SY-5609, an orally available selective CDK7 inhibitor, demonstrates broad anti-tumor activity *in vivo*

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Abstract

Previously, we reported on a series of highly potent, selective, and non-covalent CDK7 inhibitors that demonstrated antiproliferative activity against triple-negative breast cancer (TNBC) and ovarian cancer (OVA) cell lines and tumor growth inhibition in cell line-derived (CDX) and patient-derived (PDX) mouse xenograft models. Here, we report on the *in vitro* and *in vivo* profile of our development candidate, SY-5609.

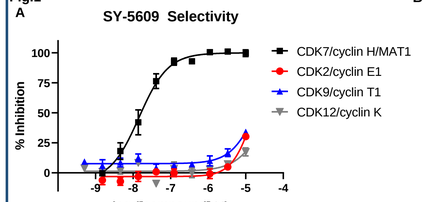
Methods: **Kinase inhibition assays at both K_m and 2 mM [ATP] were used to assess inhibition of CDK2, CDK7, CDK9, and CDK12. SPR was used to determine the K_d, k_{on}, and k_{off} binding characteristics of SY-5609 to immobilized CDK7/Cyclin H dimer. CDK7 compound occupancy was determined using a biotinylated small molecule probe to pull down free CDK7 following incubation of HL60 cells with SY-5609. Inhibition of tumor cell line growth was assessed following 72 hrs of incubation with SY-5609. Flow cytometry was used to assess apoptosis and cell cycle modulation after 48 hrs of treatment. Effects on DNA damage and repair were assessed by immunofluorescence staining for γH2AX and RAD51 proteins. To assess *in vivo* effects, mice were implanted subcutaneously and randomized for treatment when tumors reached 150-200 mm^3 and dosed orally for 3 weeks by both QD and BID dosing regimens. Collected tumor tissue samples were analyzed for protein levels of MCL1, pCDK2, MYC, and RNA Pol II CTD pSer5 by western blot.**

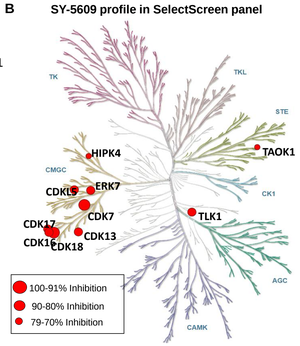
Results: **SY-5609 bound CDK7/Cyclin H with a K_d of 0.059 nM and occupied CDK7 in HL60 cells with an EC_{50} of 33 nM. Cell growth inhibition EC_{50} values were 6-17 nM in a panel of solid tumor cell lines. Selectivity of SY-5609 over CDK12, CDK9, and CDK2 was 13,000-, 16,000-, and 49,000-fold, respectively. SY-5609 led to induction of apoptosis, cell cycle arrest, and inhibition of DNA damage repair in tumor cell lines. Significant growth inhibition was observed in a panel of CDX and PDX solid tumor models with both QD and BID dosing of SY-5609 with resulting decreases in direct (pCDK2, RNA Pol II CTD pSer5) and indirect (MCL1, MYC) protein biomarkers.**

In summary, we describe SY-5609, an orally available, potent, and selective CDK7 inhibitor that drives robust TGI as well as inhibition of downstream CDK7 markers in CDX and PDX tumor models. These data support the rationale for advancing SY-5609 into IND-enabling studies.

SY-5609 is a potent and selective oral CDK7 inhibitor

Fig.1





Potency and selectivity over closely related CDKs

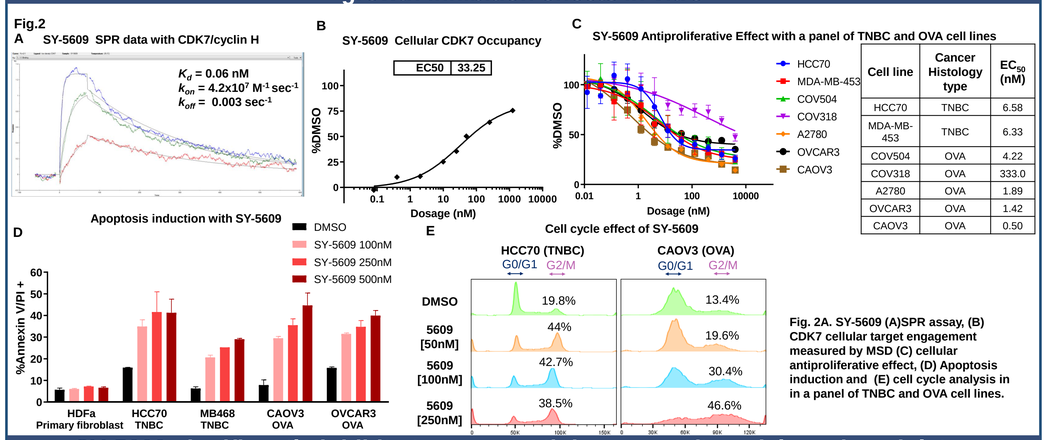
	Potency	Selectivity
CDK7	0.06 nM*	---
CDK2	2900 nM†	49,000x
CDK9	970 nM†	16,000x
CDK12	770 nM†	13,000x

* K_d determined by SPR
† K_i determined by activity assay

Fig 1A. SY-5609 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. SY-5609 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 6 nM enzyme.
1B. SY-5609 was profiled in the SelectScreen panel of 485 kinases (ThermoFisher). Kinases that were inhibited 70% or greater by 1 μM SY-5609 are displayed.

Biochemical potency of SY-5609 translates into cellular target engagement and robust growth inhibition effects *in vitro*

Fig.2



Cell line	Cancer Histology type	EC_{50} (nM)
HCC70	TNBC	6.58
MDA-MB-453	TNBC	6.33
COV504	OVA	4.22
COV318	OVA	333.0
A2780	OVA	1.89
OVCAR3	OVA	1.42
CAOV3	OVA	0.50

Fig. 2A. SY-5609 (A)SPR assay, (B) CDK7 cellular target engagement measured by MSD (C) cellular antiproliferative effect, (D) Apoptosis induction and (E) cell cycle analysis in in a panel of TNBC and OVA cell lines.

SY-5609 significantly inhibits tumor growth in xenograft models and modulates downstream markers of CDK7 activity

Fig.3

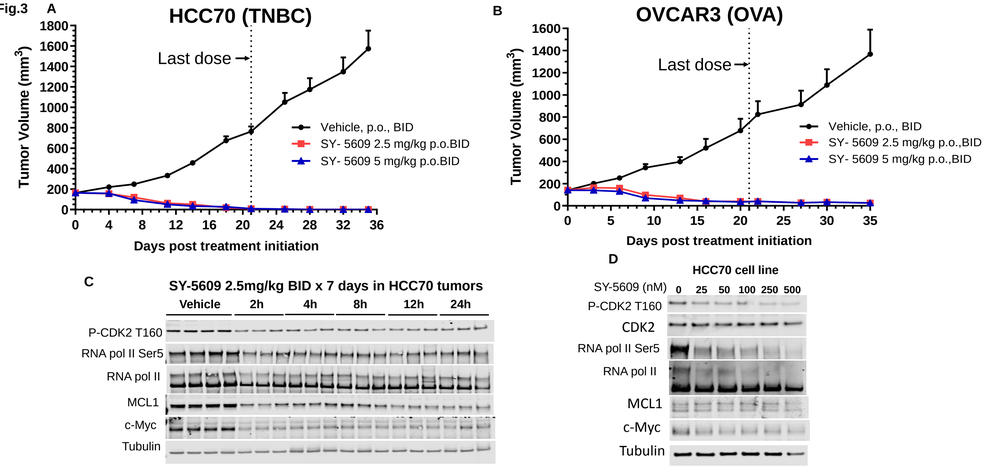


Fig.3 A and B. Tumor growth inhibition in cell line derived TNBC and OVA mouse xenograft models upon SY-5609 treatment. Tumor bearing mice were treated twice daily with vehicle, SY-5609 at 2 doses for 21 days and followed by 14 days observation. 3C. Tumors treated with SY-5609 BID after 7 days were harvested at indicated timepoint post last dose and various protein markers were assessed by immunoblotting. 3D. HCC70 cells were treated with SY-5609 with indicated doses for 48hrs and protein lysates were collected at indicated timepoints and various protein markers were assessed by immunoblotting.

SY-5609 demonstrates substantial tumor growth inhibition and is well tolerated in multiple PDX models

Fig.4

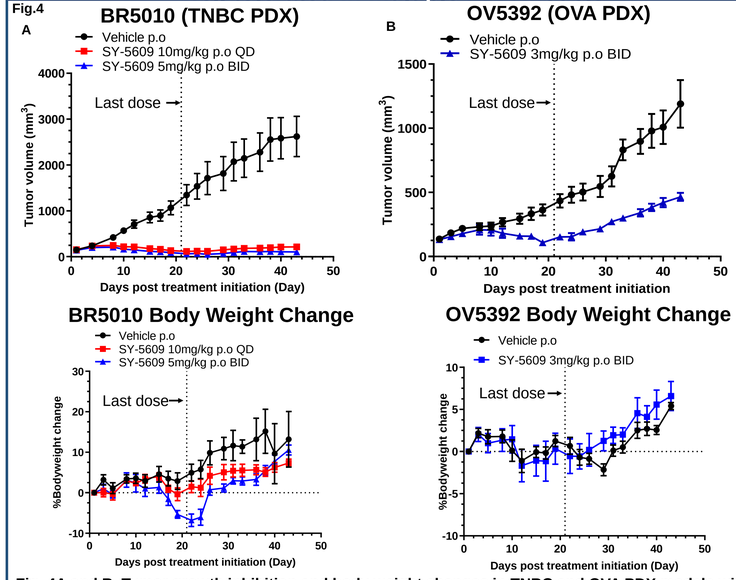


Fig. 4A and B. Tumor growth inhibition and body weight changes in TNBC and OVA PDX models with SY-5609 treatment. Tumor bearing mice were treated twice daily with vehicle or SY-5609 for 21 days and followed by 21 days observation. Minimum weight loss was observed in both models.

Conclusions

- **SY-5609 is a potent, selective and orally available CDK7 inhibitor with at least 13,000-fold selectivity over other CDKs.**
- **Robust antiproliferative effects *in vitro* were generally seen at low nM drug concentrations and associated with induction of apoptosis and cell cycle arrest; apoptosis induction was not observed in a primary normal cell line.**
- **Complete tumor regressions as a monotherapy were observed in multiple TNBC and OVA cell line derived xenograft models with doses below the maximum tolerated dose; modulation of downstream markers of CDK7 activity, including MCL1, was observed in tumor tissues, confirming CDK7 inhibition *in vivo*.**
- **Substantial tumor growth inhibition was also observed in multiple PDX models with doses below MTD, supporting the potential for a therapeutic window that supports clinical development in patients**
- **SY-5609 is progressing through IND-enabling studies to support initiation of a Phase 1 oncology trial in early 2020.**

Disclosures:
All authors: Syros Employment and stock ownership