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NOVEL SINGLE CHAIN ANTIBODIES TO INHIBIT CCR7 MEDIATED-ENTRY OF PEDIATRIC T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA INTO THE CNS

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Aim: Approximately 30 percent of pediatric T-cell acute lymphoblastic leukemia (T-ALL) patients who undergo standard chemotherapy will suffer disease relapse within two years of completing treatment. Many of these patients develop leukemic involvement of the central nervous system (CNS). Currently, there is an urgent need to identify chemotherapeutic agents with minimal toxicity that can restrict migration of these leukemic cells into the CNS. C-C chemokine receptor 7 (CCR7) expression in T-ALL cells is both vital to and sufficient for migration of leukemic cells into the CNS.

Methods: We have genetically engineered anti-CCR7 antibodies (scAbs) against human CCR7 to block CNS entry during chemotherapy. Binding constants of the scAbs were measured by flow cytometry. Chem-1 calcium optimized cells were used to determine which scAbs block signaling through CCR7. To analyze the inhibitory effect of the scAbs on the processes required for entry to the brain, we analyzed transmigration of primary human T-ALL through a brain endothelial cell (HBEC) monolayer. To determine if the antibodies inhibit migration, in vivo, we have obtained a humanized murine model of T-ALL.

Results: We have tested a panel of eight CCR7-function scAbs for their ability to block binding of CCR7 ligand binding, and downstream signaling events (Ca2+mobilization, transmigration), and have identified a subset that can block CCR7 activation, and receptor-mediated binding to ligand in vitro, with an EC50 of 1.89nM to 5.49nM. At a 1 µM concentration, our top 2 scAbs blocked calcium mobilization in Chem-1 CCR7 expressing cells. In the presence of the EC50 concentration for each antibody, we have identified a single candidate scAb that successfully blocked transmigration of primary human T-ALL across an HBEC monolayer. At present studies are ongoing to determine if these antibodies can prevent breach of the blood brain barrier in vivo.

Conclusions: We have identified novel genetically engineered CCR7-function-blocking single-chain antibodies (scAbs) that inhibit CCR7-induced signaling and downstream migration in vitro that will serve as a platform for our studies in vivo. Ultimately, the goal of our studies is to define candidate CCR7-function blocking antibodies that can serve as potential chemotherapeutic agents to decrease relapse rates and improve overall survival.

Disclosure: E. Kim: Dr. Kim is the Chief Scientific Officer and Co-Founder of MSM Protein Technologies. He works with MSM Protein, an early drug development company and has supplied our lab with the antibodies targeting our G-protein Coupled Receptor of interest (CCR7). All other authors have declared no conflicts of interest.