



Contents

I	INTRODUCTION	1
1	Equine malignant melanoma	1
1.1	Clinical presentation	1
1.2	Current therapies for equine malignant melanoma.....	2
2	Immune-mediated melanoma therapy	2
3	Interleukin-12 in cancer therapy	4
3.1	IL-12 in melanoma therapy.....	5
3.2	Adjuvant effects of IL-12.....	8
3.3	DCs and the immune-mediated therapy	9
3.3.1	DCs in melanoma therapy.....	9
4	Equine malignant melanoma immune-mediated therapy.....	10
5	Recombinant IL-12 toxicity and gene therapy	12
6	Gene therapeutic approaches	12
6.1	Viral vectors	13
6.2	Non-viral vectors	13
6.2.1	Non-viral transfection methods.....	13
6.2.1.1	Naked DNA injection	13
6.2.1.2	Gene gun	14
6.2.1.3	Electroporation.....	14
6.2.1.4	Cationic lipid (liposome)-DNA complex (lipoplex)	14
6.2.1.5	Cationic polymer-DNA complex (polyplex).....	15
7	Transfection reagents.....	15
8	Transfection efficiency.....	15
8.1	Transfection efficiency enhancers	16
8.1.1	Nanoparticles (NPs)	16
8.1.2	Gold Nanoparticles (AuNPs)	16
8.1.3	Magnetic Nanoparticles.....	16
9	Equine IL-12 mammalian expression vectors.....	16
10	Protein Expression verification/quantification after IL-12 treatment.....	17
II	HYPOTHESIS AND AIMS	19
III	RESULTS I:.....	20



Comparison of nanoparticle-mediated transfection methods for DNA expression plasmids: efficiency and cytotoxicity	20
Manuscript I	21
IV RESULTS II:.....	43
Establishment and evaluation of a bead-based Luminex assay allowing simultaneous quantification of equine IL-12 and IFN-gamma.....	43
Manuscript II	44
V RESULTS III:	74
Enhanced protocol for CD14+ cell enrichment from equine peripheral blood via anti-human CD14 mAb and automated magnetic activated cell sorting	74
Manuscript III	75
VI GENERAL DISCUSSION	88
VII FINAL REMARKS.....	93
VIII SUMMARY	94
IX ZUSAMMENFASSUNG.....	96
X REFERENCES	98
XI ANNEX	116
Materials and methods	116
Mammalian expression vectors.....	116
Cell lines	117
Cell transfection	118
Protein Expression.....	121
Results.....	125
Protein expression after transfection.....	125
Transfection efficiency and toxicity	129
XII ACKNOWLEDGMENTS	131