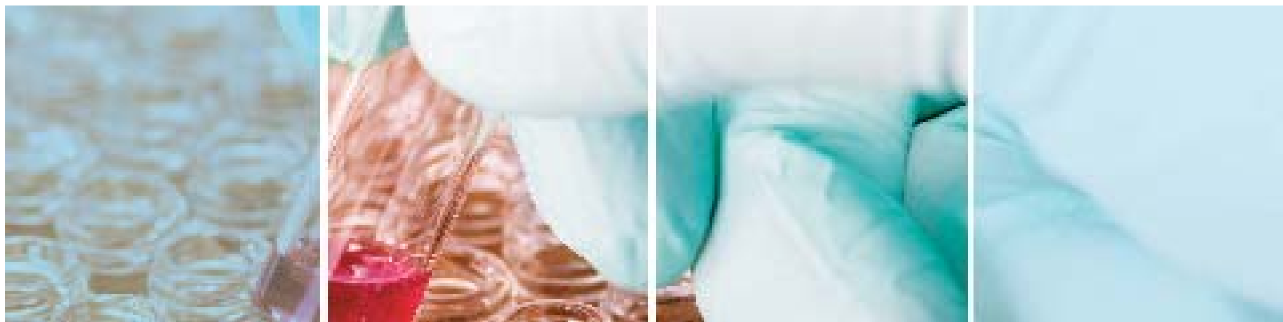
CRT Licensing Opportunity



Inhibitors of Cdc7

- Two lead series with low nM biochemical and sub-μM PD biomarker activities
- Inhibition of Cdc7 leads to tumour cell-specific apoptotic response *in vitro*
- Drug-like molecules with excellent selectivity and *in vitro* ADME profiles

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Background and Therapeutic Rationale

Deregulated cell cycle progression is a hallmark feature of several tumour types and the DNA replication licensing system has emerged as a powerful mechanism for controlling cell proliferation. Cdc7 is a highly conserved serine/threonine protein kinase which forms a complex with the regulator protein ASK required for kinase activity. Cdc7 is a core component of the licensing machinery and critical for G1/S phase transition and S phase progression. As cells progress to S phase, Cdc7 phosphorylates MCM2 leading to activation of MCM2 helicase activity and thereby the loading on to the origins of replication of additional factors including Cdc45 and the GINS complex that collectively promote origin unwinding of the DNA and recruitment of DNA polymerases required for DNA synthesis. Cdc7 not only lies at an integration point for mitogenic signalling pathways, but also plays a role in maintaining genomic stability through intra-S-phase checkpoint pathways in response to DNA damage and stalled replication forks.

Correct processing of stalled or damaged replication forks is essential for cell viability. It is proposed that in untransformed cells inhibition of origin firing may trigger a "licensing checkpoint", leading to a block to DNA replication initiation and stable G1 arrest, whereas cancer cells seem to have lost this checkpoint function and initiate DNA synthesis with only a subset of replication origins primed. This may result in the stalling and collapse of replication forks and double-strand breaks, triggering intra-S phase and/or G2-M checkpoints and apoptosis in cancer cells. Depletion of Cdc7 in cancer cell lines has been demonstrated to cause an abortive S phase leading to p53-independent apoptotic cell death or aberrant mitosis. In contrast, in untransformed cells Cdc7 depletion results in a reversible arrest in G1 and cells remain in a viable nonproliferative state [1-2].

Increased expression of Cdc7 (and ASK), has been reported in human cancer cell lines and primary tumour samples. CRT's academic collaborators have demonstrated that increased Cdc7 expression is associated with conventional biological indicators of poor clinical outcome, accelerated cell cycle progression and reduction of G1 phase (indicative of increasingly aggressive tumour behaviour) in epithelial ovarian tumours and is an independent prognostic indicator of both relapse and overall survival [1].

Inhibition of the catalytic activity of Cdc7 represents an attractive strategy for an anti-tumour therapeutic (reviewed in [3]). Small molecule inhibitors of Cdc7 are anticipated to result in anti-proliferative and tumour selective pro-apoptotic response.

Potent and Selective Cdc7 Inhibitors

The CRT Discovery Laboratories identified several distinct preliminary Cdc7 inhibitor hit series and two series have been prioritised for lead optimisation. Current data demonstrates that representatives of both series inhibit Cdc7 kinase activity with nM potency (sub-10nM biochemical; direct mode of action pharmacodynamic biomarker (Cdc7-dependent phosphorylation of MCM2 (S53), Table 1)), show excellent selectivity against a diverse panel of kinases (Table 1), and display drug-like

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physiochemical properties, good in vitro ADME properties and *in vivo* pharmacokinetic profile. A representative compound from Lead Series A has demonstrated i*n vivo* pharmacodynamic modulation of signalling through Cdc7.

Table 1: Cellular activities and selectivity of representatives of CRT's Cdc7 inhibitors.

	Series A Representative	Series B Representative
MW	<500	<300
PD biomarker IC_{50} (nM)	95	760
Colo205 GI50 (μM)	4.7	9
S(90) at 1μM (80 kinase panel)	0.025	0

In phenotypic assays, Cdc7 inhibition in colorectal cancer cells leads to (i) a pronounced decrease in proliferation (Table 1) and (ii) an abortive S phase progression followed by an increase in cells with a sub-G1 DNA content indicative of apoptosis (as detected by FACS analysis, Figure 1). In contrast, normal fibroblasts treated with compounds remain viable and show no evidence of apoptosis, indicating that inhibition of Cdc7 with CRT compounds results in tumour cell-specific killing.

Commercial Opportunity

CRT seeks a commercial partner for collaborative research and/or exclusive licensing for the further development of these Cdc7 inhibitors.

Academic Collaborators

The project is run in collaboration with Professor Gareth Williams and Dr Kai Stoeber from UCL CRUK Cancer Centre, UCL's Department of Pathology and Wolfson Institute for Biomedical Research. They bring extensive experience and expertise to the programme in the fields of chromosomal DNA replication biology and diagnostic biomarker development. They have ongoing research interests in the molecular function of Cdc7 and diagnostic strategies for cell cycle therapy.

Cancer Research Technology

CRT is an oncology-focussed development and commercialisation company. Identification of small molecule inhibitors of Cdc7 is one of a robust pipeline of projects currently underway in CRT's Discovery Laboratories (CRTDL). CRTDL bridges the gap between academia and industry by working in collaboration with the originating academic laboratories and enabling their discoveries to be turned into projects that are readily recognisable and valued by the pharmaceutical industry.

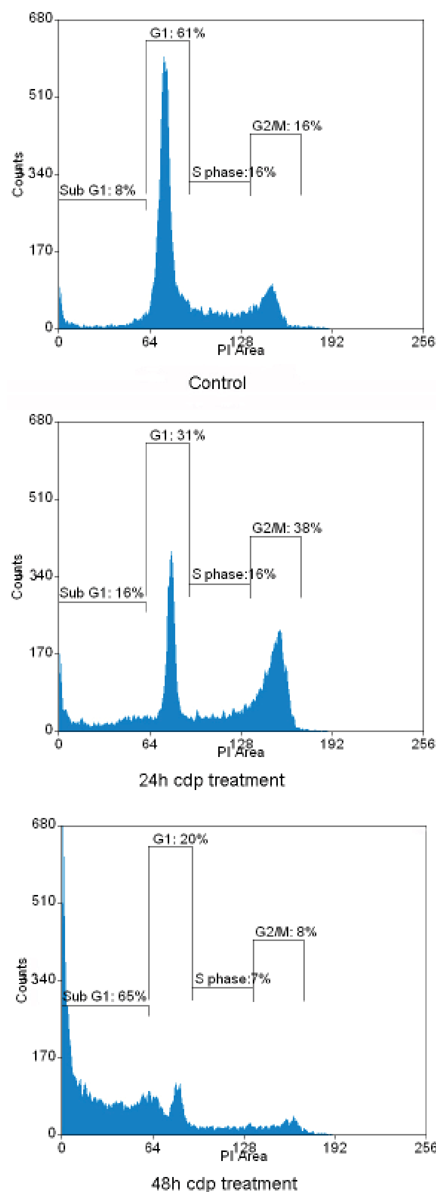

Figure 1: CRT Series A inhibitor induces an accumulation of Colo205 colorectal adenocarcinoma cells in late S- and G2/M phases of the cell cycle followed by apoptosis. Cells were treated with either 3μM of a representative CRT Series A compound or DMSO (control) for the indicated times. The DNA content was analysed by flow cytometry using propidium iodide.

References

1. Kulkarni AA *et al.*, (2009) Clin. Cancer Res. 15(7):2417-2425
2. Montagnoli, A. *et al.*, (2004) Cancer Res. 64:7110-7116
3. Swords E *et al.*, (2010) Eur. J. Cancer. 46:33-40

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