oNKo-001: A reduced potency IL-12 Fc fusion protein with enhanced therapeutic index

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Introduction

Interleukin-12 (IL-12) is a potent inflammatory cytokine that exhibits broad acting immunomodulatory effects including the activation and proliferation of T and NK cells, induction of Th1 differentiation, inhibition or reprogramming of suppressive cells such as TAMs and Tregs, and induction of MHC-I expression on tumour cells (Figure 1). While IL-12 displays remarkable anti-tumour activity in syngeneic tumour models, its clinical development has been hampered by severe toxicities associated with the activation of circulating immune cells. Here, we present a series of reduced potency IL-12 Fc fusion proteins engineered to minimise toxicity whilst retaining on-tumour activity and exhibiting an enhanced pharmacokinetic profile.

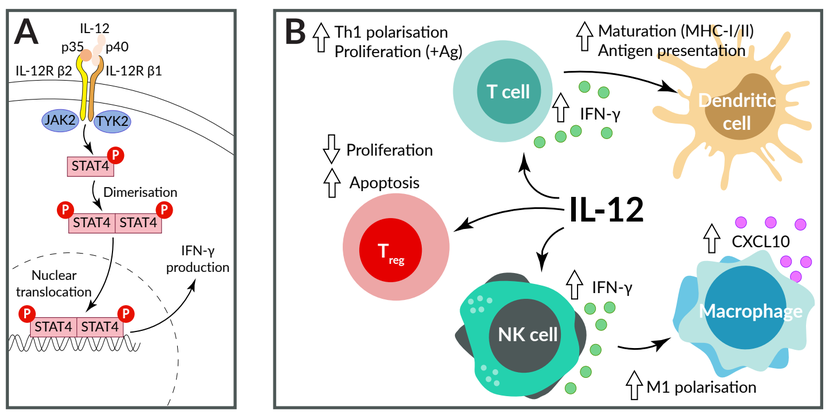

Figure 1. (A) Signalling pathway and (B) biological effects of IL-12 within the tumour microenvironment

Rationale for reduced potency IL-12 Fc

Circulating immune cells express lower levels of IL-12 receptor subunits than activated (tumour infiltrating) immune cells. Reduced potency IL-12 variants are designed to bias activity away from cells with low receptor expression, thereby avoiding systemic toxicity.

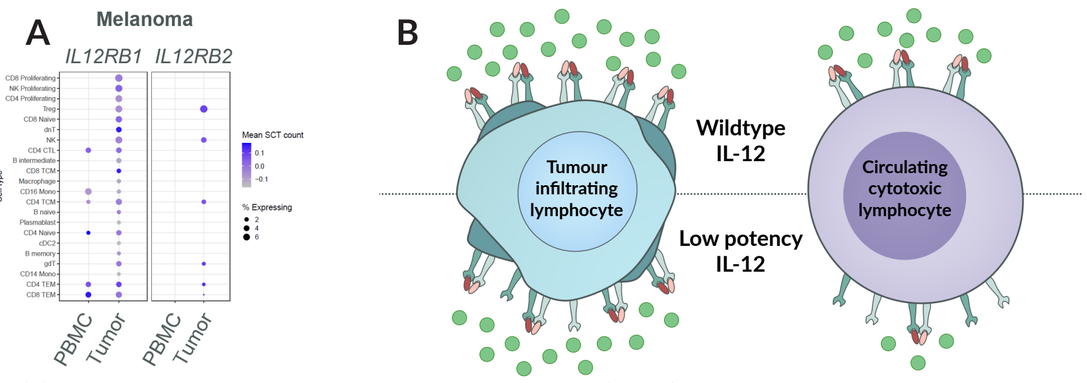

Figure 2. (A) Expression of IL12RB1 and IL12RB2 mRNA in circulating (PBMC) and tumour-infiltrating immune cell subsets as determined by scRNA-seq. (B) Proposed MOA of low potency IL-12 Fc variants

Engineering of reduced potency IL-12 Fc

A series of reduced potency IL-12 p35 mutants were designed and expressed as monovalent Fc fusion proteins. IL-12 p35 mutants exhibited reduced capacity to stimulate IFNγ production by primary T cells.

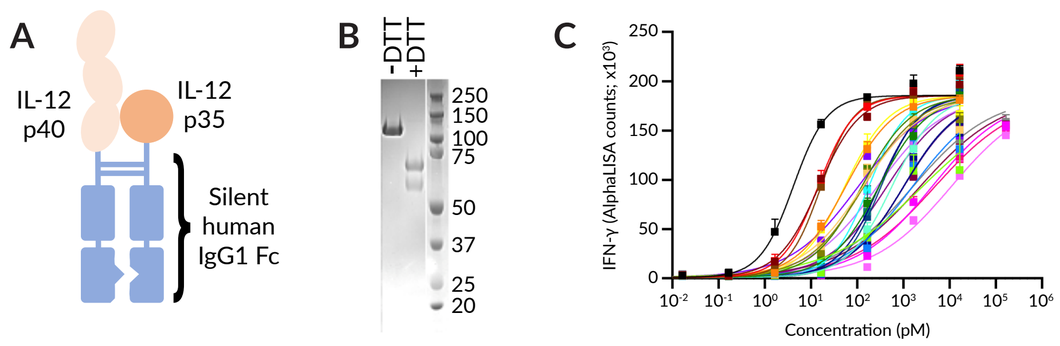

Figure 3. (A) Schematic representation of IL-12 Fc construct, (B) SDS-PAGE analysis of purified IL-12 Fc, (C) IFNγ production by activated primary T cells in response to incubation with WT IL-12 Fc(black line) and selected IL-12 Fc variants (coloured lines).

Favourable in vivo characteristics of reduced potency variants in a human PBMC adoptive transfer model

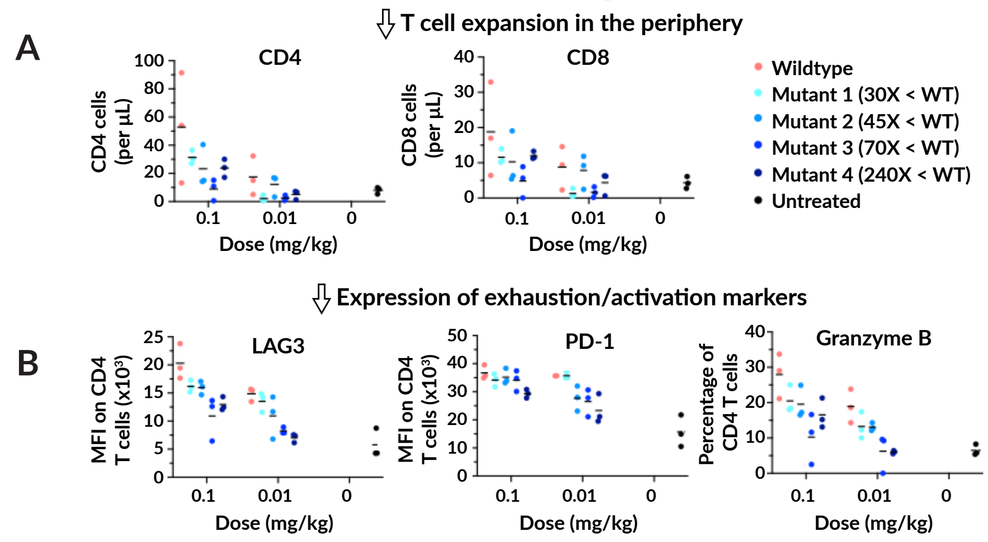

Figure 4. NSG mice were injected with 10x10⁶ human PBMC and were treated with WT IL-12-Fc or variants thereof on day 0 and 7. The number of CD4+ and CD8+ T cells (A) and the expression of activation/exhaustion markers on CD4+ T cells (B) in the blood is depicted on day 12. Solid horizontal bars indicate the mean.

In vivo anti-tumour activity of 240X reduced potency human IL-12 Fc

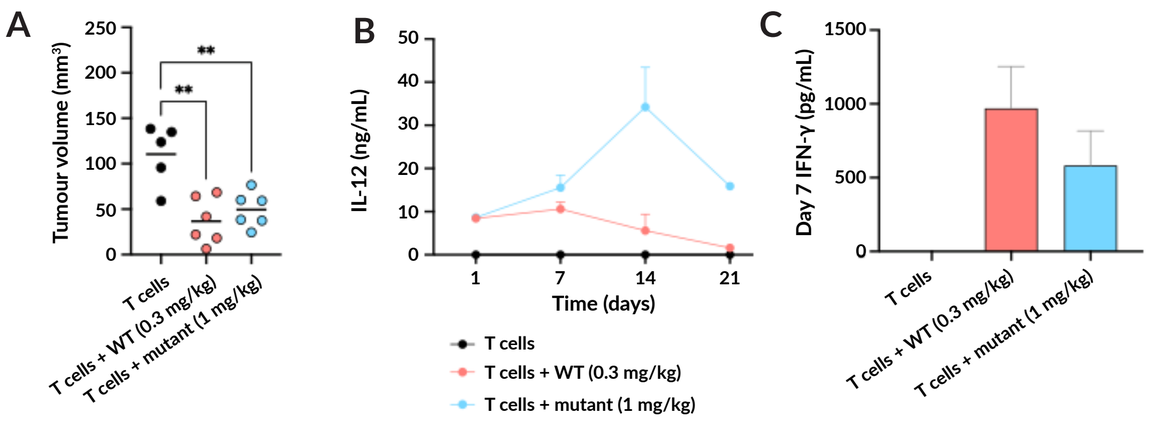

Figure 5. Fourteen days after engraftment of NY-ESO expressing HCT116 tumour cells, NSG mice were injected with NY-ESO reactive primary T cells and IL-12-Fc WT or mutant on day 0 and 7. Tumour volume (A), circulating IL-12 levels (B) and plasma IFNγ (C) are depicted. Error bars represent SEM.

Reduced potency mouse IL-12 Fc surrogate in a CT26 syngeneic tumour model

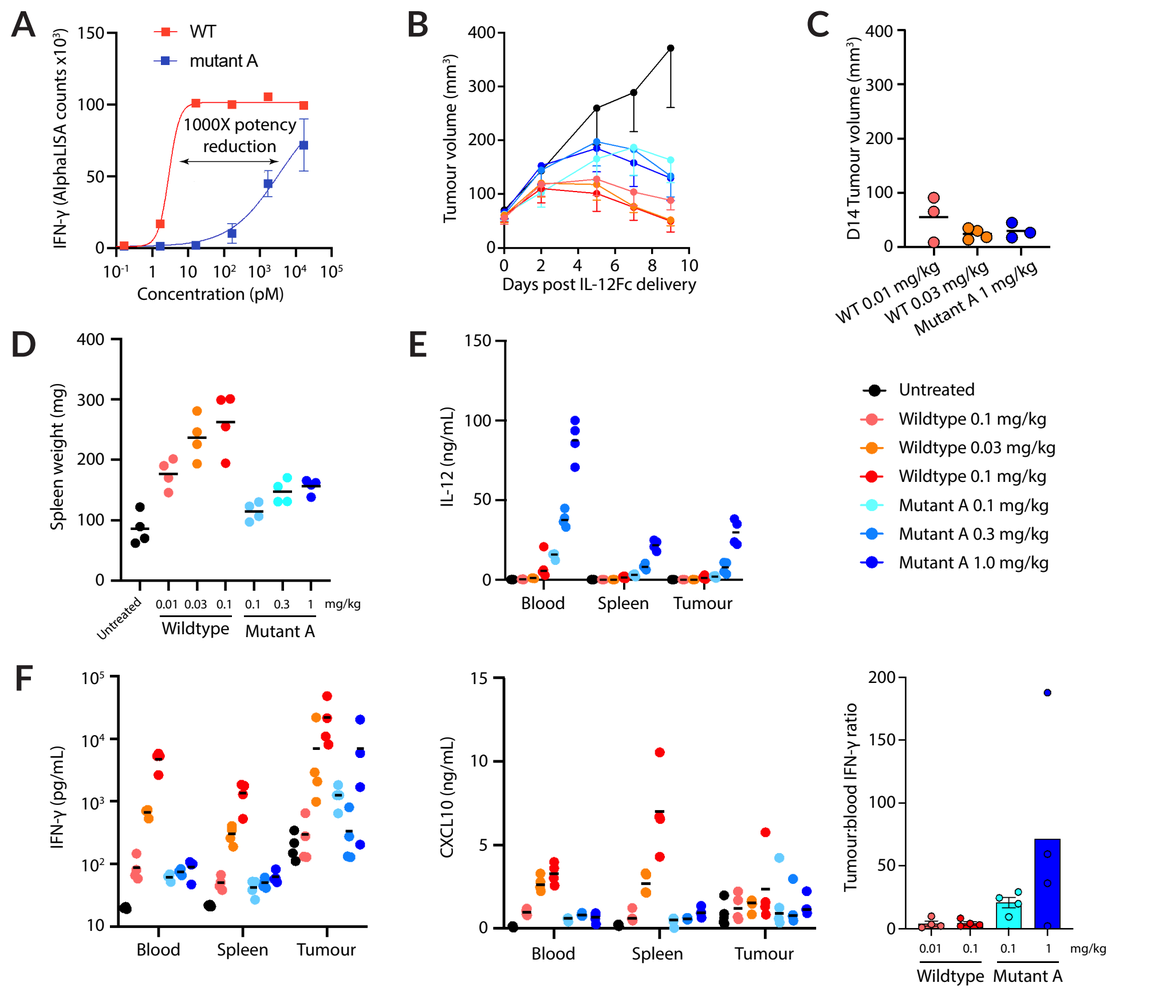

Figure 6. (A) Potency measurement of mouse IL-12 Fc mutant assessed by T cell IFNγ assay. (B) Growth curves for CT26 tumours following treatment on day 0 and 7 with mIL-12-Fc WT or mutant as indicated. Tumour growth curves are plotted until D9 due to ulceration in a subset of mice across all groups. Error bars represent SEM (n=6 per group). (C) Tumour volume on D14 post treatment for groups with at least three mice remaining. In a separate experiment, mice were harvested 5 days after treatment and organs were harvested for analysis. Spleen weights (D), IL-12 levels in the blood (E), and measurements of secreted inflammatory markers (F) are shown. In D-F, solid horizontal lines/bars represent the mean (n=4 per group).

Conclusions

- A series of human IL-12 Fc reduced potency variants were generated in a monovalent Fc fusion format.
- Reduced potency IL-12 Fc variants demonstrate superior pharmacokinetics and exhibit robust anti-tumour activity in in vivo models, whilst reducing toxicity associated with the activation of circulating immune cells.
- Compared to WT IL-12 Fc, reduced potency variants increase the levels of proinflammatory cytokines (e.g. IFN-ɣ) in the tumour relative to the periphery, indicating that potency reductions in IL-12 are associated with an increase in its therapeutic index.