CDK12 and CDK13 regulate expression of large transcripts requiring substantial processing to produce mature mRNA. This transcriptional regulation is constitutively phosphorylated on specific residues within the C-terminal domain of RNA polymerase II and associated with RNA processing factors (Chila, 2016). RNAi knockdown of CDK12 in cell culture decreases expression of DNA damage response genes, including BRCA1 and AT10, while enhancing sensitivity to DNA damaging agents (Brazel, 2011; Liang, 2015). Recently THZ531, a selective covalent inhibitor of CDK12 and CDK13, was shown to potentiate DNA damage response genes in cancer cells. 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 DNA-PK inhibitors or DNA damage agents for difficult-to-treat cancers such as high-grade serous ovarian cancer and triple-negative breast cancer.

Using THZ531 as a benchmark, we developed assays capable of discriminating sub-nM inhibitors, including those with low levels of pan-CDK activity and cell-based CDK occupancy assays. Expression profiling of THZ531 treated cell lines resulted in different sets of genes being affected than was observed following treatment with inhibitors targeting CDK7, CDK9 or BET-bromodomain proteins. Additional assays developed including biochemical potency, mimic phospho-CDK selectivity and selectivity of pan-CDK for CDK12, CDK9 and CDK7. Syros continues to optimize potent, selective, and orally bioavailable CDK12 and CDK7 inhibitors suitable for clinical development.

While the pharmacokinetic properties of THZ531 preclude adequate target engagement in tumor tissue, data from HL-60 cells (leukemia-derived, suspension) suggest that THZ531 exposure, dose & time with CDK12 occupancy for mechanistic studies. 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 CDK7 on the 6-12 hour timescale (dose-dependent) but does not covalently occupy CDK7 at these doses after 6 hours.

Conclusions and Future Work

• Syros has developed a suite of assays capable of discriminating sub-nM CDK inhibitors, quantifying the impact of CDK12, CDK9, and pan-CDK inhibition, assessing cellular activity of CKD12 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 THZ531, SY-4240, and JQ1 across multiple cell lines demonstrated differential sensitivity of CDK12i vs. other CDKi.
• Examination of transcriptional changes and consequential cellular effects with benchmark inhibitors revealed important insights into the activity of CDK12i vs. other CDKi.
• Sampling 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.
• 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 provided the basis of understanding the efficacies and tolerability of pan-CDK inhibitors in preclinical models of breast and ovarian cancer.