

Accepted as poster #3521 at the American Association for Cancer Research (AACR) Annual Meeting 2020, June 22–24, 2020

Transcriptome Analysis of TCGA Prostate Cancer Samples Identifies an Association of Poorer Survival and Aggressive Disease Biology with CDC-like Kinase (CLK) Expression and Spliceosome Regulation

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In prostate cancer, alternative splicing of mRNA and spliceosome activity are implicated in several areas of disease pathogenesis. This is exemplified by the strong association of androgen receptor splice variants with treatment resistance and poor clinical outcome in castration-resistant disease. Therefore, pharmacologic targeting of spliceosome-regulating proteins such as CLKs and serine/arginine-rich splicing factors (SRSFs) represents a novel treatment approach for prostate cancer. To evaluate the therapeutic potential of inhibiting CLK activity in prostate cancer, the association between splicing-related gene expression and survival was investigated in The Cancer Genome Atlas Prostate Adenocarcinoma (TCGA-PRAD) data collection (N=495).

Survival analysis of RNA-seq data assessed 17,879 genes to measure their association with progression-free interval (PFI). Using transcript per million as the metric for normalized gene expression, age-adjusted Cox proportional hazards regression models were performed for each gene (R v3.6.0, coxph v2.43-3). A total of 3,145 genes significantly correlated with worse prognosis (P -adj<0.10, Cox coefficient >0). *CLK1* (P -adj=0.0218, HR=1.5939), *CLK2* (P -adj=0.001298, HR=2.1393), and *SRSF2* (P -adj=0.00167, HR=3.2917) were found to be positively associated with poorer PFI, ranking 1202, 400, and 437, respectively. Reactome pathway analysis of the significant gene set showed that mRNA splicing and processing accounted for 5 of the 19 pathways that were strongly associated with poorer PFI.

An additional pathway analysis (GSEA v3.0, MSigDB v6.2) of tumors categorized by *PTEN* status to assess relationship with disease severity showed that mRNA splicing (P -adj=0.0243, NES=1.7714) was enriched in *PTEN*-null versus *PTEN*-wt tumors. Other pathways of interest, including Wnt signaling (P -adj=0.0187, NES=1.846), cell cycle (P -adj=0.0124, NES=1.974), chromatin remodeling (P -adj=0.0135, NES=1.901), DNA damage repair (P -adj=0.013974, NES=1.8934), and *PTEN* regulation (P -adj=0.0230, NES=1.7861), were also enriched in *PTEN*-null tumors.

Lastly, a survival analysis within all TCGA-PRAD patients showed that low *CLK1* (P =0.03) and *CLK2* (P =0.0004) expression were individually associated with better prognosis versus their high-expressing counterparts. Analysis of *CLK3* and *CLK4* expression did not reach statistical significance.

Collectively, these findings revealed an association of spliceosome activity and *CLK1/2* expression with aggressive disease biology in prostate cancer. A Phase 1 study of SM08502, a novel, small-molecule pan-CLK inhibitor, in subjects with advanced solid tumors is ongoing (NCT03355066). This analysis nominates prostate cancer as a tumor type worth further exploring for the clinical activity of SM08502.

References:

1. Paschalis A, et al. *Nat Rev Clin Oncol*. 2018 Nov;15(11):663-675. doi: 10.1038/s41571-018-0085-0.
2. Ku Y, et al. *Nat Rev Urol*. 2019 Nov;16(11):645-654. doi: 10.1038/s41585-019-0237-8.
3. Armenia J, et al. *Nat Genet*. 2018 May;50(5):645-651. doi: 10.1038/s41588-018-0078-z.
4. Colwill K, et al. *EMBO J*. 1996 Jan 15;15(2):265-75.
5. Duncan P, et al. *Exp Cell Res*. 1998 Jun 15;241(2):300-8.
6. Stojdl DF and Bell JC. *Biochem Cell Biol*. 1999;77(4):293-8.