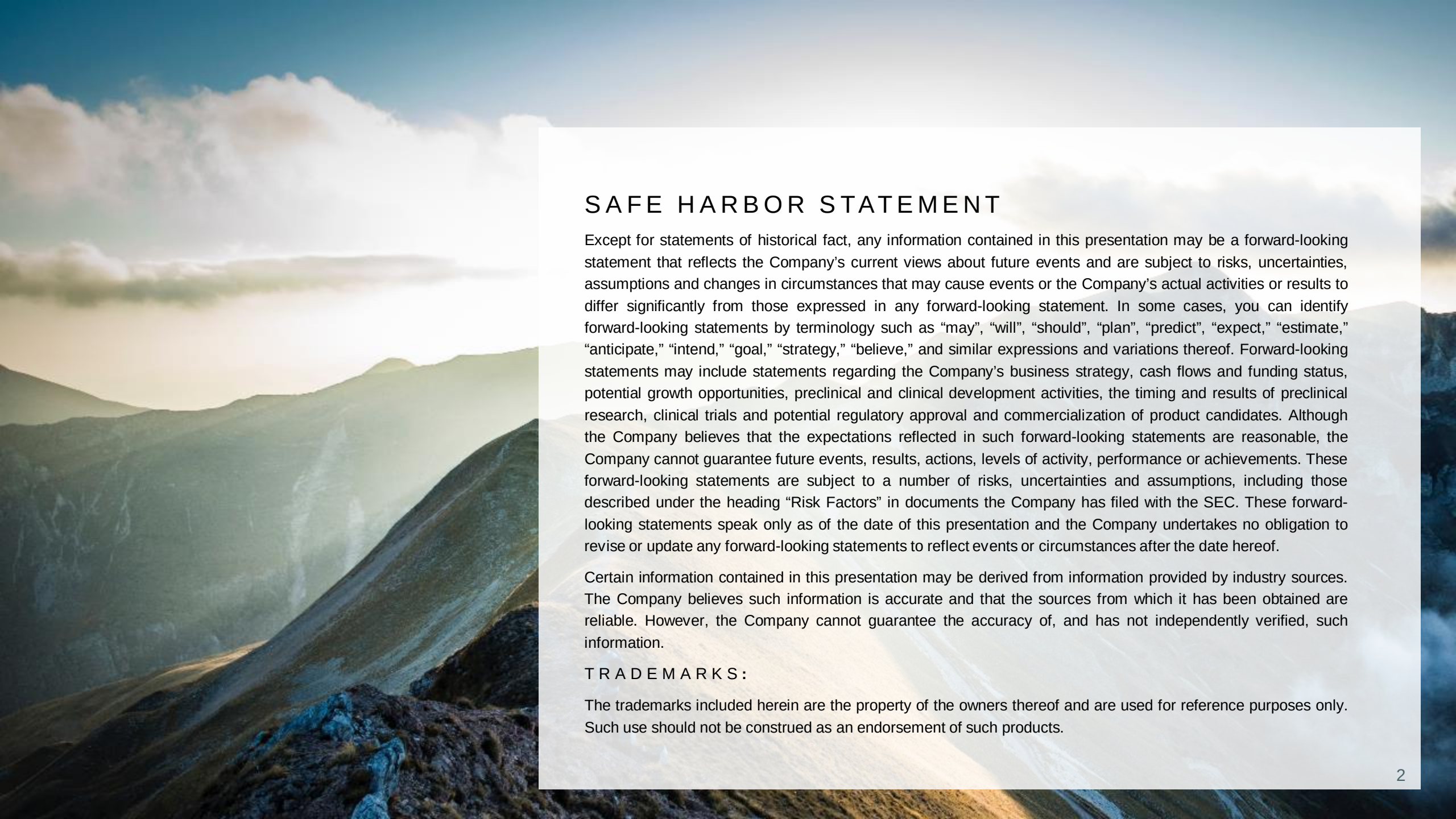
SIERRA ONCOLOGY

Charting the path from pioneering biology to impactful therapeutics

AACR 2019 Late-Breaking Data for SRA141 targeting Cdc7

NASDAQ: SRRA



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Sierra is Advancing Two Drug Candidates Targeting the DNA Damage Response (DDR)

SRA737

TARGETING Chk1

THERAPEUTIC FOCUS

High Grade Serous Ovarian Cancer, Squamous Cell Carcinoma, SCLC & Other Solid Tumors

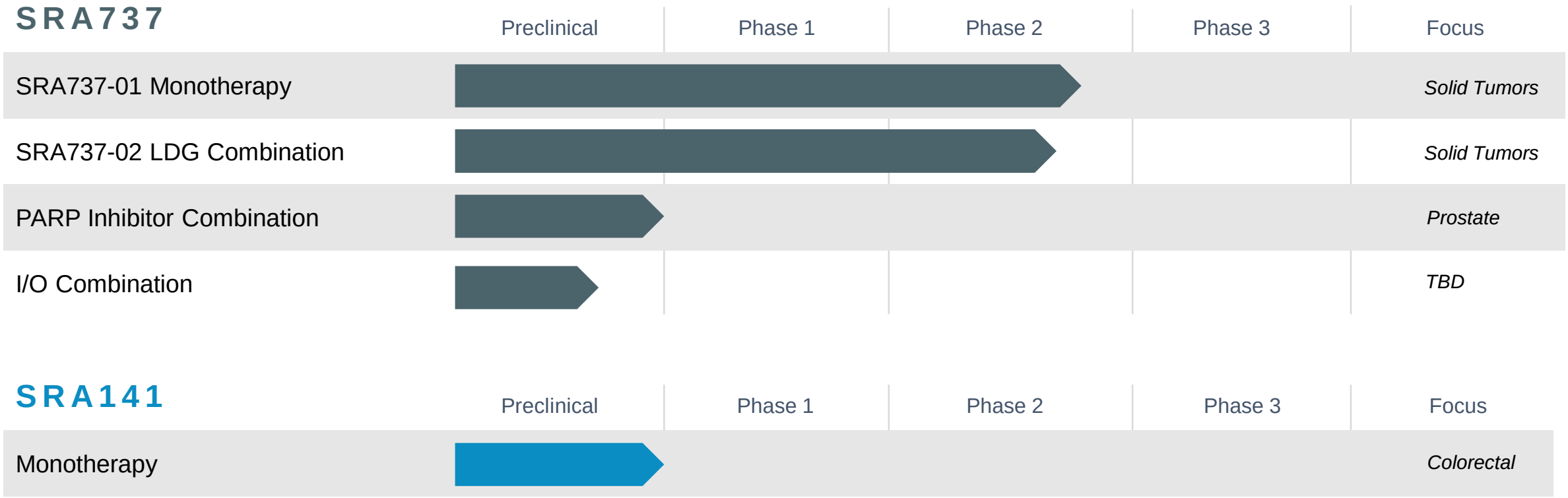
SRA141

TARGETING Cdc7

THERAPEUTIC FOCUS

Colorectal Cancer

Our Pipeline* Of ‘Next Generation’ DDR Therapeutics



*Sierra is also developing its Phase 3 asset, momelotinib, for the treatment of myelofibrosis.

Executive Summary

- SRA141 is a potent, selective small molecule inhibitor of Cdc7 with favorable pharmaceutical properties possessing best in class potential
- Cdc7 is a ‘next-generation’ DDR target for cancer, with a key role in controlling the initiation of DNA replication
  - Cdc7i-mediated cell killing is driven through a **unique apoptotic mechanism of action** that may involve mitotic catastrophe in cancer cells
  - Clinical validation is emerging with clinical stage Cdc7i programs from Takeda and CRUK/Eli Lilly
- Cdc7 inhibition has particular relevance in microsatellite-stable (MSS) metastatic colorectal cancer (mCRC), based on preclinical data from Sierra, Takeda and Eli Lilly
- Sierra has prepared for a potential Phase 1/2 trial of SRA141 in patients with advanced colorectal cancer
  - An Investigational New Drug Application (IND) filing has been accepted by the FDA

SRA141

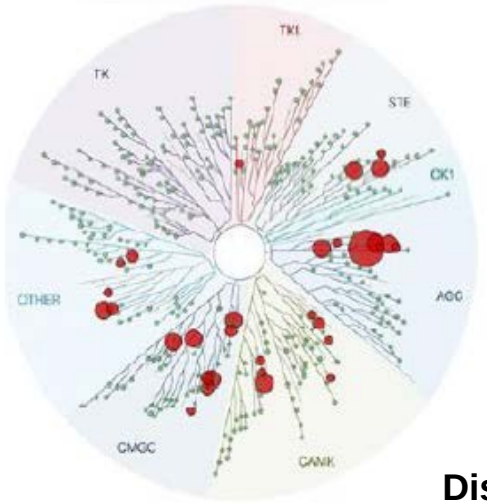
Potent, Highly Selective, Orally Available Inhibitor of Cdc7

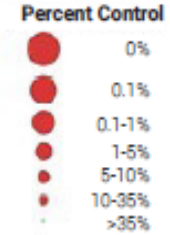
SRA141:
Potential Best-in-Class Cdc7 Inhibitor

SRA141 is highly selective:

- Minimal off-target kinase activity observed (e.g., GSK3α, GSKβ, PIM1; 500nM)
- Cellular potency confirmed limited off-target effects
- More selective than TAK-931

SRA141 @500nM

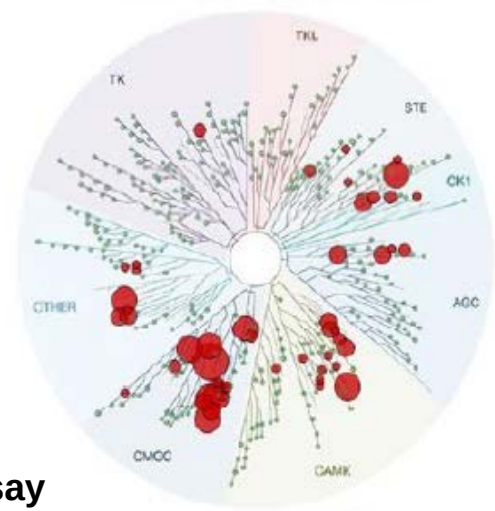




Summary of Hits

% of Control	SRA141	TAK-931
Less < 35%	38	52
Less < 20%	26	39
Less < 10%	21	29
Less < 5%	14	20
Less < 1%	1	11

TAK-931 @500nM

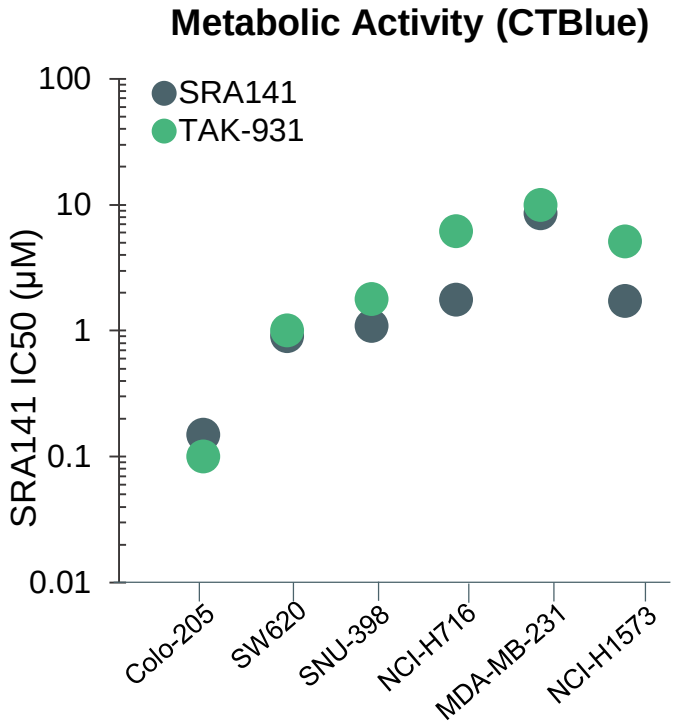


DiscoverX Kinome Screening Assay

Note: Cdc7 not included in this KINOMEscan profiling assay of 468 kinases

SRA141 is potent:

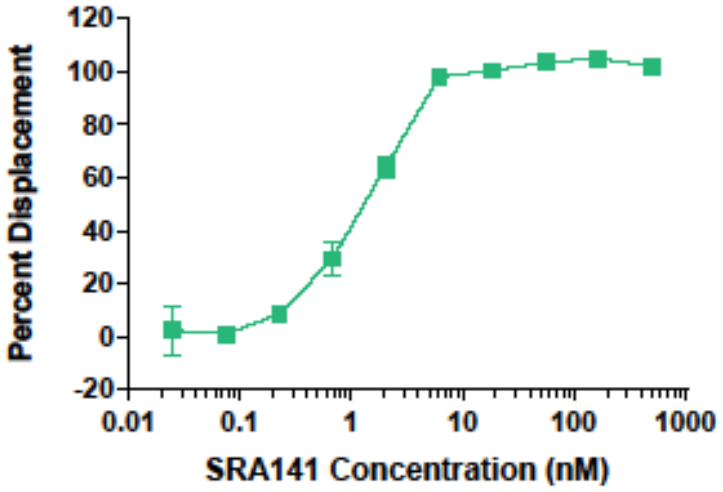
- Low μM potency in a variety of cell lines, particularly CRC
- Generally equipotent with TAK-931 *in vitro*



SRA141:
Potent, Highly-Selective, Small Molecule Inhibitor of Cdc7

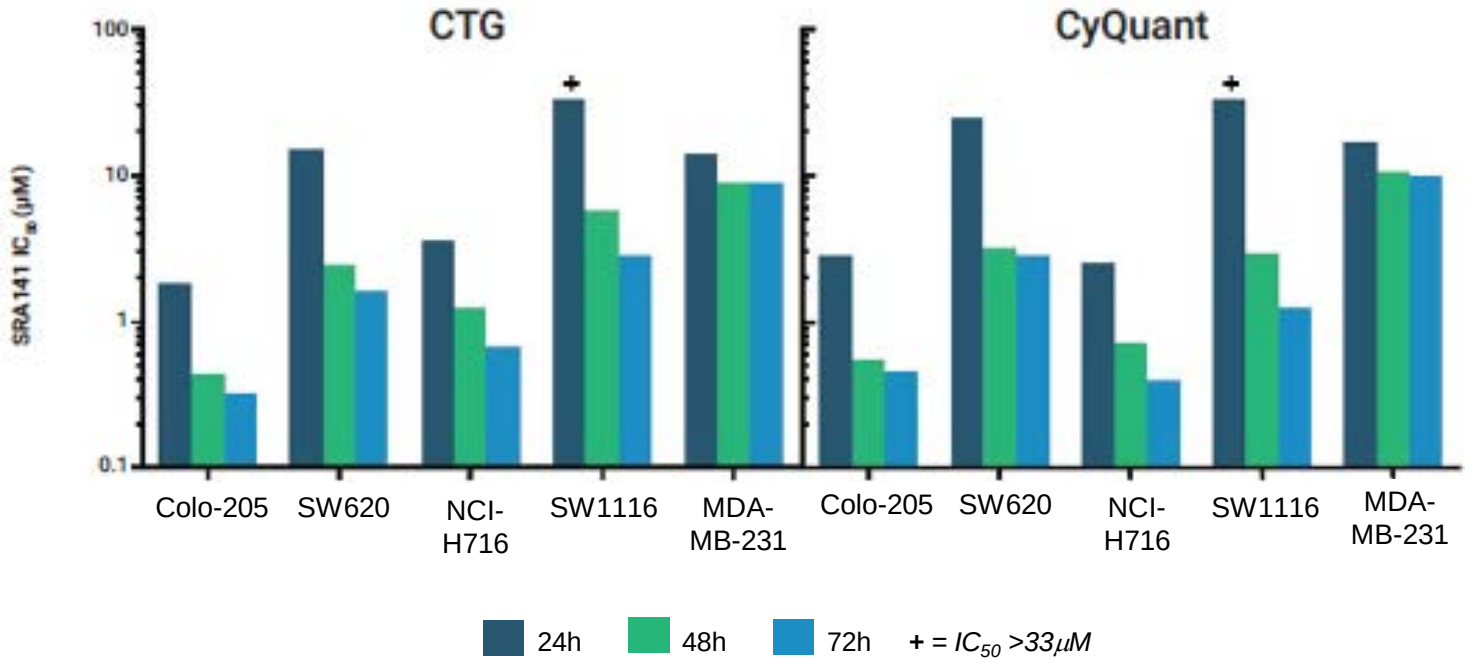
BIOCHEMICAL ACTIVITY

Cdc7 IC50	1.4 nM
Residence time	$t_{1/2}$ = 215 min



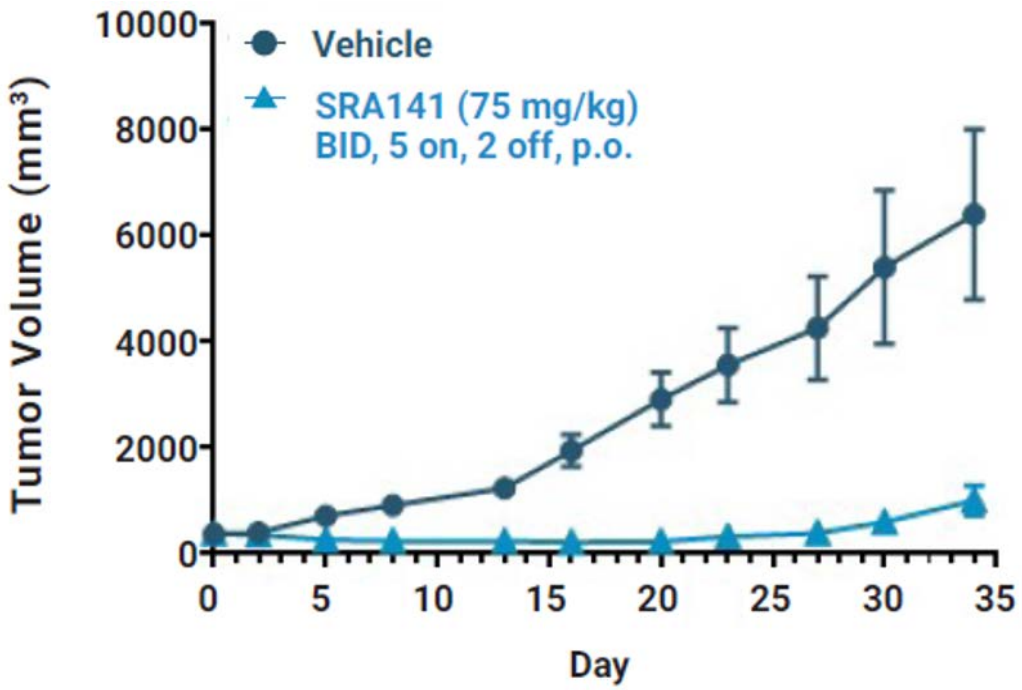
CELLULAR ACTIVITY

SRA141 demonstrates potent anti-proliferative activity in a range of cell lines, which increases with longer treatment durations



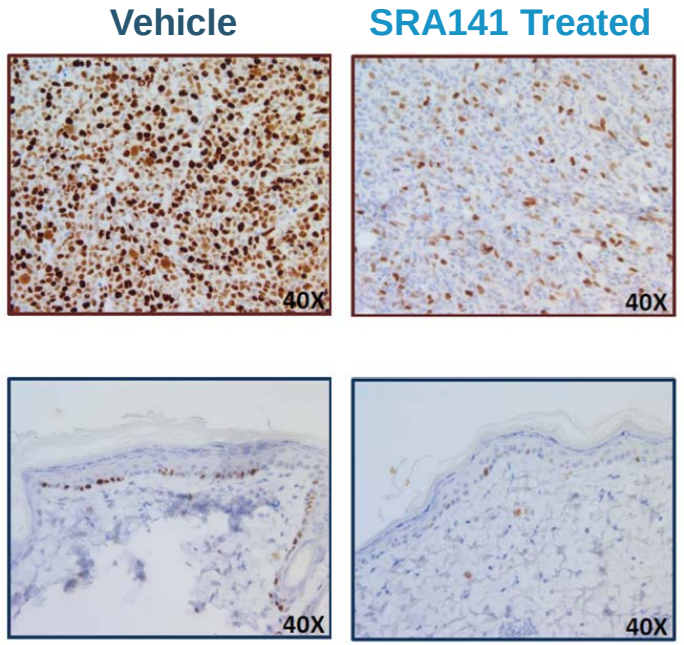
SRA141:
Demonstrated *In Vivo* Efficacy and On-Target PD Marker

MV-4-11 AML Xenograft Model



- Significant tumor growth inhibition (92% at day 27 and 85% at day 34)
- A complete tumor regression (1/7) and a partial regression (1/7) were achieved at study completion

PD marker pMCM2



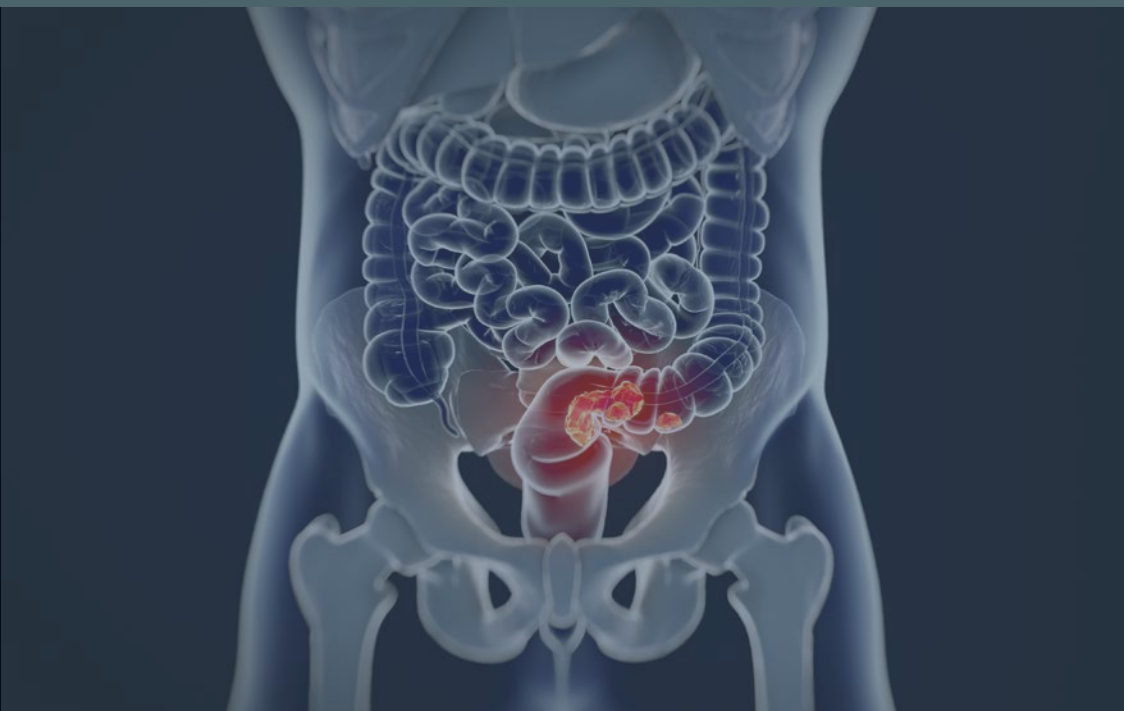
- Both tumor and skin demonstrate inhibition of pMCM2 at steady state (5 doses) following treatment with SRA141
- PD marker and assay validated for clinical use

SRA141

Attractive Development Opportunity in MSS Colorectal Cancer

Large Opportunity in Metastatic Colorectal Cancer

MSS Phenotype



- Colorectal cancer (CRC) is the 3rd most common cancer and 2nd leading cause of cancer deaths
  - WHO estimates 1.8M cases and ~860k deaths worldwide
- Metastatic colorectal cancer (mCRC) is one of the most deadly cancers: **5-year survival rate of only 12%**
  - 50% of diagnosed CRC patients will present with or eventually develop metastatic disease
- Microsatellite-stable (MSS) is the dominant phenotype of mCRC, comprising up to 80-90% of cases
  - MSS mCRC is a mutually exclusive phenotype with the MSI-high type
  - There are limited treatment options for late-line MSS mCRC; current IO agents have demonstrated efficacy in MSI-high mCRC but not the MSS phenotype

MSS Metastatic Colorectal Cancer: **Limited Treatment Options in 2nd and 3rd-Line+ Patients**

- Treatment of mCRC primarily includes established targeted TKI's + Chemotherapy in the 1st and 2nd-lines, influenced by *RAS* status; and in later lines of therapy, by genetic biomarker populations (e.g., MSI-H, *BRAF* or *RAS* mutations)

Metastatic CRC

- 100-110K cases of recurrent or metastatic CRC annually*
- Prevalent population of 200-210K
- ~80% are drug-treated

1st and 2nd-Line

Treatment dominated by VEGFRi / EGFRi + Chemotherapy combinations

- Currently a $4B+ global market

2nd and 3rd-Line +

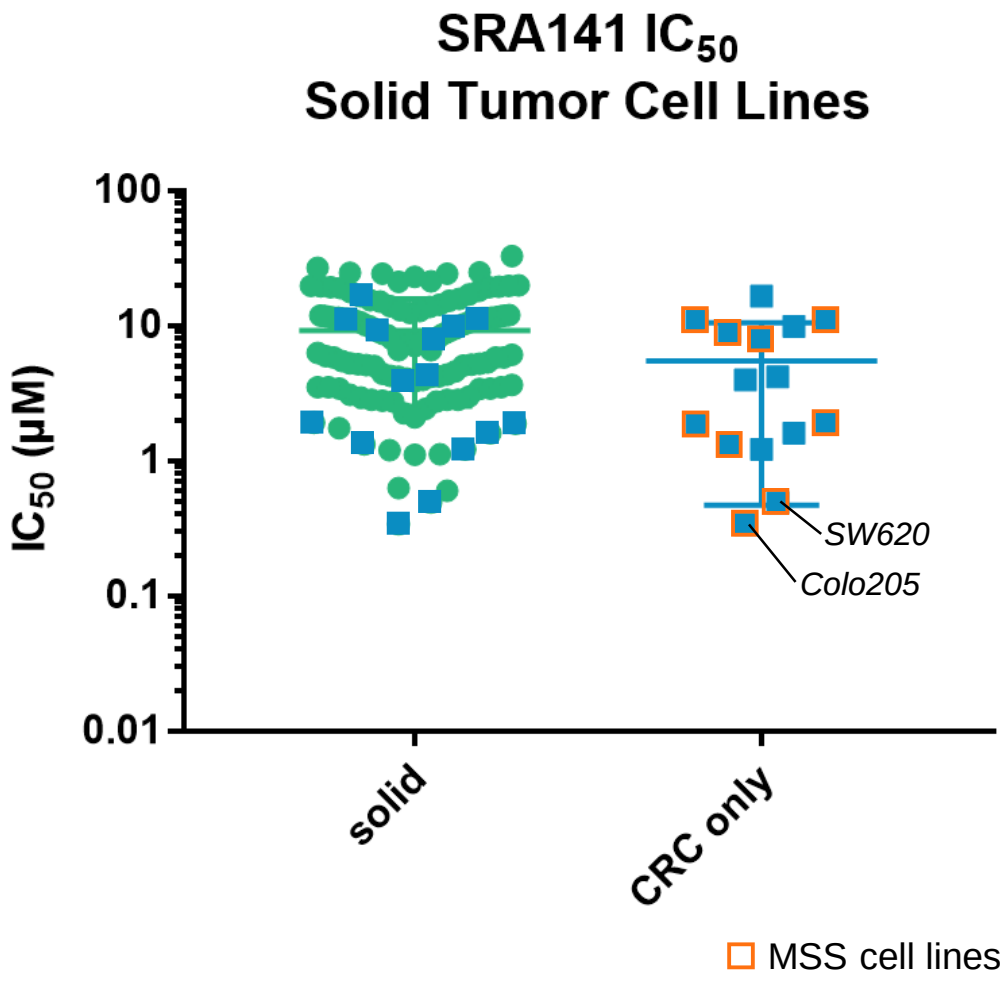
~150K drug-treated population in 2nd and 3rd-line; therapies driven by biomarker selection

Type	Therapy	Patient Population	Efficacy
MSI	Anti-PD-1 (IO)	MSI-High (~10%)	Mod-High
MSS	BRAFi/MEKi	BRAF-mutant (~8%)	Mod-High
	2nd-gen EGFRi	RAS-wt (~28%)	Moderate
	Limited options	RAS-mutant (~55%)	Low

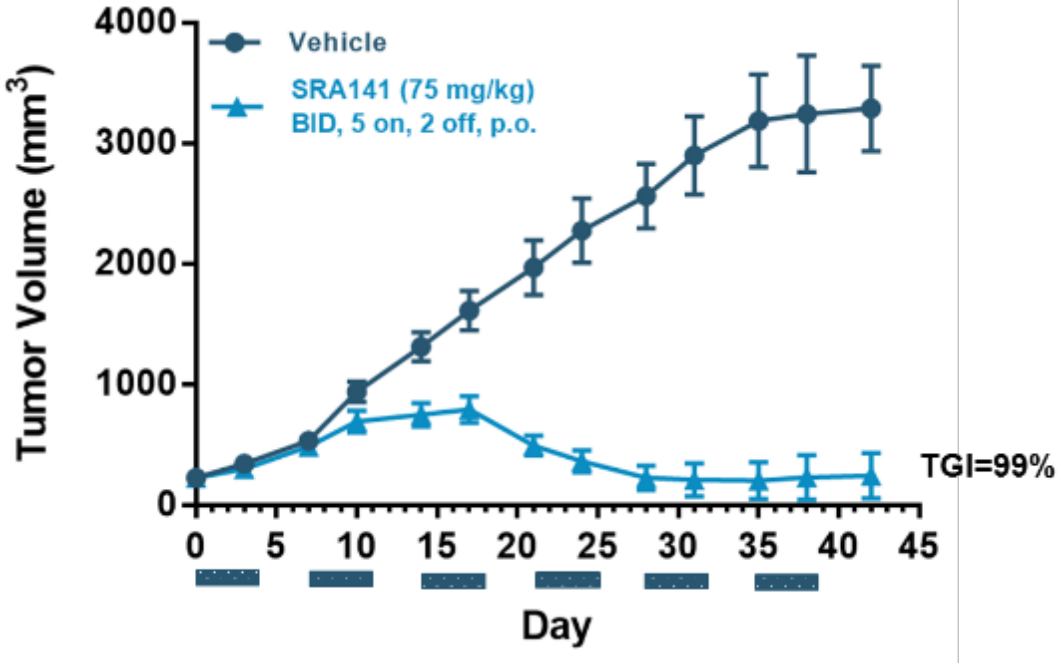
*In US, EU5, and Japan
Source: Decision Resources

MSS Metastatic Colorectal Cancer:
Differential Sensitivity to SRA141 in MSS CRC Cell Lines

- SRA141 has potent anti-cellular activity in a range of cell lines
  - E.g., bladder, sarcoma, colon, renal, head-and-neck, melanoma, gastric
- Colorectal cancer lines among the most sensitive to SRA141
  - More than half of the most sensitive (IC_{50} ≤2μM) solid tumor cell lines are colorectal
  - 5/7 of the most sensitive CRC cell lines are of MSS phenotype, including Colo205 and SW620
- Cdc7 overexpression has been observed in CRC and is frequently associated with the presence of mutations in *TP53*



Colo-205, A MSS Phenotype Colorectal Xenograft: **SRA141 Elicits Complete and Partial Tumor Regressions**



Dosing initiated when tumor volumes reached 225mm^3
TGI calculated on Day 31, final day all Vehicle control rats remained on study

- Colo-205 model genetics: *TP53* & MSS (relevant genetics for Cdc7i)
- 4/7 (57%) Complete tumor regression
  - All 4 persisted to study completion
- 1/7 (14%) Partial tumor regression

PD marker pMCM2

- Following a single dose, SRA141 resulted in an approximate 50% decrease in pMCM2 after 12 hours

Cdc7 Preclinically and Clinically Validated: **Supports An MSS Colorectal Cancer Opportunity**

- CRC also identified as an opportunity for Cdc7 inhibitors by other drug developers such as Takeda (TAK-931, Phase 2) and CRUK/Eli Lilly (LY3143921, Phase 1)

- Preclinically, both companies have demonstrated *in vitro* activity of Cdc7 inhibitors across multiple CRC cell lines and *in vivo* xenograft and PDX models of CRC
  - Takeda presented data on TAK-931 in 40 CRC PDX models, in which **MSS** and **p53** status was a key biomarker of sensitivity

- Clinically, Takeda observed initial signs of efficacy for TAK-931 in their Phase 1 trial with responses in a variety of tumor types and has since initiated a Phase 2 efficacy study **focused on p53 mutant and/or MSS cancers**, including CRC
  - Additionally, Takeda clinically validated the use of skin punch biopsies for a pMCM2 PD biomarker assay in their Phase 1 trial

Collectively, this robust preclinical and clinical validation of Cdc7 establishes a clear expeditious development path for SRA141 in MSS mCRC

Support for Targeting Cdc7 in a Range of Tumors

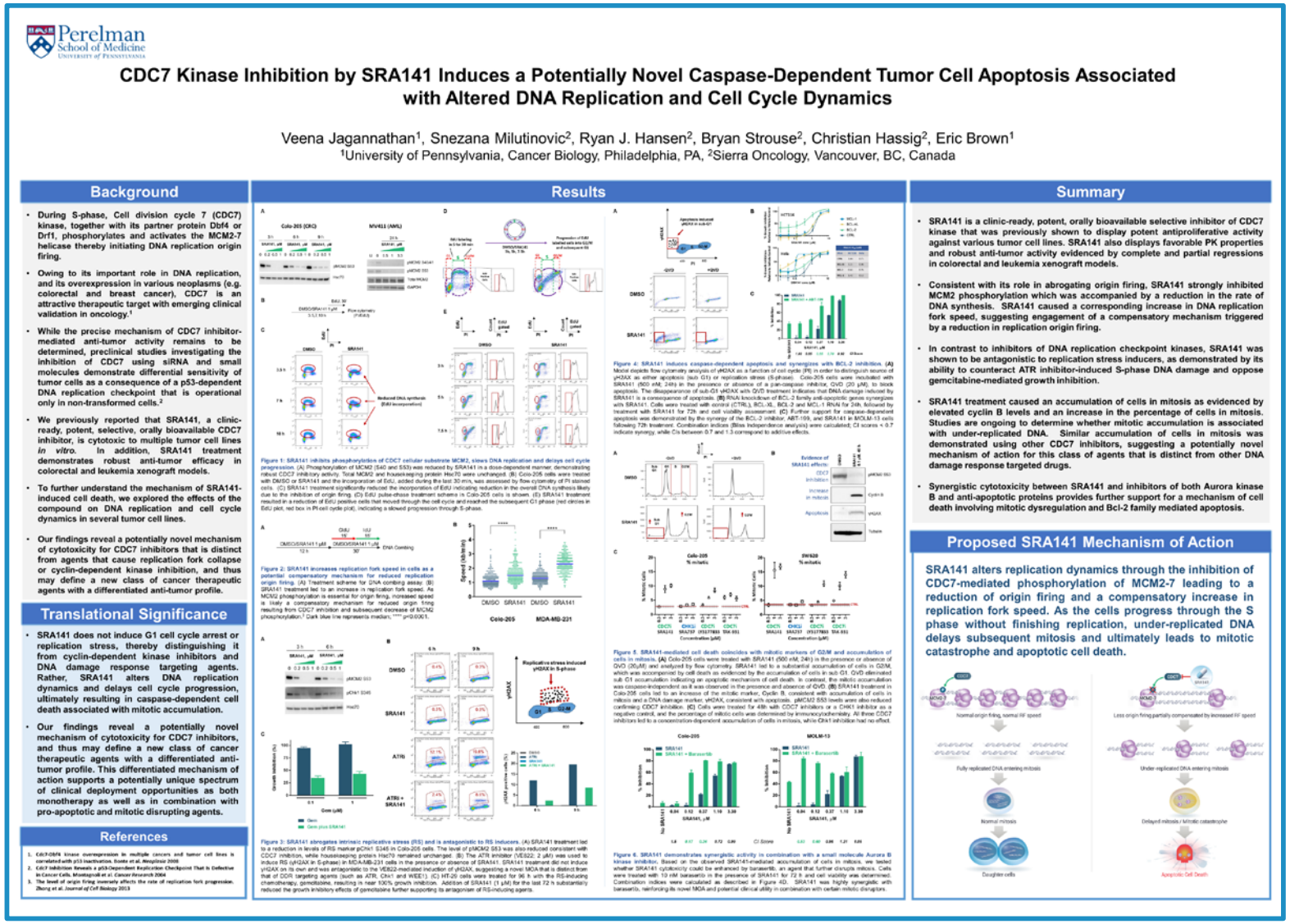
- In addition to CRC, Cdc7 overexpression correlating to poor prognosis has been observed in a range of tumor types including lung, melanoma, breast, DLBCL, oral squamous cell carcinoma, and ovarian[1-4]
- Preclinical evidence of Cdc7i efficacy includes animal models of breast, AML, lung and pancreatic tumors has been generated by Sierra, Takeda, and Eli Lilly[5-7]
- Additionally, in their FIH Phase 1 trial, Takeda saw preliminary signs of efficacy in cervical, esophageal, and duodenum cancers[8]

1. Bonte et al. Neoplasia (2018)
2. Hou et al. Medical Oncology (2012)
3. Jin et al. Journal of Molecular Medicine (2018)
4. Kulkarni et al. Clinical Cancer Research (2009)
5. Sierra Oncology. Unpublished data (2018)
6. Iwai et al. AACR Poster Presentation, Abstract #3073 (2017)
7. Xiang et al. AACR Oral Presentation (2016)
8. Shimizu et al. ASCO Oral Presentation (2018)

SRA141 Late-Breaking Data - AACR 2019

Cdc7 Kinase Inhibition by SRA141 Induces a Potentially Novel Caspase-Dependent Tumor Cell Apoptosis Associated with Altered DNA Replication and Cell Cycle Dynamics

AACR 2019
SRA141 Late-Breaking Poster Presentation



Perelman School of Medicine, University of Pennsylvania

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

Veena Jagannathan[1], Snezana Milutinovic[2], Ryan J. Hansen[2], Bryan Strouse[2], Christian Hassig[2], Eric Brown[1]
[1]University of Pennsylvania, Cancer Biology, Philadelphia, PA, [2]Sierra Oncology, Vancouver, BC, Canada

Background

- During S-phase, Cell division cycle 7 (CDC7) kinase, together with its partner protein Dbf4 or Drf1, phosphorylates and activates the MCM2-7 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 emerging clinical validation in oncology.[1]
- While the precise mechanism of CDC7 inhibitor-mediated anti-tumor activity remains to be determined, 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.[2]
- We previously reported that SRA141, a clinic-ready, potent, selective, orally bioavailable CDC7 inhibitor, is cytotoxic to multiple tumor cell lines *in vitro*. In addition, SRA141 treatment demonstrates robust anti-tumor efficacy in colorectal and leukemia xenograft models.
- To further understand the mechanism of SRA141-induced cell death, we explored the effects of the compound on DNA replication and cell cycle dynamics in several tumor cell lines.
- 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.

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 targeting 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, and thus may define a new class of cancer therapeutic agents with a differentiated anti-tumor profile. This differentiated mechanism of action supports a potentially unique spectrum of clinical deployment opportunities as both monotherapy as well as in combination with pro-apoptotic and mitotic disrupting agents.

References

1. Cdc7-Dbf4 kinase overexpression in multiple cancers and tumor cell lines is correlated with p53 inactivation. Bonte et al. *Neoplasia* 2008
2. Cdc7 Inhibition Reveals a p53-Dependent Replication Checkpoint That is Defective in Cancer Cells. Montagnoli et al. *Cancer Research* 2004
3. The level of origin firing inversely affects the rate of replication fork progression. Zhong et al. *Journal of Cell Biology* 2013

Results

Figure 1: SRA141 inhibits phosphorylation of CDC7 cellular substrate MCM2, slows DNA replication and delays cell cycle progression. (A) Phosphorylation of MCM2 (S40 and S53) was reduced by SRA141 in a dose-dependent manner, demonstrating robust CDC7 inhibitory activity. Total MCM2 and housekeeping protein Hsc70 were unchanged. (B) Colo-205 cells were treated with DMSO or SRA141 and the incorporation of EdU, added during the last 30 min, was assessed by flow cytometry of PI stained cells. (C) SRA141 treatment significantly reduced the incorporation of EdU indicating reduction in the overall DNA synthesis likely due to the inhibition of origin firing. (D) EdU pulse-chase treatment scheme is Colo-205 cells is shown. (E) SRA141 treatment resulted in a reduction of EdU positive cells that moved through the cell cycle and reached the subsequent G1 phase (red circles in EdU plot, red box in PI cell cycle plot), indicating a slowed progression through S-phase.

Figure 2: SRA141 increases replication fork speed in cells as a potential compensatory mechanism for reduced replication origin firing. (A) Treatment scheme for DNA combing assay. (B) SRA141 treatment led to an increase in replication fork speed. As MCM2 phosphorylation is essential for origin firing, increased speed is likely a compensatory mechanism for reduced origin firing resulting from CDC7 inhibition and subsequent decrease of MCM2 phosphorylation.[3] Dark blue line represents median; **** p<0.0001.

Figure 3: SRA141 abrogates intrinsic replicative stress (RS) and is antagonistic to RS inducers. (A) SRA141 treatment led to a reduction in levels of RS marker pChk1 S345 in Colo-205 cells. The level of pMCM2 S53 was also reduced consistent with CDC7 inhibition, while housekeeping protein Hsc70 remained unchanged. (B) The ATR inhibitor (VE822; 2 μM) was used to induce RS (γH2AX in S-phase) in MDA-MB-231 cells in the presence or absence of SRA141. SRA141 treatment did not induce γH2AX on its own and was antagonistic to the VE822-mediated induction of γH2AX, suggesting a novel MOA that is distinct from that of CDR targeting agents (such as ATR, Chk1 and WEE1). (C) HT-29 cells were treated for 96 h with the RS-inducing chemotherapy, gemcitabine, resulting in near 100% growth inhibition. Addition of SRA141 (1 μM) for the last 72 h substantially reduced the growth inhibitory effects of gemcitabine further supporting its antagonism of RS-inducing agents.

Figure 4: SRA141 induces caspase-dependent apoptosis and synergizes with BCL-2 inhibition. (A) Model depicts flow cytometry analysis of γH2AX as a function of cell cycle (PI) in order to distinguish source of γH2AX as either apoptosis (sub G1) or replication stress (S-phase). Colo-205 cells were incubated with SRA141 (500 nM; 24h) in the presence or absence of a pan-caspase inhibitor, QVD (20 μM), to block apoptosis. The disappearance of sub-G1 γH2AX with QVD treatment indicates that DNA damage induced by SRA141 is a consequence of apoptosis. (B) RNAi knockdown of BCL-2 family anti-apoptotic genes synergizes with SRA141. Cells were treated with control (CTRL), BCL-XL, BCL-2 and MCL-1 RNAi for 24h, followed by treatment with SRA141 for 72h and cell viability assessment. (C) Further support for caspase-dependent apoptosis was demonstrated by the synergy of the BCL-2 inhibitor ABT-199, and SRA141 in MOLM-13 cells following 72h treatment. Combination indices (Bliss Independence analysis) were calculated; CI scores < 0.7 indicate synergy, while CIs between 0.7 and 1.3 correspond to additive effects.

Figure 5: SRA141-mediated cell death coincides with mitotic markers of G2/M and accumulation of cells in mitosis. (A) Colo-205 cells were treated with SRA141 (500 nM; 24h) in the presence or absence of QVD (20μM) and analyzed by flow cytometry. SRA141 led to a substantial accumulation of cells in G2/M, which was accompanied by cell death as evidenced by the accumulation of cells in sub G1. QVD eliminated sub G1 accumulation indicating an apoptotic mechanism of cell death. In contrast, the mitotic accumulation was caspase-independent as it was observed in the presence and absence of QVD. (B) SRA141 treatment in Colo-205 cells led to an increase of the mitotic marker, Cyclin B, consistent with accumulation of cells in mitosis and a DNA damage marker, γH2AX, consistent with apoptosis. pMCM2 S53 levels were also reduced confirming CDC7 inhibition. (C) Cells were treated for 48h with CDC7 inhibitors or a CHK1 inhibitor as a negative control, and the percentage of mitotic cells was determined by immunocytochemistry. All three CDC7 inhibitors led to a concentration-dependent accumulation of cells in mitosis, while Chk1 inhibition had no effect.

Figure 6. SRA141 demonstrates synergistic activity in combination with a small molecule Aurora B kinase inhibitor. Based on the observed SRA141-mediated accumulation of cells in mitosis, we tested whether SRA141 cytotoxicity could be enhanced by barasertib, an agent that further disrupts mitosis. Cells were treated with 10 nM barasertib in the presence of SRA141 for 72 h and cell viability was determined. Combination indices were calculated as described in Figure 4D. SRA141 was highly synergistic with barasertib, reinforcing its novel MOA and potential clinical utility in combination with certain mitotic disruptors.

Summary

- 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 increase 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 S-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.

Proposed SRA141 Mechanism of Action

SRA141 alters replication dynamics through the inhibition of CDC7-mediated phosphorylation of MCM2-7 leading to a reduction of origin firing and a compensatory increase in replication fork speed. As the cells progress through the S phase without finishing replication, under-replicated DNA delays subsequent mitosis and ultimately leads to mitotic catastrophe and apoptotic cell death.

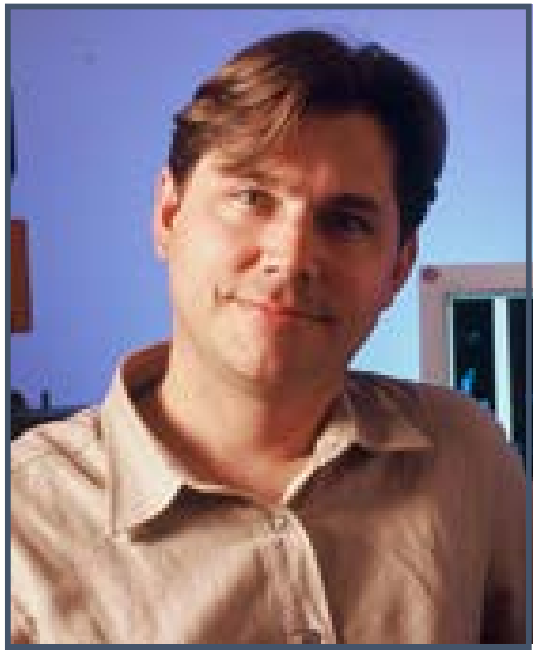
AACR American Association for Cancer Research ANNUAL MEETING 2019 ATLANTA

Cdc7 Kinase Inhibition by SRA141 Induces a Potentially Novel Caspase-Dependent Tumor Cell Apoptosis Associated with Altered DNA Replication and Cell Cycle Dynamics

Presented April 3, 2019

Eric J. Brown, PhD
Perelman School of Medicine at the University of Pennsylvania

- Dr. Brown's laboratory examines how ATR signaling maintains genome stability during DNA synthesis and how this function is essential to cancer cells
- Dr. Brown's laboratory was the first to report that oncogenic stress is sufficient to cause selective sensitivity to ATR inhibition, and is currently identifying predictive biomarkers of therapeutic benefit and the mechanisms of action of these drugs through a combination of genome-wide breakpoint mapping and replication fork proteomics. Collectively, the Brown laboratory seeks both to define the mechanisms of action of ATR/CHK1 inhibitors and to identify their optimal uses in cancer therapies
- Dr. Brown received his BA (Genetics) from the University of California at Berkeley (1989) and his PhD (Immunology) from Harvard University (1996). He performed his doctoral research with Dr. Stuart Schreiber at Harvard University, where he purified and cloned the mammalian target of rapamycin (mTOR). In his postdoctoral research in Dr. David Baltimore's laboratory at the California Institute of Technology, Dr. Brown investigated the impact of ATR suppression on genome stability and checkpoint signaling in response to replication stress



Eric J. Brown, PhD

Associate Professor of Cancer Biology, Perelman School of Medicine at the University of Pennsylvania

Member of Sierra's DNA damage response (DDR) Advisory Committee.

Background

- During S-phase, Cell division cycle 7 (Cdc7) kinase, together with its partner protein Dbf4 or Drf1, phosphorylates and activates the MCM2-7 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 emerging clinical validation in oncology
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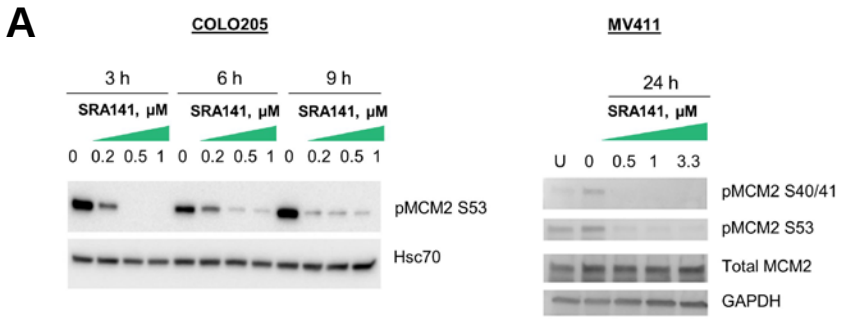
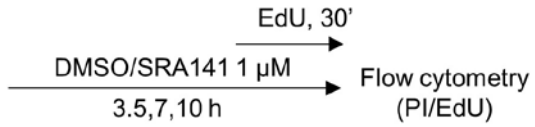
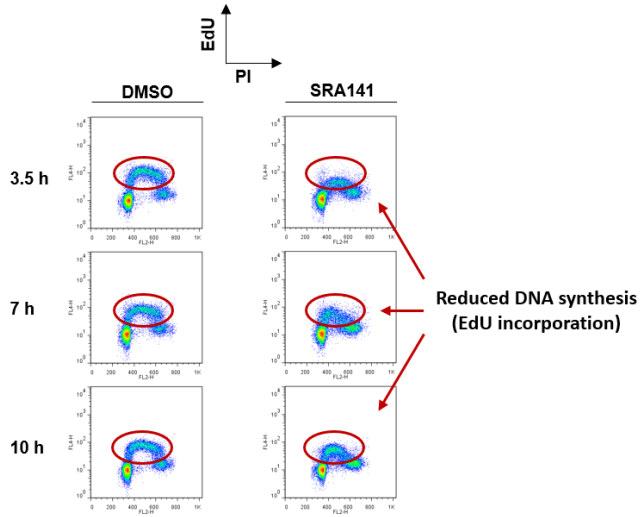
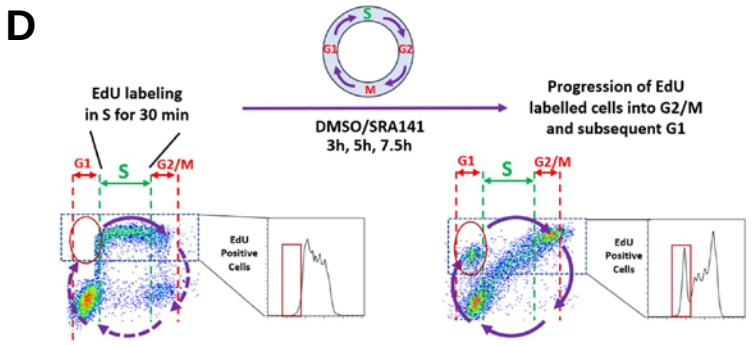
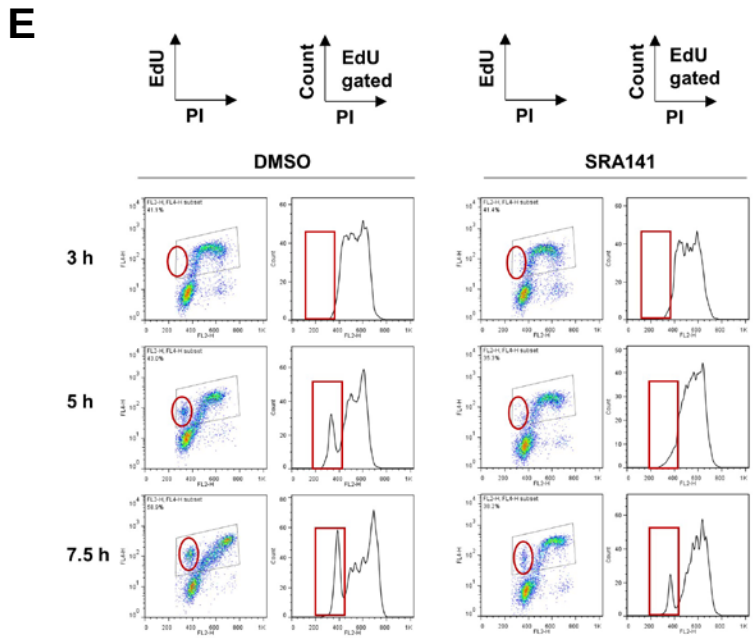
SRA141 inhibits phosphorylation of Cdc7 cellular substrate MCM2, slows DNA replication and delays cell cycle progression











(A) Phosphorylation of MCM2 (S40 and S53) was reduced by SRA141 in a dose-dependent manner, demonstrating robust Cdc7 inhibitory activity. Total MCM2 and housekeeping protein Hsc70 were unchanged.

(B) Colo-205 cells were treated with DMSO or SRA141 and the incorporation of EdU, added during the last 30 min, was assessed by flow cytometry of PI stained cells.

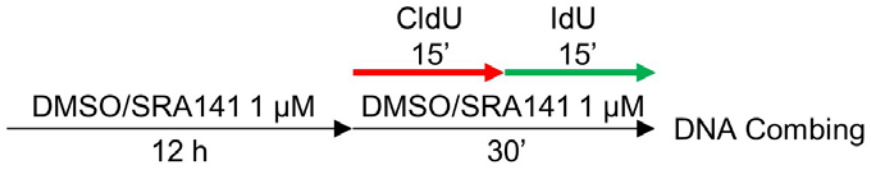
(C) SRA141 treatment significantly reduced the incorporation of EdU indicating reduction in the overall DNA synthesis likely due to the inhibition of origin firing.

(D) EdU pulse-chase treatment scheme in Colo-205 cells is shown.

(E) SRA141 treatment resulted in a reduction of EdU positive cells that moved through the cell cycle and reached the subsequent G1 phase (red circles in EdU plot, red box in PI cell cycle plot), indicating a slowed progression through S-phase.

SRA141 increases replication fork speed in cells as a potential compensatory mechanism for reduced replication origin firing

A



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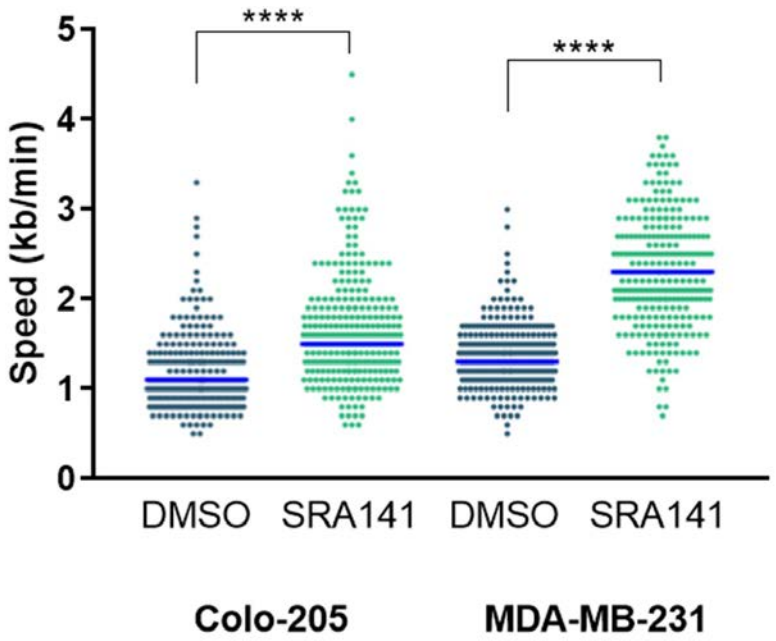
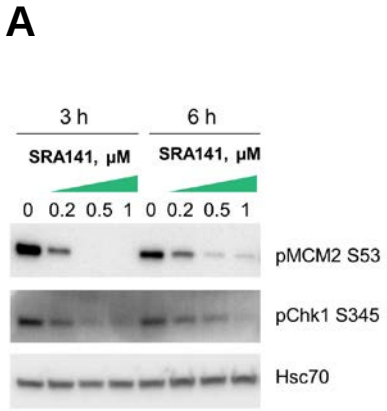


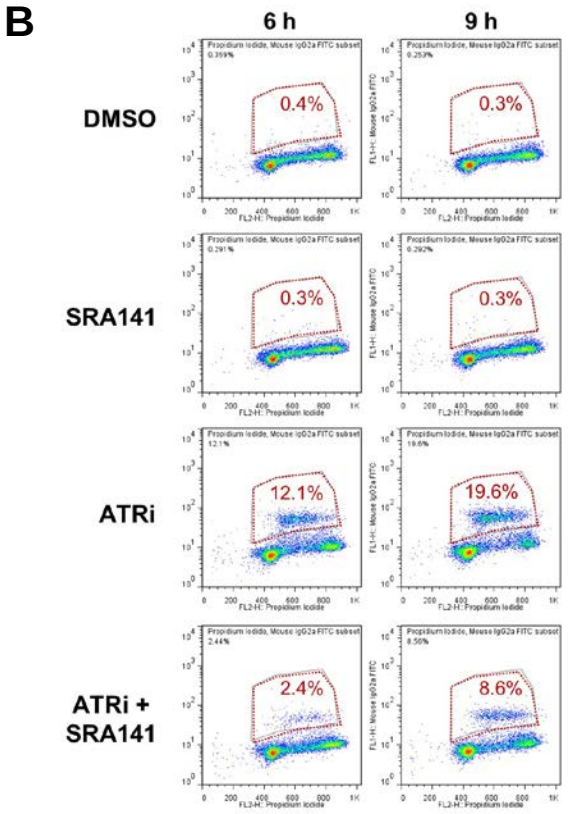
(A) Treatment scheme for DNA combing assay.

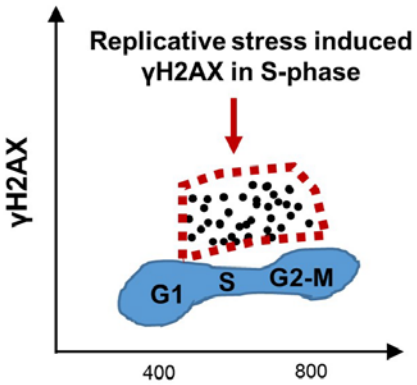
(B) SRA141 treatment led to an increase in replication fork speed. As MCM2 phosphorylation is essential for origin firing, increased speed is likely a compensatory mechanism for reduced origin firing resulting from Cdc7 inhibition and subsequent decrease of MCM2 phosphorylation.

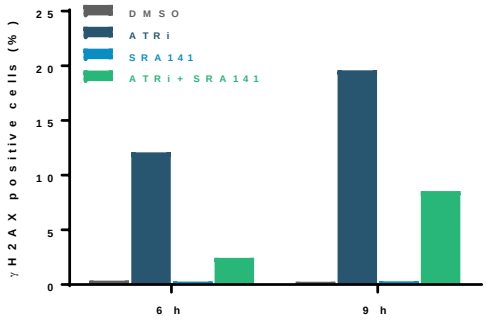
Dark blue line represents median
**** $p<0.0001$.

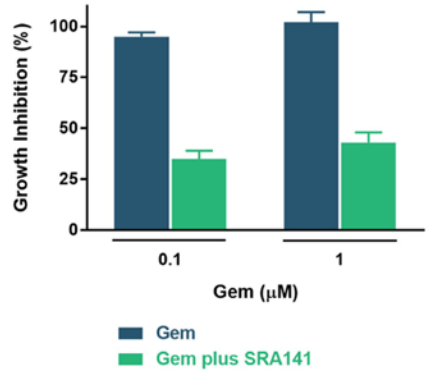
SRA141 abrogates intrinsic replicative stress (RS) and is antagonistic to RS inducers









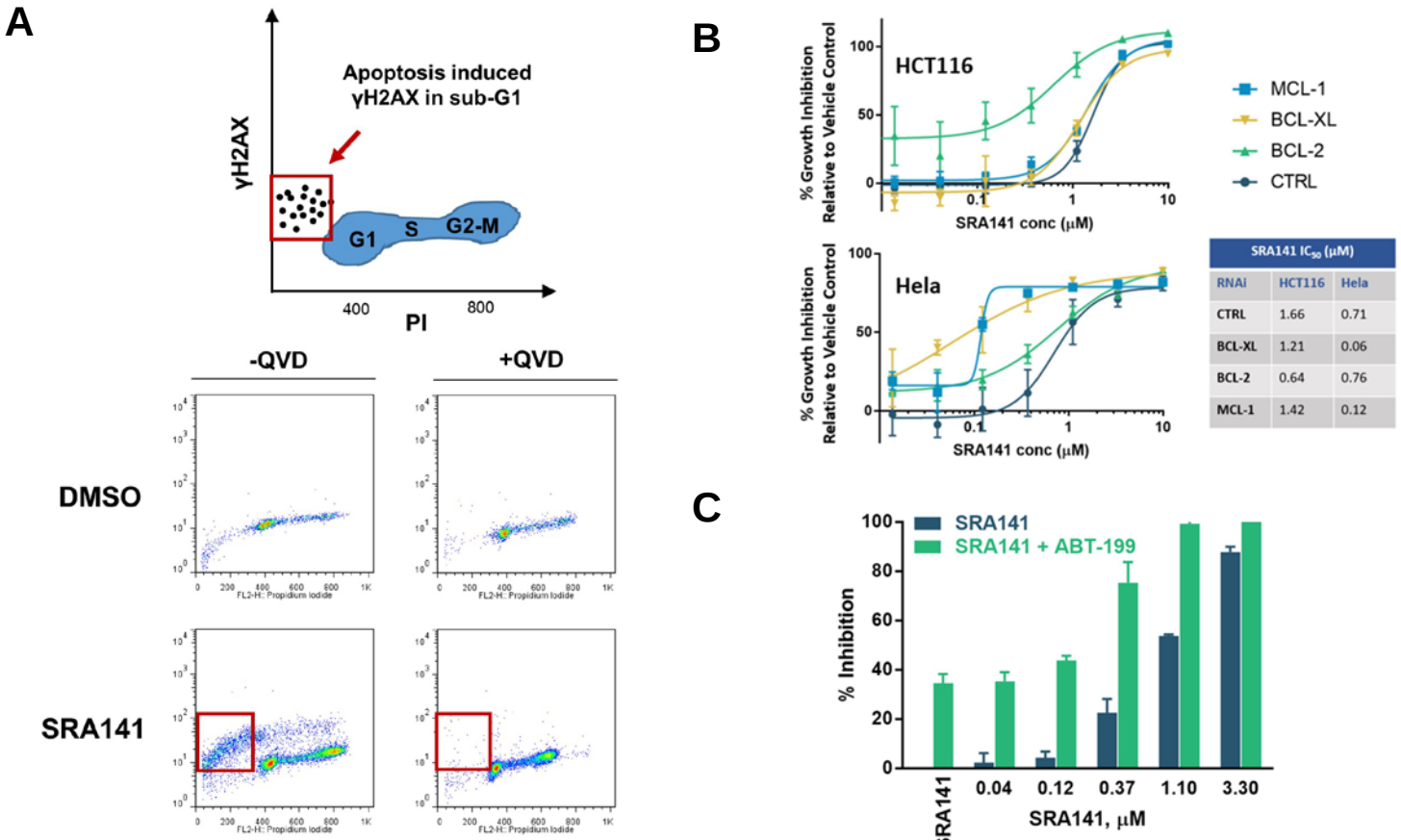


(A) SRA141 treatment led to a reduction in levels of RS marker pChk1 S345 in Colo-205 cells. The level of pMCM2 S53 was also reduced consistent with Cdc7 inhibition, while housekeeping protein Hsc70 remained unchanged.

(B) The ATR inhibitor (VE822; 2 µM) was used to induce RS (γH2AX in S-phase) in MDA-MB-231 cells in the presence or absence of SRA141. SRA141 treatment did not induce γH2AX on its own and was antagonistic to the VE822-mediated induction of γH2AX, suggesting a novel MOA that is distinct from that of DDR targeting agents (such as ATR, Chk1 and WEE1).

(C) HT-29 cells were treated for 96 h with the RS-inducing chemotherapy, gemcitabine, resulting in near 100% growth inhibition. Addition of SRA141 (1 µM) for the last 72h substantially reduced the growth inhibitory effects of gemcitabine further supporting its antagonism of RS-inducing agents.

SRA141 induces caspase-dependent apoptosis and synergizes with BCL-2 inhibition



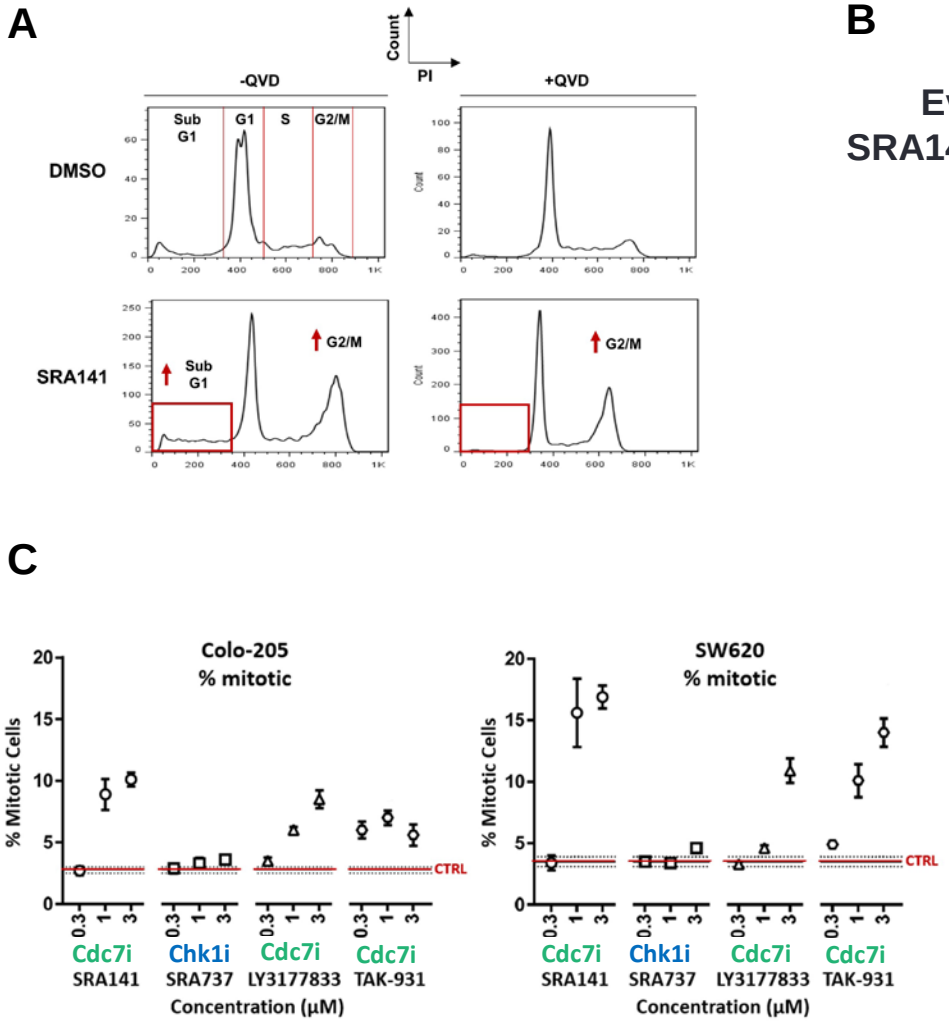
SRA141 IC_{50} (μM)		
RNAi	HCT116	Hela
CTRL	1.66	0.71
BCL-XL	1.21	0.06
BCL-2	0.64	0.76
MCL-1	1.42	0.12

(A) Model depicts flow cytometry analysis of γH2AX as a function of cell cycle (PI) in order to distinguish source of γH2AX as either apoptosis (sub G1) or replication stress (S-phase). Colo-205 cells were incubated with SRA141 (500 nM; 24h) in the presence or absence of a pan-caspase inhibitor, QVD (20 μM), to block apoptosis. The disappearance of sub-G1 γH2AX with QVD treatment indicates that DNA damage induced by SRA141 is a consequence of apoptosis.

(B) RNAi knockdown of BCL-2 family anti-apoptotic genes synergizes with SRA141. Cells were treated with control (CTRL), BCL-XL, BCL-2 and MCL-1 RNAi for 24h, followed by treatment with SRA141 for 72h and cell viability assessment.

(C) Further support for caspase-dependent apoptosis was demonstrated by the synergy of the BCL-2 inhibitor, ABT-199, and SRA141 in MOLM-13 cells following 72h treatment. Combination indices (Bliss Independence analysis) were calculated; CI scores < 0.7 indicate synergy, while CIs between 0.7 and 1.3 correspond to additive effects.

SRA141-mediated cell death coincides with mitotic markers of G2/M and accumulation of cells in mitosis



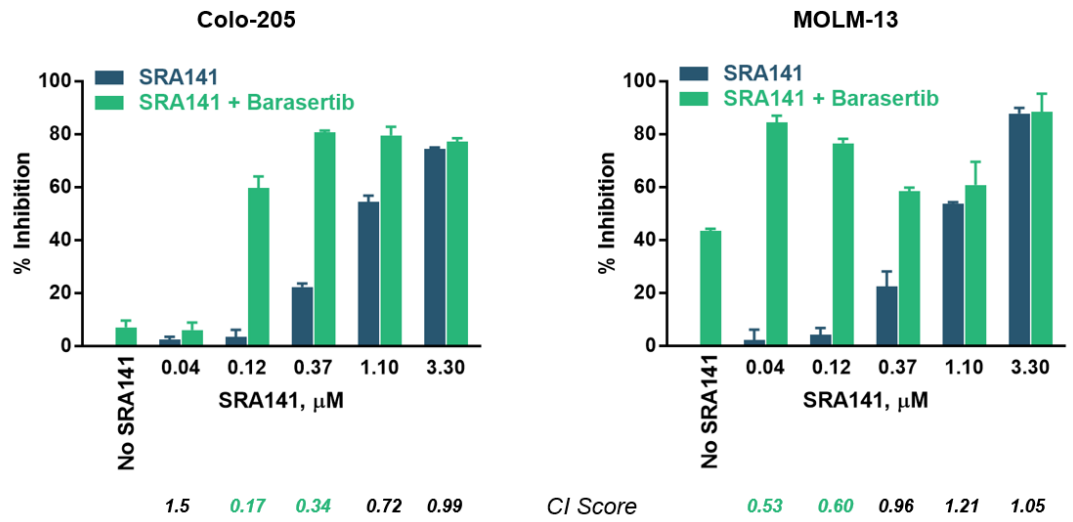
(A) Colo-205 cells were treated with SRA141 (500 nM; 24h) in the presence or absence of QVD (20µM) and analyzed by flow cytometry. SRA141 led to a substantial accumulation of cells in G2/M, which was accompanied by cell death as evidenced by the accumulation of cells in sub G1. QVD eliminated sub G1 accumulation indicating an apoptotic mechanism of cell death. In contrast, the mitotic accumulation was caspase-independent as it was observed in the presence and absence of QVD.

(B) SRA141 treatment in Colo-205 cells led to an increase of the mitotic marker, Cyclin B, consistent with accumulation of cells in mitosis and a DNA damage marker, γH2AX, consistent with apoptosis. pMCM2 S53 levels were also reduced confirming Cdc7 inhibition.

(C) Cells were treated for 48h with Cdc7 inhibitors or a CHK1 inhibitor as a negative control, and the percentage of mitotic cells was determined by immunocytochemistry. All three Cdc7 inhibitors led to a concentration-dependent accumulation of cells in mitosis, while Chk1 inhibition had no effect.

SRA141 demonstrates synergistic activity in combination with a small molecule Aurora B kinase inhibitor

A



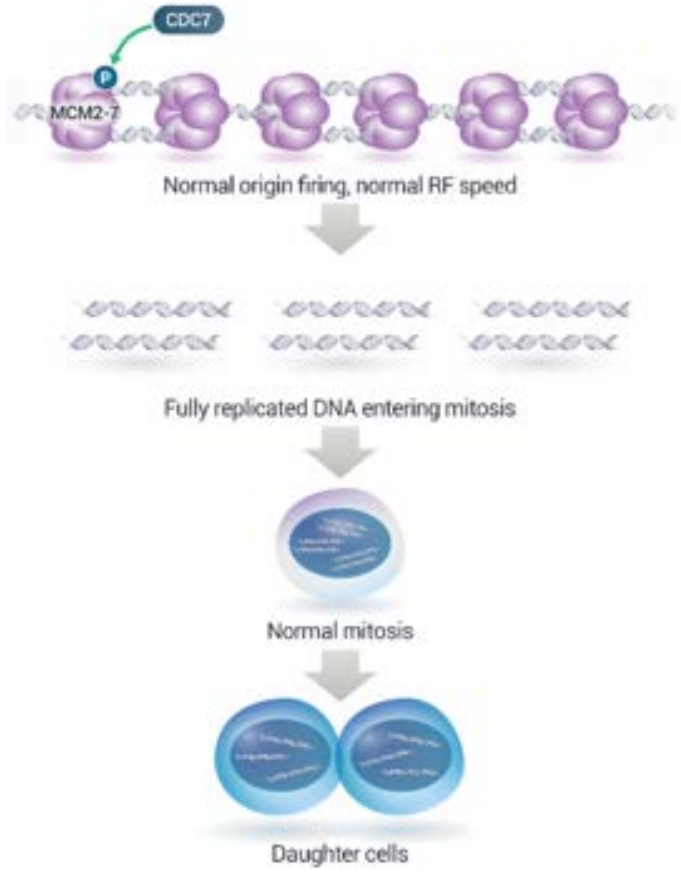
(A) SRA141 demonstrates synergistic activity in combination with a small molecule Aurora B kinase inhibitor. Based on the observed SRA141-mediated accumulation of cells in mitosis, we tested whether SRA141 cytotoxicity could be enhanced by barasertib, an agent that further disrupts mitosis. Cells were treated with 10 nM barasertib in the presence of SRA141 for 72h and cell viability was determined. SRA141 was highly synergistic with barasertib, reinforcing its novel MOA and potential clinical utility in combination with certain mitotic disruptors.

Proposed Model for Cdc7i-mediated Tumor Cell Death

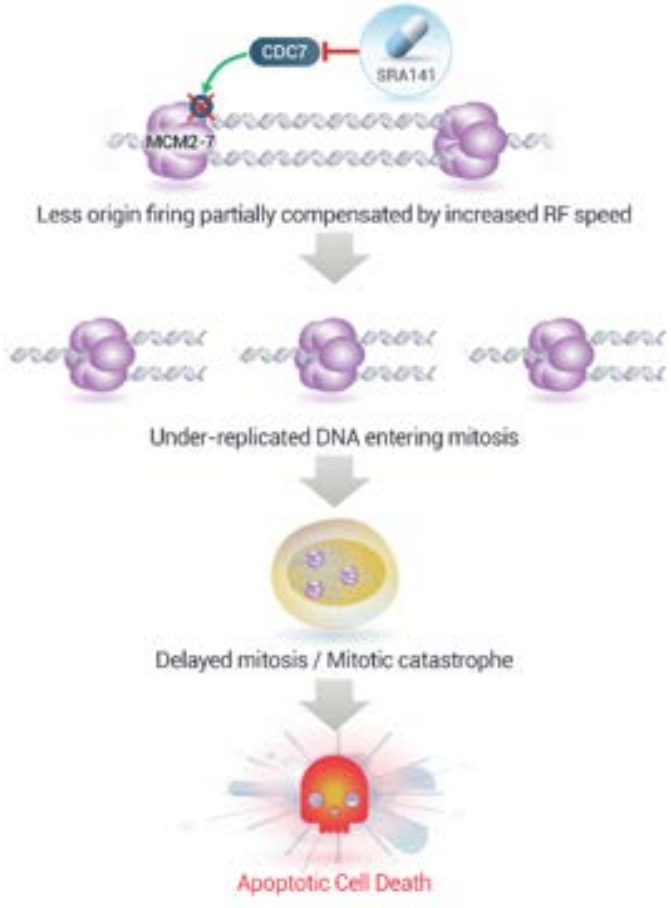
Unique Cdc7i-mediated cell death driven by mitotic catastrophe

- During S-phase, the serine-threonine kinase Cdc7 is responsible for initiating DNA replication by phosphorylating MCM2 at replication origins throughout the genome
- Inhibition of Cdc7 in tumor cells results in reduced origin firing, and premature mitosis with under-replicated DNA, leading to apoptosis
- In contrast, inhibition of Cdc7 in healthy non-transformed cells activates a transient p53-dependent cell cycle arrest to avoid apoptosis (not shown)

Normal Cdc7 Function in DNA Replication



Inhibition of Cdc7 with SRA141



Summary

- 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 increase 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 S-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.

Conclusions

Conclusions

- Previous studies demonstrated that SRA141 potently and selectively inhibits Cdc7, resulting in robust anti-tumor efficacy in colorectal xenograft models, however the mechanism of action had not been characterized
- Our findings reveal a potentially **novel mechanism of cytotoxicity** for Cdc7 inhibitors that is distinct from other agents, and thus SRA141 may herald a **new class of cancer therapeutic agents** with a differentiated anti-tumor profile
    - SRA141 does not induce G1 cell cycle arrest or replication stress, thereby distinguishing it from drugs like palbociclib or SRA737. Rather, SRA141 **alters DNA replication dynamics** and **delays cell cycle progression**, ultimately resulting in **caspase-dependent cell death** associated with mitotic accumulation
    - Promisingly, this mechanism appears to synergize with anti-apoptotic drugs, such as venetoclax and dysregulators of mitosis, like barasertib
    - This differentiated mechanism of action supports a potentially unique spectrum of clinical deployment opportunities for SRA141 as both monotherapy as well as in combination with pro-apoptotic and mitotic disrupting agents
- An Investigational New Drug Application (IND) filing has been accepted by the U.S. Food and Drug Administration (FDA) for SRA141, and Sierra has prepared for a potential Phase 1/2 trial of the drug candidate in patients with advanced colorectal cancer. Sierra is currently evaluating the optimal timing to commence this trial within the context of our recently expanded portfolio
- Sierra Oncology retains the global commercialization rights to SRA141

Targeted Hematology and Oncology Therapeutics

We are a clinical stage drug development company advancing targeted therapeutics for the treatment of patients with unmet medical needs in hematology and oncology

- Bold drug development company oriented to registration and commercialization
- Lead asset, momelotinib, for the treatment of myelofibrosis with large 2nd-line market opportunity
- Two assets focused on DNA Damage Response (DDR) targeting: SRA737 and SRA141
- Highly experienced management team with proven track record in drug development
- Strong financial standing:
  - Shares (as of December 31):
    - 74.4M outstanding
    - 84.9M fully diluted
  - $106.0M in cash and cash equivalents (as of December 31)
  - $5M borrowed in structured debt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