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An oral and selective CDK12 inhibitor demonstrates robust anti-tumor activity

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Abstract

Background: CDK12 is an attractive cancer target due to its role in transcription and DNA damage repair regulation. Here we profiled a new oral and selective small-molecule CDK12 inhibitor. In preclinical models this compound has demonstrated promising antitumor activity especially in combination with DNA damaging agents.

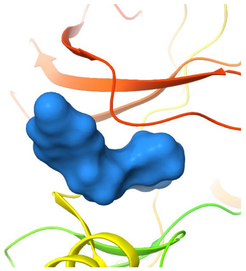
Material and methods: ADP-glo™ assays, Kinome screen and Immunofluorescence (IF) assays see figure legends. Cell proliferation was determined using cell titer glo after 48-72hrs compound treatment. Apoptosis was measured by annexin V/PI staining and flow cytometry analysis after 24-72hrs of treatment. Cell cycle profile was evaluated with Click-iT-EdU, pHH3 staining and FxCycle violet stain and flow cytometry analysis following 24hrs of treatment. Mouse xenograft study see figure legends.

Results: A series of CDK12 inhibitors were designed and profiled in biochemical and cellular assays. A representative member of the class, compound A, exhibited selectivity over CDK2, CDK7, and CDK9 of 46-, 27-, and 9-fold, respectively. Compound A inhibited proliferation in a panel of cell lines with EC_{50} in the low nanomolar range. Compound A treatment led to dose dependent apoptosis in multiple cancer cell lines and induced G2/M arrest in cancer cell lines. In vitro, combination treatment with compound A and lurbinectedin or olaparib led to increased DNA damage accumulation, decreased homologous recombination repair and overall enhanced antiproliferative effect. Dose dependent tumor growth inhibition was observed in multiple cell line derived mouse xenograft models with Compound A treatment. Enhanced and durable antitumor effect was observed with lurbinectedin and compound A combination compared to single agent treatment in vivo. Compound A in combination with Olaparib treatment led to enhanced anti tumor efficacy in a PARPi resistant patient derived xenograft model.

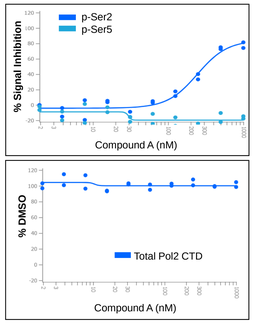
Conclusions: We designed and profiled orally available, CDK12 selective inhibitors with potent activity as single agent in vitro and in vivo in multiple cancer models. Compound A demonstrated enhanced antitumor effect in vitro and in vivo when combined with DNA damaging agents. Compound A can sensitize PARPi resistant model to Olaparib treatment in vitro and in vivo. These data support the rationale for advancing one or more members of this class toward clinical development.

Compound A is a potent and selective oral CDK12 inhibitor

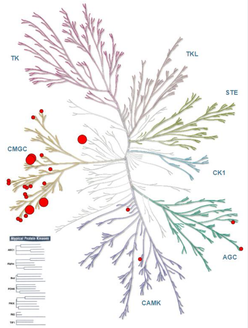
A CDK12 Crystal Structure with Compound A



C RNA pol II Ser2, Ser5 phosphorylation and total RNA pol II level change upon Compound A treatment



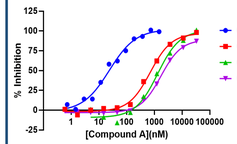
D Compound A profile in SelectScreen panel



Large Circle = > 90% inhibition @ 1 uM <100 nM est. IC50

Small Circle = > 50% inhibition @ 1 uM (100-1000 nM est. IC50)

B ADP-Glo™ Kinase Assay @ 20x K_M ATP

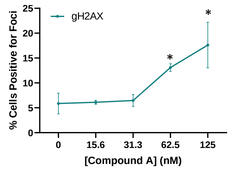


Activity	
ADP-Glo™ Activity Assay IC50@ 20x K_M ATP (nM) CDK12|CDK2|CDK7|CDK9	23.9 | 1737| 1321| 715
RNA Pol2 Inhibition (nM) p-Ser2 | p-Ser5	280 | >1000

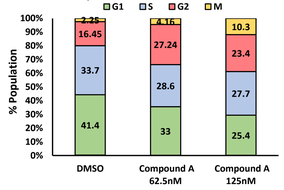
A. CDK12 crystal structure with compound A. **B**. Inhibition of recombinant CDK12/CyclinK, CDK2/CyclinE, CDK7/CyclinH/MAT1 and CDK9/CyclinT2 with compound A was measured by assessing ADP converted from ATP via luminescent signal at 20x K_M ATP using the ADP-Glo™ kit. **C**. RNA pol II Ser2, Ser5 phosphorylation change and total RNA pol II level upon 4hr compound A treatment in immunofluorescent assay in H1048 cell line. **D**. Compound A was profiled in duplicate against 370 kinases at 10 uM ATP in the radiometric HotSpot™ assay.

Compound A treatment induces DNA damage, cell cycle dysregulation and genomic instability ultimately leading to growth inhibition and apoptosis in cancer cells

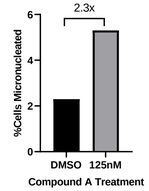
A Compound A treatment (24hr) leads to DNA damage accumulation in H1048 cells



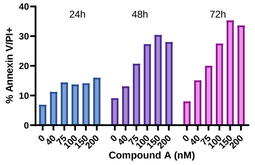
B Compound A treatment (24hr) leads to G2 and M phase arrest in H1048 cell line



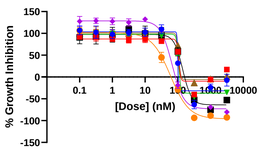
C Compound A treatment (24hr) leads to micronuclei accumulation in H1048 cells



D Compound A treatment induces apoptosis in H1048 cells



E Compound A treatment leads to antiproliferative effect in multiple cancer cell lines

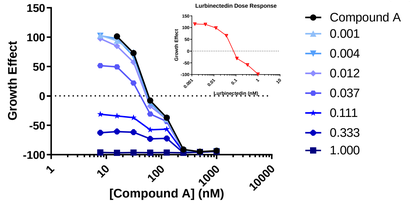


Cell Line	IC50 nM
A673	100
A2780	106
MB-231	106
MB-468	67
H1048	60
DMS-53	141
LN299	107

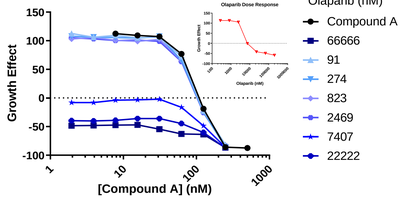
A. DNA damage accumulation measured by γH2AX IF signal in H1048 cells treated with Compound A for 24hrs. **B**. cell cycle analysis in H1048 cells treated with Compound A for 24hrs. **C**. Percentage of cells positive of micronuclei in H1048 cells treated with Compound A for 24hrs. **D**. Apoptosis induction in H1048 cells treated with Compound A. **E**. Cellular antiproliferative effect of Compound A in a panel of cancer cell lines.

Compound A shows synergistic/additive anti-proliferative effects in combination with lurbinectedin and olaparib with increased DNA damage and impaired DNA damage repair

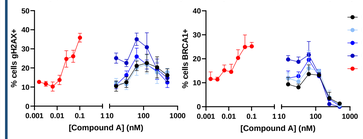
A Lurbinectedin + Compound A Combination in H1048 (3 day)



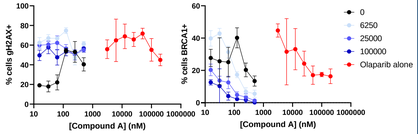
B Olaparib + Compound A combination in MDA-MB-468 (5 day)



C H1048 gH2AX Signal Compound A + lurbinectedin (24hr) / H1048 BRCA1 Signal Compound A + lubinectedin (24hr)



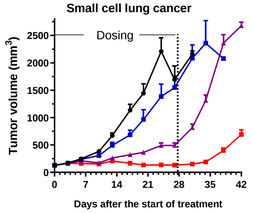
D MDA-MB-468 gH2AX signal Compound A + olaparib (24hr) / MDA-MB-468 BRCA1 signal Compound A + olaparib (24hr)



A, B. Growth inhibition of A) H1048 cells treated with Compound A, lurbinectedin or combination after 72hrs and B) MDA-MB-468 cells treated with Compound A, olaparib or combination after 120hrs. **C**. DNA damage accumulation measured by γH2AX foci signal (left) and homologous recombination repair measured by BRCA1 foci signal (right) in H1048 cells treated with Compound A, lurbinectedin or combination for 24hrs. **D**. DNA damage accumulation measured by γH2AX foci signal (left) and homologous recombination repair measured by BRCA1 foci signal (right) in MDA-MB-468 cells treated with Compound A, olaparib or combination for 24hrs.

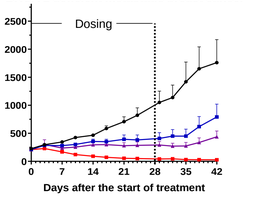
CDK12 inhibition demonstrates strong tumor growth inhibition and is well tolerated in multiple CDX models

H1048
Small cell lung cancer



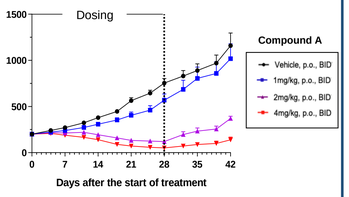
Group	TGI D24	BWC D28
1mg/kg	40%	+14%
2mg/kg	83%	-1%
4mg/kg	100%	-4%

MDA-MB-436
BRCA 1 deficient metastatic breast cancer



Group	TGI D28	BWC D28
1mg/kg	76%	+4%
2mg/kg	90%	+2%
4mg/kg	121%	-4%

MDA-MB-468
BRCA WT Triple negative breast cancer

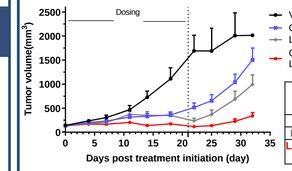


Group	TGI D29	BWC D28
1mg/kg	34%	+5%
2mg/kg	115%	+2%
4mg/kg	127%	-6%

Tumor growth inhibition in H1048, MDA-MB-436 and MDA-MB-468 CDX models with Compound A treatment. Balb/c mice were implanted subcutaneously with indicated cells and randomized for treatment with test drug or vehicle when tumors reached 150-200mm³. Tumor bearing mice were treated twice daily with vehicle or Compound A for 28 days and followed by 14 days observation. Minimum weight loss was observed in all three models. TGI: tumor growth inhibition. TGI (%) = (1 - ($TV_{Treatment/Dn}$ - $TV_{Treatment/D0}$)/ ($TV_{Control/Dn}$ – $TV_{Control/D0}$)) × 100%. BWC: body weight change.

Compound A + lurbinectedin shows enhanced, durable antitumor effect in H1048 model

H1048 Small cell lung cancer

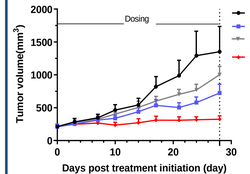


Group	TGI D18	BWC D21
Compound A	77%	+2%
Lurbinectedin	78%	+3%
Lurbinectedin + Compound A	96%	+2%

Tumor growth inhibition in H1048 SCLC CDX model with Compound A and lurbinectedin treatment. Balb/c mice were implanted subcutaneously with H1048 cells and randomized for treatment with test drug or vehicle when tumors reached 100-150mm³. Tumor bearing mice were treated twice daily with vehicle or Compound A, once weekly with lurbinectedin or combination for 21 days and followed by 14 days observation. Minimum weight loss was observed.

Compound A + Olaparib shows enhanced antitumor effect in a PARPi resistant PDX model

ST3308 High-grade serous ovarian cancer



Group	TGI D28	BWC D28
Compound A	55%	+5%
Olaparib	30%	+12%
Olaparib + Compound A	91%	+7%

Tumor growth inhibition in ST3308 PDX model (homozygous mutation in BRCA1 with patient treatment history with olaparib) with Compound A and olaparib treatment. Athymic Nude, Outbred Homozygous (Crl:NU(NCr)-Foxn1^{nu}) mice were implanted subcutaneously with cells and randomized for treatment with test drug or vehicle when tumors reached 200mm³. Tumor bearing mice were treated twice daily with vehicle or Compound A, once daily with olaparib or combination for 28 days. Minimum weight loss was observed.

Conclusions

- **We have identified a potent, selective, orally available CDK12 inhibitor compound A.**
- **Compound A demonstrated strong antiproliferative effect in a panel of cells, inducing DNA damage accumulation, cell cycle arrest and apoptosis as single agent.**
- **Compound A showed enhanced antiproliferative effect in vitro when combined with DNA damaging agent lurbinectedin or Olaparib with increased DNA damage and impaired DNA damage repair machinery.**
- **Compound A showed robust and dose dependent antitumor effect in multiple cancer cell line derived xenograft models with tolerated doses.**
- **Compound A showed enhanced and durable antitumor effect in vivo when combined with lurbinectedin compared to single agent treatment.**
- **Compound A sensitized a PARPi resistant patient derived xenograft model to olaparib treatment in vivo.**
- **These data support the rationale for advancing one or more members of this class toward clinical development.**

Disclosures:
All authors: Syros Employment and stock ownership