**PRE-CLINICAL CHARACTERIZATION OF G1T28, A NOVEL CDK4/6 INHIBITOR FOR PROTECTION OF BONE MARROW FROM CYTOTOXIC CHEMOTHERAPIES**

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**ABSTRACT**

G1T28 is a clinical-stage, oral, novel inhibitor of cyclin-dependent kinases 4 and 6 (CDK4/6). Preclinical evaluation of G1T28 identified its unique preclinical profile as a first-in-human (FIH) compound with clinical potential. It is a novel CDK4/6 inhibitor that shows potent and selective activity against CDK4 and CDK6, the key drivers of human cancer, and is a robust chemoprotectant. The results presented include a comprehensive assessment of the safety and pharmacology of G1T28 in CDK4/6-dependent and CDK4/6-independent xenograft models. G1T28 was found to be a potent, selective, and effective chemoprotectant in bone marrow, with potent in vitro activity against CDK4/6-dependent cells. In vivo, G1T28 showed promising pharmacokinetic properties, with a mean time to peak plasma concentration (Tmax) of 0.5 hours and a mean residence time (MRT) of 3.2 hours. G1T28 demonstrated a clean, robust G1 arrest in CDK4/6-dependent fibroblasts treated with 300 nM G1T28 for up to 24 hours. Following treatment, the cell cycle distribution returned to control levels 16−24 hours after treatment with G1T28, returning to control levels 16−24 hours after G1T28 washout as well. The G1 cell cycle arrest observed in CDK4/6-dependent fibroblasts treated with G1T28 was confirmed in vivo using microfluidic kinase detection technology (Caliper Assay Platform). The compound was tested in multiple xenograft models representing CDK4/6-dependent and CDK4/6-independent cancers, and the compound showed promise as a targeted bone marrow chemoprotectant.

**METHODS**

**Cell Cycle Analysis:** Determination of cell fractions in various stages of the cell cycle following treatment with either vehicle or indicated dose of G1T28 was performed using Flow Jo 7.3.2 software analysis.

**Western Blot Analysis:** Cells were incubated with G1T28 for varying times. whole-cell extracts were harvested using RIPA buffer (ThermoFisher Scientific, Waltham, MA). Protein concentrations were determined using the DC Protein Assay Kit (Bio-Rad, Hercules, CA). Equal amounts of protein were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane. After blocking, membranes were probed with a monoclonal antibody against phospho-Rb on Ser807/Ser811 (Cell Signaling Technology, Danvers, MA), followed by horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Membranes were washed and developed using the ECL Plus system (Amersham Biosciences, Piscataway, NJ).

**RESULTS**

**FIGURE 1. G1T28 is a Potent and Selective Inhibitor of CDK4/6**

**FIGURE 2. In vivo Selectivity for CDK4 in DiscoverX® Panel**

**SUMMARY**

G1T28 is a potent and selective inhibitor of CDK4/6, with a unique pharmacological profile that suggests clinical potential. It shows promise as a targeted bone marrow chemoprotectant.

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