



Index

Index	V
Abbreviations.....	X
1 Introduction.....	17
2 Literature	18
2.1 Equine melanoma.....	18
2.1.1 Incidence and classification	18
2.1.2 Clinical signs and diagnosis	19
2.1.3 Histology and immunohistology	19
2.1.4 Etiology	21
2.1.5 Therapy.....	22
2.2 Importance of immunity against neoplasia	23
2.2.1 Properties of cancer immunity.....	23
2.2.2 Tumor escape	25
2.2.3 Tumor antigens	26
2.3 Immunologic Therapy against cancer.....	27
2.3.1 Autologous tumor cells for immunological treatment of cancer	27
2.3.2 Peptides used as tumor vaccines	28
2.4 DNA vaccines.....	29
2.4.1 Overcoming self tolerance by xenovaccination	30
2.4.2 DNA vaccination using minimalistic immunologically defined gene expression (MIDGE®) vectors	30
2.5 Immunologic therapy against melanoma	31
2.5.1 The use of gp100 in immunotherapy.....	32
2.5.2 The use of tyrosinase in immunotherapy	33
2.6 Cytokines.....	35
2.6.1 Interleukin 12	36
2.6.1.1 Antitumoral effects of IL12.....	37
2.6.2 Interleukin 18	38
2.6.2.1 Antitumoral effects of IL18.....	39

2.6.3	Combination of IL12 and IL18	40
2.6.4	Adjuvant effects of cytokines.....	41
2.7	Determination of tumor regression	41
2.8	Detection of specific effector cells	42
2.8.1	<i>In vivo</i> detection of specific CTL	42
2.8.2	<i>In vitro</i> CTL assays	42
2.8.2.1	^{51}Cr release-assay.....	42
2.8.2.2	ELISPOT (Enzyme linked immunospot)	43
2.8.2.3	Intracellular cytokine staining (ICS)	44
2.8.2.4	Tetramer-Assay.....	46
2.8.2.5	CTL detection in neoplasia.....	46
2.9	Antibody detection.....	47
2.10	Luciferase assay	48
3	Material und Methods.....	49
3.1	Patients	49
3.2	Groups.....	52
3.3	Gene synthesis.....	54
3.3.1	Synthesis of MIDGE-Th1 [®] vectors	58
3.4	Preparation of SAINT-18/DNA complexes.....	58
3.5	Control of <i>in vivo</i> transfection by luciferase assay	59
3.5.1	Luciferase assay	60
3.6	Injection of MIDGE-Th1 [®] coding for differentiation antigens (hgp100 and htyr) and interleukins (eqIL12 and eqILRAP-IL18)	60
3.7	Evaluation of unspecific side effects of treatment with MIDGE-Th1 [®] vectors and SAINT18 alone or in combination.....	62
3.8	Clinical evaluation for determination of response to treatment	63
3.8.1	Clinical evaluation	63
3.8.2	Hematology and blood biochemistry	63
3.8.3	Measurement of melanomas	63
3.8.4	Calliper measurements	64
3.8.5	Ultrasonographic measurements	64

3.8.6 Calculation of tumor volume	64
3.8.7 Photographic documentation	66
3.9 Cell culture and transfection of human embryonic kidney cells (HEK 293)..	66
3.10 Measurement of the humoral immune response induced by vaccination .	67
3.11 Indirect evaluation of cytotoxic response by IFNy production of specifically stimulated PBMCs using flow cytometry	70
3.11.1 Dermal cell isolation, culture and cryopreservation	70
3.11.2 Transfection of Equine Dermal Cells	71
3.11.3 Isolation and culture of peripheral blood mononuclear cells	72
3.11.4 IFNy staining.....	73
3.12 Statistical analysis.....	75
4 Results	76
4.1 Control of <i>in vivo</i> transfection	76
4.2 Therapy Control.....	78
4.2.1 Clinical examination of treatment groups IL12/18, gp100 and tyr	78
4.2.2 Clinical examination of horses treated for evaluation of unspecific side effects (group SAINT-18/luc, group SAINT-18/- and group -/IL12/18) ..	79
4.2.3 Macroscopic evaluation and photographic documentation	80
4.2.4 Hematology and blood biochemistry analysis	83
4.2.5 Absolute tumor volume	83
4.2.5.1 Differences between the treatment groups.....	83
4.2.6 Relative tumor volume	83
4.2.6.1 Calliper measurements.....	83
4.2.6.2 Ultrasonographic measurements.....	87
4.2.7 Correlations between measurement methods and examiners	92
4.2.8 Evaluation of the response to treatment according to the RECIST criteria	92
4.3 Measurement of the humoral response induced by vaccination.....	93
4.4 Evaluation of cytotoxic response by IFNy detection using flow cytometry ...	96
4.4.1 EDC isolation, culture and cryopreservation; Transfection of EDCs	96
4.4.2 IFNy staining.....	97

5	Discussion	100
5.1	Antitumoral effect: possible influencing factors.....	100
5.1.1	Antitumoral effect of IL 12 and 18 DNA.....	100
5.1.2	Further factors possibly influencing the antitumoral effect	103
5.1.2.1	The antitumoral effect of the combination of DNA and transfection agent	103
5.1.2.2	The antitumoral effect of unmethylated CpG-motifs	104
5.2	Absent antitumoral effect of DNA coding for xenogenic melanoma antigens ..	105
5.2.1	<i>In vivo</i> transfection	105
5.2.2	Influence of the dosage.....	105
5.2.3	Influence of the proteins chosen for vaccination	106
5.2.4	Influence of the grade of similarity	107
5.2.5	Influence of the vaccination interval	107
5.2.6	Influence of the route of administration	108
5.3	Correlation of measurements between measurement methods and examiners ..	110
5.3.1	Evaluation of the response to treatment according to the RECIST criteria	112
5.4	Measurement of the humoral response induced by vaccination.....	113
5.5	Evaluation of cytotoxic response by IFN γ detection using flow cytometry .	113
5.6	Evaluation of unspecific side effects.....	115
5.6.1	Increased body temperature	115
5.6.2	Local skin reactions	116
5.7	Conclusions limitations and outlook.....	117
6	Summary	120
7	Zusammenfassung.....	123
8	References	126
9	Appendix	174
9.1	Materials	174
9.1.1	Instruments	174

9.1.2 Clinical materials	176
9.1.3 Laboratory materials	179
9.1.4 Laboratory reagents.....	181
10 Acknowledgements	187