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SY-1365, a potent and selective CDK7 inhibitor, exhibits promising anti-tumor activity in multiple preclinical models of aggressive solid tumors

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Abstract

CDK7 has recently emerged as an attractive target in cancer since its inhibition decreases the transcript levels of oncogenic transcription factors, especially those driven by super-enhancers (SEs). Cancers have been hypothesized to be addicted to SE regulated genes and simultaneous suppression of multiple SE associated genes through CDK7 inhibition might represent a novel, powerful way to selectively kill cancer cells.

Previously, we reported that SY-1365, a highly selective covalent CDK7 inhibitor, induces apoptosis in leukemia cells, but not in non-malignant cells and demonstrates anti-tumor activity in *in vivo* models of leukemia. In this study, we extend these findings to the identification of multiple solid tumors that are susceptible to SY-1365 and compare the effects of SY-1365 on gene expression to other gene control agents.

SY-1365 was screened in a panel of solid tumor cell lines, revealing activity in breast, ovarian, colorectal and lung cancer cells that exhibited low nM EC_{50} and rapid induction of apoptosis. In breast cancer, a subset of triple negative breast cancer (TNBC) cell lines were found to be more sensitive than luminal breast cancer cell lines, so we extended our studies to *in vivo* models and show substantial tumor growth inhibition in multiple patient-derived xenograft models of TNBC.

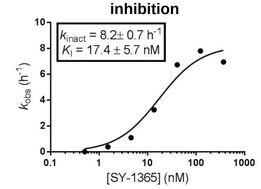
Since other compounds have been reported to modulate the expression of SE regulated genes, we compared the transcriptional effects of SY-1365 treatment with those of a pan-CDK inhibitor (flavopiridol), a CDK9 inhibitor (NVP2) and a BRD4 inhibitor (JQ1) using microarray analysis.

Applying principal component analysis, we observed a unique transcriptional response elicited by SY-1365 compared to the other inhibitors. NVP2 and flavopiridol inhibited an overlapping and much larger gene set than either JQ1 or SY-1365. Interestingly, SY-1365 treatment decreased the expression of oncogenic transcription factors, cell cycle checkpoint regulators and DNA damage response genes. The downregulation of transcripts involved in apoptosis and DNA damage response by SY-1365 suggests that CDK7 inhibition might synergize with targeted agents that affect these processes. Indeed, we observed that SY-1365 was synergistic with the PARP inhibitor niraparib and the Bcl2 inhibitor venetoclax in triple negative breast cancer and AML cell lines, respectively.

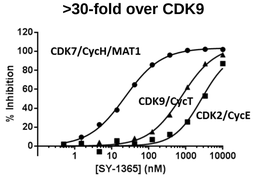
In summary, we have identified TNBC, ovarian and small cell lung cancer cell lines as highly sensitive to SY-1365 *in vitro* and have observed substantial tumor growth inhibition in PDX models of TNBC. SY-1365 induced a distinct transcriptional response compared with other transcriptional inhibitors, with apoptotic and DNA damage pathways being central. Finally, these mechanism of action studies support a rationale for investigating combinations of SY-1365 with inhibitors of PARP and Bcl-2.

SY-1365 is a potent, selective CDK7 inhibitor

Fig. 1A



1B



1C

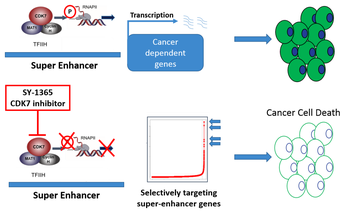


Fig 1A. CDK7 Activity Assay. The loss of CDK7 activity as a function of pre-incubation time was fit to determine the k_{obs} rate constants, which are plotted as a function of SY-1365 concentration. These data were fit to obtain K_I (the apparent inhibitor affinity) and K_{inact} (the maximal CDK7 inactivation rate constant). **1B. CDK2 and CDK9 kinase activity inhibition assays.** Percent inhibition values were calculated relative to the uninhibited enzyme for each CDK and plotted as a function of SY-1365 concentration to generate IC_{50} curves with the following best-fit IC_{50} values: CDK7/CycH/MAT1 = 20 ± 1.4 nM, CDK2/CycE = 2,600 ± 270 nM and CDK9/CycT = 670 ± 40 nM,. **1C. Schematic of potential SE-gene targeting leading to cancer cell death.**

SY-1365 shows potent antiproliferative effect in solid tumor cell lines

Fig.2

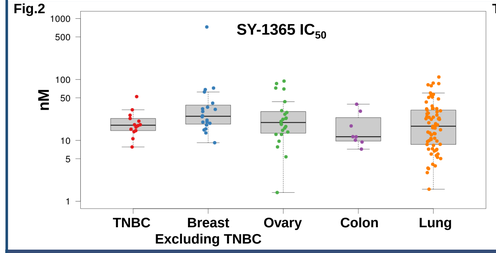


Table.1

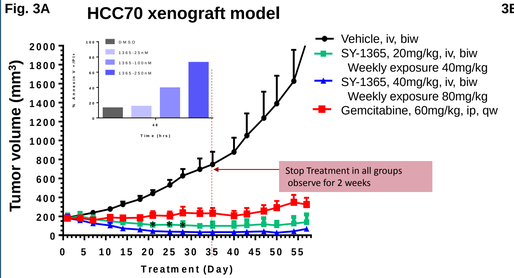
	Lines tested (N)	Mediam IC_{50} (nM)	% Cytotoxic
TNBC	14	17.75	100
Breast excluding TNBC	20	24.89	95
Ovary	24	19.65	70.83
Colon	8	11.49	75
Lung	70	17.1	91.43

Fig 2.SY-1365 antiproliferation IC_{50} in various solid tumor cell lines. Antiproliferation effect of SY-1365 was analyzed across a broad cell line panel including breast, ovary, colon and lung cancer cell lines.

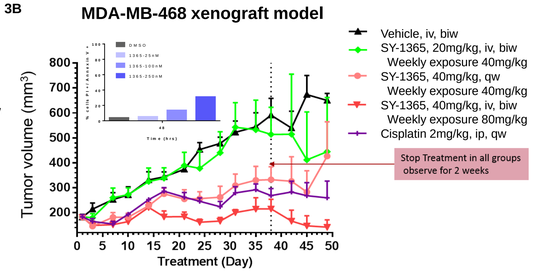
Table 1. Number of cell lines tested in the listed tumor type and median IC50 of SY-1365 observed in the cell lines in Fig 2. Percentage of cell lines that showed cytotoxic effect is listed as %Cytotoxic

SY-1365 inhibits tumor growth in TNBC *in vivo* models

Fig. 3A



3B



3C

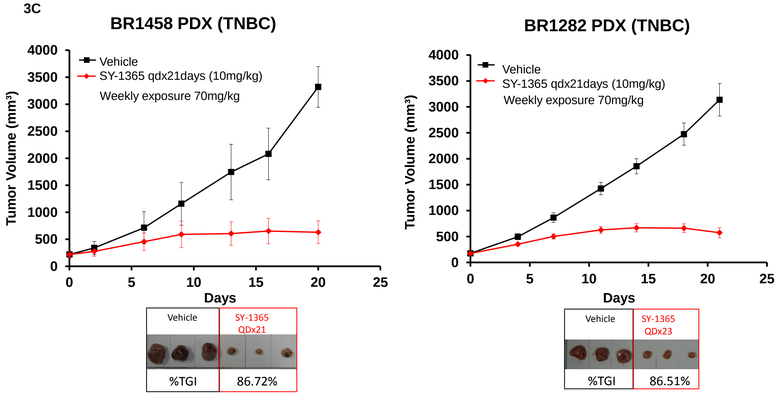


Fig.3 A and 3 B. Tumor growth inhibition in cell line derived TNBC mouse xenograft models and in vitro apoptosis response upon SY-1365 treatment. Tumor bearing mice were treated biw with vehicle, SY-1365 at 2 doses, and gemcitabine (3 A) and cisplatin (3 B) (n=6/group) for 35 days. SY-1365 were well tolerated in both doses in mice with minimum body weight change (data not shown). Inset: In vitro apoptosis induction by 1h treatment with SY-1365 measured by flow cytometry for annexin V/PI 48h after start of treatment.

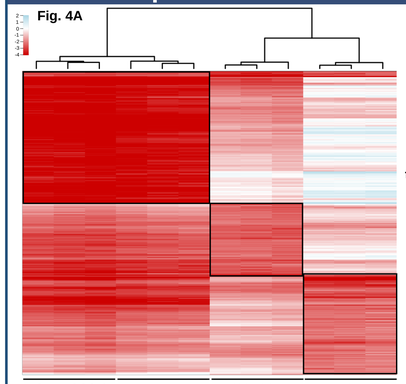
3 C. Tumor growth inhibition in TNBC PDX models. Tumor bearing mice were treated qd with vehicle or SY-1365 (10mg/kg) for 21 days. Treatment daily at 10mg/kg provided a weekly drug administration of 70mg, overall in the same range as 40mg/BIW (80 mg weekly). Pictures of individual tumors harvested after 21 days of treatment with either vehicle or SY-1365 show a striking difference in overall tumor size.

* = $p < 0.05$;

biw = twice weekly, qw = once weekly, qd = once daily

SY-1365 induces unique transcriptional signature compared to other transcriptional inhibitors

Fig. 4A



4B

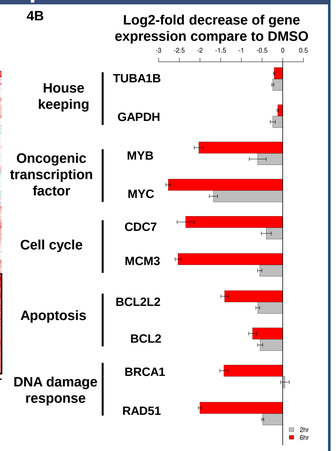


Fig 4A. Heatmap of gene expression changes after treatment with different transcriptional inhibitors. Gene expression changes were measured (in triplicate) 6 hours post treatment in THP1 cell line (AML). The union of the top 200 most down-regulated genes upon each treatment vs DMSO are shown. Hierarchal clustering of gene expression changes reveals clear differences in transcriptional effects between inhibitors (top). Heatmap coloring indicates the log2-fold change vs DMSO of each probe (rows) in each sample (columns). **4B. Treatment with SY-1365 causes gene expression changes in key cellular processes.** Barplot of gene expression changes of select genes after treatment with SY-1365 at 2 hours (gray) and 6 hours (red). Height of each bar is the average log2-fold change vs DMSO over three replicates. Error bars, standard error of three replicates.

SY-1365 is synergistic with venetoclax *in vitro*

Fig. 5

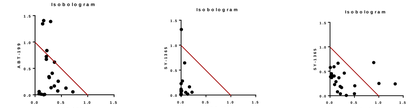


Fig 5. Combination of SY-1365 with venetoclax (ABT-199, BCL-2 inhibitor) demonstrates synergy. Isobolograms of combinations of SY-1365 with ABT-199. Data was generated from co-treatment in indicated AML cell lines and assayed for viability on day 3. The red line indicates additive effects, points below the line indicate synergism.

Conclusions

- SY-1365, a first-in-class selective CDK7 inhibitor, demonstrated antiproliferative and apoptotic effects in solid tumor cell lines, including triple negative breast, ovarian and small cell lung cancers.
- SY-1365 inhibited tumor growth in TNBC CDX and PDX mouse models with minimum body weight change.
- Twice weekly regimen planned for clinical trial showed substantial anti-tumor effect *in vivo*.
- SY-1365 induced a distinct, more selective transcriptional response compared with other transcriptional CDK inhibitors.
- In AML *in vitro* models, SY-1365 demonstrated synergistic activity with venetoclax; this data provides a rationale for further investigating the combination of SY-1365 with inhibitors targeting apoptotic pathway.

Disclosures:
All authors: Syros Employment and stock ownership