Summary English

The main goal of the thesis is the, “Generation of antibody fragments toward the tumour-associated Thomsen-Friedenreich antigen” using recombinant antibody technologies. The TF disaccharide is a tumour-specific pan-carcinoma marker, which occurs only on tumours and virtually not on normal tissues. The exceptional tumour specificity renders it a prime goal for tumour therapy and diagnosis. Despite the thorough investigation of TF, the only TF-specific antibodies reported so far were of the IgM type which are not suitable for the treatment of small primary carcinomas, metastasis or minimal residual disease.

In contrast to protein epitopes against which scFv can readily be generated by using phage display systems, it was not possible to obtain scFv against the small non-charged carbohydrate TF epitope. This was shown to be due to the lower intrinsic affinity of a single anti-TF binding site. The breakthrough was achieved by establishing a new multivalent scFv phage-display format based on the display of scFvs with dramatically shortened linkers (1aa) using a phagemid system. scFv(1aa)-libraries were generated using spleenocytes from immunised mice. TF-specific multivalent scFvs were successfully selected and the most promising clones were evaluated in ELISA, immunocytology, -histology, surface plasmon resonance and size exclusion chromatography. Successive shortening of the scFv linker from 18 to 0 amino acids directed the formation of multimeric scFv complexes from dimers, trimers to tetramers in solution, which correlated with an increasing functional affinity, and thus indicates that multimers are also present on the phage particle. The most promising clone showed an outstanding TF-specificity and an affinity of ~20 nM, which is very high for a carbohydrate specific antibody fragment. Interestingly the sequence variability of the clones generated was very low and the affinity could not be improved by affinity maturation indicating a small optimal sequence window for TF-specific antibodies. Another breakthrough was the successful generation of stable multimers, their purification, conjugation with the chelator DTPA, ^{111}In labelling, and in vitro characterisation. Two different xenograft mice tumour models were used showing for the first time, successful in vivo targeting of TF with good tumour-uptake. The kidney burdens were low compared to other recombinant antibody fragments from the literature, particularly for the tetrameric scFv(0aa) construct. This shows that these multimeric scFvs are highly tumour-specific and suitable for the development of RAIT.

In summary, the first stable multivalent TF-specific antibody fragments were generated as a basis for the development of highly tumour-specific RAIT. A novel multimeric phage display methodology was established which is likely to be useful for the generation of carbohydrate-specific antibodies and other antibodies with lower affinities in single binding domains.