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Generation and *in vitro* characterisation of anti-CD22 recombinant and chemically linked ONC-based immunoRNases

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In this thesis, chemically linked and recombinant anti-CD22 immunoRNases carrying the amphibian RNase Onconase (ONC) as a payload were generated, purified and *in vitro* characterised. A whole IgG and a small sized diabody fragment were previously engineered from the humanised anti-CD22 RFB4 scFv. Immunoconjugation was performed with the standard heterobifunctional crosslinkers SMCC, SMPT and SPDP in different conjugational strategies, investigating Des[30-75]-ONC and pyridyl disulfide-ONC as conjugational payload formats. The chemically linked pyridyl disulfide-ONC and the recombinant dimeric huCD22-Db-ONC were purified to homogeneity. Both recombinant and chemically linked pyridyl disulfide-ONC immunoRNases demonstrated a high dose-dependent anti-CD22 specific *in vitro* cytotoxic activity. The missing cytotoxicity of the Des[30-75]-ONC immunoRNases was the outcome of the low conjugational reactivity and impaired IEX-matrix-binding of Des[30-75]-ONC and probably of the impaired intracellular functioning of the anticipated Lys-MCC-Des[30-75]-ONC adducts, as well. In contrast, the highly *in vitro* cytotoxicity of the chemically linked pyridyl disulfide-ONC immunoRNases with favourably low DARs resulted from optimal crosslinking reactions, efficient purification techniques and intract intracellular functioning of pyridyl disulfide-ONC. In addition, the successful purification of pyridyl disulfide-ONC immunoRNases according to their DAR constitutes a major advantage for their further clinical development, since this is at present only possible for dipeptide-linked ADCs. Thus, chemically linked ONC-based immunoRNases seem to be feasible and appealing therapeutic alternative for immunoconjugates with conventional payloads.

However, current crosslinking techniques allow only for generation of heterogeneous immunoconjugates. This could be overcome thorugh development of genetically engineered unpaired cysteine or selenocysteine residues within the IgGs, allowing for a site-directed chemical payload attachment. Another option is genetically engineered fusion proteins, like huCD22-Db-ONC, with the significant advantage of a structural homogeneity through a defined antibody to payload stoichiometry. However, considering the superior IC_{50} values of the 170 and 180 kDa chemically linked pyridyl disulfide-ONC immunoRNases over huCD22-Db-ONC and the currently limited number of antibody fragment-based anti-tumour fusion proteins within phase I-II trials, clinical development of IgG-based chemically linked immunoRNases should at present be prioritised.