Abstract 1143
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Targeting the transcriptional kinases CDK12 and CDK13 in breast and ovarian cancer

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Disclosures:
All authors: Syros Pharmaceuticals employment and stock ownership

Abstract

CDK12 and CDK13 regulate expression of large transcripts requiring substantial processing to produce mature mRNA. This transcriptional regulation includes coordinated phosphorylation of specific repeats within the C-terminal domain of RNA polymerase II and association with RNA processing factors (Chila, 2016). RNAi knockdown of CDK12 in cell culture decreases expression of DNA damage response genes, including BRCA1 and ATR, while enhancing sensitivity to DNA damaging agents (Blazek, 2011; Liang, 2015). Recently THZ531, a selective covalent inhibitor of CDK12 and CDK13, was shown to decrease expression of DNA damage response genes in cell culture (Zhang, 2016). Here we present further studies with THZ531 to guide our discovery program toward molecules suitable for clinical development and to explore mechanistic rationales for combining a CDK12/13 inhibitor with PARP inhibitors or DNA damaging agents for difficult-to-treat cancers such as high-grade serous ovarian cancer and triple-negative breast cancer (TNBC).

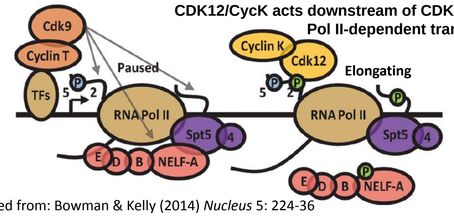
Using THZ531 as a benchmark, we developed assays capable of discriminating sub-nM inhibitors, including quantifying time-dependent covalent inhibition and cell-based CDK occupancy. Since CDK7, like CDK12 and CDK13, contains a cysteine residue proximal to the kinase active site, these approaches are critical to understand covalent inhibitor selectivity. Furthermore, we performed kinome paneling studies to better understand selectivity of this scaffold in support of our ongoing efforts to optimize CDK12/13 potency and selectivity.

To pharmacologically investigate the previously reported effects of CDK12 RNAi, growth inhibition of a panel of ovarian and breast cancer cell lines was assessed following treatment with THZ531 (OVA EC_{50} = 50-200 nM (n=6); BRCA EC_{50} <50 nM (n=4)). Expression profiling revealed that THZ531 treatment resulted in different sets of genes being affected than was observed following treatment with inhibitors targeting CDK7, CDK9 or BET-bromodomain proteins. Additionally, CDK12/13, CDK7 and CDK9 inhibitors were profiled in a broad cell line panel (n>400) to reveal relationships between inhibitor sensitivity, mutation status, gene expression, and potential oncology indications that may be addressed by these different mechanisms. Finally THZ531 was synergistic with both PARP inhibitors and DNA damaging agents in ovarian and breast cancer cell lines. These data highlight cancer indications and combinations that may be particularly amenable to treatment with CDK12/13 inhibitors.

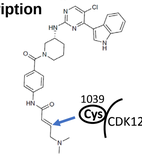
While the pharmacokinetic properties of THZ531 preclude adequate target engagement in tumor tissue at tolerated doses in mouse model systems, our ongoing medicinal chemistry program is progressing to identify and optimize CDK12/13 inhibitors suitable for clinical evaluation.

CDK12/CycK regulates transcriptional elongation with RNA Pol II

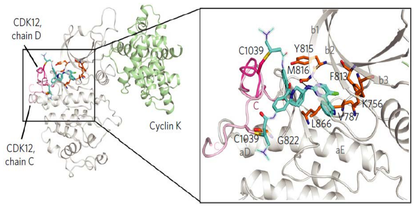
CDK12/CycK acts downstream of CDK9/CycT in regulation of RNA Pol II-dependent transcription



Adapted from: Bowman & Kelly (2014) *Nucleus* 5: 224-36

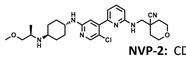


THZ531
Covalent CDK12 inhibitor from the Gray lab at the Dana Farber Cancer Institute

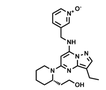


Adapted from: Zhang et. al. (2016) *Nat Chem Biol 12*: 876-84

Other Transcriptional CDK inhibitors used in this study:



NVP-2: CDK9-selective inhibitor from Novartis

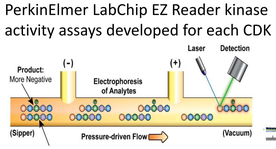


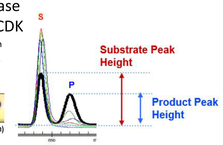
dinaciclib: Pan-CDK inhibitor from Merck

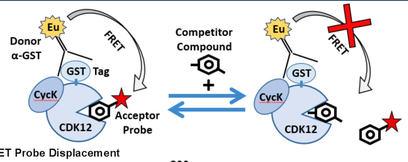
SY-4240: Proprietary highly selective CDK7 inhibitor from Syros

Biochemical assays allow interpretation of the SAR of time-dependent covalent CDK12 inhibition

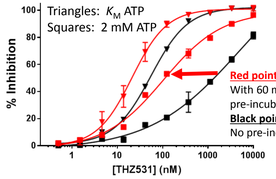
PerkinElmer LabChip EZ Reader kinase activity assays developed for each CDK



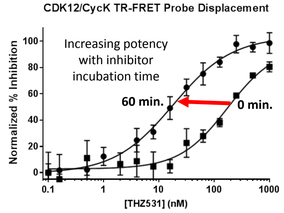


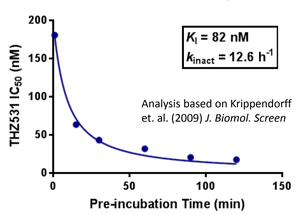


CDK12/CycK Kinase Inhibition

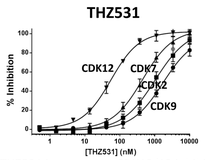


CDK12/CycK TR-FRET Probe Displacement

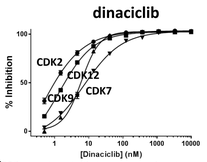




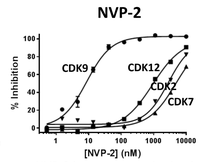
A suite of CDK assays enables evaluation of the CDK selectivity profile for each inhibitor and supports ongoing discovery



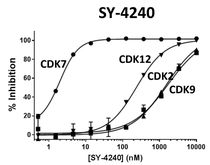
THZ531 is a covalent CDK12/13 inhibitor



Dinaciclib is a non-selective pan-CDK inhibitor



NVP-2 is a potent and selective CDK9 inhibitor

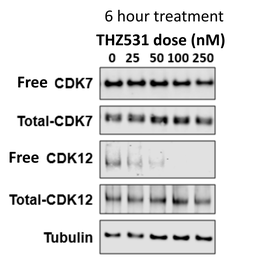


SY-4240 is a highly potent and selective CDK7 inhibitor

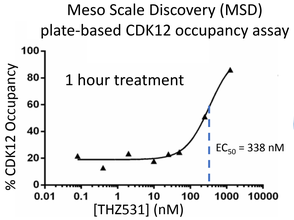
PerkinElmer LabChip EZ Reader kinase assays, all using K_M **ATP** for each CDK

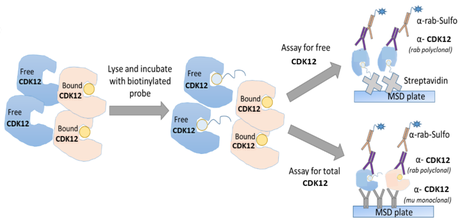
Additional assays developed but not shown: CDK1, CDK3, CDK4, CDK5, CDK6; All exhibit IC_{50} values > 2 μM except with dinaciclib

Cellular target engagement assay elucidates the relationship between CDK12i exposure, dose & time with CDK12 occupancy for mechanistic studies



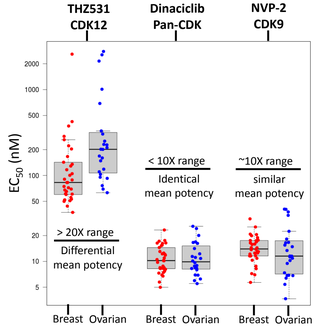
Western blot using same reagents as MSD assay except chemiluminescent detection





- Data from HL-60 cells (leukemia-derived, suspension). These assays have also been adapted to adherent cell lines and tumor tissue samples.
- THZ531 fully occupies CDK12 on the 1-6 hour timescale (dose-dependent) but **does not covalently occupy CDK7** at these doses after 6 hours

A large panel of breast and ovarian cancer cell lines exhibit differential sensitivity to CDK12i vs. other CDKi



Anti-proliferation assays using ATPlite

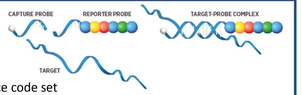
- Anti-proliferation potency ranges reflective of biochemical potency for each compound
- Range of sensitivities observed with THZ531 likely demonstrating differential sensitivity to varying levels of CDK12 inhibition
- Range of sensitivities to dinaciclib is narrow with identical means between breast and ovarian, likely due to polypharmacology
- Range of sensitivities to NVP-2 is narrow and likely reflective of similar impacts due to CDK9 inhibition across multiple cell lines

Changes in transcription with inhibitor treatment reveal distinct effects of inhibiting different transcriptional CDKs

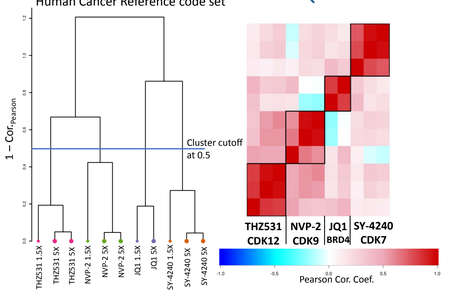
Samples clustered according to change in gene expression upon treatment with different transcriptional inhibitors

- HCC70 TNBC cell line
- Treatment for 6 hours at 1.5X EC_{50} and 5X EC_{50} for each inhibitor
- Quantification of changes in transcript abundance relative to DMSO control
- Correlation in transcript changes calculated across 157 detectably expressed genes in the DMSO set
- Pearson correlation coefficients based on transcript abundance changes were calculated between all sets of different treatment pairs, and 1 – Cor. Coef. Was used as a distance metric for clustering

Cartoon courtesy of:



Human Cancer Reference code set



Conclusions and Future Work

- Syros has developed a suite of assays capable of discriminating sub-nM CDK inhibitors, quantifying time-dependent covalent inhibition, assessing CDK family selectivity, and evaluating cell-based CDK12 and CDK7 occupancy.
- Profiling a large set of cell lines derived from breast and ovarian cancer revealed important differences between selective inhibition of CDK12, CDK9, and pan-CDK inhibition.
- Expression profiling of a TNBC cell line treated for 6 hours with THZ531 (CDK12i), SY-4240 (CDK7i), NVP-2 (CDK9i), and JQ1 (BRD4i) revealed distinct transcriptional changes
- Syros continues to optimize potent, selective, and orally bioavailable CDK12 and CDK7 inhibitors suitable for clinical development.
- Examination of transcriptional changes and consequential cellular effects with benchmark inhibitors form the basis of understanding the determinants of efficacy and tolerability in pre-clinical models of breast and ovarian cancer.