The Novel Orally Available Sub-Nanomolar Potent and Selective Checkpoint Kinase 1 (Chk-1) Inhibitor CASC-578 is Highly Active in Mantle Cell Lymphoma as a Single Agent and in Combination With Wee-1 Inhibition

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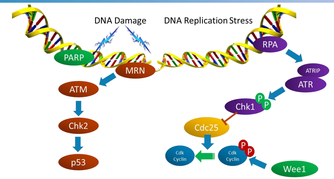
Targeting the DNA Damage Response and Cell Cycle Control Axis in Cancer



- 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
- Wee1 functions in parallel with Chk1 to regulate Cdk/Cyclin activity

CASC-578

- CASC-578 has been designated as a development candidate and is well-positioned for IND-enabling studies
  - Sub-nanomolar Chk-1 inhibitor, limited off-target activities
  - Good drug-like properties (ADME/PK, oral bioavailability)
  - Completed 7-day repeat dose tolerability studies in mice, rats and cynomolgus monkeys
  - No findings in GLP cardiovascular safety study completed in NHP, including QTc and LVP/contractility endpoints
  - Active in multiple tumor models as a single agent and in combination with chemotherapy or Wee1 inhibitor

CASC-578 Sensitivity is Enriched in Hematological Tumor-Derived Cell Line Subsets, Including MCL and AML

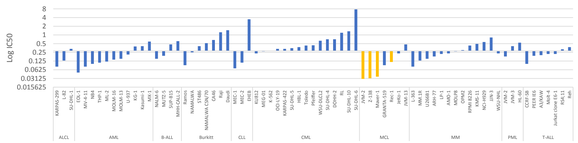


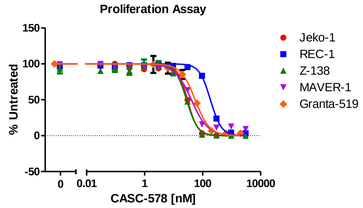
Figure 1. A panel of ~70 hematopoetic cell lines were screened for sensitivity to CASC-578 in a 72 hour proliferation assay (CrownBio Omnipanel). Bars in yellow represent additional Cascadian Therapeutics in-house data. Log IC50 values are plotted with the horizontal axis intersecting at the median IC50 value for the total population.

Genotype of Mantle Cell Lymphomas (MCL) May Predict Sensitivity to Chk1 Inhibition by CASC-578

- Mantle Cell Lymphoma (MCL) is a rare and usually aggressive form of Non-Hodgkin Lymphoma that affects around 15,000 patients in the United States.
- The majority of MCL patients display the phenotype of a chromosomal translocation at t(11;14)(q13;q32) that leads to the over-expression of cyclin D1.
- Since Chk-1 and Wee-1 kinases are regulators of Cdk/Cyclin activity, MCL could be uniquely sensitized to Chk-1 inhibitors alone or in combination with Wee-1 inhibitors as a novel therapeutic approach.

CASCADIAN THERAPEUTICS

CASC-578 Demonstrates Potent Single Agent Anti-Proliferative Activity in Mantle Cell Lymphoma Cell Lines



Cell Line	CASC-578 IC50 (nM)
Z-138	28
Jeko-1	30
MAVER-1	41
Granta-519	55
REC-1	185
MEAN	67

Figure 2. MCL cell lines were treated with serial half-log dilutions of CASC-578 in 96-well format and assayed 72 hours later for proliferation using CellTiter-Glo® Assay (Promega). Data are expressed as percent of untreated control.

CASC-578 is Active and Well-Tolerated as a Single Agent in Mantle Cell Lymphoma Tumor Xenograft Models

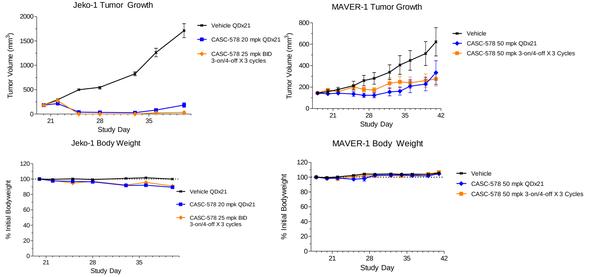
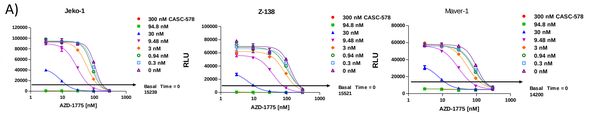


Figure 3. Jeko-1 and MAVER-1 cells were inoculated subcutaneously in the flank of CB17.SCID (Jeko-1) or athymic nude (MAVER-1) mice. Once tumors reached a volume of ~200 mm³ mice were randomized into study groups (N=10). Mice were treated with CASC-578 by oral gavage with the dosing schedules indicated above. Data expressed as mean +/- SEM.

CASC-578 in Combination with a Wee-1 Inhibitor Shows Synergistic Anti-Proliferative Effects in Mantle Cell Lymphoma Cell Lines

A)



B)

Cell Line	Combination Index
Jeko-1	0.231
Z-138	0.542
Maver-1	0.573

Figure 4 A). Mantle cell lymphoma cell lines were treated with a titration of the Wee-1 inhibitor AZD-1775 in combination with increasing concentrations of CASC-578 and proliferation was measured at 72-hours. B). Mantle cell lymphoma cell lines were treated with equipotent ratios of CASC-578 and AZD-1775 and Combination Index (CI) values were calculated using the Chou-Talalay method and CalcuSyn software (Cancer Res. 2010 Jan 15;70(2):440-6).

CASC-578 Induces DNA Damage in Mantle Cell Lymphoma Cell Lines Which is Enhanced with Concurrent Wee-1 Inhibition

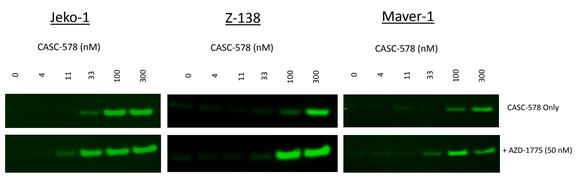


Figure 5. MCL cell lines were treated for 18 hours with a titration of CASC-578 alone or in combination with the Wee-1 inhibitor AZD-1775 (50 nM). Cells were lysed and phospho-H2A.X (S139) levels were assayed by immunoblot and detected on a LI-COR Odyssey imager.

CASC-578 Induction of Apoptosis in Mantle Cell Lymphoma Cell Lines is Increased with Concurrent Wee-1 Inhibition

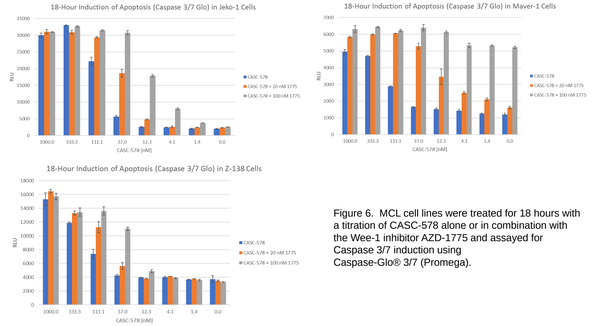


Figure 6. MCL cell lines were treated for 18 hours with a titration of CASC-578 alone or in combination with the Wee-1 inhibitor AZD-1775 and assayed for Caspase 3/7 induction using Caspase-Glo® 3/7 (Promega).

The Anti-Tumor Activity of CASC-578 is Enhanced When Combined with a Wee-1 Inhibitor in a Jeko-1 Mantle Cell LymphomaTumor Model

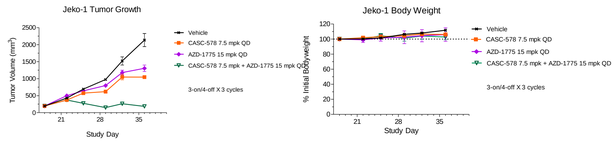
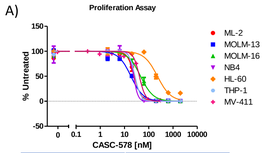


Figure 7. Jeko-1 cells were inoculated subcutaneously into the flank of CB17.SCID mice. Once tumors reached a volume of ~200 mm³ mice were randomized into study groups (N=10). Mice were treated with CASC-578, AZD-1775, or both by oral gavage at the doses and schedules indicated. Data expressed as mean +/- SEM.

CASC-578 Demonstrates Anti-Proliferative Activity and Induces DNA Damage in AML Cell Lines

A)



Cell Line	CASC-578 IC50 (nM)
ML-2	26
MOLM-13	18
MOLM-16	46
NB4	24
HL-60	228
THP-1	27
MV-411	41

B)

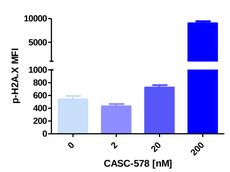


Figure 8 A). AML cell lines were treated with serial half-log dilutions of CASC-578 in a 96-well format and assayed for proliferation after 72 hours using CellTiter-Glo® Assay (Promega). Data are expressed as percent of untreated control. B). THP-1 cells were treated with CASC-578 for 18 hours at the doses indicated and assayed for phospho-H2A.X by Luminex Assay (Millipore).

CASC-578 is Active and Well-Tolerated as a Single Agent in an MV-411 AML Tumor Xenograft Model

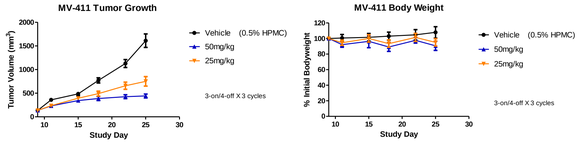


Figure 9. MV-411 cells were mixed 1:1 with Matrigel and injected subcutaneously into the right flank of female NOD.SCID mice. Once tumors reached ~100-200 mm³ mice were randomized into groups (N=10) and dosed with CASC-578 by oral gavage according to the above schedule. Data expressed as mean +/- SEM.

Conclusions

- CASC-578, a highly potent and specific inhibitor of Chk-1, demonstrates compelling single agent activity on mantle cell lymphoma cell lines both *in vitro* and *in vivo,* including complete tumor regression in a Jeko-1 xenograft model.
- CASC-578 synergizes with the Wee-1 inhibitor AZD-1775 to induce DNA damage, apoptosis, and tumor control.
- CASC-578 shows strong anti-proliferative activity and induction of DNA damage in AML-derived cell lines, as well as single agent activity in an MV-411 tumor xenograft model.
- Targeting the DNA Damage Response (DDR) axis with the Chk-1 inhibitor CASC-578 alone or in combination with Wee-1 inhibition presents a promising therapeutic approach to treating mantle cell lymphoma and other hematological cancers.
- Taken together, these data support the advancement of CASC-578 into IND-enabling studies.