Background Tumour Necrosis Factor (TNF) is a pro-inflammatory cytokine capable of activating anti-cancer immune cell responses and directly inducing cancer cell necrosis. Although a very potent molecule its application as anti-cancer therapeutic has been hampered by severe toxicity, which is often characterised by cytokine release syndrome (CRS). To overcome the toxicity challenge and harness its therapeutic value, TNF must be localised to the tumour to minimise toxicity. One strategy is to use tumour extracellular matrix proteins such as collagen, as a reservoir, and exploit the leaky nature of tumour vasculature within the tumour microenvironment to specifically deliver therapeutic payloads. Our lab has previously shown that recombinant fusion of a collagen-binding domain (CBD), based on the A3 domain from blood protein von Willebrand factor (VWF), facilitates the localised delivery of cytokine payloads to the tumour microenvironment. Encouraged by this, we have fused the CBD domain to TNFα, and hypothesise that this fusion protein would show a favourable toxicity profile compared to WT-TNFα.

Methods Recombinant mouse TNFα and a CBD fusion version were produced using a transient mammalian protein production platform, purified using a histidine affinity column and size exclusion chromatography, then characterised by SDS-PAGE (figure 1A,B). Proteins were functionally characterised in vitro using binding ELISAs against collagen III and TNF-receptor 1 (TNFRI) (figure 1C,D). The efficacy and toxicity profiles of the fusion proteins were evaluated in vivo using a murine breast cancer model (figures 2 and 3).

Results Here we have shown that CBD-TNFα fusion proteins can be successfully produced, and retain their ability to bind to TNFRI and can bind to collagen III - figure 1. Furthermore, in a murine breast cancer model intravenous administration of CBD-TNFα drives complete remission (3/5 and 3/4 mice, with 2 µg and 5µg CBD-TNFα respectively) (figure 2). Crucially, we observe a remarkable reduction in toxicity compared to WT-TNFα as indicated by minimal weight loss and reduction in plasma interleukin-6 (a cytokine which is strongly upregulated during CRS) (figure 3).

Conclusions The fusion protein remains biologically functional and, initial results, demonstrate a remarkable improvement in the toxicity profile of TNFα in EMT6 bearing mice. CBD-TNFα may open a new avenue for clinical translation of TNFα as an intravenously injected therapeutic.

REFERENCES
CBD-TNFα drives complete remission and improves survival in EMT6 bearing mice

(A) Tumour growth curves of Balb/c mice (female, 8–12wk) bearing 5×10⁵ EMT6 breast cancer cells subcutaneously on the back skin, were treated with PBS (n=5) 2ug CBD-TNFα (n=5) or 5ug CBD-TNFα (n=4) via intravenous injection on day 5 and day 9. Average growth curves (A) and individual growth curves (B) are shown - where 3/5 and 3/4 animals demonstrated CR (complete response) with 2ug CBD-TNFα and 5ug CBD-TNFα, respectively. Data from single experiment, presented as average ± SEM. (C) Survival curve of Balb/c mice (female, 8–12wk) bearing 5×10⁵ EMT6 breast cancer cells subcutaneously on the back skin, treated with PBS (n=5), 2ug WT-TNFα (n=5)*, 5ug WT-TNFα (n=5)*, 2ug CBD-TNFα (n=5, TNFα molar equivalent) or 5ug CBD-TNFα (n=4, TNFα molar equivalent) via intravenous injection on day 5 and day 9. *, WT treated groups were culled after administration of second dose due to toxicity humane endpoint criteria being met, Statistical analysis was performed using a log-rank (Mantel-Cox) test using GraphPad prism (v9.5.1).

CBD fusion proteins shows favourable toxicity profile compared to WT-TNFα

(A) Body weight change of EMT6 bearing mice treated with PBS (n=5), 2ug WT-TNFα (n=5), 5ug WT-TNFα (n=5), 2ug CBD-TNFα (n=5, TNFα molar equivalent) or 5ug CBD-TNFα (n=4, TNFα molar equivalent) via IV injection normalised to first treatment (day 5). Average ± SEM (B) plasma IL6 levels from blood taken 2 hours post first treatment with either PBS (n=6) 2ug WT-TNFα (n=6), 5ug WT-TNFα (n=6), 2ug CBD-TNFα (n=5, TNFα molar equivalent) or 5ug CBD-TNFα (n=6, TNFα molar equivalent), quantified using murine IL6 ELISA kit. Average ± SEM, one-wav ANOVA was performed using Graphpad prism (p<0.05).

http://dx.doi.org/10.1136/jitc-2023-SITC2023.1201