Therapeutically targeting cd64 in acute myeloid leukemia via single-chain based antibody immunotoxin

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Cancer immunotherapy is a promising innovative and effective treatment for many forms of cancer. Among hematologic malignancies, acute myeloid leukemia (AML) remains an unmet medical need as it is primarily treated with chemotherapy, which is characterised by severe side effects. H22(scFv)- ETA' is an immunotherapeutic recombinant protein that has been shown in this study to be highly effective in selectively destroying CD64-positive dysfunctional myeloid tumour cells in AML. CD64 is highly expressed on monocytic blast cells in patients with AML and not in normal hematopoietic stem cells or non-hematopoietic tissues. The overexpression of CD64 and its rapid internalization make it a suitable target antigen for antibody-based targeted therapies. H22(scFv)- ETA' has only been produced in shake flasks, a scale that does not provide sufficient quantity to conduct preclinical and/or clinical studies. Therefore, the current phase of this study is focused on optimizing the productivity of H22(scFv)- ETA' and conducting a scale-up production from the shake flask to a 5 L stirred tank reactor (STR). This enables us to conduct comprehensive preclinical studies to further evaluate the therapeutic efficacy of H22(scFv)- ETA' against AML. H22(scFv)- ETA' is recombinantly expressed in *E. coli* Bl21 (DE3) and purified by metal ion affinity chromatography and size exclusion chromatography. The volumetric mass transfer coefficient (kLa) is used as a scale-up criterion to achieve effective batch and fed-batch fermentation processes. The therapeutic efficacy of H22(scFv)- ETA' is evaluated by several biological assays, including binding assays using flow cytometry and cytotoxicity using Annexin-V bioassay. The development of successful scale-up production of H22(scFv)- ETA' is critical as it provides insight into a process that can be established at pilot scale and eventually at commercial scale in the context of biopharmaceutical manufacturing.

Keywords: AML; Immunotherapy; Immunotoxin; Process development; Scale-up