# Identifying Inhibitors and Inducers of the Alternative Lengthening of Telomeres (ALT) Pathway Through the Development of a High-throughput Phenotypic Screen

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In healthy human cells, telomeres are end caps on DNA strands to protect against degradation. However, as cell replication increases, telomeres progressively shorten, eventually triggering cell death. In cancer cells, telomeres lengthen to evade senescence. While such uncontrollable proliferation of 85-90% of tumors is sustained by reactivation of telomerase, an enzyme responsible for telomeric DNA sequences synthesis, 10-15% of human cancers rely on a telomerase-independent mechanism called the alternative lengthening of telomeres (ALT). There're many biomarkers of ALT, but C-circles, partially single-stranded telomeric DNA circles, are the most robust due to their quantifiability and specificity to ALT. Though the pathway isn't fully understood, ALT cancers are known to be more aggressive. Additionally, due to the lack of ALT-targeted treatments, there's a pressing need for us to develop therapeutic options to selectively inhibit this mechanism. By testing different assay conditions, I've optimized an existing low-throughout assay into a high-throughput screen that's significantly more time and cost efficient and sensitive for large scale screening of ALT activity inhibitors. I screened ALT positive cells against an epigenetic chemical compound library in this platform and measured relative C-circle level as an indicator of ALT activity changes in response to compound treatment. From the screen, I've detected and evaluated a handful of inhibitors that alter ALT activity in neuroblastomas and osteosarcomas. Additionally, I tested for cytotoxic effects of the inhibitors and found varying concentrations at which hit compounds maintained cell viability. In the future, I'll conduct orthogonal assays to validate the inhibitors as potential therapeutics.