AACR 2023
815

Improved Tolerability and Tumor Specific Delivery of a Therapeutic Bispecific T Cell Engager Using a pH-Sensitive Nanoparticle Platform

Qingtai Su, Stephen Gutowski, Gaurav Bharadwaj, Austin Burcham, Bhargavi Allu, Irina Kalashnikova, Zirong Chen, Ruolan Han, Jason B. Miller, Tian Zhao

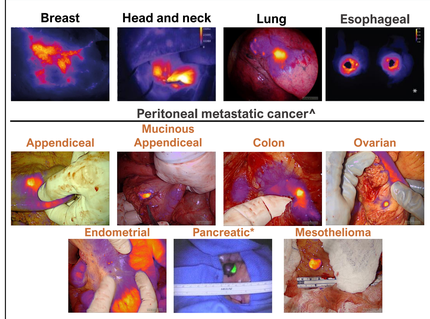
[1]OncoNano Medicine, Inc., Southlake, TX 76092

Introduction

Bispecific antibodies are an emerging class of therapeutics for immune-oncology applications. T cell engagers (TCEs) target tumor-associated antigens (TAA) and T cells to eradicate antigen-expressing cancer cells. TCEs for solid tumors have demonstrated encouraging preclinical efficacy but development has been challenging resulting in dose-limiting toxicities due to on-target/off-tumor effects. To increase tumor specificity, we have developed ON-BOARD, an ultra-pH sensitive nanoparticle platform for masked and targeted delivery to the acidic tumor microenvironment (TME). Herein we report the ON-BOARD platform for the efficacious masked delivery of TAA targeting TCEs to tumors in mice demonstrating significantly improved tolerability and potential for clinical translation of this platform.

TAA-targeting TCEs were encapsulated in ON-BOARD nanoparticles and these formulations were characterized for particle properties and drug loading. ON-BOARD•TCE nanoparticles were assessed *in vitro* under neutral pH or acid-activated conditions in TDCC assays. *In vivo* studies were performed in mice bearing "immune desert" pancreatic cancer tumors. ON-BOARD tumor localization was measured by fluorescence while unencapsulated TCE and ON-BOARD-TCE pharmacokinetics was also evaluated. Pharmacodynamic studies evaluated immune-phenotype changes in the TME and draining lymph nodes, and systemic cytokine levels following a single injection. Efficacy studies demonstrated that ON-BOARD-TCE achieved comparable tumor growth inhibition to unencapsulated TCE while significantly mitigating the toxicity as a monotherapy and in combination with anti-4-1BB agonistic monoclonal antibody.

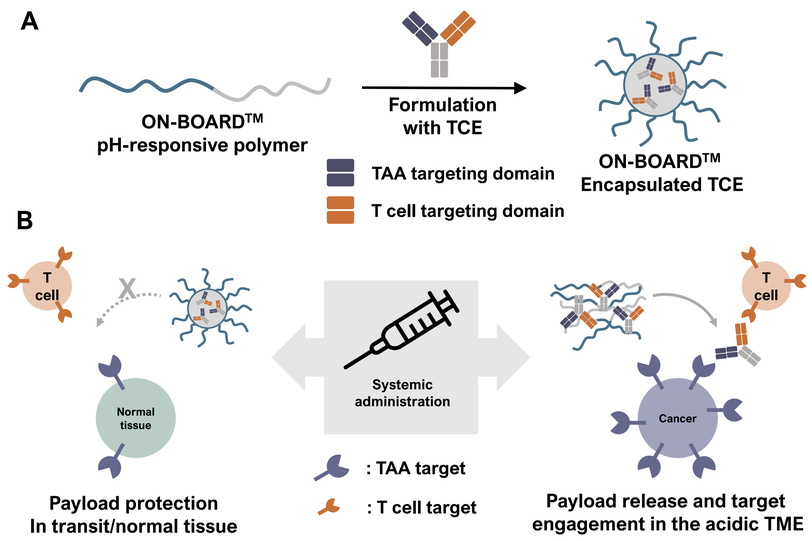
Pegsitacianine clinically validates ON-BOARD™ specificity in multiple solid tumor types in patients



- Pegsitacianine demonstrated tumor agnostic targeting and high specificity using ON-BOARD™ micelles
- Lighting up of 11 different tumor types with the same agent in 135 patients
- No drug-related SAEs
- Detection of residual disease (missed by SOC) in 50% of peritoneal patients
- Sensitivity: 84%
- FDA granted **Breakthrough Therapy Designation** for the visualization of metastatic disease in the peritoneal cavity in patients undergoing cytoreductive surgery

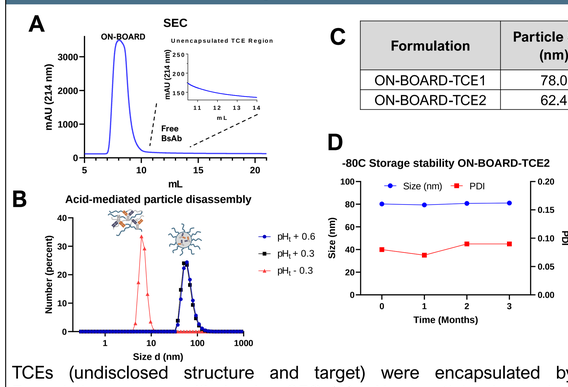
^Tissue of origin imaged during cytoreductive surgery during peritoneal metastatic cancer trial.
*Pancreatic photo is created by merge with a pseudo-colored image after imaging acquisition with Hamamatsu PDE
[1] Voskuil FJ, Steinkamp PJ, Zhao T, et al. Nat Commun, 2020; 11: 3257, [2] Clinicaltrials.gov: NCT03735680 NCT04950166, NCT05048082

ON-BOARD™ enables bispecific T cell engager encapsulation and tumor delivery with reduced systemic exposure



ON-BOARD™ can encapsulate therapeutic bispecific antibody payloads of a diverse range of protein constructs in pH-responsive nanoparticles without protein engineering of the payloads (**A**). Following systemic administration (**B**) ON-BOARD™ can reduce on-target/off-tumor interactions in normal tissue but trigger payload release and target engagement in the acidic tumor microenvironment.

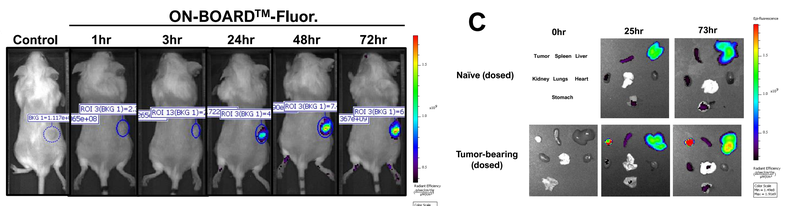
ON-BOARD™ encapsulates different bispecific T cell engagers



C	Formulation	Particle Size (nm)	PDI	EE%
	ON-BOARD-TCE1	78.0	0.043	88.9%
	ON-BOARD-TCE2	62.4	0.17	100%

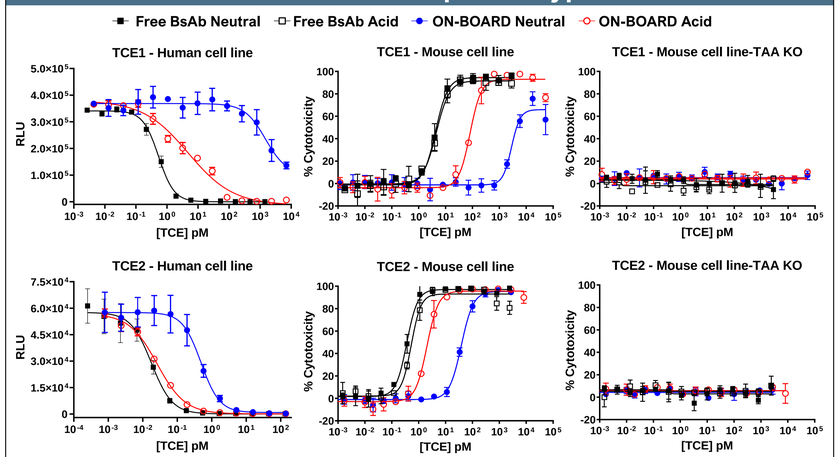
TCEs (undisclosed structure and target) were encapsulated by ON-BOARD™ nanoparticles. Formulations were purified using SEC (**A**). Nanoparticle size and pH-responsive disassembly were determined by dynamic light scattering (**B**) and the encapsulation efficiencies were quantified (**C**). ON-BAORD formulations were stable for at least 3 months in -80 ˚C storage (**D**) as evaluated by DLS and showed no TCE payload leakage or degradation as evaluated by western blot (**E**).

ON-BOARD™ effectively accumulates in "immune desert" pancreatic cancer in mice



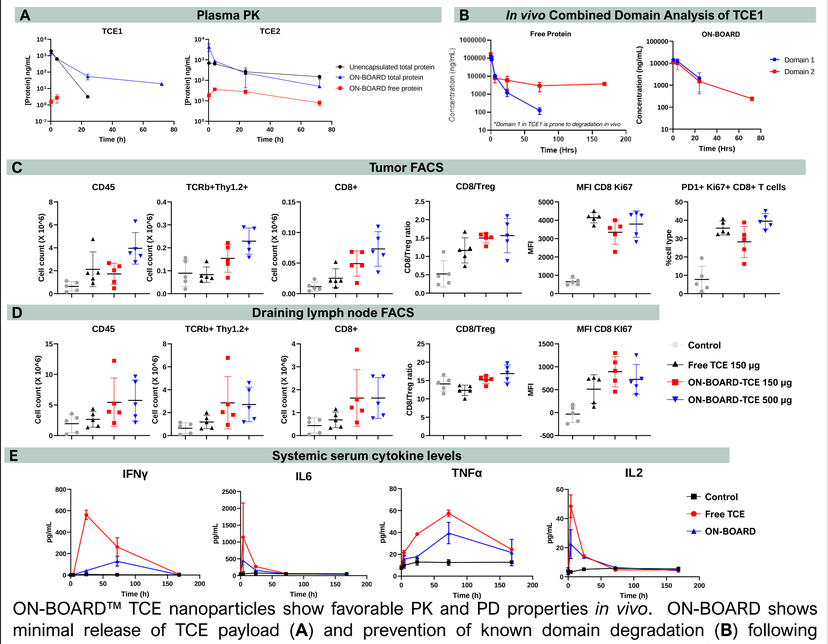
ON-BOARD™ micelles labeled with a fluorophore effectively accumulate in mouse tumors *in vivo* following systemic intravenous administration. ROI analysis of *in vivo* (**A**,**B**) and *ex vivo* (**C**,**D**) fluorescence imaging confirmed tumor uptake (N = 4-5).

ON-BOARD™ shows pH-responsive bioactivity with multiple BsAb constructs in multiple cell types *in vitro*



ON-BOARD™ formulations demonstrated protection and pH specificity in TDCC assays against mouse and human cancer cell lines as target cells. No cell killing was observed in TAA knockout cells.

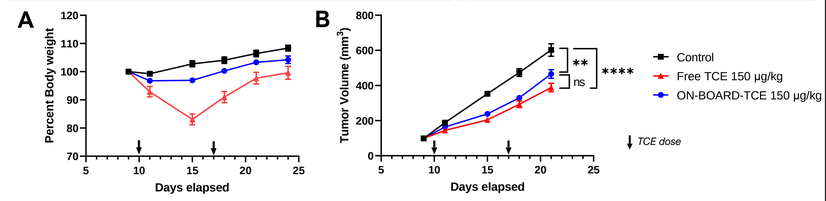
ON-BOARD™•TCE formulation improves tolerability and shows favorable PK and systemic/TME PD properties in mice



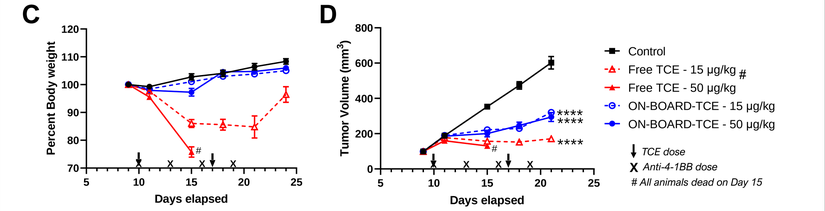
ON-BOARD™ TCE nanoparticles show favorable PK and PD properties *in vivo*. ON-BOARD shows minimal release of TCE payload (**A**) and prevention of known domain degradation (**B**) following systemic administration. Flow cytometry analysis in the tumor microenvironment (**C**) and tumor draining lymph node (**D**) shows increased cellularity, CD8+ T cells, CD8/T reg ratio and CD8 T cell activation (Ki67, PD1). ON-BOARD™ also induced lower cytokine release in serum (**E**) compared to free TCE.

ON-BOARD™•TCE formulation synergizes with anti-4-1BB to inhibit "immune desert" pancreatic cancer tumor growth in mice

Monotherapy



Anti-4-1BB combo therapy



In a poorly immunogenic tumor model, ON-BOARD™-TCE formulation significantly inhibited tumor growth and improved tolerability compared to unencapsulated TCE both as a monotherapy (**A**,**B**) and in combination with anti 4-1BB (**C**,**D**). Animals were randomized at 100 mm^3. ON-BOARD-TCE and free TCE were dosed IV on Days 10 and 17 at the indicated dose while anti-4-1BB was administered IP at 150 μg per injection on days 10, 13, 16, and 19. Statistics were performed by one-way ANOVA.

Summary

Encapsulation of therapeutic T cell engagers for tumor specific delivery and pH-dependent activation using ON-BOARD™, a clinically validated pH-sensitive nanoparticle platform, has been achieved. ON-BOARD demonstrated improved tolerability of TCE payloads *in vivo* and anti-tumor efficacy in a challenging "immune desert" tumor model. This work demonstrated great potential of using ON-BOARD™ for delivery of bispecific antibodies for oncology applications.

www.onconano.com