# CASC-578, a Novel, Orally Available Checkpoint Kinase 1 Inhibitor, is Active as a Single Agent in Solid Tumors From Diverse Histological Origins and Displays Synergistic Anti-Tumor Activity in Combination With Wee1 Inhibition

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## Background

- Chk1 is a key modulator of the cell division cycle and DNA damage response (DDR) signaling
  - Regulates the cell division cycle in response to DNA damage and DNA replication stress
  - Functions in parallel with other DDR and cell cycle regulatory pathways, many of which are deregulated in cancer cells
  - Loss of normal DDR and cell cycle regulation in cancers increases sensitivity to Chk1 inhibition
- Targeting cell cycle regulation and DDR signaling is a clinically validated approach to cancer therapy

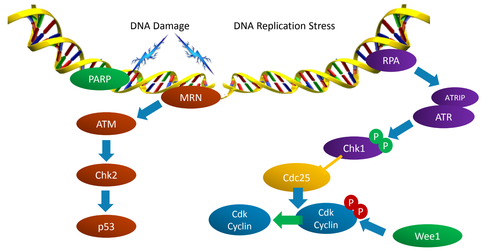
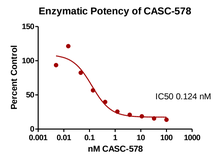
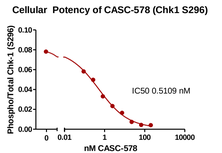



Figure 1. Chk1 inhibitors block cell cycle checkpoint activation by disrupting the control of Cdc25, leading to activation of Cdk/Cyclin activity. Inhibition of Chk1 results in the induction of DNA damage and cell death in tumor cells

## CASC-578 is a Potent and Selective Inhibitor of Chk1

A)

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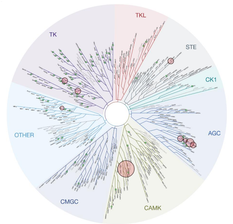


| Hit | Selectivity Kinase/Chk1 IC50 | Cellular IC50 (nM) |
|---|---|---|
| **Chk1** | **1** | **1 (HT-29 cells)** |
| Rsk3 | 36 | TBD |
| Flt3 | 32 | > 5000 (MV-411 cells) |
| Ret | 69 | 5000 (TT cells) |
| Rsk4 | 74 | TBD |
| Map4k4/Hgk | 209 | TBD |
| Rsk2 | 72 | TBD |
| Rsk1 | 134 | TBD |
| Chk2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

Figure 2. Potency and selectivity of CASC-578. A) Enzymatic and cellular potency of CASC-578. Enzymatic assays were performed using 10 μM [γ- $^{33}$P]-ATP and 20 μM of the peptide substrate KKKVSRSGLYRSPSMPENLNRPR (Reaction Biology Corp.). Cellular Chk1 potency was determined using HT-29 colon carcinoma cells in an 18 hr assay by immunoblotting with a rabbit anti-Chk1 serine 296 phospho-epitope antibody (Cell Signaling Technology Inc.). B) Enzymatic selectivity of CASC-578. A panel of 120 kinases was screened with CASC-578 using 1 μM ATP. All kinases inhibited >80% and Chk2 are shown in the table and the IC50, measured at ATP Km for each kinase, is represented relative to Chk1. Cellular IC50s were derived from a signal transduction assay in relevant cell lines using phospho-epitope specific antibodies. TBD = To be determined

## CASC-578 is Active in NSCLC Xenograft Models

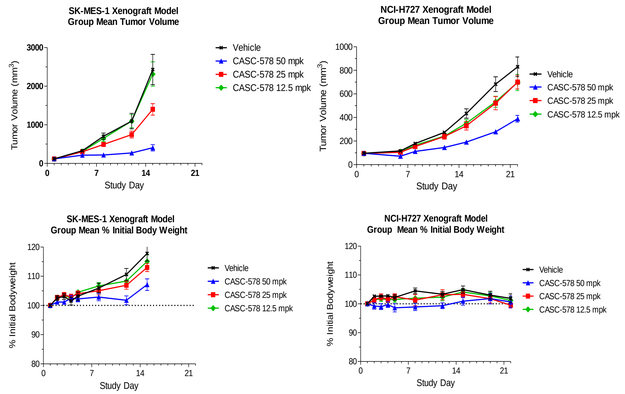


Figure 4. Activity of CASC-578 as a single agent in NSCLC tumor xenograft models. SK-MES-1 or NCI-H727 tumor cells were inoculated subcutaneously in the flank of athymic nude mice. Once tumors reached a volume of ~200 mm3 mice were randomized into study groups (N=10). Mice were treated QD by oral gavage with CASC-578 at the dose levels indicated above. Data represent group mean +/- SEM

## CASC-578 and AZD-1775 Demonstrate Enhanced Activity in NSCLC

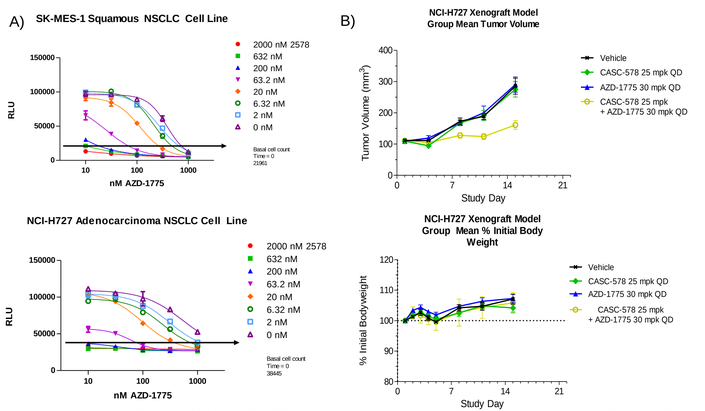


Figure 5. Activity of CASC-578 in combination with AZD-1775 in NSCLC tumor cell lines and the NCI-H727 xenograft model. A) SK-MES-1 and NCI-H727 tumor cells were treated with a titration of CASC-578 or the Wee-1 inhibitor AZD-1775 alone, or in combination, with increasing concentrations of CASC-578. Cell proliferation was measured 72 hours after drug addition using CellTiter-Glo® Assay (Promega). Black arrow represents the signal corresponding to the starting cell number (cytostatic limit). B) NCI-H727 tumor cells were inoculated subcutaneously in the flank of athymic nude mice. Once tumors reached a volume of ~200 mm$^3$ mice were randomized into study groups (N=10). Mice were treated QD by oral gavage with CASC-578, AZD-1775 or the combination of both drugs at the dose levels listed above. Data represent group mean +/- SEM

## CASC-578 Displays Excellent Drug-Like Properties

| Parameters | Results |
|---|---|
| Chk1 potency and selectivity | Chk1 enzymatic IC50 ~0.10 nM, limited cross-reactivity with other targets in multi-receptor and kinase screen<br>Potent cellular Chk1 inhibition (<1nM) |
| Caco2 bi-directional permeability | A-B/B-A: 14/16<br>Efflux =1.2 |
| Reversible Plasma Protein Binding (%) | Rat: 95%; Cynomolgus monkey: 99%; Human: 95% |
| Blood & Plasma Stability | Stable in blood & plasma; $T_{1/2}$ > 120 min |
| RBC Partitioning | Ratio: 2.6 |
| *In Vitro* Intrinsic Clearance | Microsomes $Cl_{int'}$ $_{(ml/min/kg)}$: 19/20/96/18/5 (m/r/d/c/h) |
| CYP Inhibition | Direct: IC50 > 20 μM all CYP isoforms<br>TDI: no TDI |
| *In Vitro* Inhibition of UGT1A1 | No inhibition of UGT1A1 at relevant concentration: IC50 > 100 μM<br>Not a substrate of UGT1A1 |
| Induction of CYP3A4 and 1A2 (reporter cell based assay) | At 10 μM concentration, %human PXR activation 40%; %human AhR activation 2% |
| Transporter Inhibition | No inhibition of SLC transporters at 10 μM concentration.<br>At 10 μM, BCRP & P-gp inhibition were 41% and 52% respectively |

CASCADIAN THERAPEUTICS

## CASC-578 is Active in Carcinoma Cell Lines Derived From Diverse Histological Origins

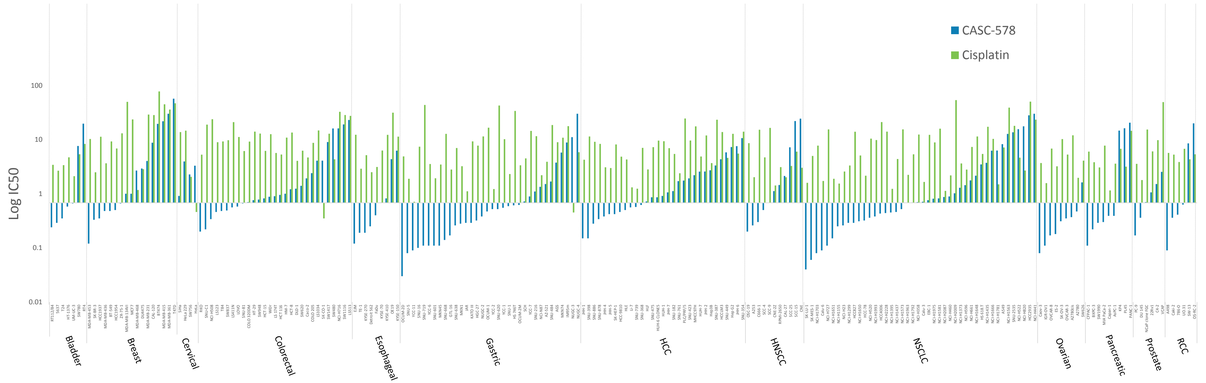


Figure 3. Large scale cell line screen with CASC-578. A panel of 232 carcinoma derived cell lines were screened in a proliferation assay using dilutions of CASC-578 or cisplatin. Cell lines were treated with serial half-log dilutions of CASC-578 or cisplatin using a starting concentration of 30 μM with half-log serial dilutions to achieve 9 dose levels and assayed 72 hours later for proliferation using CellTiter-Glo® Assay (Promega). IC50 (EC50) values were calculated by fitting the dose-response data using a nonlinear regression model. Log IC50 values are plotted with the horizontal axis intersecting at the median IC50 value for the total carcinoma population. IC50 values are plotted relative to the median IC50 for the carcinoma panel.

## CASC-578 is Positioned For Advancement Into Development

- CASC-578 is a highly potent and selective Chk1 inhibitor
  - Sub-nanomolar potency vs Chk1 in enzymatic and cellular Chk1 assays
  - Highly selective, with limited off-target kinase activity (>1000x selective vs. Chk2)
- Attractive pharmaceutical properties
  - Oral bioavailability and low efflux ratio allow for flexible dose schedule, potential for treating MDR resistant and CNS metastasized cancers
  - Good metabolic stability, no CYP inhibition liabilities and excellent hERG inhibition index
  - Low risk of cardiotoxicity based on cynomolgus monkey safety pharmacology study results, including QTc and contractility (left ventricular LVdP/$dt_{max}$) endpoints
- Active as a single agent and in combination with Wee1 inhibitor
  - High throughput cell screening has identified solid tumor disease lineages enriched for sensitivity to CASC-578
    - Ongoing assessment of biomarkers associated with sensitivity and resistance
  - Single agent activity in NSCLC tumor models and enhanced activity with Wee1 inhibitor *in vitro*, and *in vivo*