24 SMALL-MOLECULE REGULATED SAFETY SWITCH FOR IMPROVED SAFETY OF CAR CELL THERAPIES IN THE BRAIN

[1]Rebecca Cottman*, [1]Vanessa Johnson, [1]Michelle Hung, [1]Yin Yin Chong, [1]Eren Chu, [1]Assen Roguev, [1]Marcus Gainer, [1]Joseph Sperling, [1]Monika Avina, [1]Brett Kiedaisch, [2]Raghav Kannan, [2]Chandra Verma, [3]Chew-Li Soh, [3]Conor McAuliffe, [3]Mark Tomishima, [1]Russell M Gordley, [1]Timothy K Lu. *[1]Senti Biosciences, South San Francisco, CA, USA; [2]Aplomex, Queenstown, Rajah Crescent, Singapore; [3]BlueRock Therapeutics, New York, NY, USA*

Background The application of CAR T cell therapies have demonstrated success in the clinic in select hematologic malignancies. Their broad utilization has been limited to select centers with specialized care due to risk of Cytokine Release Syndrome (CRS) and Immune effector cell-associated neurotoxicity (ICANS). Longer term effects include a potential for clonal dominance and tumorigenicity. Small molecule (SM) regulated safety switches have been developed that enable quick clearance of engineered cells at the onset of adverse symptoms that threaten the health of the patient. These platforms largely rely on rapalogs, which are unable to cross the blood-brain barrier (BBB), providing poor protection from CAR-T toxicity in the brain. To address this need, we developed a safety switch inducible by clinical levels of Tamoxifen, a FDA approved small molecule that crosses the BBB.

Methods The safety switch includes an inactive monomer of a pro-apoptotic factor, Caspase-9 (Casp9) fused to ERT2, a tamoxifen-triggered dimerization domain, such that SM induces Casp9 dimerization and apoptosis. Cells transduced with the safety switches were labeled with mCherry, and killing efficiency was evaluated by imaging mCherry+ cells in the SM-treated versus drug-free condition via Incucyte.

Results Safety switches using the previously reported ERT2 achieved 30% killing within 72 hours of treatment with 2.5 nM tamoxifen metabolites, the estimated concentration in the brain from a clinical dose of Tamoxifen, falling short of key safety metrics of complete (>95%) and quick (<48 hours) clearance of engineered cells. Computational simulation of ERT2-ligand complexes revealed mutations likely to enhance drug sensitivity. These were built into a combinatorial library of ERT2 variants where their SM sensitivity was linked to induced fluorescent reporter expression. When libraries were screened using FACS, and cells that turned on the reporter at desired SM concentrations were sequenced, we identified ERT2 variants with enhanced SM sensitivity. Safety switches built using these variants achieved complete killing of primary T cells within 48 hours of induction at concentrations of tamoxifen metabolites estimated in the brain.

Conclusions SM regulated safety switches can rapidly reverse CAR-T mediated toxicity in patients, but current switches are regulated by drugs that are incapable of crossing the BBB. We have addressed this challenge through the development of safety switches responsive to pharmacologically relevant concentrations of a FDA approved SM that penetrates the BBB. This technology can be applied to mitigate CAR-T induced neurotoxicity, potentially expanding the therapeutic window for brain-targeted therapies.

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