An mRNA-encoded masked IL-12 improves systemic tolerability while maintaining anti-tumor efficacy in preclinical studies

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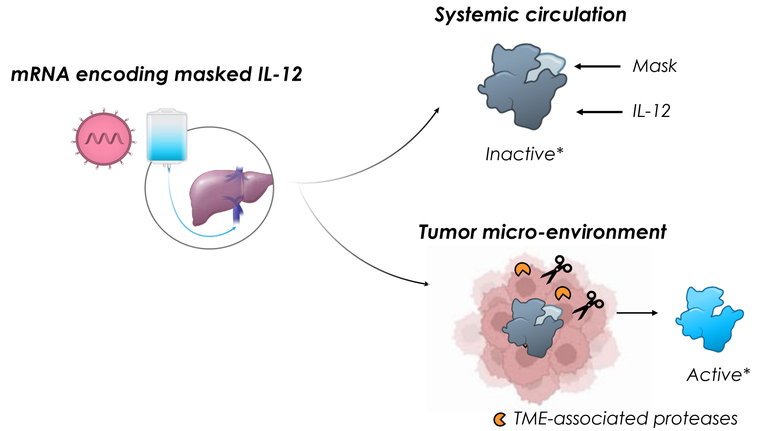
Poster #3127

Introduction

IL-12 is a potent pro-inflammatory cytokine with promising anti-tumor activity, driven by its ability to activate both innate and adaptive immune cells. However, systemic administration of IL12 is limited by significant inflammatory toxicity and a short half-life, resulting in a narrow therapeutic margin. To overcome these challenges, strategies to reduce systemic exposure and localize IL-12 activity within the tumor microenvironment (TME) have been explored

Here, we introduce a novel approach leveraging CytomX's PROBODY® Therapeutics masking technology and Moderna's mRNA platform. Our mRNA-encoded masked IL-12 molecule is designed to remain inactive in circulation, reducing systemic toxicity, while selectively activating in the TME via elevated protease activity. This preferential activation enhances anti-tumor efficacy while improving tolerability. We demonstrate preclinical proof-of-concept in a tumor-bearing mouse model, showing that mRNA-encoded masked IL-12 is both well-tolerated and elicits robust anti-tumor responses



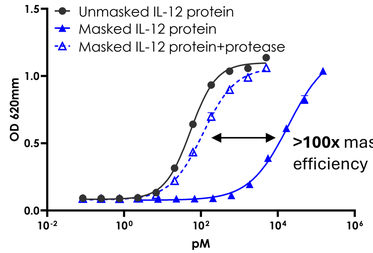
Objectives / parameters investigated for masked IL-12 design :

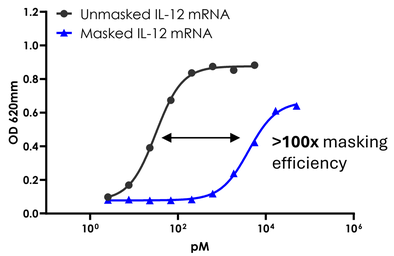
1. Affinities such that mask remains intact in circulation and dissociates rapidly with protease cleavage in TME
2. Protease linkers stable when mRNA is translated in vivo and protein enters systemic circulation while preferentially activated in solid tumors
3. Half-life extension in circulation to extend duration of action and decrease dosing frequency

*IL12 cartoon for illustration purposes only

Lead mask provides >100X masking efficiency in vitro both as protein and mRNA

- Masked IL-12 constructs were first characterized in vitro as recombinant proteins for protease activation and their impact on IL-12 signalling in a HEK reporter cell line (left)
- Lead masked IL-12 molecules were evaluated as mRNA (right) prior to in vivo efficacy studies



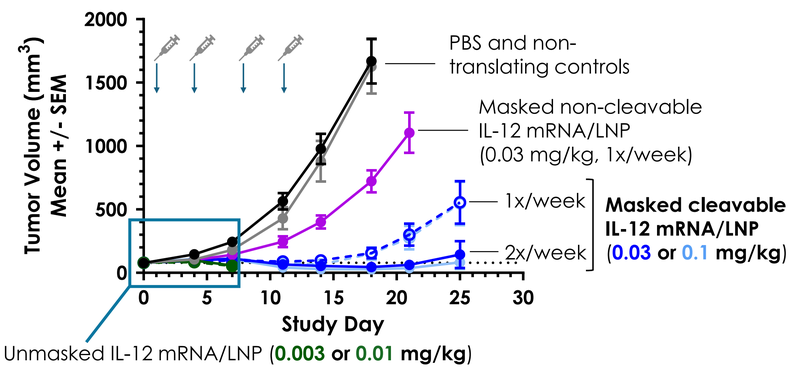


mRNA-encoded masked IL-12 demonstrates strong anti-tumor activity

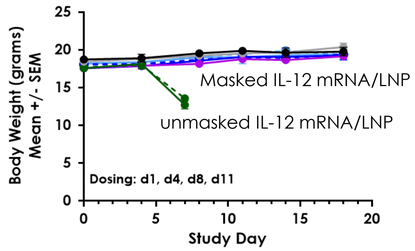
Efficacy of masked IL-12 mRNA/LNP in MC38 tumor mice model

- MC38 tumor-bearing mice were dosed with 0.03 or 0.1 mg/kg masked IL-12 mRNA/LNP 1x/week for 2 weeks or 2x/week for 2 weeks
- Unmasked IL-12 mRNA/LNP was evaluated at 10X lower doses (0.003 2x/week and 0.01 mg/kg 1x/week)
- Masked IL-12 mRNA/LNP demonstrated strong and long-lasting anti-tumor efficacy (animals remained disease-free 5 weeks post treatment)

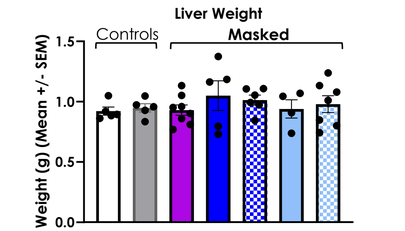
Intravenous mRNA/LNP



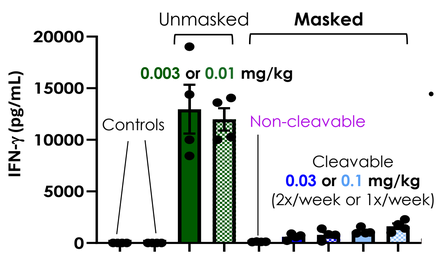
mRNA-encoded masked IL-12 is well tolerated at 30X higher doses than mRNA-encoded unmasked IL-12



- No body weight loss in any group treated with masked IL-12 mRNA/LNP
- Mice treated with unmasked IL-12 mRNA/LNP did not survive post d7



- Animals treated with masked IL-12 mRNA/LNP have normal liver weights



- Minimal peripheral activation of masked IL-12 post mRNA/LNP dosing (Plasma IFN-γ levels on day 4 post IL-12 mRNA/LNP dose)

Conclusions

- An mRNA-encoded masked IL-12 demonstrates >100x masking in vitro compared to mRNA-encoded unmasked IL-12
- The mRNA-encoded masked IL12 is systemically well-tolerated while showing robust and long-lasting anti-tumor efficacy in a tumor bearing mouse model
- These findings support the potential of mRNA-based masked IL12 as a safe and effective immunotherapy

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