospective commercial partners global rights to the Chk1 programme on an exclusive basis for all fields. The programme is a collaborative discovery programme between The Institute of Cancer Research, Sareum Ltd and CRT. Details of unpublished results relating to the cellular and *in vivo* efficacy of Lead Series compounds is available under CDA.



Intellectual Property

There is a strong patent portfolio protecting the Lead Series and surrounding chemical space with both composition of matter and medical use claims to the key compounds. Key patents include: PCT/GB2007/004819; PCT/GB2008/002259; PCT/GB2008/003362; PCT/GB2009/000438. An additional patent application has been filed to protect a back-up series which exhibits a different binding mode (PCT/GB2009/001786).

References

1. Collins I, Garrett MD. *Curr. Opin. Pharmacol.* 2005 5:366-373.
2. Chen Z, *et.al.* *Mol. Cancer Ther.* 2003, 2:543-548.
3. Matthews *et.al.* *J. Med. Chem.* 2009, 52:4810-4819.

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