Background

Triple negative breast cancer (TNBC) remains a challenge to treat due to its heterogeneous tumour microenvironment, rapid metastasis, and high recurrence rates. TNBCs are currently still treated with chemo- and radiotherapy, both of which cause significant side effects. Interleukin-12 (IL-12) is a powerful anti-tumour cytokine but its use in the clinic is limited by significant toxicity. We bioengineered IL-12 by conjugating it to both a collagen-binding domain (CBD) and a mask. The former delivers the IL-12 payload into the collagenous stroma of solid tumours, whilst the latter temporarily suppresses IL-12 activity in the bloodstream to suppress toxicity. The mask is linked to CBD-IL12 using a protein linker, that is cleaved by proteases upon infiltrating the tumour stroma. The removal of the mask activates masked CBD-IL12 (M-CBD-IL12) in a tumour-specific manner.

Methods

M-CBD-IL12 with a protease-sensitive linker was produced in HEK293F cells and purified using immobilised metal affinity and size exclusion chromatography. Murine tumour lysate and serum were used to assess the cleaving of the linker through western blotting and flow cytometry. M-CBD-IL12 was tested in two murine models of TNBC (EMT6/4T1), each with unique immune landscapes. Plasma was collected for analysis of cytokine markers and tumours were analysed using flow cytometry.

Results

M-CBD-IL12 significantly prolonged the survival of mice with orthotopic EMT6 tumours, which are considered more myeloid cell infiltrated. A complete remission was seen in 4 out of 6 mice treated with M-CBD-IL12, each of which remained tumour-free for 284 days following tumour inoculation. In an intervention setting, the IL-12- and M-CBD-IL12-treated mice had significantly smaller tumours than PBS controls. Mice treated with IL-12 or M-CBD-IL12 had significantly higher levels of intra-tumoural IFN-gamma with significantly fewer T regulatory cells. The CD8+ T-cells expressed significantly less PD-1 following M-CBD-IL12; this suggests M-CBD-IL12 reduces immunosuppressive pathways in the EMT6 tumour microenvironment. Despite similar efficacies, the mice treated with M-CBD-IL12 had significantly less plasma IFN-gamma (toxicity marker) than mice treated with IL-12, suggesting the mask and CBD are suppressing IL-12-related toxicity. The 4T1 model of TNBC is thought to be more T-cell inflamed; M-CBD-IL12 did not prolong survival of mice with 4T1 tumours, suggesting a selective efficacy of M-CBD-IL12 for TNBC.

Conclusions

M-CBD-IL12 cures murine EMT6 TNBC with low toxicity and prevents its recurrence long-term. Further studies are required to understand the immunological mechanism behind the observed responses.

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REFERENCES

