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SY-5609, a Potent and Selective CDK7 Inhibitor, Potentiates BTK Inhibitor Activity in Mantle Cell Lymphoma Preclinical Models

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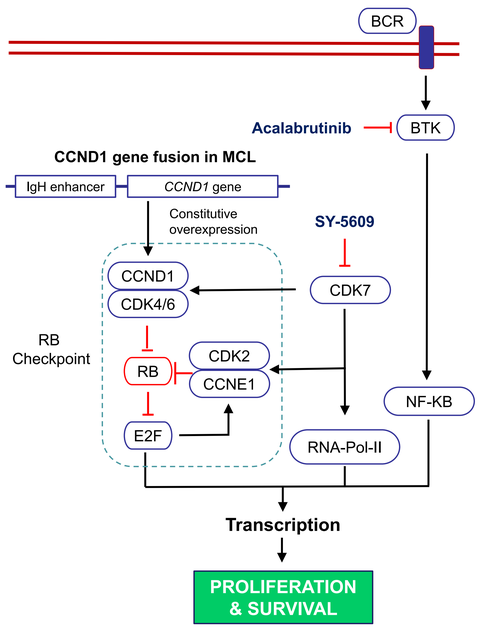
Background and rationale

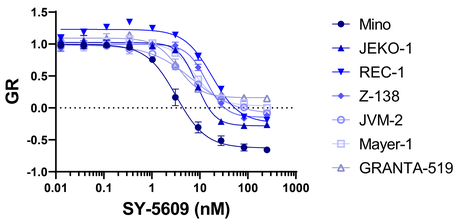
Schematic of targets, pathways, biology and inhibitors evaluated in this study



- CDK7 is a key regulator of transcription and cell cycle progression and has been implicated in multiple tumor types driven by aberrant transcriptional (MYC, ESR1) and/or aberrant cell cycle control (loss of RB pathway checkpoint function) mechanisms
- SY-5609 is a potent, selective, and oral CDK7 inhibitor in development in patients with advanced solid tumors, including patients with RB pathway alterations (NCT04247126)
- Mantle cell lymphoma (MCL) is an aggressive B cell lymphoma:
  - Characterized by t(11;14)(q13;q32) translocation that leads to constitutive overexpression of *CCND1* and suppression of RB checkpoint function
  - Dependent on B-cell receptor (BCR) signaling through Bruton's Tyrosine Kinase (BTK), a strong activator of downstream transcriptional programs that drive cell proliferation and survival (e.g. NF-KB)
- Here we report on the activity of SY-5609 in models of MCL, providing rationale for the evaluation of SY-5609, including in combination with BTK inhibitors, in patients with MCL

SY-5609 potently inhibits proliferation of MCL cell lines in vitro

Proliferation Assay (day 5)



GR Curve Metrics

Cell line	GR50	GRmax	GEC50
Mino	1.4	-0.65	3.2
GRANTA-519	4.0	0.15	3.5
JEKO-1	5.1	-0.29	8.6
JVM-2	5.3	-0.09	7.2
Maver-1	6.3	-0.01	7.8
Z-138	8.3	-0.17	13.5
REC-1	15.6	-0.20	17.8

- SY-5609 in vitro antiproliferative activity was assessed using CellTiter-Glo assay
- GR (normalized growth rate inhibition): ratio between growth rates under treated and vehicle treated control conditions, therefore accounting for variable growth rates between cell lines
- GR50: concentration of SY-5609 that inhibits growth rate by 50% (GR = 0.5)
- GRmax: minimum GR value
- GEC50 (relative GR50): concentration at point midway between top and bottom asymptote of fitted curve

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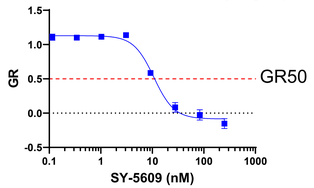
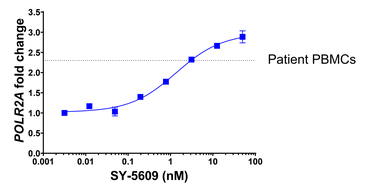
SY-5609 inhibits Mino cell proliferation at concentrations that also induce *POLR2A* to levels observed in SY-5609-treated patient PBMCs

Mino Proliferation (day 5)



- CellTiter-Glo assay
- Data points represent mean +/- SEM (n=3)

Mino POLR2A PD response (24hrs)

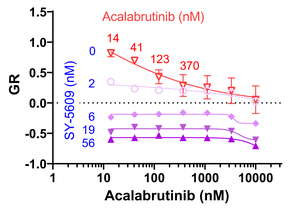


- Dashed horizontal line represents trough POLR2A fold change in PBMCs from patients dosed with 3mg SY-5609 QD at steady state (ENA 2020)
- Data points represent mean +/- SEM (n=3)

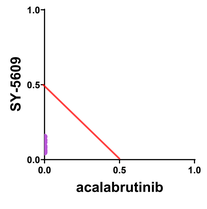
SY-5609 is synergistic with BTK inhibitor acalabrutinib in Mino cells, and potentiates antitumor activity of acalabrutinib in Mino xenografts

SY-5609 is synergistic with acalabrutinib in Mino cells in vitro

Proliferation (day 5)

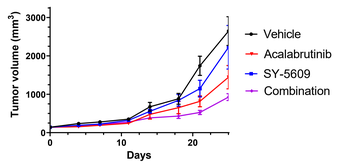


Isobologram



- Data points on isobologram represent combinations of 14-370nM acalabrutinib and 2-56nM SY-5609
- Results shown are representative of 3 independent experiments

SY-5609 potentiates acalabrutinib antitumor activity in Mino xenografts



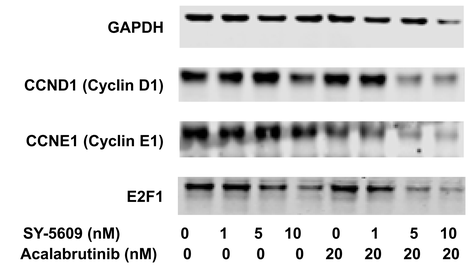
Treatment	Treatment Regimen	Growth Rate Inhibition
SY-5609	0.5 mg/kg BID 7 days on/7 days off	8.47*
Acalabrutinib	15 mg/kg QD Continuous	17.6*
Combination	As above	37.8

*p<0.01, single agent vs combination

- N=5 per group, mean+/-SEM shown in figure
- All regimens well-tolerated: no body weight loss observed at end of treatment (day 25)

Combination of SY-5609 and acalabrutinib decreases expression of key regulators of RB checkpoint function and cell cycle progression in Mino cells

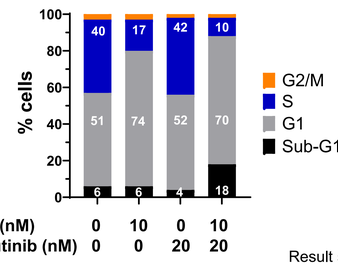
Protein Expression by Western Blot (72 hours)



Result shown is representative of 2 independent experiments, similar results observed at 24 and 48 hours

Combination of SY-5609 and acalabrutinib leads to increase in proportion of Mino cells with sub-G1 DNA content, a marker of cell death

Cell Cycle Distribution (72 hours)



- SY-5609 (10nM) induces G1 arrest
- Acalabrutinib (20nM) does not induce cell cycle changes by 72 hours
- Combination induces an increase of cells with sub-G1 DNA content (a marker of dead or dying cells)

Result shown is representative from 3 independent experiments

Conclusions

- SY-5609 potently inhibits proliferation of MCL cell lines in vitro
- SY-5609 antiproliferative activity in MCL cell line Mino is associated with *POLR2A* PD changes comparable to those observed in PBMCs from patients with advanced solid tumors treated with SY-5609 at a tolerable dose and regimen
- SY-5609 shows synergistic antiproliferative activity with the BTK inhibitor acalabrutinib in Mino cells in vitro, and potentiates acalabrutinib antitumor activity in Mino xenografts in vivo
- The combination of SY-5609 and acalabrutinib in Mino cells in vitro, at subtherapeutic concentrations of either single agent, is associated with:
  - Decreased expression of CCND1, CCNE1, and E2F1 proteins, key regulators of RB checkpoint function and cell cycle progression
  - Increased proportion of cells with sub-G1 DNA content, a marker of cell death
- A Phase 1b safety and preliminary efficacy study of SY-5609, including in combination with a BTK inhibitor, is planned for patients with relapsed/refractory MCL