Abstract # 96

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An oral and selective CDK7 inhibitor demonstrates substantial anti-tumor effect in breast and ovarian cancer models

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Abstract

Background: CDK7 has emerged as an attractive cancer target due to its role in transcriptional control and cell cycle regulation, and demonstration of tumor cell killing in pre-clinical models with small molecule inhibitors. SY-1365 is an IV administered CDK7 inhibitor and currently in phase I clinical study (NCT03134638). Here we profile new oral and selective CDK7 inhibitors.

Material and methods: CDK2,7,9, and 12 inhibition assays: Each enzyme was incubated with a fluorescently-labelled peptide substrate, 2 mM ATP, and an inhibitor, with product conversion measured by Perkin Elmer LabChip EZ Reader II. SPR assay: CDK7/cyclin H dimer was immobilized on a CM5 chip and each compound was titrated over the immobilized protein and response units used to determine K_d, off- and on-rates. CDK7 occupancy assay: Cells were treated with compounds for 1hr, lysed, and incubated with biotinylated small molecule probe to pull down free CDK7, and total and unoccupied CDK7 quantitated. Cellular assays: Cell lines were incubated with compounds for 72hrs and cell number determined using CyQUANT™ Direct Cell Proliferation Assay kit. Cells were stained for annexin V and PI and analyzed by flow cytometry to assess apoptosis after 48hrs of treatment. Cells were fixed and stained with FxCycle violet stain and analyzed by flow cytometry to assess cell cycle following 48hrs of treatment. Mouse xenograft: balb/c mice were implanted subcutaneously with HCC70 cells or patient-derived breast cancer cells and randomized for treatment with test drug or vehicle when tumors reached 150-200mm^3. Mice were dosed BID through oral administration for 3 weeks.

Results: A series of CDK7 inhibitors were designed and profiled in biochemical assays and tumor cell lines. Analysis of 467 compounds revealed a correlation between CDK7 K_d, CDK7 occupancy (EC_{50}) and cell growth inhibition (EC_{50}). A representative member of the class, SY-5102, exhibited selectivity over CDK12, CDK9, and CDK2 of 236-, 1174-, and 1202-fold, respectively. In addition, SY-5102 inhibited proliferation of triple negative breast cancer (TNBC) and ovarian (OVA) cells, with EC_{50} in the low nanomolar range. SY-5102 induced apoptosis in a dose-dependent manner in multiple TNBC and OVA cell lines and also induced G2/M arrest. Strong tumor growth inhibition in breast cancer CDX and PDX models was observed when SY-5102 was dosed orally at 4mg/kg BID.

Conclusions: We designed and profiled orally available CDK7 selective inhibitors with potent activity against TNBC and OVA cells and induced tumor growth inhibition in breast cancer cell and patient derived xenograft models. These data support the rationale for advancing one or more members of this class toward clinical development.

SY-5102 is a selective oral CDK7 inhibitor

Fig. 1

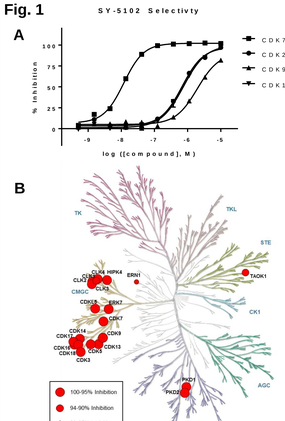


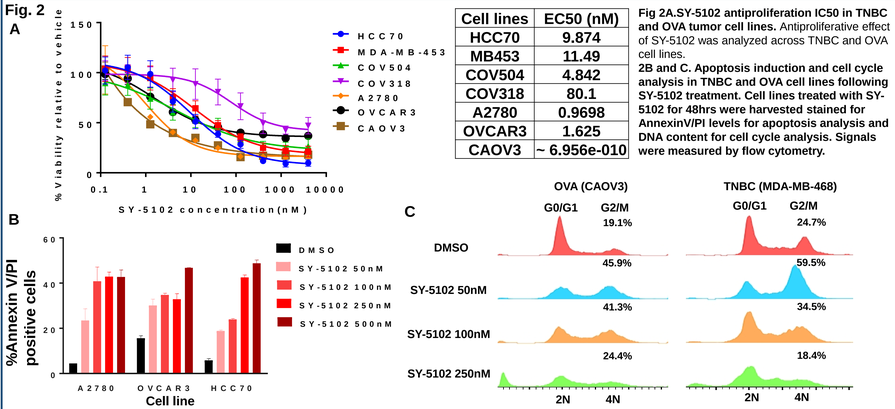
Table 1

	IC$_{50}$ (nM)	
	SY-5102	Dinaciclib
CDK7/CycH/MAT1	0.042 *	222.7
CDK16/CycY	12.86	174
CDK12 R722C/CycK	14.26	7.152
CDK12wt/CycK	21.4	7.874
CDK13/CycK	46.77	35.99
CDK9/CycK	64.04	5.223
CDK9/CycT1	71.64	3.72
CDK3/CycE1	122.7	16.91
CDK2/CycE1	142.7	14.66
CDK5/p35NCK	153.4	8.821
CDK1/CycE1	219.2	57.18
CDK5/p25NCK	284.9	19.91
CDK2/CycA2	328.4	23.01
CDK3/CycC	421.1	290.7
CDK17/p35NCK	467.8	1332
CDK1/CycA2	508.5	51.13
CDK4/CycD2	560.6	58.02
CDK2/CycD1	576.4	75.4
CDK4/CycD1	765.4	65.07
CDK19/CycC	905.4	1450
CDK1/CycB1	955.5	57.54
CDK4/CycD3	1391	121.7
CDK8/CycC	1490	3180
CDK6/CycD1	1908	35.51
CDK6/CycD2	4264	352.6
CDK6/CycD3	5375	751.4
CDK20/CycH	5762	5711
CDK20/CycT1	8335	4379
Selectivity over closest off-target:	306-fold	2-fold

Fig 1A. SY-5102 is potent against CDK7/Cyclin H/MAT1 and selective over CDK2, CDK9, and CDK12. For each CDK, the inhibition of CDK activity was determined with 2mM ATP.
1B. SY-5102 was profiled in the SelectScreen panel of 485 kinases (ThermoFisher). Kinases that were inhibited 85% or greater by 1 µM Compound A are displayed.
Table 1. SY-5102 was profiled in the CDK/Cyclin-IC50-Profiler of 28 CDK/Cyclin complexes (ProQinase). IC_{50} values were for each CDK with Compound A and control compound Dinaciclib are displayed.
*SY-5102 potency for CDK7/CycH/MAT1 is reported as SPR K_d as the IC_{50} is below the level of detection in this assay with 2.4 nM enzyme.

SY-5102 induces robust antitumor effects *in vitro*

Fig. 2

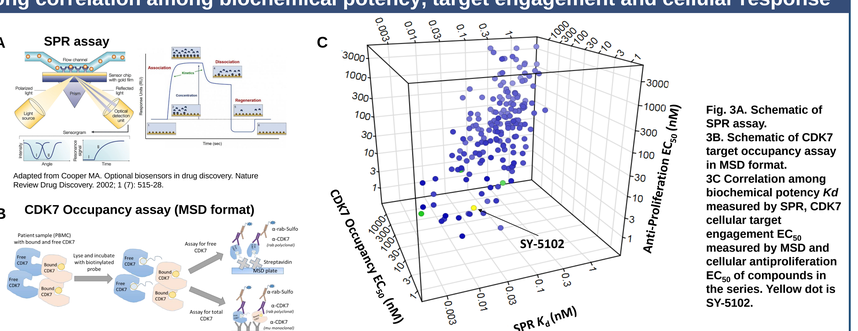


Cell lines	EC50 (nM)
HCC70	9.874
MB453	11.49
COV504	4.842
COV318	80.1
A2780	0.9698
OVCAR3	1.625
CAOV3	~ 6.956e-010

Fig 2A.SY-5102 antiproliferation IC50 in TNBC and OVA tumor cell lines. Antiproliferative effect of SY-5102 was analyzed across TNBC and OVA cell lines.
2B and C. Apoptosis induction and cell cycle analysis in TNBC and OVA cell lines following SY-5102 treatment. Cell lines treated with SY-5102 for 48hrs were harvested stained for AnnexinV/PI levels for apoptosis analysis and DNA content for cell cycle analysis. Signals were measured by flow cytometry.

Strong correlation among biochemical potency, target engagement and cellular response

Fig.3

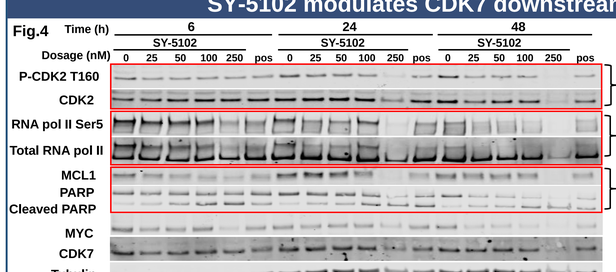


Adapted from Cooper MA. Optional biosensors in drug discovery. Nature Review Drug Discovery. 2002; 1 (7): 515-28.

Fig. 3A. Schematic of SPR assay.
3B. Schematic of CDK7 target occupancy assay in MSD format.
3C Correlation among biochemical potency *Kd* measured by SPR, CDK7 cellular target engagement EC_{50} measured by MSD and cellular antiproliferation EC_{50} of compounds in the series. Yellow dot is SY-5102.

SY-5102 modulates CDK7 downstream targets

Fig.4



Inhibition of T-loop phosphorylation Direct substrate

Inhibition of RNA Pol II CTD Ser5 phosphorylation Direct substrate

Apoptosis induction

Fig 4. Time and dose dependent molecular consequences of CDK7 inhibition. HCC70 cells were treated with SY-5102 with indicated doses, protein lysates were collected at indicated timepoints and various protein markers were assessed by immunoblotting.

Substantial antitumor effects observed in xenograft models

Fig. 5

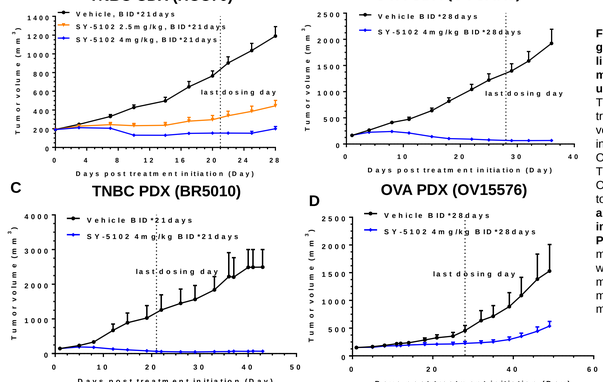


Fig.5 A and B. Tumor growth inhibition in cell line derived TNBC and OVA mouse xenograft models upon SY-5102 treatment. Tumor bearing mice were treated twice daily with vehicle, SY-5102 at 2 doses in TNBC model and 1 dose in OVA model for 21 days for TNBC model and 28 days for OVA models followed by up to 14 days observation. **5C and D. Tumor growth inhibition in TNBC and OVA PDX models.** Tumor bearing mice were treated twice daily with vehicle or SY-5102 (4 mg/kg) for 21 days for TNBC model and 28 days for OVA model.

Advanced leads for development candidate selection

Fig.6

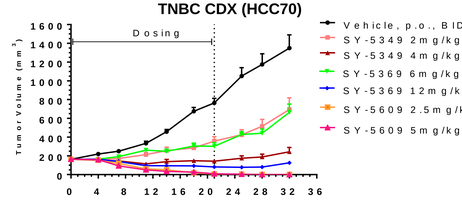


Table 3

	SY-5102	SY-5349	SY-5369	SY-5609
CDK7 K_d K_{off}	0.015 nM 0.0006 s^{-1}	0.0022 nM 0.0002 s^{-1}	0.014 nM/ 0.0005 s^{-1}	0.059 nM 0.0032 s^{-1}
CDK12 IC_{50} (fold over CDK7)	206 nM (188x)	3486 nM (23,305x)	1874 nM (2775x)	>10,000 nM (>2492x)
CDK2 IC_{50} (fold) CDK9 IC_{50} (fold)	710 nM (2329x) 2112 nM (2147x)	2366 nM (51,207x) 6157nM (41,312x)	2546 nM (7055x) 6233 nM (5637x)	>10,000 nM (>8068x) >10,000 nM (>2508x)

Fig.6. Tumor growth inhibition in cell line derived TNBC xenograft models upon three compounds treatment. Tumor bearing mice were treated twice daily with vehicle or indicated compounds at 2 doses each in TNBC model for 21 days followed by 14 days observation.
Table 3. Biochemical potency and selectivity profile of SY-5102 and three potential development candidates.

Conclusions

- A series of highly selective and orally available CDK7 inhibitors were discovered and profiled.
- Robust antiproliferative effects in ovarian and TNBC cell lines were seen and associated with induction of apoptosis and cell cycle arrest.
- There was a strong correlation among biochemical potency, CDK7 target engagement and cell growth inhibition which indicated CDK7-driven effects.
- Substantial anti-tumor effects were observed in multiple TNBC and OVA cell-line and patient-derived xenograft models.
- SY-5609 is progressing into IND-enabling studies.

Disclosures:
All authors: Syros employment and stock ownership