

# CX-5461 a Novel, Orally Bioavailable and Selective Small Molecule Inhibitor of RNA Polymerase I Transcription Induces Autophagy and Shows Potent Anti-tumor Activity

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## Abstract

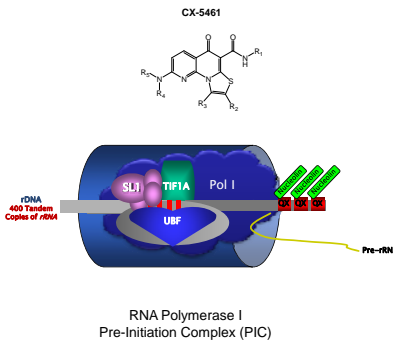
Cancer is a disease of uncontrolled proliferation. The rate of cellular growth and proliferation is directly proportional to the rate of ribosome synthesis. The rate-limiting regulatory process in ribosome formation is transcription of the ribosomal RNA (rRNA) genes in ribosomal DNA (rDNA) by RNA Polymerase I (Pol I). Pol I transcription is initiated by SL1, a five-subunit protein complex that together with UBF anchors Pol I to rDNA promoter and is required for specific initiation of rRNA synthesis. Knock down of SL1 subunit expression inhibits rRNA synthesis. Mitotic silencing of rRNA synthesis occurs through inactivation of SL1. In addition, tumor suppressors p53, Rb, and PTEN are often lost during tumorigenesis and have been shown to control rRNA synthesis by interfering with SL1 function. Collectively these findings underscore the importance of SL1 and Pol I function in regulating cell proliferation via initiation of rRNA synthesis. It follows that inhibitors of the SL1/Pol I complex may be effective anticancer agents.

We employed a nuclear lysate-based cell-free system to identify selective Pol I inhibitors. CX-5461 was identified as a potent inhibitor of Pol I that exhibited more than ten-fold selectivity against Pol I versus RNA Polymerase II (Pol II). Further characterization of CX-5461 in cell culture confirmed potent inhibition of Pol I and showed antiproliferative activity with  $IC_{50}$  < 100 nM for multiple cell lines. qRT-PCR analysis of pancreatic carcinoma MIA PaCa-2 and melanoma A375 cells treated with CX-5461 demonstrated that CX-5461 inhibited rRNA synthesis with  $IC_{50}$  50-100 nM and exhibited ~200-fold selectivity over inhibition of Pol II transcription. Order of addition studies demonstrated that CX-5461 acts at the initiation step of Pol I transcription. ChIP and EMSA studies showed that CX-5461 interferes with SL1 function by disrupting SL1-rDNA promoter interaction. In vitro mechanism of action studies indicate CX-5461 induces autophagy. CX-5461 shows oral bioavailability in multiple species and demonstrated significant anti-tumor efficacy in xenografts.

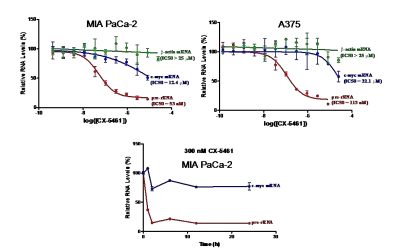
CX-5461 is a first in class agent designed to selectively inhibit Pol I transcription and represents a molecularly targeted approach to selectively kill cancer cells by halting the production of excess ribosomes and inducing autophagic cell death. The preclinical data support the development of CX-5461 as an anticancer drug with potential for activity in many types of cancer.

## RNA Polymerase I as a Cancer Target

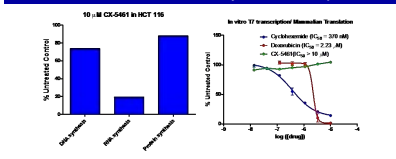
- Transformed cells over-express the rRNA products of Pol I
- Pol I is essential for ribosome biogenesis therefore essential for growth and proliferation
- Mutations affecting ribosome biogenesis directly alter differentiation and cancer susceptibility



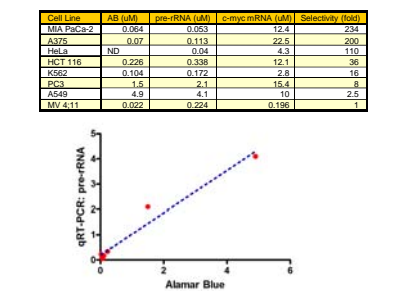
## CX-5461 Rapidly and Selectively Inhibits RNA Polymerase I (rRNA Transcription) vs Polymerase II (mRNA transcription)



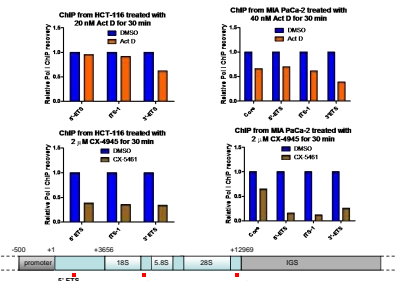
## CX-5461 Does Not Affect DNA and Protein Synthesis in Mammalian Cells and has No Effect on Viral RNA Polymerase Transcription



## Potency in inhibition of rRNA Synthesis by CX-5461 Correlates with its Activity in Alamar Blue Assay



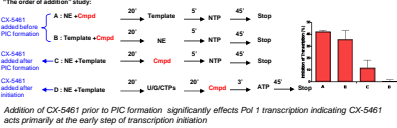
## CX-5461 does Not Block Pol I Transcription Elongation in the Cells



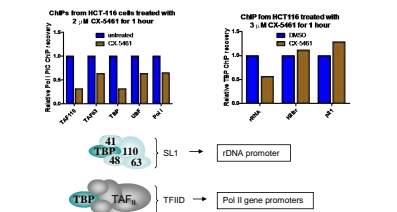
**Ribosomal DNA gene unit:**

RNAPI I loading was determined by ChIP. Consistent with the fact that Actinomycin D blocks transcription elongation, the reduction of RNAPI I loading is more pronounced at the 3' than at the 5' end of the transcribed region. In contrast, upon CX-5461 treatment RNAPI I loading is reduced to the similar level throughout the transcribed region, suggesting that CX-5461 does not block Pol I transcription elongation in the cells.

## Which Step of Transcription is Inhibited by CX-5461?

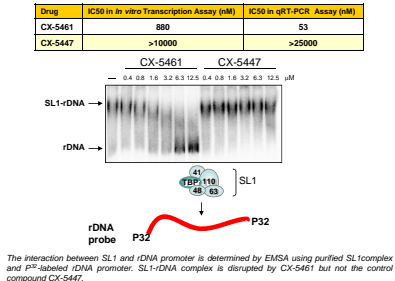


## CX-5461 Effects RNA Polymerase I PIC Assembly but has No Effect on Occupancy of TBP on RNA Polymerase II Promoters

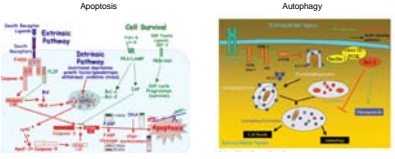


The occupancies of SL1 and TFIID at their corresponding promoters, the rDNA promoter and the promoters of Pol II-transcribed genes, are determined by ChIP. Here we show that CX-5461 at concentration of 2 μM disrupts the association of SL1 with the rDNA promoter but not the association of TFIID with histone H2B and pC1 gene promoters.

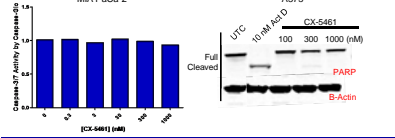
## CX-5461 Inhibits SL1-rDNA Promoter Interaction In Vitro



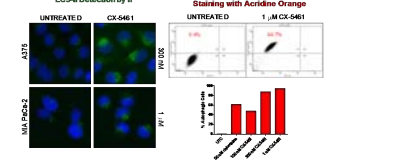
## Apoptosis Versus Autophagy



## CX-5461 Does Not Induce Apoptosis After 24 Hour Treatment



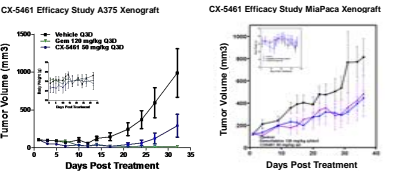
## CX-5461 Induces Autophagy After 24 Hour Treatment



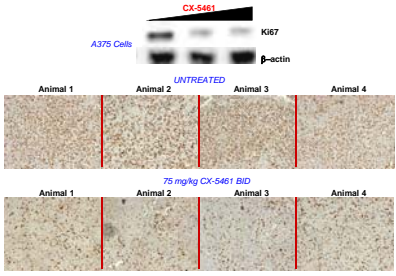
## CX-5461 PK in Multiple Preclinical Species

Species	Dose (mg/kg)	Route	CL <sub>50</sub> (L/h/kg)	V <sub>d</sub> (L/kg)	Terminal T <sub>1/2</sub> (h)	t <sub>1/2</sub> (h)
Mouse	5	IV	0.02	0.238		33.6
Mouse	25	PO				
Rat	5	IV	0.2	2.0	7.7	
Rat	10	PO			7.8	49.9
Dog	5	IV	2.1	27.8	9.1	
Dog	5	IV	1.6	20.9	8.9	
Dog	15	PO			13.5	35.8
Dog	15	PO			7.6	38.2
Monkey	5	IV	1.8	33	12.8	
Monkey	10	PO			13.6	45.1

## CX-5461 Anti-tumor Activity



## CX-5461 Causes Dramatic Reduction of Ki67 Expression in A375 Cell Culture and A375 Mouse Xenografts



## Summary

- CX-5461 selectively inhibits RNA Pol I transcription, PIC assembly and SL1-rDNA interaction
- CX-5461 shows potent antiproliferative activity in cancer cells
- CX-5461 induces autophagy in cancer cells
- CX-5461 shows potent antitumor activity in A375 and MIA PaCa xenografts
- CX-5461 is orally bioavailable in multiple preclinical species