

TABLE OF CONTENTS

ABBREVIATIONS	8
SUMMARY	10
INTRODUCTION	20
1 <i>An overview of lung cancer</i>	21
1.1 Epidemiology	21
1.2 Classification	21
1.2.1 General classification.....	21
1.2.2 New and future classifications	22
1.3 Diagnosis and treatment.....	23
1.3.1 Generalities	23
1.3.2 Chemotherapy.....	24
2 <i>Pulmonary drug delivery in lung cancer therapy</i>	38
2.1 Main advantages.....	38
2.2 Techniques, devices and formulation development	39
2.3 Main issues encountered.....	41
2.4 Inhaled nanomedicine – an ongoing concept in lung cancer therapy.....	42
3 <i>Scientific strategy</i>	49
3.1 Chosen chemotherapeutic drugs	49
3.1.1 Temozolomide.....	49
3.1.2 Paclitaxel	51
3.2 Types of folate receptor-targeted nanocarriers developed	54
3.2.1 Folate receptor-targeting	54
3.2.2 Polymeric micelles.....	55
3.2.3 Solid lipid nanoparticles	57
3.3 Pulmonary delivery – Dry powder for inhalation	59
AIMS OF THE WORK	62
EXPERIMENTAL PART	66
PART I. DEVELOPMENT OF NEW FOLATE-GRAFTED EXCIPIENTS FOR PULMONARY DRUG DELIVERY	67
1 <i>Introduction and aims</i>	68
2 <i>Materials and methods</i>	70
2.1 Materials.....	70
2.2 Synthesis of folate-grafted excipients.....	70
2.2.1 Synthesis of folate-poly(ethylene glycol)-hydrophobically-modified dextran (F-PEG-HMD) and derivatives.....	70
2.2.2 Synthesis of folate-poly(ethylene glycol)-(N-[(2-hydroxy-3-trimethyl-ammonium) propyl] chitosan) (F-PEG-HTCC) and derivatives.....	73

Table of contents

2.3	Characterization of F-PEG-HMD and F-PEG-HTCC.....	75
2.3.1	Determination of the poly(ethylene glycol)-graft ratio (PEG-GR)	75
2.3.2	Determination of the molecular weight (Mw) range	75
2.3.3	Thermal properties – Thermogravimetric analysis and Differential scanning calorimetry	75
2.3.4	Determination of the critical micellar concentration, and the size and zeta potential of F-PEG-HMD micelles Erreur ! Signet non défini.	
3	<i>Results and discussion</i>	76
3.1	F-PEG-HMD	76
3.2	F-PEG-HTCC.....	79
4	<i>Conclusions</i>	80
PART II. DEVELOPMENT OF NANOCARRIER-BASED DRY POWDER FORMULATIONS FOR INHALATION USING THE CHEMOTHERAPEUTIC DRUG TEMOZOLOMIDE		82
1	<i>Introduction and aims</i>	83
2	<i>Materials and methods</i>	83
2.1	Materials	83
2.2	Determination of temozolomide content	83
2.3	Preformulation investigation – micellar solubilisation of temozolomide	84
2.4	Preparation of F-PEG-HMD micelles containing temozolomide	85
2.5	Preparation and characterization of dry powders for inhalation containing the polymeric micelles.....	85
2.5.1	Preparation of dry powders	85
2.5.2	Re-dispersibility of the initial micelles in aqueous media	86
2.5.3	<i>In vitro</i> pulmonary deposition – aerodynamic performance.....	86
2.5.4	<i>In vitro</i> release profile of temozolomide from the dry powders	86
2.6	Cell culture.....	87
2.7	<i>In vitro</i> anti-proliferative properties of the dry powders.....	87
3	<i>Results and discussion</i>	87
3.1	Preformulation investigation – micellar solubilization of TMZ	87
3.2	Preparation and characterization of F-PEG-HMD micelles containing temozolomide.....	88
3.3	Production and characterization of the dry powders for inhalation containing the polymeric micelles	89
3.4	<i>In vitro</i> release profile of TMZ from the dry powders.....	92
3.5	<i>In vitro</i> anti-proliferative properties of the dry powders.....	93
4	<i>Conclusions</i>	94
PART III. DEVELOPMENT OF NANOCARRIER-BASED DRY POWDER FORMULATIONS FOR INHALATION USING THE CHEMOTHERAPEUTIC DRUG PACLITAXEL		95
1	<i>Introduction and aims</i>	96
2	<i>Materials and methods</i>	96
2.1	Materials	96
2.2	Determination of paclitaxel content.....	97
2.3	Preparation and characterization of nanovectors using the new folate-grafted copolymers	97
2.3.1	Preparation.....	97
2.3.2	Determination of particle size distribution, zeta potential and morphology	98

Table of contents

2.3.3	Determination of paclitaxel entrapment efficiency	99
2.3.4	<i>In vitro</i> release profile of paclitaxel from the paclitaxel-loaded nanocarriers.....	99
2.4	<i>In vitro</i> studies in folate receptor-expressing cancer cell lines	100
2.4.1	Cell culture	100
2.4.2	Western blot analysis - Folate receptor α protein expression	100
2.4.3	<i>In vitro</i> cell binding and uptake of the nanocarriers.....	Erreur ! Signet non défini.
2.4.4	<i>In vitro</i> anti-proliferative properties of the paclitaxel-loaded nanocarriers.....	102
2.5	Preparation and characterization of dry powders for inhalation containing the nanocarriers.....	102
2.5.1	Preparation of dry powders	102
2.5.2	Re-dispersibility of the nanocarriers in aqueous media	103
2.5.3	Size and morphology – environmental scanning electron microscopy	103
2.5.4	<i>In vitro</i> pulmonary deposition – aerodynamic performance.....	103
2.6	Preclinical investigation	104
2.6.1	Animals and husbandry conditions	104
2.6.2	Pulmonary administration of formulations to mice	104
2.6.3	Local pulmonary tolerance of nanocarrier-based dry powder formulations in healthy mice	104
2.6.4	Development of an orthotopic lung cancer mouse model from an folate receptor-expressing cell line – the M109 model.....	106
2.6.5	Determination of the dose of paclitaxel to administer to mice by inhalation.....	107
2.6.6	<i>In vivo</i> lung tumor distribution of the nanocarriers after pulmonary delivery.....	107
2.6.7	<i>In vivo</i> anti-cancer activity.....	108
3	Results and discussion	109
3.1	Preparation and characterization of the nanocarriers.....	109
3.1.1	Polymeric micelles.....	109
3.1.2	Coated solid lipid nanoparticles	111
3.2	<i>In vitro</i> release profile of paclitaxel from the nanocarriers.....	112
3.3	Folate receptor α protein expression measurements in HeLa and M109-HiFR	114
3.4	<i>In vitro</i> cell binding and uptake of the nanocarriers	114
3.5	<i>In vitro</i> anti-proliferative properties of the nanocarriers.....	117
3.6	Preparation and characterization of dry powders for inhalation containing the nanovectors.....	120
3.7	Local pulmonary tolerance of nanocarrier-based dry powder formulations in healthy mice.....	124
3.8	Development of an orthotopic lung cancer mouse model from a folate receptor-expressing cell line – the M109 model	127
3.9	Determination of the dose of paclitaxel to administer by inhalation	128
3.10	<i>In vivo</i> lung tumor distribution of the nanocarriers after pulmonary delivery	129
3.11	<i>In vivo</i> anti-cancer activity	130
4	Conclusions	134
	GENERAL CONCLUSIONS AND PERSPECTIVES	136
	BIBLIOGRAPHY	142