Abstract 3585

# Activity of SY-5609, an oral, noncovalent, potent, and selective CDK7 inhibitor, in preclinical models of colorectal cancer

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## Introduction

- Colorectal cancer (CRC) is driven by genetic alterations that result in constitutive activation of oncogenic transcription factors (eg β-catenin, cMYC) and of mitogenic signaling and cell cycle progression (eg KRAS, BRAF)
- CDK7 is a key regulator of transcription, through phosphorylation of the CTD domain of RNA Polymerase II, and cell cycle progression, through phosphorylation of cell cycle kinases CDK1, 2, 4, and 6, suggesting CDK7 inhibitors may be effective in the treatment of CRC, where inhibiting both processes may be important
- SY-5609 is an oral, non-covalent, potent and highly selective CDK7 inhibitor in phase 1 clinical development (NCT04247126, Abstract TPS3662, ASCO 2020)
- Here we report on the:
  - activity of SY-5609 on proliferation and cell cycle arrest in CRC cell lines in vitro
  - relationship between SY-5609-induced tumor growth inhibition (TGI) and pharmacodynamic effects in CRC patient derived xenograft (PDX) tissue
  - activity of SY-5609 in a panel of 30 independent PDX models of CRC
- The results provide initial insights into the role of SY-5609 in driving anti-tumor activity in preclinical models of CRC and support the development of SY-5609 in CRC patients

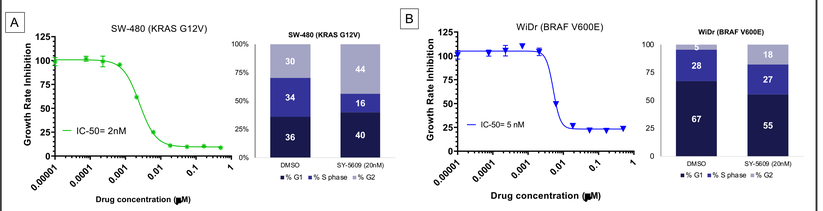
## Methods

In vitro Cellular Assays: Antiproliferative activity were assessed in SW-480 (KRAS mutant CRC) and WiDR (BRAF mutant CRC) cell lines using Cell Titer Glo 2.0 following manufacture protocol. SY-5609 was plated in 10 point dose curve and normalized to DMSO and a Day zero plate. Cellular EC-50 was calculated on Day 5 using GraphPad Prism. Cell cycle effects of SY-5609 on SW-480 and WiDR were analyzed at 72 HRS using PI/Edu staining via manufacture protocol and analyzed using FlowJo.

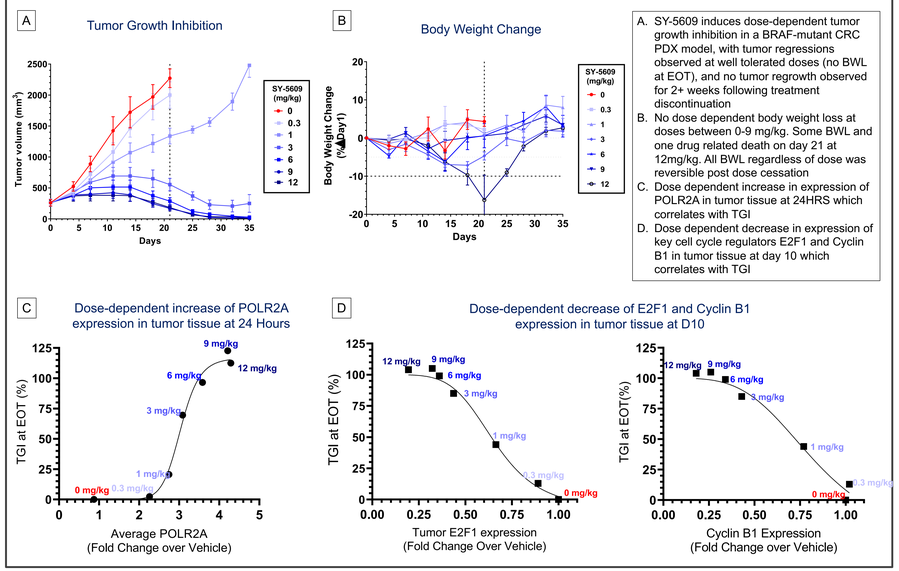
PK/PD/TGI relationship: The relationship between SY-5609 dose, pharmacodynamic (PD) changes in xenograft tissue, tumor growth inhibition (TGI), and mouse body weight (BW) was evaluated in a BRAF-mutant CRC model across a range of doses. SY-5609 was administered once daily (QD) by oral gavage for 21 days (EOT) at 0 mg/kg, 0.3 mg/kg, 1 mg/kg, 3 mg/kg, 6 mg/kg, 9 mg/kg and 12 mg/kg. Tumors were harvested 24 HRS post the first dose and just prior to receiving the 10th dose to analyze for transcriptional changes using custom designed Nanostring codeset via Nanostring nCounter Element XT system.

CRC PDX models: SY-5609 TGI activity at sub-maximum-tolerated-dose levels was evaluated across a panel of 30 independent CRC models including BRAF-mutant, KRAS-mutant, or non-BRAF/KRAS mutant (wild type) models (10 of each). SY-5609 was administered by oral gavage at 6 mg/kg/day for 21 days (end of treatment, EOT) and body weight change assessed over course of treatment at EOT (day 21) and end of study (EOS, Day 28).

## SY-5609 potently inhibits proliferation and induces G2/M arrest in KRAS- and BRAF-mutant CRC cell lines in vitro

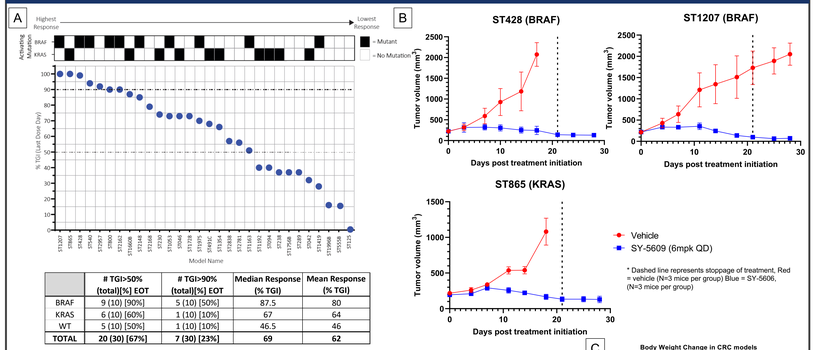



## SY-5609 induces dose-dependent tumor growth inhibition and pharmacodynamic effects in tumor tissue in CRC PDX models




A. SY-5609 induces dose-dependent tumor growth inhibition in a BRAF-mutant CRC PDX model, with tumor regressions observed at well tolerated doses (no BWL at EOT), and no tumor regrowth observed for 2+ weeks following treatment discontinuation
B. No dose dependent body weight loss at doses between 0-9 mg/kg. Some BWL and one drug related death on day 21 at 12mg/kg. All BWL regardless of dose was reversible post dose cessation
C. Dose dependent increase in expression of POLR2A in tumor tissue at 24HRS which correlates with TGI
D. Dose dependent decrease in expression of key cell cycle regulators E2F1 and Cyclin B1 in tumor tissue at day 10 which correlates with TGI

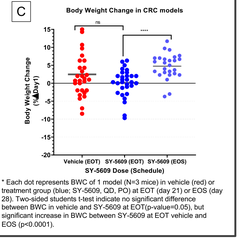
## Once daily oral SY-5609 dosing induces robust responses in CRC PDX models at well tolerated doses




| | # TGI>50% (total)[%] EOT | # TGI>90% (total)[%] EOT | Median Response (% TGI) | Mean Response (% TGI) |
|---|---|---|---|---|
| BRAF | 9 (10) [90%] | 5 (10) [50%] | 87.5 | 80 |
| KRAS | 6 (10) [60%] | 1 (10) [10%] | 67 | 64 |
| WT | 5 (10) [50%] | 1 (10) [10%] | 46.5 | 46 |
| TOTAL | 20 (30) [67%] | 7 (30) [23%] | 69 | 62 |

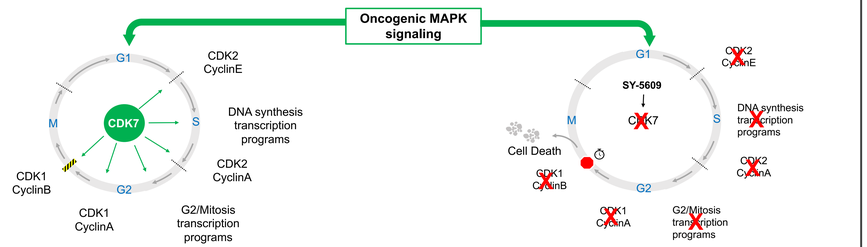
* Dashed line represents stopgage of treatment, Red = vehicle (N=3 mice per group) Blue = SY-5606, (N=3 mice per group)

A. SY-5609 shows robust response in CRC PDX models
   i. ≥50% TGI at end of treatment in 67% (20/30) of models.
   ii. ≥90% TGI or regression at end of treatment in 23% (7/30) of models
   iii. Enrichment for deep responses in BRAF mutant models (50%, 5/10) relative to KRAS mutant (10%, 1/10), and wild-type (10%, 1/10) models.
B. Sustained regressions observed in 2 BRAF and 1 KRAS mutant CRC model
C. SY-5609 was well tolerated in study
   i. Average body weight change of 0% at end of treatment across all 30 PDX models tested
   ii. Body weight loss, when observed, was rapidly reversed with in one week upon treatment cessation




* Each dot represents BWC of 1 model (N=3 mice) in vehicle (red) or treatment group (blue; SY-5609, QD, PO) at EOT (day 21) or EOS (day 28). Two-sided students t-test indicate no significant difference between BWC in vehicle and SY-5609 at EOT(p-value>0.05), but significant increase in BWC between SY-5609 at EOT vehicle and EOS (p<0.0001).

## Putative role for CDK7 inhibition on cell cycle and transcription in CRC




- Perpetual commitment to S-phase of the cell cycle
- Dependence on CDK7 activity for successful transition through S-phase, G2, and mitosis

- DNA replication and mitosis disrupted through inhibition of CDK2, CDK1, and cell-cycle-specific transcription programs
- Induces G2 arrest and subsequent cell death

## Conclusions

- SY-5609 potently inhibited proliferation and induced G2/M arrest in BRAF-mutant (WiDr) and KRAS-mutant (SW-480) CRC cell lines in vitro
- Once daily oral dosing of SY-5609 in a BRAF-mutant CRC PDX model induced dose-dependent tumor growth inhibition (TGI), including regressions that are sustained after treatment discontinuation, at well tolerated doses; dose-dependent TGI is associated with dose-dependent expression changes of cell cycle markers *E2F1* and *CCNB1* and the transcriptional marker *POLR2A*
- Once daily oral dosing of SY-5609 in a panel of 30 CRC PDX models resulted in ≥ 50% TGI in 67% (20/30) and ≥ 90% TGI (deep responses) in 23% (7/30) of models, including in models derived from heavily pre-treated patients, at well tolerated doses
  - Deep responses were enriched in BRAF mutant (50%, 5/10) vs. KRAS mutant or wild-type models (10%, 1/10 each); tumor regressions were observed in 2 BRAF and 1 KRAS mutant model
- These results highlight the therapeutic potential of SY-5609 in CRC and support the evaluation of SY-5609 in CRC in early phase clinical trials
- SY-5609 is in phase 1 clinical development for patients with advanced solid tumors including CRC (NCT04247126; Abstract TPS3662, ASCO 2020)

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