Engaging Bicycle® NK-TICA™ Drives Targeted Tumor Cytotoxicity

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Background: Natural killer (NK) cells are immune cells that can detect and eliminate tumor cells and bridge innate to adaptive immune responses. Bicycles are small (ca. 1.5 kDa), chemically synthetic, structurally constrained peptides discovered via phage display and optimized using structure-driven design and medicinal chemistry approaches. We have now applied this technology to identify Bicycles that bind specifically to a key activating receptor, NKp46. We term this new class of fully synthetic molecules Natural Killer tumor-targeted immune cell agonists (NK-TICA™) and herein we will describe their in vitro evaluation.

Methods: Using our unique phage display screening platform, we have identified high affinity, selective binders to NKp46. By conjugating the Bicycle® NK cell-engaging binders to model tumor antigen EphA2-binding Bicycle®, we have developed a bifunctional Bicycle® NK-TICA™ molecule. In vitro functional assays, we evaluated the ability of the Bicycle® NK-TICA™ to induce NK cell activation and proliferation as well as cell-mediated cytotoxicity and cytokine production in NK tumor co-culture assays. We similarly evaluated changes in EphA2 tumor binding affinity of the Bicycle® NK-TICA™s in their ability to enhance NK immune response.

Results: We have developed novel modular compounds with high affinity and selectivity to NK cell receptors with specific tumor targeting capability. We demonstrate potent, selective binding of our Bicycles to receptor-expressing cells and the capability of the bifunctional molecule to induce NK cell function. With Bicycle’s novel NK-TICA™ compounds, we demonstrate engagement of NK cells, specific activation and function of NK cells, and enhanced tumor cytotoxicity across multiple tumor antigens, in a dose dependent manner. With changes in tumor binding affinity, we found changes in the activation of the NK cells in vitro.

Conclusions: We have shown that when chemically coupled to a tumor antigen binding Bicycle®, the result is a bifunctional molecule (NK-TICA™) capable of directing potent and specific NK cell activation and kill in vitro.