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A Clinically Validated pH-Sensitive Nanomedicine Platform for Encapsulating Therapeutic Bispecific T Cell Engagers for Tumor Specific Delivery and Activation

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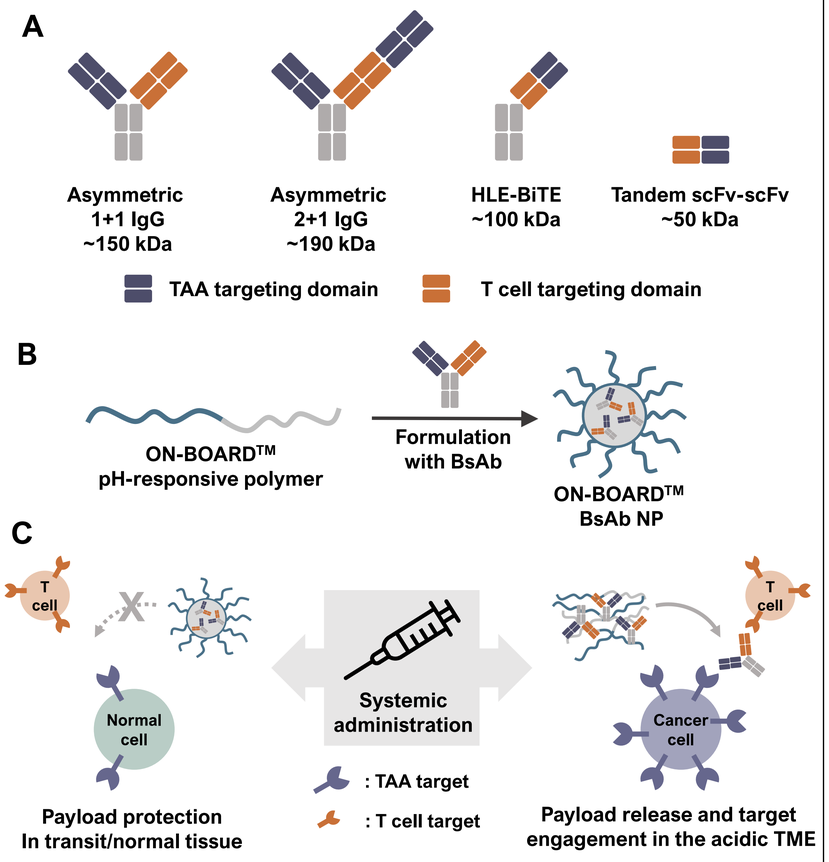
Introduction

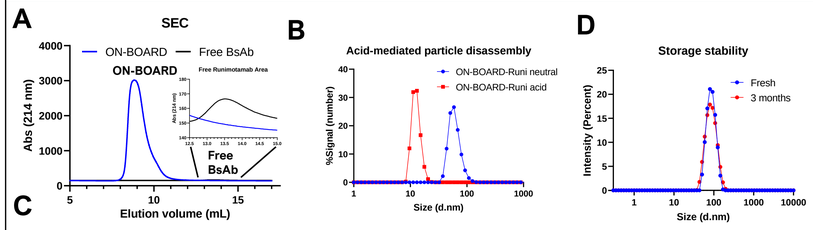
Bispecific antibodies (BsAbs) are an important class of therapeutics for immune-oncology applications. T cell engagers (TCEs) target tumor-associated antigens and cytotoxic T cells to eradicate antigen-expressing tumor cells. Blinatumomab (CD19 X CD3 bispecific) is approved for CD19-positive B cell acute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

ON-BOARD™ enables bispecific antibody encapsulation and tumor delivery with reduced systemic exposure



ON-BOARD™ can encapsulate therapeutic bispecific antibody payloads of a diverse range of protein constructs (**A**) in pH-responsive nanoparticles without protein engineering of the payload (**B**). Following systemic administration (**C**) 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



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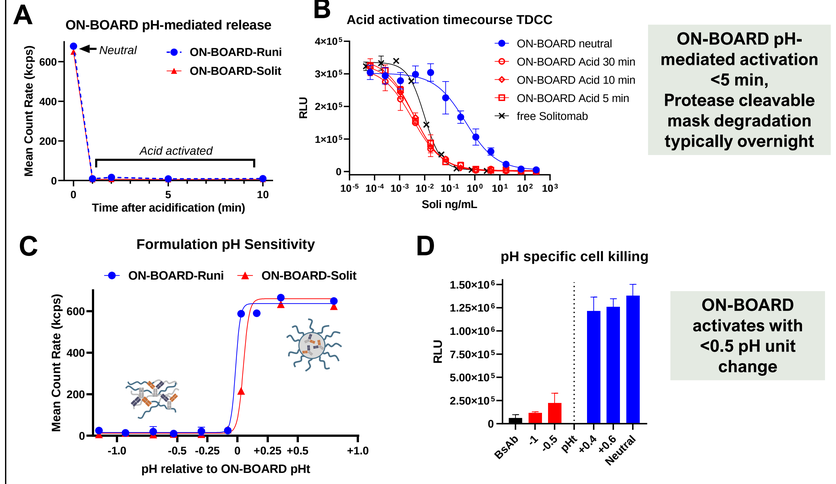
Bispecific T cell engager	TAA target	T cell target	BsAb structure	Particle Size (nm)	PDI	EE%
Blinatumomab	CD19	CD3	Tandem scFv-scFv	63	0.085	91.6%
Solitomab	EPCAM	CD3	Tandem scFv-scFv	60.6	0.12	74.5%
Cibisatamab	CEA	CD3	Asymmetric 2+1 IgG	54.3	0.076	92.1%
Runimotamab	HER2	CD3	Asymmetric 1+1 IgG	65.2	0.045	69.6%
Odronextamab	CD20	CD3	Asymmetric 1+1 IgG	56.1	0.09	98.3%
Gresonitamab	CLDN18.2	CD3	HLE-BiTE	71.8	0.081	90.2%
Glofitamab	CD20	CD3	Asymmetric 2+1 IgG	68.1	0.139	100%
Undisclosed BsAb1	Undisclosed			51.4	0.16	77.0%
Undisclosed BsAb2	Undisclosed			78.0	0.043	88.9%
Undisclosed BsAb3	Undisclosed			62.4	0.17	100%

Bispecific antibodies consisting of different structures and targets 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 storage (**D**).

ON-BOARD™ BsAb formulations show rapid release and high pH specificity

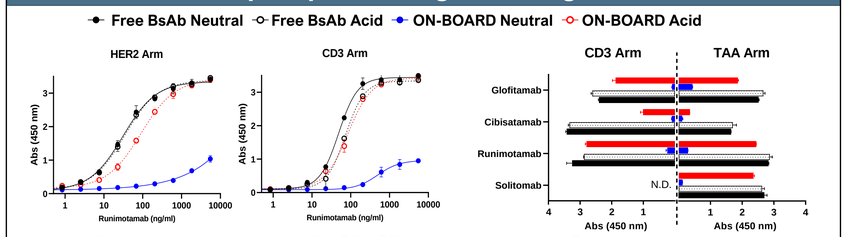


ON-BOARD pH-mediated activation <5 min, Protease cleavable mask degradation typically overnight

ON-BOARD activates with <0.5 pH unit change

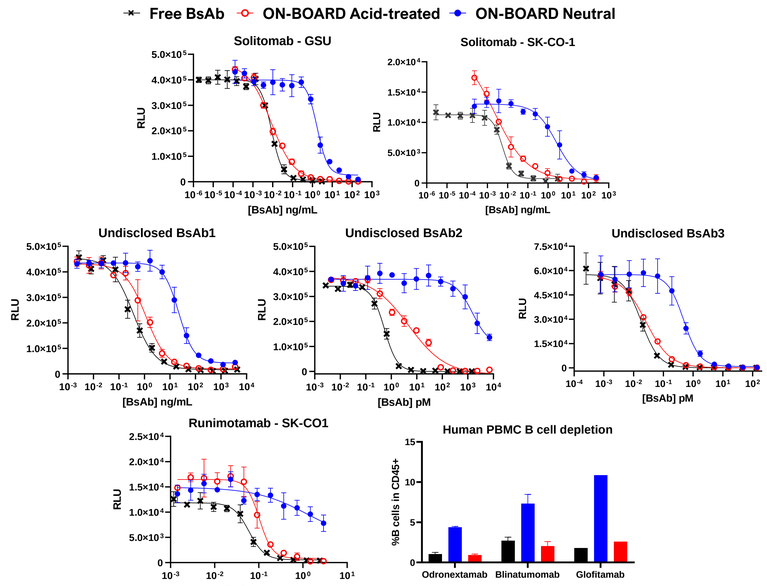
ON-BOARD™ rapidly and specifically releases BsAb payloads following acid-mediated activation. ON-BOARD formulations demonstrated acid-mediated activation time <5 mins in both DLS (**A**) and target cancer cell killing in TDCC assays (**B**). ON-BOARD formulations show specific pH-triggered nanoparticle disassembly (**C**) and target cancer cell killing in TDCC assays (**D**). Representative data using ON-BOARD formulations with runimotamab (DLS) and solitomab (DLS and TDCC) is shown.

ON-BOARD™ payload BsAbs retain function and formulations demonstrate pH-specific target binding from each arm



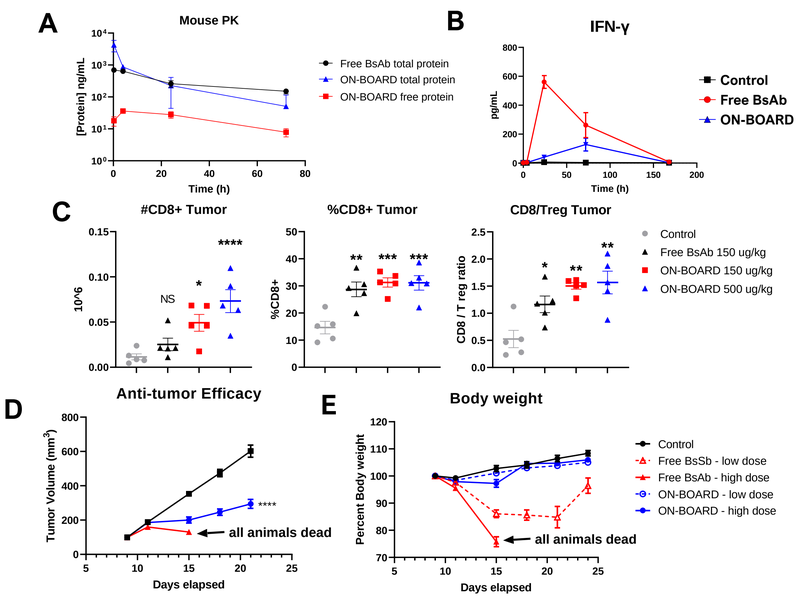
pH-specific target engagement of ON-BOARD™ formulations with BsAb ligands was evaluated by ELISA. ON-BOARD TAA and CD3 arms demonstrated good protection under neutral conditions (blue) but target binding on acid-mediated payload release (red) similar to free BsAb (black).

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 various cancer cell line as target cells including SK-CO-1 large colon and GSU gastric cancer lines. B cell depletion assays in human PBMCs was also demonstrated using CD19 and CD20 BsAbs.

ON-BOARD™ formulation inhibits growth of poorly immunogenic, "cold" tumor and improves tolerability of BsAb



ON-BOARD™ formulation (undisclosed BsAb) demonstrates reduced systemic exposure of BsAb *in vivo* (**A**) and reduction in systemic proinflammatory cytokines compared to unencapsulated BsAb (**B**). In a poorly immunogenic "cold" tumor model, ON-BOARD™-BsAb formulation showed favorable PD markers in the TME (**C**) and significantly inhibited tumor growth when combined with a T cell targeted immunotherapy (**D**) and significantly improved tolerability compared to unencapsulated BsAb (**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 bispecific antibodies of different structures and targets without sophisticated protein engineering. This work demonstrated great potential of using ON-BOARD™ for delivery of bispecific antibodies for oncology applications.