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Preclinical evaluation of PK, PD, and anti-tumor activity of the oral, non-covalent, potent and highly selective CDK7 inhibitor, SY-5609, provides rationale for clinical development in multiple solid tumor indications

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Summary

- CDK7 is a key regulator of transcription and cell cycle progression and has been implicated in multiple tumor types driven by aberrant transcriptional control (e.g. *MYC*-, *ESR1*-activation) and/or aberrant cell cycle control (e.g. *RB1*, *CCNE1*, *CDKN2A* alterations)
- SY-5609 is an oral, non-covalent, potent, and highly selective CDK7 inhibitor that is advancing through IND-enabling studies to support initiation of a planned Phase 1 oncology trial in Q1 of 2020
- Here we report on the:
  - potency and selectivity of SY-5609 in *in vitro* CDK activity assays
  - relationship between SY-5609-induced tumor growth inhibition (TGI), pharmacokinetics (PK), and tumor tissue pharmacodynamic (PD) effects in xenograft models of high-grade serous ovarian cancer (HGSOC) and triple negative breast cancer (TNBC)
  - activity of SY-5609 in patient-derived xenograft (PDX) models from tumor types with transcriptional and/or cell cycle aberrations including HGSOC, TNBC, small cell lung cancer (SCLC), and estrogen receptor positive breast cancer (ER+BC)
- The results highlight the broad potential for SY-5609 across multiple difficult to treat cancers and support the development of SY-5609 in patients with advanced solid tumor malignancies

Methods

In vitro CDK activity assays: For each CDK2, CDK9, and CDK12, 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. SY-5609 was profiled in the SelectScreen panel of 485 kinases (ThermoFisher).

PK/PD/TGI relationship: BALBc nude mice bearing TNBC (HCC70) or HGSOC (OVCAR3) xenografts were treated with SY-5609 by oral gavage days over a range of doses. Tumor volume was evaluated during the 21-day dosing period and for 14 days after treatment discontinuation. Plasma and tumor samples were collected from separate cohorts of mice for all doses tested. SY-5609 plasma PK was assessed using a validated LC/MS-MS assay. PD in xenograft tissue was evaluated using a Nanostring codeset that evaluated *E2F1* expression relative to an empirically determined set of control genes with invariable expression changes in response to CDK7 inhibition across multiple preclinical models.

TNBC, HGSOC, and SCLC PDX models: Tumor-bearing NODSCID mice were dosed orally with vehicle (Veh) or SY-5609 at a total daily dose of 6 mg/kg for 21 days in all models except TNBC-1, which received 10 mg/kg daily. %TGI at end of treatment (EOT, day 21) was calculated as: 1- [(Mean TV SY-5609 @ EOT – Mean TV SY-5609 @ Day 0)/(Mean TV Veh @ EOT – Mean TV Veh @ Day 0)] x 100. % regression was calculated as: (Mean TV SY-5609 @ EOT)/(Mean TV SY-5609 @ Day 0) x 100. The same calculations were used for end of study (EOS; day 42). DNA extracted from passage matched PDX tumor tissue was sequenced using Agilent's SureSelectXT Human All Exon V6 kit, to a depth of ~300X. Variants were called using Sention's Haplotyper tool, and CNVs were detected using CNVkit (Wuxi NextCode). RB pathway alterations were defined based on analysis of core RB pathway genes recurrently altered in these tumor types: RB1-, CDKN2A-mutation and/or deletion (copy number ≤ 1); CCNE1-, CCND1/2-, CDK4-, CDK6-amplification (copy number ≥ 6).

ER+ breast cancer PDX models: ER+BC models were established as previously described (Wick et al., SABCS Annual Meeting abstract P3-03-04, 2015). Palbociclib-resistance (PBR) was developed in these models by treatment with palbociclib over several passages in vivo. TGI for the various treatments were evaluated in athymic nude mice as described above.

SY-5609 is a potent and highly selective CDK7 inhibitor

- SY-5609 is more potent and selective than other oral, non-covalent CDK7 inhibitors in clinical development [a]
  - SY-5609 has high affinity for CDK7 (K_d = 0.059 nM) and inhibits 4/485 (0.8%) kinases with ≥ 90% inhibition at 1μM - CDK7 (>99%), CDK18 (93%), MAPK15 (90%), CDK17 (90%) [b]

A

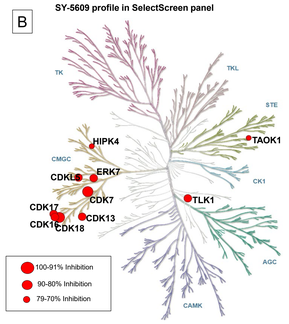
CDK Complex	Potency	Selectivity
CDK7	0.06 nM*	---
CDK2	2900 nM†	49,000x
CDK9	970 nM†	16,000x
CDK12	770 nM†	13,000x

* K_d determined by SPR; † K_i determined by activity assay

(A) SY-5609 is potent against CDK7/Cyclin H/MAT1 (K_d = 0.06nM) and selective over CDK2, CDK9, and CDK12[b]

(B) 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 [b]

B



[a] Patel et al., Molecular Cancer Therapeutics, 2018; [b] Hu et al., AACR Annual Meeting abstract 4421, 2019

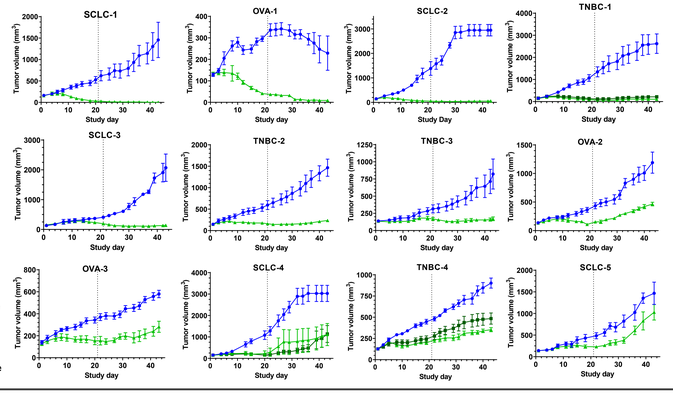
Deep and sustained SY-5609 responses in TNBC, HGSOC, and SCLC PDX models are associated with RB pathway alterations

- 12/12 (100%) PDX models demonstrated ≥70% TGI at end of SY-5609 treatment
- 7/12 (58%) PDX models demonstrated deep and sustained responses at end of study (≥95% TGI or regression 21 days after treatment discontinuation)
- Deeper and more sustained responses were associated with RB pathway alterations

PDX model	End of Treatment Day 21		Post-treatment Day 42		RB pathway genetics
	% TGI	% Regression	% TGI	% Regression	
SCLC-1	>100	80	>100	98	Altered
OVA-1	>100	72	>100	93	Altered
SCLC-2	>100	64	>100	73	Altered
TNBC-1	>100	55	>100	27	Altered
SCLC-3	82	0	>100	8	Altered
TNBC-2	90	0	95	0	Altered
TNBC-3	82	0	95	0	Altered
OVA-2	94	0	69	0	Not Altered
OVA-3	91	0	67	0	Not Altered
SCLC-4	92	0	64	0	Not Altered
TNBC-4	70	0	71	0	Not Altered
SCLC-5	74	0	33	0	Not Altered

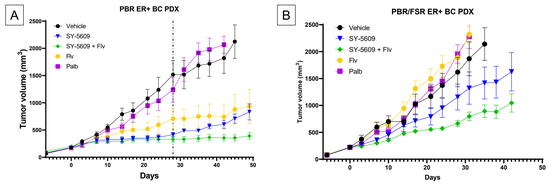
SY-5609 was administered at a total daily dose of 6 mg/kg by oral gavage, except for model TNBC-1, which received 10 mg/kg. Body weight changes at end of treatment were indistinguishable between vehicle and 10 mg/kg SY-5609 daily, with no BWL at end of treatment, below the MTD for the model (≥ 10mg/kg QD)

No differences in response to SY-5609 were observed in divided doses twice a day (light green curves) or as a total dose once daily (dark green curves) dosing schedules. Vehicle (blue).



SY-5609 induces robust responses in treatment-resistant ER+BC PDX models

- SY-5609 induces deep and sustained TGI in combination with fulvestrant in palbociclib-resistant (PBR) ER+BC PDX tumors (A)
- SY-5609 resensitizes palbociclib- and fulvestrant-resistant (PBR/FSR) ER+BC PDX tumors to fulvestrant treatment (B)



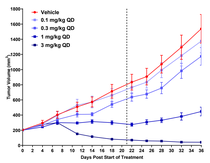
Palb: palbociclib, 50mg/kg once daily, oral; Flv: fulvestrant, 2.5mg/kg once weekly, sub-cutaneuous, SY-5609: 6 mg/kg once daily, oral

Treatment	PBR ER+BC PDX % TGI		PBR/FSR ER+BC PDX % TGI	
	Day 28	Day 42	Day 28	Day 42
Palbociclib	21	0	0	0
Fulvestrant	61	65	0	0
SY-5609	83	74	33	27
SY-5609 + Fulvestrant**	89	89	68	57

**P<0.001 vs fulvestrant

Daily oral administration of SY-5609 is associated with dose-dependent plasma exposures, transcriptional PD change in xenograft tissue, and TGI

A



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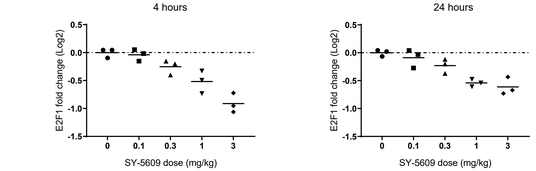

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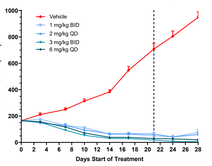

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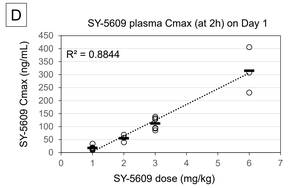

(A) Sustained regressions observed with 3 mg/kg daily dosing, at least 3-fold lower than MTD (≥ 10mg/kg QD). Dotted line represents last day dosing.

(B) Data represents E2F1 expression in tumor tissue after 4 days of daily dosing. Similar results were observed in tumor tissue after a single dose of SY-5609.

(C) Regressions observed with 2 mg/kg daily dosing, at least 5-fold lower than MTD (≥ 10mg/kg QD). Similar TGI observed if same total dose administered once (QD) or twice (BID) daily. Dotted line represents last day dosing.

(D) Dose proportional increases in plasma Cmax and AUC (not shown) at therapeutic doses (1-6 mg/kg) in mice. No accumulation of SY-5609 was observed in plasma with repeated daily dosing, however SY-5609 was still detectable at trough in plasma at concentrations that were effective in vitro (not shown), supporting QD dosing regimen.

Conclusions

- SY-5609 is an oral, non-covalent, potent and highly selective CDK7 inhibitor
- Daily oral dosing of SY-5609 induces dose-dependent TGI in ovarian and breast tumor xenografts with tumor regressions observed at doses as low as 1/5th of MTD
- SY-5609 plasma exposures are dose proportional and do not accumulate with repeated daily dosing at therapeutic doses in mice (1-6 mg/kg)
- SY-5609 induces rapid (4 hours) and sustained (24 hours) dose-dependent transcriptional PD responses in xenograft tumor tissue that correlated with TGI, supporting a QD dosing regimen
- SY-5609 induces regressions, which are sustained after treatment discontinuation, at well-tolerated doses in multiple PDX models from SCLC, TNBC, and HGSOC; sustained regressions are associated with RB pathway alterations
- SY-5609 induces robust anti-tumor activity in combination with fulvestrant in treatment-resistant PDX models of ER+ breast cancer
- These results highlight the broad potential for SY-5609 across a variety of solid tumor types
- A Phase 1 trial of SY-5609 is planned to initiate in Q1 of 2020, with inclusion of breast, ovarian, and lung cancer patients, and patients with solid tumors with RB pathway alterations irrespective of tumor type

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