

**Identification and Functional
Characterization of Novel Plasma Cell-
Specific Surface Antigens in
Multiple Myeloma**

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Abstract

Multiple myeloma (MM) is a clonal B-cell tumour of differentiated and usually slowly proliferating plasma cells, mainly located in the bone marrow. MM is still an incurable disease with a median survival of about 3 to 5 years, and it is responsible for about one percent of all cancer-related deaths in Western countries. However, the precise molecular events causing multiple myeloma are still not fully understood.

Immunotherapeutic approaches are playing an increasing role in the development of novel treatment strategies of various malignancies. In particular, the use of recombinant bispecific single-chain antibodies (bsc-ABs) is gaining in importance, because these molecules possess exceptional biological properties. However, to date the lack of suitable plasma cell-specific surface antigens has hindered the development of antibody-based treatment strategies for MM. In order to identify such plasma cell-specific antigens hybridoma supernatants (generated by Dr. Axel Greiner, University of Würzburg) were screened by flow cytometry with a panel of human multiple myeloma-, plasma cell leukaemia-, and B-cell lymphoma cell lines. Three supernatants were found reactive with human MM cell line RPMI-8226. Western blot analysis revealed a band of ~25kDa. A single specific spot was identified with two-dimensional SDS-PAGE and Western blotting of RPMI-8226 membrane proteins and this protein-spot was further analysed by MALDI/MS. Database comparison of the peptide sequence identified the putative plasma cell-specific antigen as human lambda (λ)-light chain.

Recently a novel monoclonal antibody (mAB), designated anti-Wue-1, has been generated which specifically binds to the cell surface of normal and malignant human plasma cells (PC) and mucosa-associated lymphoid tissue (MALT) lymphoma with PC differentiation. On basis of the anti-Wue-1 mAB, a novel MM directed recombinant bispecific single-chain antibody was engineered, designated bsc-anti-Wue-1xCD3 or MT105. Part of this project was to analyze the biological properties of bsc-anti-Wue-1xCD3, using the MM cell line NCI-H929 co-cultured with effector T-cells, isolated from buffy coats of healthy donors. It was demonstrated that bsc-anti-Wue-1xCD3 induces efficient T-cell mediated cell death of NCI-H929 cells. In contrast to conventional bispecific antibodies, bsc-anti-Wue-1xCD3 was efficacious at low effector to target (E:T) ratios and without any additional T-cell stimulation.

The third part of this PhD project was dedicated to the identification, cloning and functional characterization of the novel WUE-1 antigen. First, WUE-1 positive MM cell lines were

identified, using flow cytometry. However, further characterization of the antigen by standard methods such as Western blot analysis or immunoprecipitation kept failing. Subsequently, the attempt was undertaken to isolate the antigen by means of expression cloning and immunoselection with anti-Wue-1 mAB ("panning"). The isolated clones were analyzed by sequencing, Northern blotting, and flow cytometry, but the clones turned out to contain only non-specific inserts.

Since the efficacy of the single-chain (sc) anti-Wue-1 antibody and its advantages over the parental mAB were successfully shown, a recombinant chimeric T-cell receptor (TCR) was generated, comprising the variable single chain (Fv sc) anti-Wue-1 antibody domain, attached to a human IgG Fc sequence, the transmembrane CD28 moiety, and an intracellular CD3 ζ signalling domain. This WUE-1 specific TCR was used to develop a novel assay for use in a multiple myeloma expression library screen. Jurkat (T-) cells transfected with the chimeric TCR served as bioindicators. Specific TCR crosslinking with an antigen results in MHC-independent effector cell activation, which can be monitored by cytokine ELISA. Here, expression of the WUE-1 specific TCR on grafted Jurkat cells was successfully demonstrated by triggering the interferon gamma (IFN- γ) release by addition of Fc specific anti-human IgG antibody. However, IFN- γ could not be detected in co-cultures of transfected Jurkat effector cells and WUE-1 positive target cells.

Although the molecular structure of the WUE-1 antigen is still unclear, it was shown that its expression profile and biochemical characteristics discriminate WUE-1 from other plasma cell-associated antigens described so far. Moreover, it was demonstrated that bssc-anti-Wue-1xCD3 induces efficient T-cell mediated cell death at low E:T ratios, and without any additional T-cell stimulation. WUE-1 therefore represents a very promising candidate for use in the development of novel immunotherapeutic treatment strategies of multiple myeloma.

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