

Over the last few years there has been an increased interest in Immunotherapeutic procedures for the treatment of cancer. Immunotoxins (IT), which consist of a tumor-specific antibody or antibody fragment and a highly active toxic domain, represent a promising concept for such a therapy. Therapy success is determined mainly by (1) the selectivity of the cell-specific binding structure, (2) the cytotoxic activity of the toxin, and (3) effective tumor penetration dependent on IT size.

In this work two different recombinant IT were constructed specific for Hodgkin lymphoma, which is well suited for targeted immunotherapy since lymphocyte activation markers are expressed on H-RS cells in high copy numbers as the CD30 antigen. CD30 is cleaved from the surface of target cells and the resulting soluble ectodomain (sCD30) protected the tumor cells from antibody binding. The first step was the isolation of an anti-CD30 single chain variable fragment (scFv) with the Phage-display technique, by which peptides are displayed on the surface of the phage allowing the isolation of high affinity antibody fragments. Coupling the anti-CD30 scFv with the toxin *Pseudomonas Exotoxin A* (ETA') leads to the IT Ki-3(scFv)ETA'. The other IT, consisting of a different anti-CD30 scFv Ki-4 and a newly constructed recombinant Ricin A-chain, showed specific anti-tumor activity against HR-S cells *in vitro* and in a mouse model. This was in contrast to Ki-3(scFv)ETA' and was most probably because of different cluster-binding on the CD30 receptor and also because of shedded CD30. CD30 shedding can be inhibited by hydroxamate inhibitors of metalloproteinases such as BB-3644. The influence of BB-3644 on the efficacy of the Ki-3(scFv)-ETA' was therefore evaluated. *In vitro*, the addition of BB-3644 augmented the antitumor effect of Ki-3(scFv)-ETA' against Hodgkin-derived L540 cells. SCID-mice challenged with CD30-positive L540rec cells were treated with the IT. One single non-toxic dose of BB-3644 increased the mean survival time of animals treated concomitantly with Ki-3(scFv)-ETA' to 92 days compared with 35 days in the control ($P < 0.01$). When BB-3644 was continuously delivered using subcutaneously implanted pumps, there was no observed tumor growth in the animals within 200 days.