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Encapsulation of IL-12 with an ultra pH-sensitive nanoparticle platform improves tolerability and promotes antitumor response in mice

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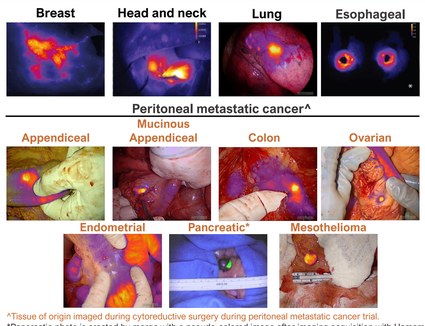
Introduction

Interleukin-12 is a potent proinflammatory cytokine that proliferates and activates T cells, NK cells and differentiates Th1 cells. Translation of IL-12 for cancer treatment has been hindered by insufficient therapeutic index and there are currently no approved IL-12 therapies. To minimize the severe toxicities while maintaining potency, we have developed ON-BOARD, an ultra-pH sensitive nanoparticle platform for masked and targeted delivery of payloads to the acidic tumor microenvironment. The clinical feasibility of ON-BOARD has been demonstrated by high tumor specificity of pegsitacianine in multiple tumor types from the Phase I and II clinical trials. Herein we report encapsulation and masked delivery of IL-12 to tumor-bearing mice using ON-BOARD, demonstrating significantly improved tolerability, anti-tumor efficacy, and potential for clinical translation.

IL-12 fused with Fc was formulated in ON-BOARD nanoparticles. Particle properties were characterized, and lead formulations were identified by in vitro screening to determine pH-mediated bioactivity in cell-based reporter assays.. Since human IL-12 is not cross-reactive with mouse IL-12 receptor, *in vivo* studies were performed using a murine surrogate IL-12Fc payload, to compare the activity of unencapsulated IL-12Fc to ON-BOARD•IL-12Fc formulations. PD response was evaluated by measuring systemic cytokine levels in plasma, while clinical chemistry was performed to evaluate liver and kidney functions. Anti-tumor efficacy of ON-BOARD•IL-12Fc formulations was performed in mice bearing syngeneic MC38 colorectal cancer tumors compared to unencapsulated IL-12.

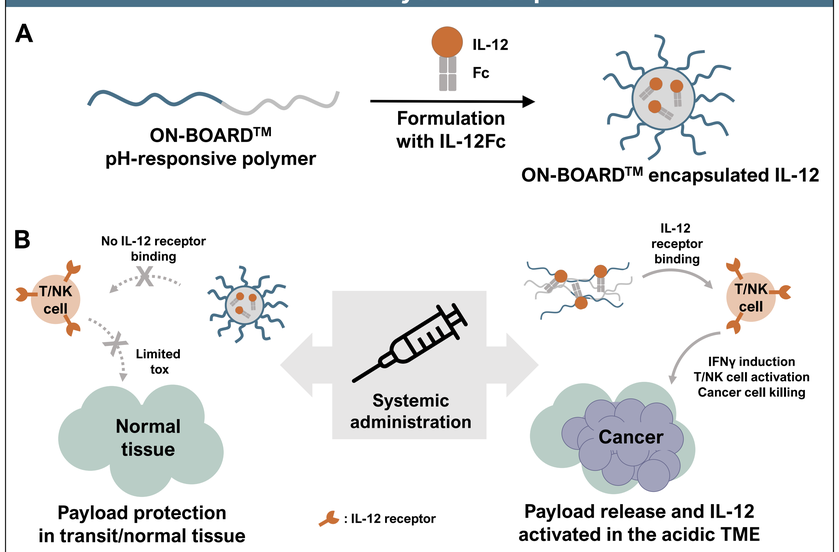
Pegsitacianine clinically validates ON-BOARD™ specificity in multiple solid tumor types in patients



- 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 IL-12Fc encapsulation and tumor delivery with reduced systemic exposure

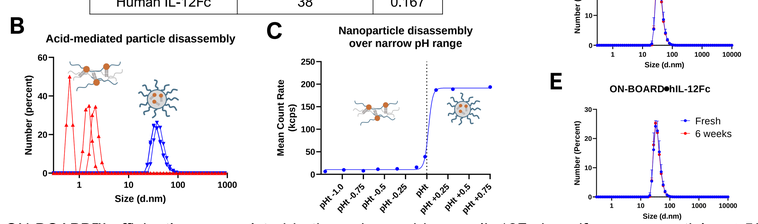


ON-BOARD™ can encapsulate therapeutic proteins like IL-12 in pH-responsive nanoparticles without protein engineering of the payload (**A**). Following systemic administration (**B**) ON-BOARD™ can reduce on-target/off-tumor interactions - T/NK cell activation and subsequent associated toxicities in normal tissue but trigger payload release in acidic TME resulting in target engagement and potent cancer cell killing.

ON-BOARD™ efficiently encapsulates IL-12Fc in pH-responsive nanoparticles with favorable stability

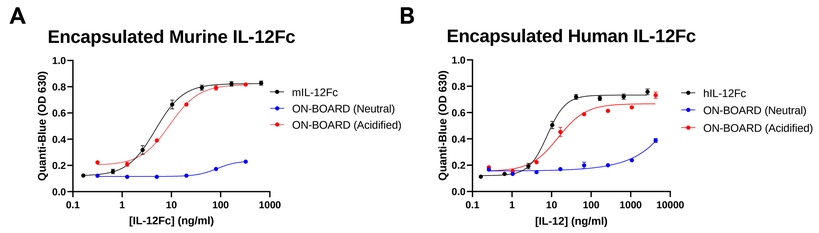
A

Payload	Particle Size (nm)	PDI
Murine IL-12Fc	37	0.167
Human IL-12Fc	38	0.167



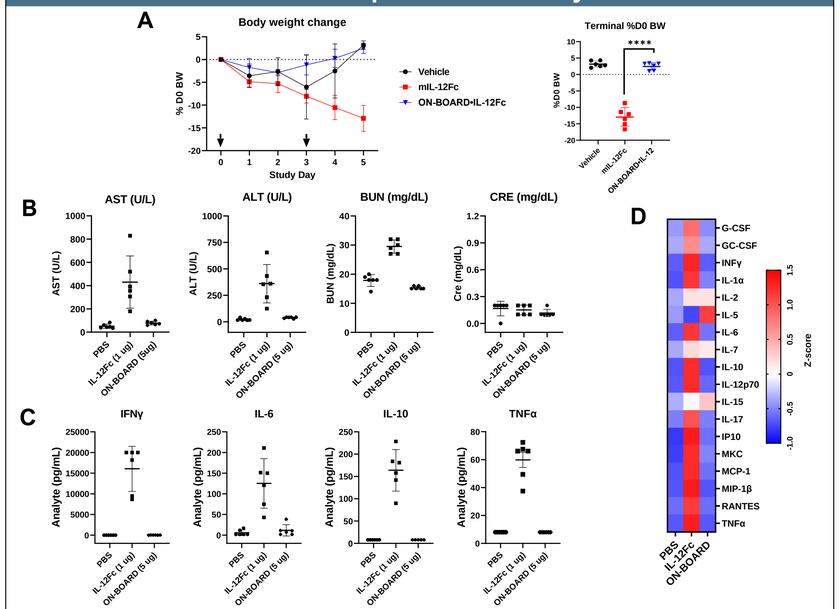
ON-BOARD™ efficiently encapsulated both murine and human IL-12Fc in uniform nanoparticles < 50 nm in size with narrow particle size distribution (**A**). ON-BOARD•mIL-12 demonstrated acid-mediated disassembly (**B**) and sharp pH responsiveness (**C**) as evaluated by dynamic light scattering. ON-BOARD encapsulated mIL12Fc (**D**) and hIL-12Fc (**E**) formulations could be frozen and reconstituted with particle properties maintained for at least 6 weeks.

ON-BOARD™ is able to encapsulate both murine and human IL-12Fc and shows pH-responsive IL-12 bioactivity *in vitro*



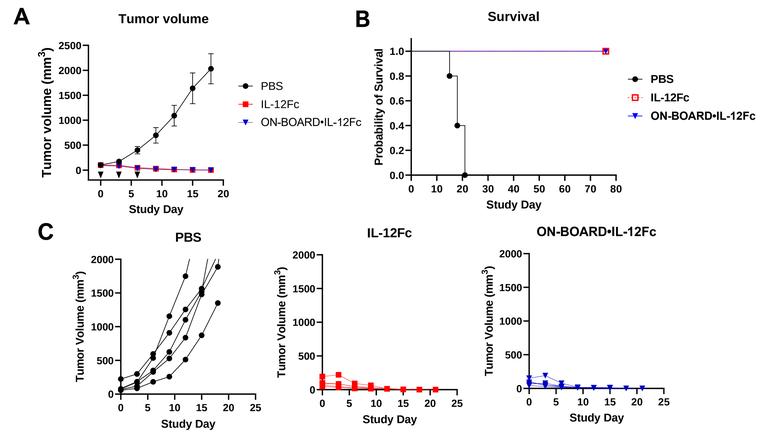
Both murine and human IL-12Fc can be encapsulated by ON-BOARD™ pH-sensitive micelles. ON-BOARD•IL-12Fc formulations demonstrated protection and pH specificity in a cell-based reporter assays with a large activation window for both encapsulated murine and human IL-12Fc.

ON-BOARD•IL-12Fc demonstrates *in vivo* tolerability at 10X MTD of free IL-12Fc protein in healthy mice



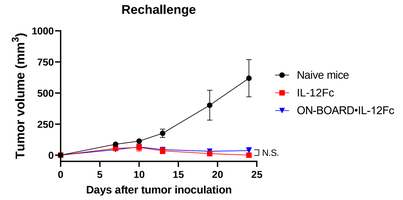
In vivo tolerability of ON-BOARD•IL-12Fc was determined following systemic administration via tail vein injection (*N=6*) to healthy female BL6 mice. Free IL-12Fc (1 ug) or ON-BOARD•IL-12Fc formulations (5 ug) were injected on Days 0 and 3 and takedown was performed on Day 5. ON-BOARD significantly reduced toxicity of IL-12 by mitigating body weight loss (**A**), terminal (Day 5) liver (AST, ALT), kidney (BUN, CRE) function markers (**B**) and systemic plasma cytokine levels (**C,D**). The heat map was generated by processing cytokine concentrations to *Z*-score and statistical analyses were performed by one-way ANOVA.

ON-BOARD•IL-12Fc formulation shows potent anti-tumor efficacy and prolonged survival in MC38 tumor-bearing animals



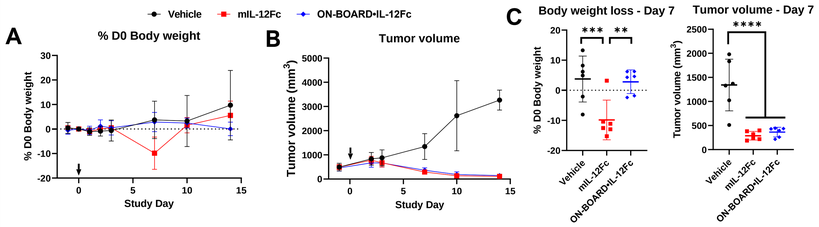
ON-BOARD•IL-12Fc nanoparticles demonstrated anti-tumor efficacy in MC-38 tumor-bearing mice. Animals were treated with IL-12Fc (0.5 µg) or ON-BOARD•IL-12Fc (5 µg) on Days 0, 3 and 6 and monitored for tumor growth (**A**) and survival (**B**) for >75 days. Individual animal tumor growth curves are shown in panel **C**. All animals treated with ON-BOARD•IL-12Fc and IL-12Fc were tumor free (*N = 5*) with 100% tumor-free survival.

ON-BOARD•IL-12Fc produces a durable antitumor memory effect



Animals cured of MC38 tumors following ON-BOARD•IL-12Fc or IL-12Fc treatment were rechallenged with 5X10^6 MC38 cells >75 days after the first treatment and monitored for tumor regrowth. Naïve mice were used as a control. Both IL-12Fc and ON-BOARD•IL-12 treated mice demonstrated memory effect by preventing regrowth of MC38 tumors. Statistics performed on Day 24 following rechallenge using one-way ANOVA.

ON-BOARD•IL-12Fc shows antitumor efficacy in mice with large established MC38 tumors following a single injection



Animals bearing large MC38 tumors (TV ~500 mm^3) were treated with a single injection on Day 0 of mIL-12Fc (5 µg) or ON-BOARD•IL-12Fc (5 µg) and monitored for body weight loss (**A**) and tumor volume (**B**). Tumor regression was observed in all animals following a single injection with no body weight loss for ON-BOARD IL-12Fc while significant body weight loss was observed for free mIL-12Fc. Statistical analyses (**C**) were performed on Day 7 for body weight and tumor volume by one-way ANOVA (*N = 6*).

Summary

Encapsulation of IL-12 for tumor specific delivery and pH-dependent activation using ON-BOARD™, a clinically validated pH-sensitive nanoparticle platform, has been achieved. ON-BOARD demonstrated significantly improved tolerability of mIL-12Fc *in vivo,* potent anti-tumor efficacy in MC38 tumors and a durable memory effect. This work demonstrated great potential of using ON-BOARD™ for delivery of IL-12 for oncology applications.

Acknowledgment

The authors acknowledge the Mendelson-Young Endowment in Cancer Therapeutics to J.G. for support on part of the animal studies presented in this poster. OncoNano Medicine has the exclusive right from UT Southwestern Medical Center of using ON-BOARD platform for delivery of therapeutics including cytokines.

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