
Table of Contents

1 Introduction	7
1.1 Cutaneous Melanoma Is a Significant Public Health Problem	7
1.2 Melanoma Is Immunogenic and an Important General Model For Cancer Immunology	7
1.3 Melanoma Associated Antigens Are Targets for the Immune Response	9
1.4 Monitoring Tumour Antigen-Specific T Cells <i>In Vivo</i>	12
1.5 Concerted Cellular and Humoral Responses Against Tumour-Associated Antigens Are A Rare Phenomenon	13
1.6 Tumour Growth In the Presence of TAA-Specific T Cells	14
1.7 Novel Therapeutic Methods Targeting Melanoma	15
1.8 Development of a GM-CSF-Based Cellular Melanoma Vaccine	16
1.9 A Trial with GM-CSF-Based Vaccines Stimulates Potent and Long-Lasting Immune Responses in Late Stage Melanoma Patients	18
1.10 The GM-CSF-Based Cancer Vaccine is the Basis for the Identification of Tumour-Associated Antigens	20
2 Materials and Methods	22
2.1 Phage Library Screening	22
2.2 Plasmid Excision	23
2.3 Total RNA Isolation from Cultured Cells or Tissues	23
2.4 Reverse Transcriptase Reaction	23
2.5 Northern Blot	24
2.6 Southern Blot	24
2.7 Nucleic Acid Transfer	25
2.8 Hybridisation	25
2.9 Colony Hybridisation Screening	26
2.10 Recombinant Glutathione S-Transferase Fusion Protein	27
2.11 Enzyme-Linked Immuno Sorbent Assay (ELISA)	27
2.12 T Cell Assays	28

2.13 Construction of a Retroviral Vector and Production of VSV-G-Pseudotyped Retroviral Particles	30
2.14 Anti-ML-IAP Monoclonal Antibody and Immunohistochemistry	31
2.15 Whole Cell Lysates	32
2.16 SDS Polyacrylamide Gel Electrophoresis (SDS PAGE)	32
2.17 Immunoblotting (Western)	33
3 Results	34
3.1 Patient K030 Displayed Potent Anti-Tumour Immune Responses Following Vaccination	34
3.2 Identification of the Novel IAP Family Member ML-IAP as a Target for the Immune Response	37
3.3 Vaccination Increases ML-IAP-Specific Antibody Titres and Induces Isotype Switching	44
3.4 Vaccine-Induced CD4 ⁺ TILs Show Strong Proliferative Response in the Presence of Recombinant ML-IAP	47
3.5 Vaccine Induced CD8 ⁺ TIL Are ML-IAP-Specific And Kill ML-IAP Positive Target Cells	48
3.6 Emergence of ML-IAP Loss Tumour Cell Variants Correlates with Lack of TILs, Absence of Tumour Necrosis and Overall Clinical Deterioration	51
3.7 ML-IAP Is Widely Expressed In Neoplasms	54
3.8 Cancer Patients Have Elevated Anti-ML-IAP Antibody Levels	55
3.9 Identification of a Novel RING-less ML-IAP Splice Variant	60
3.10 Identification of Murine ML-IAP	60
4 Discussion	65
4.1 Dysregulation of Apoptosis in Cancer	65
4.2 Vaccine Enhances ML-IAP-Specific Humoral Response – The Important Role of Anti-Tumour Antibodies	67
4.3 CD4 ⁺ T Cells are Significantly Contributing to Tumour Rejection and Recognise ML-IAP	69

4.4 CD8 ⁺ TILs Can Kill ML-IAP Expressing Cells Through Caspase-Independent Pathways	70
4.5 Loss of Tumour-Associated Antigen During Course of Treatment	71
4.6 Implications for Future Vaccine Development	72
4.7 Investigating the Distribution and Biological Function of ML-IAP Splice Variants	74
4.8 Importance of Identifying the Murine Form of ML-IAP	74
4.9 Concluding Remarks	75
5 Acknowledgements	76
6 References	77
7 Abbreviations	100
8 Appendix	102
8.1 Summary	102
8.2 Zusammenfassung (Summary in German)	104
8.3 <i>Curriculum Vitae</i>	106
8.4 Lebenslauf (<i>Curriculum Vitae</i> in German)	109