AACR 2024
LB438

Preclinical characterization of ONM-412, an ultra-pH sensitive nanoparticle encapsulated IL-12 fusion protein

Qingtai Su, Stephen Gutowski, Austin Burcham, Bhargavi Allu, Zirong Chen, Fiona Stavros, Ruolan Han, Jason Miller, Tian Zhao

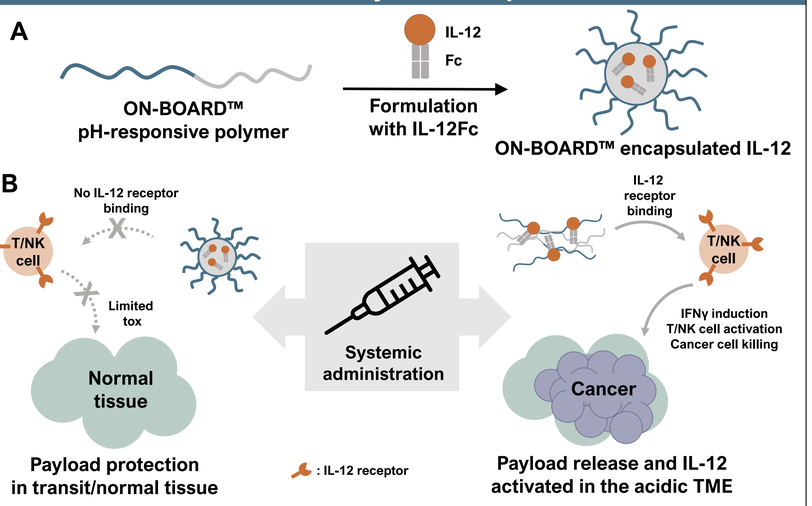
OncoNano Medicine, Inc., Southlake, TX 76092

Introduction

Interleukin-12 is a potent pleiotropic cytokine, but its clinical translation has been hindered by toxicities. To deliver IL-12 to tumors with high spatial and temporal precision while minimizing off-tumor effects, we have developed an ultra-pH sensitive nanoparticle platform - ON-BOARD™ - that shields payloads from systemic exposure and targets solid tumors by responding to tumor acidity. Herein, we report the preclinical efficacy and safety characterization of muONM-412, an ON-BOARD™ nanoparticle-encapsulated murine IL-12Fc fusion protein, and *in vitro* characterization of ONM-412, encapsulating human IL-12Fc.

Properties and stability profiles of ONM-412 were characterized. pH-specific bioactivity was determined in cell-based reporter and IFNγ induction assays while the activation of ONM-412 was compared to a protease-cleavable IL-12 prodrug. Efficacy and tolerability of muONM-412 were studied *in vivo* after intravenous injection in mice. Pharmacodynamic response was evaluated by measuring cytokine levels in plasma and tissue, and the changes in tumor immune microenvironment were characterized. Toxicity was measured by body weight loss, systemic cytokine levels, and clinical chemistry. Anti-tumor efficacy of muONM-412 was evaluated in MC38 tumor-bearing mice. The safety of ONM-412 is also undergoing evaluation in an ongoing toxicology study with cynomolgus monkeys where body weight, hematology, clinical chemistry and urine analysis are being evaluated.

ON-BOARD™ enables IL-12Fc encapsulation and tumor delivery with reduced systemic exposure

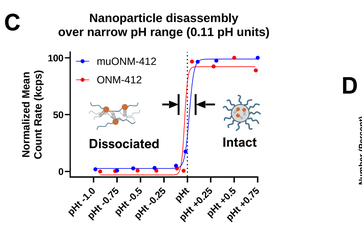


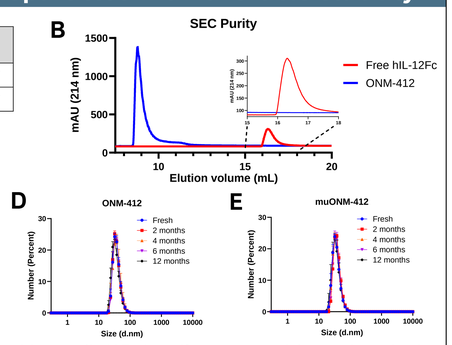
ON-BOARD™ can encapsulate therapeutic proteins like IL-12 in pH-responsive nanoparticles without protein engineering of the payload (**A**). Following systemic administration (**B**) ON-BOARD™ can reduce on-target/off-tumor interactions - T/NK cell activation and subsequent associated toxicities in normal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

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

A

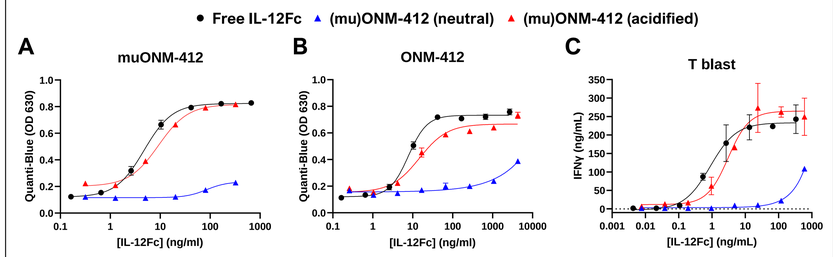
Product	Payload	Particle Size (nm)	PDI
muONM-412	Murine IL-12Fc	37	0.167
ONM-412	Human IL-12Fc	38	0.167





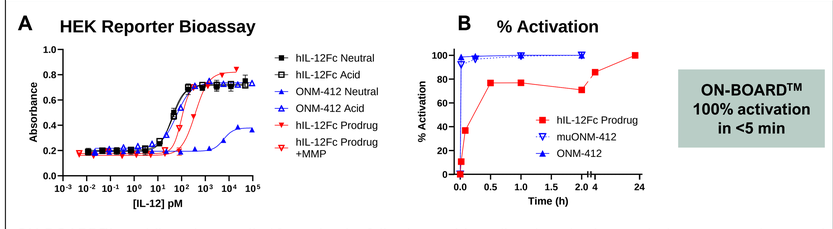
ON-BOARD™ efficiently encapsulated both murine and human IL-12Fc in uniform nanoparticles < 50 nm in size with narrow particle size distribution (**A**). No free IL-12Fc payload was detected in ONM-412 formulation by FPLC (**B**). muONM-412 and ONM-412 demonstrated comparable acid-mediated disassembly and sharp and specific pH responsiveness (**C**). ON-BOARD™ formulations ONM-412 (**D**) and muONM-412 (**E**) could be frozen and reconstituted with particle properties maintained for at least 12 months.

ONM-412 shows pH-responsive *in vitro* IL-12 bioactivity in a HEK reporter assay and in primary cell IFNγ induction



Both murine and human IL-12Fc can be encapsulated by ON-BOARD™ pH-sensitive micelles. ON-BOARD™ formulations of murine IL-12Fc (muONM-412, **A**), and human IL-12Fc (ONM-412, **B**), demonstrated protection and pH specificity in a HEK cell-based reporter assay with a large activation window. (**C**) T blasts were generated from human PBMCs by stimulating with 5 µg/mL PHA for 72h and subsequently treated with muONM-412 and free IL-12Fc protein for 3 days. ON-BOARD™ showed payload protection and pH-specific IL-12 bioactivity as measured by IFNγ induction.

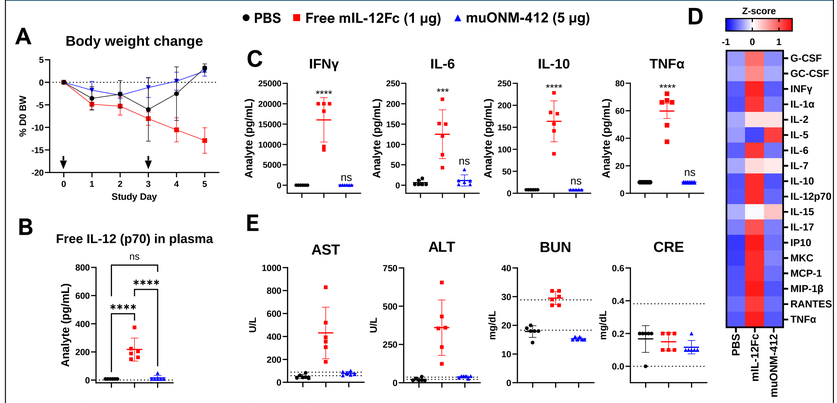
ONM-412 and muONM-412 shows rapid and complete IL-12 activation compared to protease cleavable masked IL-12 prodrug



ON-BOARD™ 100% activation in <5 min

ON-BOARD™ rapidly releases IL-12 payloads following acid-mediated activation and demonstrate improved protection of IL-12 bioactivity compared to an IL-12 prodrug. ON-BOARD™ demonstrated a wider inactive-active EC50 window (**A**) and more rapid activation of IL-12 bioactivity (**B**) compared to an IL-12 prodrug with an MMP14 cleavable mask which has been reported in the literature. (Xue D et al, Sci Immunol. 2022 Jan 7; 7(67): eabi6899)

muONM-412 demonstrates improved *in vivo* safety compared to free mIL-12Fc protein in healthy immunocompetent mice



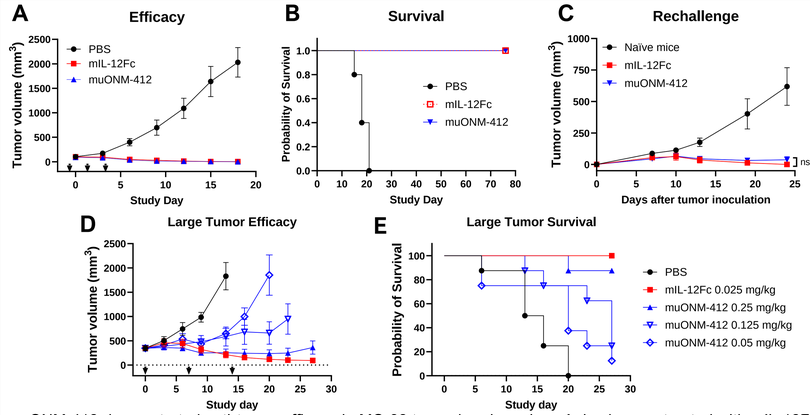
In vivo safety and stability of muONM-412 was determined following systemic administration via tail vein injection (*N=6*) to healthy female BL6 mice. On Days 0 and 3 free mIL-12Fc was administered at 1 µg/injection while muONM-412 was administered at 5 times higher mIL-12Fc equivalent dose and analysis was performed on Day 5. ON-BOARD™ significantly mitigated IL-12 related toxicity with muONM-412 treatment showing reduced body weight loss (**A**) and minimal *in vivo* IL-12 leakage (**B**) as determined by Luminex detection of free IL-12 (p70) in plasma on Day 5. Evaluation of terminal systemic plasma cytokine levels (**C**,**D**) and liver (AST, ALT), kidney (BUN, CRE) functional parameters (**E**) revealed a favorable safety profile of ON-BOARD™ and improved tolerability compared to free mIL-12Fc even at 5 times the dose delivered. The heat map was generated by processing cytokine concentrations to *Z*-score and statistical analyses were performed by one-way ANOVA.

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. muONM-412 demonstrated a favorable safety profile *in vivo*, potent anti-tumor efficacy in MC38 tumors and a durable memory effect. This work demonstrates the potential of using ON-BOARD™ for delivery of anti-cancer payloads for oncology therapeutic applications.

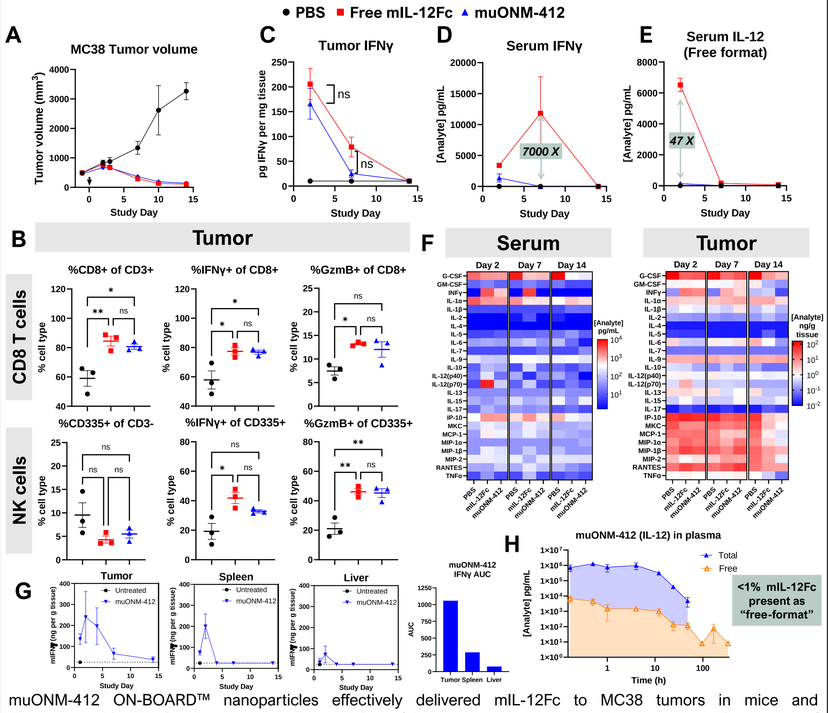
www.onconano.com

muONM-412 formulation shows potent anti-tumor efficacy and prolonged survival in MC38 tumor-bearing animals



muONM-412 demonstrated anti-tumor efficacy in MC-38 tumor-bearing mice. Animals were treated with mIL-12Fc (0.5 µg) or muONM-412 (5 µg of IL-12Fc) on Days 0, 3 and 6 and monitored for tumor growth (**A**) and survival (**B**) for >75 days. All animals treated with muONM-412 and mIL-12Fc were tumor free (*N = 5*) with 100% tumor-free survival. Animals cured of MC38 tumors following treatment were rechallenged (**C**) with MC38 cells >75 days after the first treatment and monitored for tumor regrowth compared to naïve mice. Both mIL-12Fc and muONM-412 treated mice demonstrated memory effect by preventing regrowth of MC38 tumors. Dose responsive anti-tumor efficacy and survival was also demonstrated in large established tumors (~350 mm^3) (**D**,**E**) with muONM-412 (0.25, 0.125, 0.05 mg/kg) or IL-12Fc (0.025 mg/kg) dosed weekly on Days 0, 7 and 14 (*N = 8*). Statistics were performed using one-way ANOVA.

muONM-412 demonstrates tumor immune remodeling and attenuated systemic immune response in tumor-bearing mice



muONM-412 ON-BOARD™ nanoparticles effectively delivered mIL-12Fc to MC38 tumors in mice and demonstrated IL-12 related activation and remodeling of the tumor immune microenvironment. A single injection of mIL-12Fc or muONM-412 (5 µg of IL-12Fc each) was performed on Day 0 (**A**) and demonstrated comparable tumor regression. Tumor immunophenotyping was performed by FACS (**B**) on Day 2 and showed similar increase in activated CD8 T and NK cells. ON-BOARD™ showed potent induction of tumor IFNγ (**C**) comparable to free IL-12Fc but minimized systemic exposure with decreased serum IFNγ levels (**D**) and minimal leaked IL-12 detected (**E**) suggesting productive delivery of bioactive IL-12 to tumors. Longitudinal analysis of cytokine/chemokine levels by Luminex showed comparable activation profiles in tumor homogenate but lower activation in serum by muONM-412 versus mIL-12Fc (**F**). Following a single injection of muONM-412 (0.25 mg/kg) to MC38 tumor-bearing mice, induction of IFNγ was quantitated in tumor, spleen, and liver homogenate (**G**) while plasma levels of leaked mIL-12Fc (Luminex) and total mIL-12Fc (ELISA) were measured (**H**). Data are plotted as mean +/- SEM with statistical analysis by one way ANOVA.