CDC7 Kinase Inhibition by SRA141 Induces a Potentially Novel Caspase-Dependent Tumor Cell Apoptosis Associated with Altered DNA Replication and Cell Cycle Dynamics

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Background

- During 5-phase, Cell division cycle 7 (CDC7) kinase, together with its partner protein DBD4 or Drt1, phosphorylates and activates the MCCM-7 helicase thereby initiating DNA replication origin firing.
- Owing to its important role in DNA replication, and its overexpression in various neoplasms (e.g. colorectal and breast cancer), CDC7 is an attractive therapeutic target with clinical validation in oncology.
- While the precise mechanism of CDC7 inhibitor-mediated anti-tumor activity remains to be defined, preclinical studies investigating the inhibition of CDC7 using siRNA and small molecules demonstrate differential sensitivity of tumor cells as a consequence of a p53-dependent DNA replication checkpoint that is operational only in non-transformed cells.
- We previously reported that SRA141, a clinic-ready, potent, orally bioavailable selective inhibitor of CDC7 kinase that was previously shown to display potent antiproliferative activity against various tumor cell lines. SRA141 also displays favorable PK properties and robust anti-tumor activity evidenced by complete and partial regressions in colorectal and leukemia xenograft models.
- SRA141 treatment caused an accumulation of cells in mitosis as evidenced by elevated cyclin B levels and an increase in the percentage of cells in mitosis. Studies are ongoing to determine whether mitotic accumulation is associated with under-replicated DNA. Similar accumulation of cells in mitosis was demonstrated using other CDC7 inhibitors, suggesting a potentially novel mechanism of action for this class of agents that is distinct from other DNA damage response targeted drugs.
- Synergistic cytotoxicity between SRA141 and inhibitors of both Aurora kinase B and anti-apoptotic proteins provides further support for a mechanism of cell death involving mitotic dysregulation and Bcl-2 family mediated apoptosis.

Translational Significance

- SRA141 does not induce G1 cell cycle arrest or replication stress, thereby distinguishing it from cyclin-dependent kinase inhibitors and DNA damage response targets agents. Rather, SRA141 alters DNA replication dynamics and delays cell cycle progression, ultimately resulting in caspase-dependent cell death associated with mitotic accumulation.
- Our findings reveal a potentially novel mechanism of cytotoxicity for CDC7 inhibitors that is distinct from agents that cause replication fork collapse or cyclin-dependent kinase inhibition, and thus may define a new class of cancer therapeutic agents with a differentiated anti-tumor profile.

Proposed SRA141 Mechanism of Action

SRA141 alters replication dynamics through the inhibition of CDC7-mediated phosphorylation of MCCM-7 leading to a reduction of origin firing and a compensatory increase in replication fork speed. As the cells progress through S-phase without finishing replication, under-replicated DNA delays subsequent mitosis and ultimately leads to mitotic catastrophe and apoptotic cell death.

Results

- SRA141 is a clinic-ready, potent, orally bioavailable selective inhibitor of CDC7 kinase that was previously shown to display potent antiproliferative activity against various tumor cell lines. SRA141 also displays favorable PK properties and robust anti-tumor activity evidenced by complete and partial regressions in colorectal and leukemia xenograft models.
- Consistent with its role in abrogating origin firing, SRA141 strongly inhibited MCM2 phosphorylation which was accompanied by a reduction in the rate of DNA synthesis. SRA141 caused a corresponding decrease in DNA replication fork speed, suggesting engagement of a compensatory mechanism triggered by a reduction in replication origin firing.
- In contrast to inhibitors of DNA replication checkpoint kinases, SRA141 was shown to be antagonistic to replication stress inducers, as demonstrated by its ability to counteract ATR inhibitor-induced 5-phase DNA damage and oppose gemcitabine-mediated growth inhibition.
- SRA141 treatment caused an accumulation of cells in mitosis as evidenced by elevated cyclin B levels and an increase in the percentage of cells in mitosis. Studies are ongoing to determine whether mitotic accumulation is associated with under-replicated DNA. Similar accumulation of cells in mitosis was demonstrated using other CDC7 inhibitors, suggesting a potentially novel mechanism of action for this class of agents that is distinct from other DNA damage response targeted drugs.
- Synergistic cytotoxicity between SRA141 and inhibitors of both Aurora kinase B and anti-apoptotic proteins provides further support for a mechanism of cell death involving mitotic dysregulation and Bcl-2 family mediated apoptosis.

Summary

- SRA141 alters replication dynamics through the inhibition of CDC7-mediated phosphorylation of MCCM-7 leading to a reduction of origin firing and a compensatory increase in replication fork speed. As the cells progress through S-phase without finishing replication, under-replicated DNA delays subsequent mitosis and ultimately leads to mitotic catastrophe and apoptotic cell death.