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Encapsulating therapeutic antibodies for tumor specific activation and delivery using a clinically validated pH-sensitive nanoparticle platform

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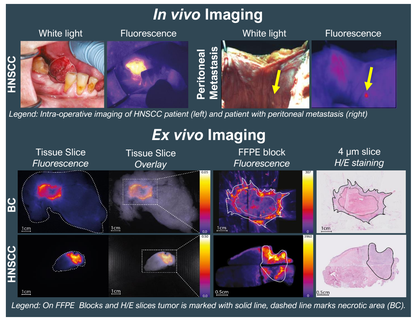
Introduction

Though antibodies for cancer treatment have achieved clinical and commercial success over the past few decades, a large portion are limited by suboptimal efficacy and on-target / off-tumor toxicity due to ubiquitous expression of their targets. A plethora of molecular engineering approaches have been introduced to restrict the activity of antibodies solely to the tumor instead of healthy tissue with the goal of improving the therapeutic index, such as masking the binding sites of antibodies with inhibitory domains. However, sophisticated modification is usually required while the outcomes are often mediocre. Previously we developed an ultra-pH sensitive nanoparticle platform named ON-BOARD™, which release the payload specifically in the acidic tumor microenvironment while remaining intact in normal tissue. The safety and feasibility of using this platform have been demonstrated by successful delivery of a fluorophore to tumors for imaging of multiple tumor types in Phase I and II clinical trials with pegsitacianine (formerly "ONM-100"). Pegsitacianine has been shown to be generally well-tolerated with an infusion-related reaction as the most common adverse event in the clinical trials conducted to date. Based on the clinical results, we present the ON-BOARD™ platform herein as a potential universal and effective tool for tumor-specific activation and delivery of therapeutic antibodies without the need for sophisticated antibody chemistry or engineering.

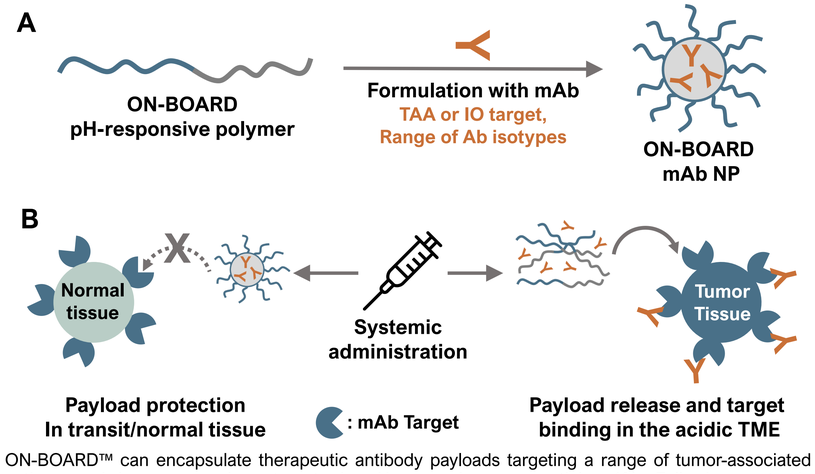
ON-BOARD™ is clinically validated by delivery of a fluorophore to multiple solid tumor types by pegsitacianine



- Pegsitacianine, a pH-responsive polymeric micelle encapsulating indocyanine green (ICG) based on ON-BOARD™ delivery technology is being investigated clinically for image-guided tumor surgery
- High specific tumor localization has been demonstrated in Phase 1 and Phase 2 trials in breast, HNSCC, esophageal, and colorectal cancers, with peritoneal metastases and lung cancer studies currently ongoing
- No SAE observed across multiple studies

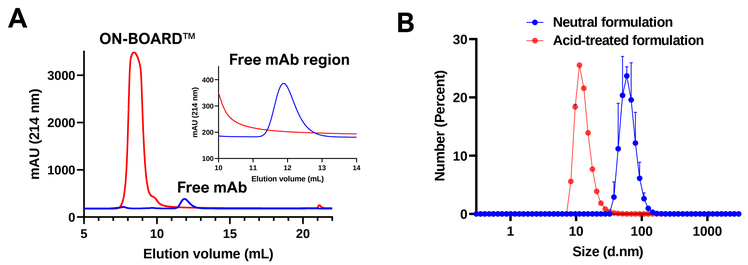
[1] Voskuil FJ, Steinkamp PJ, Zhao T, et al. Nat Commun, 2020; 11: 3257, [2] Clinicaltrials.gov: NCT03735680 NCT04950166, NCT05048082.

ON-BOARD™ enables antibody encapsulation and tumor delivery



ON-BOARD™ can encapsulate therapeutic antibody payloads targeting a range of tumor-associated antigen and immune-oncology targets in pH-responsive nanoparticles (**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™ can encapsulate biosimilar therapeutic antibodies

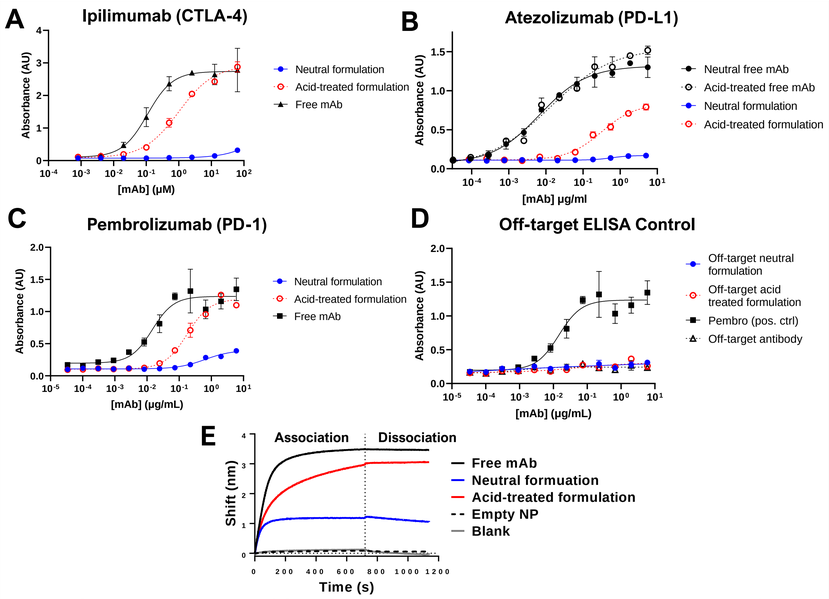


C

Biosimilar Antibody	Target	mAb Isotype	Particle Size (nm)	PDI	EE%
Atezolimumab	PD-L1	IgG1	41	0.341	59
Cetuximab	EGFR	IgG1	54	0.200	100
Pembrolizumab	PD-1	IgG4	71	0.115	70
Nivolumab	PD-1	IgG4	65	0.078	59
Trastuzumab	HER2	IgG1	54	0.240	91
Ipilimumab	CTLA-4	IgG1	63	0.204	80
Magrolimab	CD47	IgG4	64	0.071	67
Bevacizumab	VEGF	IgG1	59	0.098	58
Ramucirumab	VEGFR-2	IgG1	60	0.095	57

Biosimilar monoclonal antibodies to various TAA and IO targets, and consisting of different human IgG isotypes were encapsulated into 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-BOARD™ payload mAbs retain function and formulations demonstrate pH-specific target binding

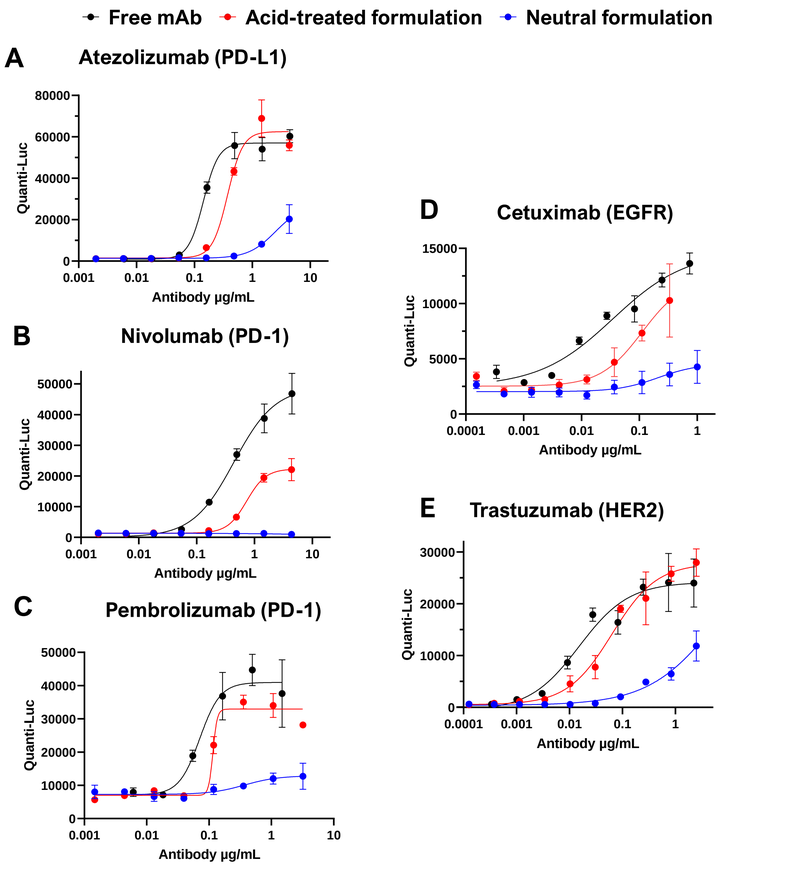


pH-specific target engagement of ON-BOARD™ NPs with mAb antigens was evaluated by ELISA and Bio-layer interferometry (BLI). ON-BOARD™ loaded with biosimilar antibodies to ipilimumab (**A**), atezolimumab (**B**), and pembrolizumab (**C**) demonstrated good protection under neutral conditions (blue curves) but target binding on acid-mediated payload release (red curves). Target specificity was demonstrated using an ON-BOARD™ formulation loaded with anti-CTLA-4 (**D**) against an irrelevant target (PD-1). ON-BOARD™ loaded with pembrolizumab biosimilar was further evaluated by BLI (**E**).

Summary

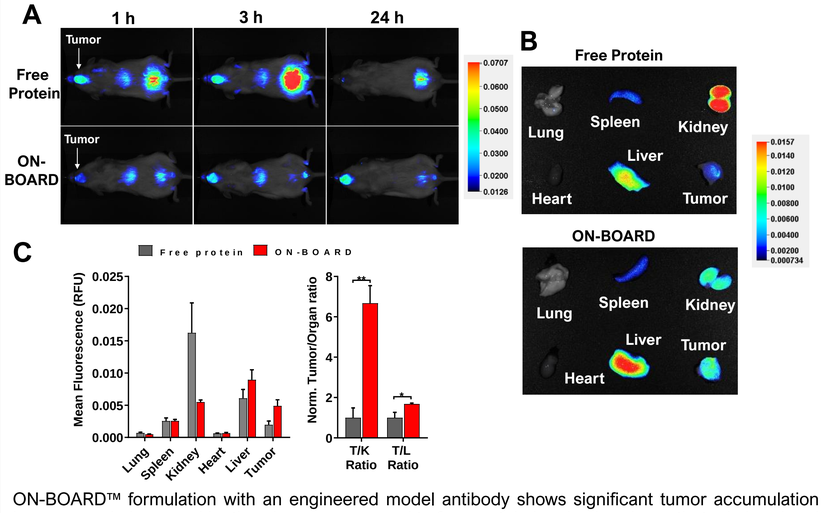
Encapsulation of a variety of therapeutic antibodies for tumor specific delivery and pH-dependent activation using ON-BOARD™, a clinically validated pH-sensitive nanoparticle platform, has been achieved. This approach is compatible with antibodies of different IgG isotypes that targets a broad range of immune-oncology targets without sophisticated protein engineering. This work demonstrated great potential of using ON-BOARD™ for delivery of antibodies for oncology applications.

ON-BOARD™ shows pH-responsive *in vitro* bioactivity



ON-BOARD™ formulations were evaluated for protection and pH specificity in *in vitro* cell-based assays compared to free antibodies. PD-1/PD-L1 interactions (**A-C**) were measured using a PD-1 reporter assay. EGFR (**D**) and HER2 (**E**) formulations were evaluating using an ADCC reporter assay using Jurkat effector reporter cells and Raji target cells overexpressing mAb antigens.

ON-BOARD™ delivers an engineered Ab to tumors



ON-BOARD™ formulation with an engineered model antibody shows significant tumor accumulation increase and pharmacokinetics change, compared to free protein in mice bearing orthotopic head and neck tumors from the biodistribution profile. Representative *in vivo* (**A**, 1h, 3h, 24h) and *ex vivo* (**B**, 24h) major organ biodistribution is shown. Quantitation of *ex vivo* organ (**C**) fluorescence was performed. Statistical analysis by student's t test (* $p<0.05$, ** $p<0.01$), $N = 3$.