B171

PK/PD modeling of the first-in-class, potent and selective covalent CDK7 inhibitor, SY-1365, provides mechanistic basis for intermittent dosing regimens in preclinical efficacy models of hematological and solid tumors

Nigel J. Waters[1], Shanhu Hu[1], Brett Matzuka[2], Graeme Hodgson[1], Yixuan Ren[1], Yoon Choi[1], Kevin Dykstra[2], Christopher Roberts[1], Kevin Sprott[1], Emmanuelle di Tomaso[1], Christian C. Fritz[1]

[1] Syros Pharmaceuticals, Cambridge, MA; [2] qPharmetra, Boston, MA

Abstract

Introduction: CDK7 has recently emerged as an attractive gene control target in cancers driven by transcriptional dependencies including triple negative breast cancer (TNBC) and acute myeloid leukemia (AML). SY-1365 is a first-in-class, potent and selective covalent CDK7 inhibitor, that has shown durable and complete responses in xenograft models of AML and various solid tumors. To advance the development of this CDK7 inhibitor in patients, the current work assessed the relationship between SY-1365 pharmacokinetics (PK), pharmacodynamics (PD) measured as CDK7 target occupancy, and efficacy in mouse xenograft models of AML (HL-60) and TNBC (HCC70).

Methods: SY-1365 was dosed intravenously in several xenograft experiments across a range of dose levels (5-40 mg/kg) and dose frequencies (daily to weekly). PK and changes in CDK7 occupancy were measured following single and repeat dosing, and efficacy studies measured the resultant dose-dependent changes in tumor volume over time after repeat dosing of SY-1365. To account for the temporal differences in PK, PD and tumor growth inhibition (TGI), a mathematical modeling approach was taken to integrate the resulting datasets. In addition, an assay to determine CDK7 occupancy in human PBMCs was developed to support the ongoing Phase 1 study.

Results: The PK in mouse was proportional with respect to dose and exhibited a terminal elimination half-life (t1/2) of 4 h. The PK/PD model was able to describe the PK, PD and efficacy in HL-60 and HCC70 xenografts, with similar exposure and CDK7 occupancy profiles observed in these two xenografts. The model demonstrated a CDK7 turnover t1/2 of 69 h, longer than that reported for other oncogenic kinases targeted with covalent inhibitors where daily dosing is required (e.g. EGFRi, BTKi), with optimal efficacy achieved when target engagement was sustained over the dosing interval. This provides a mechanistic basis for intermittent dosing based on the dose dependent TGI, including tumor regressions, observed with SY-1365 in in vivo models of TNBC and AML.

Conclusions: The PK/PD relationship for target engagement and efficacy of the first-in-class potent and selective covalent CDK7 inhibitor, SY-1365, has been characterized in preclinical models through a mathematical modeling approach. Assays were developed to enable target occupancy quantification in xenograft tumors, and in human PBMCs as a surrogate tissue to support clinical investigation. Further investigations into tumor molecular profiles driving response are currently underway. In summary, this work supported the selection of an initial twice weekly dosing regimen in the ongoing Phase 1 trial of SY-1365 in adult patients with advanced solid tumors (NCT03134638).

Inhibition of CDK7 decreases expression of oncogenic TFs

Fig. 1A

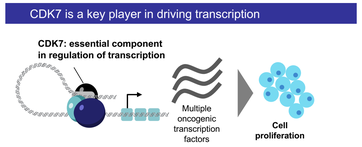
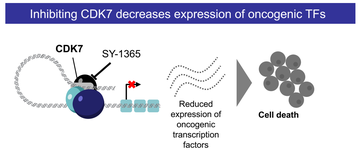
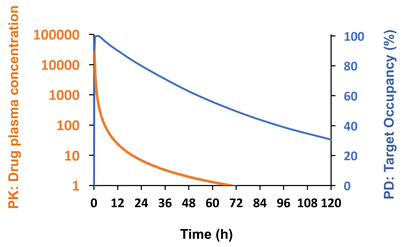


Fig. 1B



Special relationship between PK and PD for irreversible inhibitors

Fig. 2



- **For irreversible enzyme inhibitors, an initial bolus of compound exposure leads to target engagement and inactivation**
- **This leads to an extended pharmacodynamic (PD) effect even after compound exposure (PK) has decayed**
- **The decay of PD over time is related to the re-synthesis rate of the drug target**
- **SY-1365 is a covalent, irreversible inhibitor of CDK7**

A single dose of SY-1365 causes prolonged target occupancy

Fig. 3 A

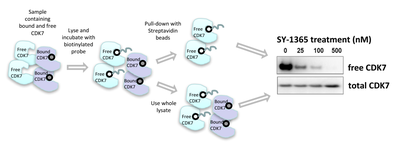


Fig. 3 B

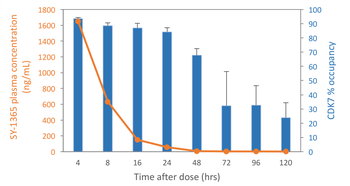


Fig 3 A) Schematic for determining target occupancy for CDK7. For higher throughput, this method was adapted to a MSD plate-based method. The % occupancy is calculated as 100 x $CDK7_{bound}$ (B) / $CDK7_{total}$ (T). **Fig 3 B)** SY-1365 was administered as a single iv dose (40 mg/kg) to HL-60 tumor-bearing mice. Plasma and tumor tissue were harvested at the time-points shown from three mice per time point. SY-1365 plasma concentrations were determined using LC-MS/MS. CDK7 tumor occupancy was determined as described in 3A.

CDK7 occupancy correlates with anti-tumor activity in HL60 xenograft

Fig. 4 A

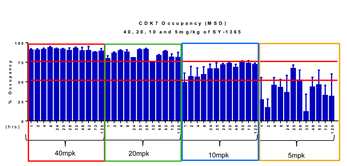


Fig. 4 B

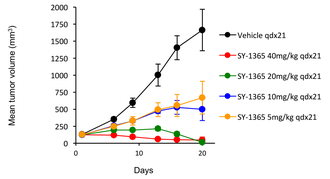


Fig 4: Target occupancy and anti-tumor activity in the human HL60 xenograft model. **A)** CDK7 occupancy in tumor was determined over the course of iv, qd dosing for five days at 5, 10, 20, & 40 mg/kg. Tumor samples were taken at the indicated times after the first dose (n=3). Samples at 24, 48, 72, and 120 h were taken immediately before dosing on that day. **B)** Tumor growth inhibition after qd, i.v. dosing with 5, 10, 20, and 40 mg/kg SY-1365 (n=8 per arm). After the establishment of tumors, SY-1365 was dosed for 21 days.

Mathematical modeling determines CDK7 resynthesis $t_{1/2}$ as 69 h

Fig. 5 A

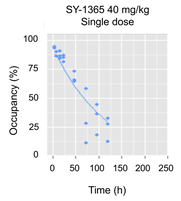
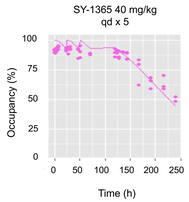


Fig. 5 B



- $\frac{dB}{dt} = k_{overall}(T - B)I - k_{out}B$ where $k_{overall} = k_{inact} \cdot K_I$
- Best fit results in:
  T = 6602 counts
  $k_{overall}$ = 6.6x10^{-5} (h^{-1}·counts^{-1})
  k_{out} = 0.01 (hr^{-1})
- $t_{1/2}$ = ln2 / k_{out} = 69 h

Fig 5: Mice containing HL60 xenograft tumors were dosed with SY-1365 at 40 mg/kg, iv either single dose (Fig 5A) or for 5 days in a row (Fig 5B). Tumor samples where taken at different times after dosing and CDK7 occupancy was determined (data represented as points in graph).
A mathematical model was derived (see formula above) with values for CDK7 total (T) and CDK7 bound (B) experimentally determined. The SY-1365 plasma concentration (I), was predicted from a separately developed PK model. A best curve fit to the data (solid line in graph) was derived from estimates for $k_{overall}$ and k_{out}. Since SY-1365 binds CDK7 covalently and total CDK7 did not change during the experiment, the disappearance rate of B, characterized by k_{out}, is considered to reflect synthesis of new, unbound CDK7.

SY-1365 can cause tumor regression in xenograft models using twice weekly dosing

Fig. 6 A

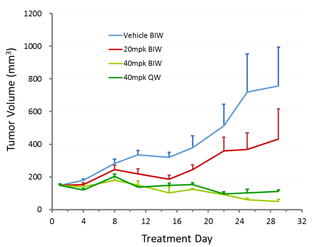


Fig. 6 B

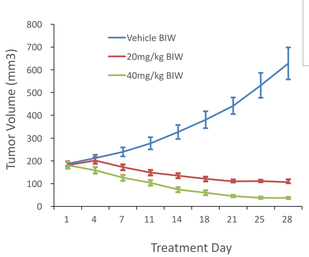


Fig 6 A) Anti tumor activity of SY-1365 in the Kasumi-1 AML xenograft model after weekly iv dosing of 40 mg/kg and biweekly dosing of 20 or 40 mg/kg (n=5 per arm).

Fig 6 B) Anti tumor activity of SY-1365 in the HCC70 (TNBC) xenograft model after biweekly iv dosing of 20 or 40 mg/kg (n=6 per arm). Insert shows CDK7 occupancy determined in tumors 4 h after a single dose.

CDK7 occupancy in PBMCs matches occupancy in tumors in mice and CDK7 occupancy can be measured in human PBMCs

Fig. 7 A

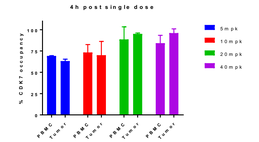


Fig. 7 B

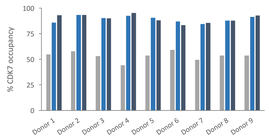


Fig 7 A) CDK7 occupancy in PBMCs and tumors from immunocompetent 4T1 mouse breast tumor xenograft-bearing mice. Data represent the mean values from 2 independent mice per group (±SD)

Fig 7 B) CDK7 occupancy in human PBMCs treated ex-vivo with varying doses of SY-1365 for 4 h. Data represent single values for each per-timepoint and donor.

Conclusions

- **SY-1365 shows a PK/PD/efficacy relationship in preclinical models of hematological and solid malignancies**
- **As a covalent inhibitor, there is a prolonged PD effect for SY-1365 (t½ ~3 days) in mice, as measured by CDK7 occupancy, despite a short plasma half life (t½ ~ 2h plasma), supporting intermittent dosing**
- **SY-1365 induces sustained regressions in a number of cancer models using intermittent dosing, providing a rationale for biweekly dosing in the clinic**
- **Target occupancy measured in mouse PBMCs and tumors is consistent, supporting the use of PBMC occupancy as a PD marker in clinical testing to establish a human PK/PD relationship and dosing regimen**

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