1200 A CLINICALLY VALIDATED PH-SENSITIVE NANOMEDICINE PLATFORM FOR ENCAPSULATING THERAPEUTIC BISPECIFIC T CELL ENGAGERS FOR TUMOR SPECIFIC DELIVERY AND ACTIVATION

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Background Bispecific antibodies (BsAbs) are an important class of therapeutics for immune-oncology applications. T cell engagers (TCEs) target tumor-associated antigens and cytotoxic T cells to eradicate antigen-expressing tumor cells. Blinatumomab (CD19 X CD3 bispecific) is approved for CD19-positive B cell acute lymphoblastic leukemia,[1] but its toxicity may be limiting, with one-third of patients in the pivotal Phase 3 study requiring treatment interruption for adverse events.[2] TCEs for solid tumors have likewise demonstrated encouraging clinical efficacy but shown dose-limiting toxicities due to on-target/off-tumor effects[3,4] For instance, patients receiving solitomab (EpCAM X CD3 bispecific) experienced severe gastrointestinal toxicity which precluded its further development.[5] To minimize the off-tumor effects, we have developed ON-BOARD, an ultra-pH sensitive nanoparticle platform, has shown utility in cytokine and monoclonal antibody encapsulation and targeted delivery to the acidic tumor microenvironment.[6,7] The clinical safety and feasibility of ON-BOARD has been demonstrated by effective delivery of fluorophores to solid tumors for imaging of multiple tumor types in Phase I and II clinical trials with pegsitacianine.[8] Herein we expand the utility of ON-BOARD platform for the encapsulation and pH-specific activation of bispecific antibodies with potential for anticancer therapy.

Methods A panel of BsAbs (including biosimilar equivalents of blinatumomab, solitomab, and others) was used to demonstrate encapsulation by the ON-BOARD platform and pH-dependent activation. Formulations of ON-BOARD with BsAbs were purified by size exclusion chromatography and the encapsulation efficiencies were quantified by HPLC. Particle size and uniformity were studied by dynamic light scattering. ON-BOARD/BsAb formulations were assessed *in vitro* under neutral pH or acid-activated conditions to determine target engagement by ELISA, bio-layer interferometry. The target-specific bioactivity and therapeutic window was determined by TDCC assays in multiple models.

Results ON-BOARD nanoparticles successfully encapsulated bispecific antibodies across a wide range of tumor-associated antigens (TAAs), including HER2, EpCAM, CEACAM5, CD19, and CD20, and structural configurations (tandem scFv and Fc-fusion). ON-BOARD formulations were stable nanoparticles with narrow size distribution (<70 nm), good encapsulation efficiency (up to 98%) and drug loading (up to 8%). Acid-mediated release and target engagement of both TAA-targeting and CD3-targeting arms was demonstrated using *in vitro* binding assays with >100-fold activation window. Further pH-specific cell killing was confirmed by TDCC assays in multiple *in vitro* models including Burkitt lymphoma, breast cancer, colorectal cancer, and lung cancer.

Conclusions The ON-BOARD pH-sensitive nanoparticle platform demonstrated potential as an effective and universal tool for solid tumor specific activation and delivery of bispecific antibody therapeutics, potentially minimizing systemic side effects.

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http://dx.doi.org/10.1136/jitc-2022-SITC2022.1200