Background Cancer immunotherapy is a promising innovative and effective treatment for many forms of cancer. Among hematological malignancies, acute myeloid leukemia (AML) remains an unmet medical need as it is mainly treated with chemotherapy, which is associated with serious side effects. Therefore, antibody-based targeted therapy is preferred as it can target and specifically eliminate malignant cells. An immunotoxin (IT) is a chimeric molecule consisting of a targeting molecule and a toxic component that specifically kills target cells. H22(scFv) ETA' is an immunotoxin consisting of a humanised single-chain fragment (scFv) antibody that targets CD64, which is overexpressed on the surface of AML cells and a truncated version of Pseudomonas exotoxin A (ETA') that kills CD64-positive AML cells. CD64 is highly expressed on monocytic blast cells in patients with AML and not on normal haematopoietic stem cells, making it a suitable target antigen.

Methods H22(scFv) ETA' was recombinantly expressed in E. coli B121 (DE3) and channelled into the periplasmic space and purified by metal ion affinity chromatography and size exclusion chromatography. The cytotoxic efficacy of H22(scFv) ETA' was assessed by the Annexin V bioassay and binding assays were assessed using flow cytometry. A diagnostic fusion protein version of H22(scFv) ETA' was constructed in which the toxic component ETA' was removed and replaced with the protein SNAP tap to generate H22(scFv) SNAP. SNAP tag enables efficient tumour targeting and diagnosis of molecular biomarkers for cancer.

Results This study showed that H22(scFv) ETA' is cytotoxic to AML cancer cells expressing CD64. H22(scFv) ETA' showed significant toxicity in vitro against CD64-positive cell lines HL-60 and U937. Binding studies showed specific binding to both cell lines HL-60 and U937. Specific binding of H22 (scFv) SNAP to HL-60 and U937 was also demonstrated.

Conclusions The results described show promising results in vitro not only for the treatment of AML, but also provide technology for the effective diagnosis of AML. The development of successful scale-up production of H22(scFv) ETA' and H22(scFv) SNAP is critical for large-scale production to enable further preclinical/clinical studies. The current phase of this study is focused on optimizing productivity and large-scale production of both fusion proteins described above.

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