1314 TRILACICLIB, AN INTRAVENOUS CYCLIN-DEPENDENT KINASE 4/6 INHIBITOR, ENHANCES ANTITUMOR RESPONSES BY MODULATING T CELLS

Sarah Ahn*, John Yi, Subing Cao. *G1 Therapeutics, Inc., Research Triangle Park, NC, USA*

Background Cyclin-dependent kinase (CDK)4/6 inhibitors, including trilaciclib, have been shown to augment antitumor immunity.[1,2] In an open-label, phase 2 trial in patients with metastatic triple-negative breast cancer (mTNBC), administration of trilaciclib prior to gemcitabine plus carboplatin improved overall survival, potentially through protection and direct activation of immune function.[3,4] Here, we report the effects of transient, trilaciclib-mediated CDK4/6 inhibition on immune function *in vitro*.

Methods Peripheral blood mononuclear cells (PBMCs) or naïve $CD4^+$ and $CD8^+$ T cells were purified from 6 healthy human donors and activated with CD2/3/28 beads with or without trilaciclib. To visualize phenotypic and functional changes, trilaciclib was added to naïve $CD4^+$ and $CD8^+$ T cells 0-, 1-, and 3-days post-activation. Activated T cells were collected and stained for flow cytometric analyses 3-, 7-, and 14-days post-activation. Supernatant from activated PBMCs was harvested after 72 hours and added to human breast cancer cells. Following 24 hours' incubation, levels of programmed death-ligand 1 (PD-L1) and human leukocyte antigen (HLA) class I and II were quantified by flow cytometry, and CXCL9 and CXCL10 chemokines by enzyme-linked immunosorbent assay.

Results Irrespective of when trilaciclib was added to $CD4^+$ and $CD8^+$ T cells, significant increases in the frequency of $CD45RO^+$ memory T cells were observed. Within $CD45RO^+$ memory T cells, T cells incubated with trilaciclib had increased frequencies of $CD62L^{lo}CD69^{hi}$ effector $CD4^+$ and $CD8^+$ T cells, with increases in $CD62L^{lo}CCR7^{lo}$ effector memory T cells also observed. Furthermore, trilaciclib significantly increased CXCL9 and CXCL10 levels ($P=0.0001$). Surface expression of PD-L1 and HLA class I and II was increased in breast cancer cell lines cultured with supernatant from T cells activated in the presence of 50 and 100 nM of trilaciclib, resulting in a greater frequency of cells being double-positive for HLA class I and II or HLA class I and PD-L1.

Conclusions Trilaciclib may enhance antitumor immunity by modulating essential steps in the cancer-immunity cycle. Our data suggest trilaciclib may increase antigen presentation by promoting HLA class I and II expression and the recruitment of T cells to the tumor site via CXCL9 and CXCL10. Trilaciclib also augments the differentiation of T cells by promoting the formation of memory T cells. These data support a role for trilaciclib in improving antitumor efficacy, as observed in the phase 2 trial in mTNBC, and provide a rationale to combine trilaciclib with immunotherapy to enhance immunogenicity within the tumor microenvironment.

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Ethics Approval This study was approved by the Wake Forest University School of Medicine's Ethics Board under IRB #00080511.

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