

Title: Investigating nanobody appended CSANs on targeting immune cells to tumor cells

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Cancer immunotherapy involves utilizing a patient's own immune system to identify and destroy cancer cells. Recent advances in this field involve bispecific monoclonal antibodies, genetically engineered T-cells, and immune cell engagers, which have shown success in clinical settings. In this regard, the Wagner Lab has shown that prosthetic antigen receptor (PAR) T-cells, which use chemically self-assembled nanorings (CSANs) can be successfully used to redirect T-cells to target antigens upregulated on cancer cells. CSANs are constituted of two dihydrofolate reductase molecules (1DD) linked via a glycine, assembled into rings using a chemical dimerizer, bis-methotrexate. Bispecific CSANs consisting of alpha CD3 single-chain variable fragment (scFv) recognizing CD3 on T-cells and alpha EpCAM scFv have been shown to successfully kill breast cancer cells. However, the scFvs, though showing excellent antigen binding capacity, pose challenges in production, stability and immunogenicity. Camelid heavy-chain variable domains, also called nanobodies, have been established as a popular alternative to the scFvs due to their smaller size, increased tissue penetration, low immunogenicity, and ease of bacterial expression, while showing similar antigen binding properties as scFvs. In this project, CSANs composed of an alpha-TCR (T-cell receptor) nanobody and an alpha-EGFR (Epidermal Growth Factor Receptor) nanobody will be evaluated to target solid tumors that overexpress EGFR. 1DD proteins will be expressed in *E. coli* as soluble proteins. Size Exclusion Chromatography, Dynamic Light Scattering, Transmission Electron Microscopy would be used as tools to evaluate CSAN formation. Flow cytometry will be used to evaluate binding properties of the CSANs to cancer cells and T-cells. Live cell imaging and cytokine release assays will be used to inspect the in vitro cytotoxic potential of CSANs. The efficacy of the nanobody based CSANs will then be evaluated in vivo. Nanobodies, due to their low immunogenicity, have a high potential to be clinically translatable. This project thus aims to develop a new generation of nanobody based CSANs.