## Contents

Foreword xv

Acknowledgments xvii

1	Antibacterial Carbohydrate Vaccines 1			
	Federica Compostella, Laura Morelli, and Luigi Lay			
1.1	Introduction 1			
1.1.1	A Brief History of Vaccines 2			
1.2	Carbohydrate-Based Vaccines 5			
1.2.1	Mechanism of the Immune Response to Carbohydrate-Based			
	Vaccines 12			
1.3	Components of Glycoconjugate Vaccines 15			
1.3.1	The Carbohydrate Antigen 16			
1.3.2	Linkers for Carbohydrate–Protein Conjugation 19			
1.3.3	The Carrier Protein 22			
1.3.4	The Adjuvant 24			
1.4	Technologies Employed for Production of Glycoconjugate Vaccines 25			
1.4.1	Traditional Glycoconjugates 26			
1.4.2	Glycoconjugates Based on Synthetic Carbohydrate Antigens 28			
1.4.2.1	Site-Selective Protein Conjugation 29			
1.4.3	Enzymatic and ChemoEnzymatic Approach 30			
1.4.4	Bioengineered Glycoconjugates 31			
1.4.5	Nanotechnology-Based Glycoconjugate Vaccines 33			
1.4.5.1	Outer Membrane Vesicles (OMVs) and Generalized Modules for			
	Membrane Antigens (GMMA) 33			
1.4.5.2				
1.4.6	Nonprotein-Based Glycoconjugates 36			
1.4.7	Noncovalent Vaccines 36			
1.5	Conclusion 37			
	Acknowledgments 38			
	References 39			

2	Antifungal Glycoconjugate Vaccines 57					
	Linda del Bino, Maria R. Romano, and Roberto Adamo					
2.1	Human Fungal Infections 57					
2.2	Immunity Against Fungal Pathogens 59					
2.3	Carbohydrate Antigens in Fungal Cell Wall 60					
2.4	Glycoconjugate Vaccines Against Candida albicans/Candida auris 61					
2.5	Glycoconjugate Vaccines Against Cryptococcus neoformans 64					
2.6	Glycoconjugate Vaccines Against Aspergillus fumigatus 66					
2.7	Universal Fungal Polysaccharide Antigens 68					
2.8	Conclusions and Future Prospects 68					
	References 69					
3	Carbohydrate-Based Antiviral Vaccines 73					
	Adrián Plata and Alberto Fernández-Tejada					
3.1	Introduction 73					
3.2	Human Immunodeficiency Virus 74					
3.2.1	Vaccine Constructs Derived from gp120 High-Mannose N-Glycan					
	Cluster 75					
3.2.1.1	Surface Oligomannose Cluster-Targeting bnAb: 2G12 Antibody 75					
3.2.1.2	Synthesis and Immunological Evaluation of 2G12 Epitope Mimics 76					
3.2.2						
	Loops (V1V2) 81					
3.2.2.1	V1V2-Targeting bnAbs 81					
3.2.2.2	Synthetic V1V2 <i>N</i> -Glycopeptide Antigens as bnAb Epitope Mimics 8.					
3.2.3	Vaccine Constructs Derived from gp120 Third Variable Loops (V3) 83					
3.2.3.1	V3-Targeting bnAbs 83					
3.2.3.2	2 Synthetic Glycoconjugates and <i>N</i> -glycopeptides as V3-Directed bnAb					
	Epitope Mimics 83					
3.2.3.3	Synthetic V3 Glycopeptides as bnAb Epitope Mimics 83					
3.3	Influenza A Virus 85					
3.3.1	Vaccine Constructs Based on Hemagglutinin (HA) 86					
3.3.1.1	Hyperglycosylated HA Vaccines 87					
3.3.1.2	α-Gal-Based Vaccine Constructs 87					
3.3.2	Vaccine Constructs Based on Neuraminidase (NA) 88					
3.3.3	Acetalated Dextran as Adjuvant Carrier 89					
3.3.4	Multivalent Constructs as Anti-Influenza Inhibitors 89					
3.4	Hepatitis C Virus 90					
3.5	Ebola Virus 91					
3.5.1	Glycoprotein-Based Vaccines 92					
3.5.2	Monoclonal Antibodies and Carbohydrate Antiviral Agents					
	as Therapeutics 92					
3.6	SARS-CoV-2 Virus 94					
3.6.1	Prospective Vaccine Constructs Based on α-Gal Epitope 94					
3.6.2	RBD-Based Constructs for Vaccine Development 95					

3.6.3	Saponins as Carbohydrate-Based Adjuvant Candidates for COVID-19 Vaccines 95					
3.7	Conclusions and Outlook 96					
	Acknowledgments 96					
	References 97					
4	Bacterial Glycolipid Lipid As and Their Potential as Adjuvants 111					
•	Atsushi Shimoyama and Koichi Fukase					
4.1	Introduction 111					
4.2	Bacterial Glycolipid Lipid A: an Innate Immune Stimulant 113					
4.3	Vaccines Containing Natural LPS as Adjuvants 117					
4.3.1	Cholera Vaccines 117					
4.3.2	Salmonella enterica Serovar Typhi Vaccines 117					
4.3.3	Other Vaccines 118					
4.4	LPS and Lipid A in the Environment or Fermented Foods as					
	Adjuvants 118					
4.5	Synthetic and Semisynthetic Lipid As as Adjuvants 120					
4.6	Developing Novel Lipid A Adjuvants 121					
4.6.1	Parasitic Bacterial Lipid As 121					
4.7	Symbiotic Bacterial Lipid As 123					
4.8	Lipid A-Based Self-Adjuvanting Vaccines 125					
4.9	.9 Conclusions 127					
	References 127					
5	Antiadhesive Carbohydrates and Glycomimetics 131					
	Jonathan Cramer, Lijuan Pang, and Beat Ernst					
5.1	Introduction 131					
5.1.1	Carbohydrate–Protein Interactions in Viral Adhesion to Host Cells 131					
5.1.2	Bacterial Adhesins and Antiadhesion Therapy 132					
5.1.3	Selected Examples 133					
5.2	DC-SIGN-Mediated Viral Adhesion and Entry into Myeloid Cells 133					
5.2.1	Introduction 133					
5.2.2	DC-SIGN Ligands Employing Natural Carbohydrate Epitopes 136					
5.2.2.1	Dendrimers 137					
5.2.2.2	Nanoparticles 137					
5.2.2.3	Polymers 138					
5.2.2.4	Other Multivalent Scaffolds 138					
5.2.3	DC-SIGN Ligands Employing Carbohydrate Derivatives or					
	Glycomimetics 139					
5.2.4	Conclusion and Perspectives 141					
5.3	The Bacterial Adhesin FimH 143					
5.3.1	UTIs and FimH 143					
5.3.2	FimH CRD 143					
5.3.3	FimH Antagonists 145					
5.3.4	Conclusion and Perspectives 147					

x	Contents					
	5.4	Pseudomonas aeruginosa Virulence Factors (PA-IL and PA-IIL) 148				
	5.4.1	Introduction 148				
	5.4.2 Mono- and Oligovalent Glycomimetic PL-Ligands 149					
5.4.3 Conclusions and Perspectives 152						
	5.5	General Aspects 152				
		References 153				
	Targeting Carbohydrates in Cancer – Analytical and Biotechnological Tools 161 Henrique O. Duarte, Joana Gomes, and Celso A. Reis					
	6.1	Aberrant Protein Glycosylation in Cancer 161				
6.2 Detection and Mapping of Carbohydrate-Based Antigens in Human						
Neoplastic Tissues 164						
	6.3	Imaging Mass Spectrometry 164				
	6.4	In Situ Proximity Ligation Assay 166				
6.5 Glycan Microarrays 169						
	6.6	Glycoengineered In Vitro, In Vivo, and Ex Vivo Models 171				
6.7 Structural Elucidation of Glycoconjugates: Glycomic and Glycopro						
	Strategies 176					
	6.8	Concluding Remarks 182				
List of Abbreviations 183						
		References 185				
	7	Carbohydrate-Specific Monoclonal Antibody Therapeutics 201				
		Matthew Lohman, Hannah Rowe, and Peter R. Andreana				
	7.1	Introduction 201				
	7.2	Types of Monoclonal Antibodies 202				
	7.2.1	IgG Antibodies 202				
	7.2.2	IgM Antibodies 203				
	7.2.3	ScFv and Fab Fragments 203				
	7.3	Humanization of Monoclonal Antibodies 204				
	CDR Grafting 204					
	7.3.2	Transgenic Animals 204				
7.4 Breakthrough Research 205						
	7.5	mAbs from Preclinical to Clinical Studies 206				
7.6 Globo Series 206						
	7.6.1	Blood Group 206				
	7.6.2	Mucin-Attached Glycans 207				
	7.7	New Treatment Options for Neuroblastoma 207				
	7.7.1	History of Unituxin 208				
	7.7.2	What is Unituxin? 209				
	7.7.3	Challenges with Unituxin 211				
	774	mAhs Rinding to Neuroblastoma 211				

Chimeric and Humanized Anti-GD2 Antibodies 212

Naxitamab as a Potential Alternative for High-Risk Patients  $\,\,\,\,212$ 

7.7.5 7.7.6

7.7.7	Chimeric Antigen Receptors (CARs) Targeting GD2 213					
7.8	Summary 214					
List of Abbreviations 215						
	References 216					
8	Carbohydrates in Tissue Engineering 223					
	Laura Russo and Francesco Nicotra					
8.1	Introduction 223					
8.2	Biomaterials and Medical Devices: Natural and Synthetic Strategies 224					
8.2.1	Carbohydrates as Building Blocks for Medical Device Formulation 224					
8.2.1.1	·					
(PGs) 225						
8.2.1.2	Polysaccharides from Plants, Algae, Animal, and Microbial					
	Fermentation 228					
8.2.2	Carbohydrates as Signaling Molecules: Opportunities in Tissue					
	Engineering and Regenerative Medicine 233					
8.3	Carbohydrates in Animal-Derived Medical Devices: Friends or					
	Foes? 234					
8.4	Glycoengineering Application to Regenerative Medicine 235					
8.5	Future Opportunities and Major Challenges 237					
	Conflict of Interest 237					
	References 237					
0	Carbohydrata Paced Therapoutics for Lycocomal Storage Disorders 245					
9	Carbohydrate-Based Therapeutics for Lysosomal Storage Disorders 245					
	Camilla Matassini, Francesca Clemente, and Francesca Cardona					
9.1	Camilla Matassini, Francesca Clemente, and Francesca Cardona An Introduction to Lysosomal Storage Disorders (LSDs) 245					
	Camilla Matassini, Francesca Clemente, and Francesca Cardona An Introduction to Lysosomal Storage Disorders (LSDs) 245 Available Treatments for LSDs: The Role of Carbohydrate-Based					
9.1 9.2	Camilla Matassini, Francesca Clemente, and Francesca Cardona An Introduction to Lysosomal Storage Disorders (LSDs) 245 Available Treatments for LSDs: The Role of Carbohydrate-Based Therapeutics 248					
9.1 9.2 9.2.1	Camilla Matassini, Francesca Clemente, and Francesca Cardona An Introduction to Lysosomal Storage Disorders (LSDs) 245 Available Treatments for LSDs: The Role of Carbohydrate-Based Therapeutics 248 Enzyme Replacement Therapy (ERT) 250					
9.1 9.2 9.2.1 9.2.2	Camilla Matassini, Francesca Clemente, and Francesca Cardona An Introduction to Lysosomal Storage Disorders (LSDs) 245 Available Treatments for LSDs: The Role of Carbohydrate-Based Therapeutics 248 Enzyme Replacement Therapy (ERT) 250 Substrate Reduction Therapy (SRT) 251					
9.1 9.2 9.2.1 9.2.2 9.2.3	Camilla Matassini, Francesca Clemente, and Francesca Cardona An Introduction to Lysosomal Storage Disorders (LSDs) 245 Available Treatments for LSDs: The Role of Carbohydrate-Based Therapeutics 248 Enzyme Replacement Therapy (ERT) 250 Substrate Reduction Therapy (SRT) 251 Pharmacological Chaperone Therapy (PCT) 252					
9.1 9.2 9.2.1 9.2.2	Camilla Matassini, Francesca Clemente, and Francesca Cardona An Introduction to Lysosomal Storage Disorders (LSDs) 245 Available Treatments for LSDs: The Role of Carbohydrate-Based Therapeutics 248 Enzyme Replacement Therapy (ERT) 250 Substrate Reduction Therapy (SRT) 251 Pharmacological Chaperone Therapy (PCT) 252 Combined ERT/PC Therapy 254					
9.1 9.2 9.2.1 9.2.2 9.2.3 9.2.4	Camilla Matassini, Francesca Clemente, and Francesca Cardona An Introduction to Lysosomal Storage Disorders (LSDs) 245 Available Treatments for LSDs: The Role of Carbohydrate-Based Therapeutics 248 Enzyme Replacement Therapy (ERT) 250 Substrate Reduction Therapy (SRT) 251 Pharmacological Chaperone Therapy (PCT) 252 Combined ERT/PC Therapy 254 Mucopolysaccharidoses 254					
9.1 9.2 9.2.1 9.2.2 9.2.3 9.2.4 9.3	Camilla Matassini, Francesca Clemente, and Francesca Cardona An Introduction to Lysosomal Storage Disorders (LSDs) 245 Available Treatments for LSDs: The Role of Carbohydrate-Based Therapeutics 248 Enzyme Replacement Therapy (ERT) 250 Substrate Reduction Therapy (SRT) 251 Pharmacological Chaperone Therapy (PCT) 252 Combined ERT/PC Therapy 254					
9.1 9.2 9.2.1 9.2.2 9.2.3 9.2.4 9.3 9.4	Camilla Matassini, Francesca Clemente, and Francesca Cardona An Introduction to Lysosomal Storage Disorders (LSDs) 245 Available Treatments for LSDs: The Role of Carbohydrate-Based Therapeutics 248 Enzyme Replacement Therapy (ERT) 250 Substrate Reduction Therapy (SRT) 251 Pharmacological Chaperone Therapy (PCT) 252 Combined ERT/PC Therapy 254 Mucopolysaccharidoses 254 Sphingolipidoses 258					
9.1 9.2 9.2.1 9.2.2 9.2.3 9.2.4 9.3 9.4 9.4.1	Camilla Matassini, Francesca Clemente, and Francesca Cardona An Introduction to Lysosomal Storage Disorders (LSDs) 245 Available Treatments for LSDs: The Role of Carbohydrate-Based Therapeutics 248 Enzyme Replacement Therapy (ERT) 250 Substrate Reduction Therapy (SRT) 251 Pharmacological Chaperone Therapy (PCT) 252 Combined ERT/PC Therapy 254 Mucopolysaccharidoses 254 Sphingolipidoses 258 Fabry Disease 258					
9.1 9.2 9.2.1 9.2.2 9.2.3 9.2.4 9.3 9.4.1 9.4.1	Camilla Matassini, Francesca Clemente, and Francesca Cardona An Introduction to Lysosomal Storage Disorders (LSDs) 245 Available Treatments for LSDs: The Role of Carbohydrate-Based Therapeutics 248 Enzyme Replacement Therapy (ERT) 250 Substrate Reduction Therapy (SRT) 251 Pharmacological Chaperone Therapy (PCT) 252 Combined ERT/PC Therapy 254 Mucopolysaccharidoses 254 Sphingolipidoses 258 Fabry Disease 258 Gaucher Disease 262					
9.1 9.2 9.2.1 9.2.2 9.2.3 9.2.4 9.3 9.4 9.4.1 9.4.2 9.4.3	Camilla Matassini, Francesca Clemente, and Francesca Cardona An Introduction to Lysosomal Storage Disorders (LSDs) 245 Available Treatments for LSDs: The Role of Carbohydrate-Based Therapeutics 248 Enzyme Replacement Therapy (ERT) 250 Substrate Reduction Therapy (SRT) 251 Pharmacological Chaperone Therapy (PCT) 252 Combined ERT/PC Therapy 254 Mucopolysaccharidoses 254 Sphingolipidoses 258 Fabry Disease 258 Gaucher Disease 262 Niemann-Pick 267					
9.1 9.2 9.2.1 9.2.2 9.2.3 9.2.4 9.3 9.4 9.4.1 9.4.2 9.4.3 9.4.4	Camilla Matassini, Francesca Clemente, and Francesca Cardona An Introduction to Lysosomal Storage Disorders (LSDs) 245 Available Treatments for LSDs: The Role of Carbohydrate-Based Therapeutics 248 Enzyme Replacement Therapy (ERT) 250 Substrate Reduction Therapy (SRT) 251 Pharmacological Chaperone Therapy (PCT) 252 Combined ERT/PC Therapy 254 Mucopolysaccharidoses 254 Sphingolipidoses 258 Fabry Disease 258 Gaucher Disease 262 Niemann-Pick 267 GM1 Gangliosidosis and Morquio B (β-Gal) 268					
9.1 9.2 9.2.1 9.2.2 9.2.3 9.2.4 9.3 9.4 9.4.1 9.4.2 9.4.3 9.4.4 9.4.5	Camilla Matassini, Francesca Clemente, and Francesca Cardona An Introduction to Lysosomal Storage Disorders (LSDs) 245 Available Treatments for LSDs: The Role of Carbohydrate-Based Therapeutics 248 Enzyme Replacement Therapy (ERT) 250 Substrate Reduction Therapy (SRT) 251 Pharmacological Chaperone Therapy (PCT) 252 Combined ERT/PC Therapy 254 Mucopolysaccharidoses 254 Sphingolipidoses 258 Fabry Disease 258 Gaucher Disease 262 Niemann-Pick 267 GM1 Gangliosidosis and Morquio B (β-Gal) 268 GM2 Gangliosidosis (β-Hexosaminidase) 272					
9.1 9.2 9.2.1 9.2.2 9.2.3 9.2.4 9.3 9.4.1 9.4.2 9.4.3 9.4.4 9.4.5 9.4.6	Camilla Matassini, Francesca Clemente, and Francesca Cardona An Introduction to Lysosomal Storage Disorders (LSDs) 245 Available Treatments for LSDs: The Role of Carbohydrate-Based Therapeutics 248 Enzyme Replacement Therapy (ERT) 250 Substrate Reduction Therapy (SRT) 251 Pharmacological Chaperone Therapy (PCT) 252 Combined ERT/PC Therapy 254 Mucopolysaccharidoses 254 Sphingolipidoses 258 Fabry Disease 258 Gaucher Disease 262 Niemann-Pick 267 GM1 Gangliosidosis and Morquio B ( $\beta$ -Gal) 268 GM2 Gangliosidosis ( $\beta$ -Hexosaminidase) 272 Krabbe 275					
9.1 9.2 9.2.1 9.2.2 9.2.3 9.2.4 9.3 9.4.1 9.4.2 9.4.3 9.4.4 9.4.5 9.4.6 9.5	Camilla Matassini, Francesca Clemente, and Francesca Cardona An Introduction to Lysosomal Storage Disorders (LSDs) 245 Available Treatments for LSDs: The Role of Carbohydrate-Based Therapeutics 248 Enzyme Replacement Therapy (ERT) 250 Substrate Reduction Therapy (SRT) 251 Pharmacological Chaperone Therapy (PCT) 252 Combined ERT/PC Therapy 254 Mucopolysaccharidoses 254 Sphingolipidoses 258 Fabry Disease 258 Gaucher Disease 262 Niemann-Pick 267 GM1 Gangliosidosis and Morquio B (β-Gal) 268 GM2 Gangliosidosis (β-Hexosaminidase) 272 Krabbe 275 Glycogen Storage Disorders 275 Pompe Disease 275 Glycoproteinoses 277					
9.1 9.2 9.2.1 9.2.2 9.2.3 9.2.4 9.3 9.4.1 9.4.2 9.4.3 9.4.4 9.4.5 9.4.6 9.5 9.5.1	Camilla Matassini, Francesca Clemente, and Francesca Cardona An Introduction to Lysosomal Storage Disorders (LSDs) 245 Available Treatments for LSDs: The Role of Carbohydrate-Based Therapeutics 248 Enzyme Replacement Therapy (ERT) 250 Substrate Reduction Therapy (SRT) 251 Pharmacological Chaperone Therapy (PCT) 252 Combined ERT/PC Therapy 254 Mucopolysaccharidoses 254 Sphingolipidoses 258 Fabry Disease 258 Gaucher Disease 262 Niemann-Pick 267 GM1 Gangliosidosis and Morquio B (β-Gal) 268 GM2 Gangliosidosis (β-Hexosaminidase) 272 Krabbe 275 Glycogen Storage Disorders 275 Pompe Disease 275					

	9.7	Conclusions 279 Acknowledgments 282 Abbreviations and Acronyms 283 References 284				
	10	Carbohydrates and Carbohydrate-Based Therapeutics in Alzheimer's  Disease 293				
	10.1	Ana M. Matos, João Barros, and Amélia P. Rauter Introduction 293				
	10.2 <i>O</i> -GlcNAc Transferase (OGT) and <i>O</i> -GlcNAc Hydrolase (OGA) in					
	10.2.1	Neurodegeneration 295 O-GlcNAc Cycling as a Therapeutic Target Against Alzheimer's Amyloid Plaques and Neurofibrillary Tangles 296				
	10.2.2	, , ,				
	10.2.2.1	PUGNAc 301				
	10.2.2.2	GlcNAcstatins 305				
	10.2.2.3	Thiazoline Inhibitors 311				
	10.3	GalNAc in Neurodegeneration 322				
	10.4	Chitosan and Derivatives in AD Brain 324				
10.5 Cholinesterase Inhibitors 325						
	10.6	Fyn Kinase Inhibitors 330				
	10.7 Amyloid Protein–Protein Interaction Inhibitors 334					
10.8 Inhibitors of Aβo and/or Oxidative Stress-Induced Neurotox						
	10.9	Carbohydrate–Protein Interactions as Potential Therapeutic Targets Against AD 341				
	10.9.1	Lipid-Raft Gangliosides as Membrane Accumulation Sites for Toxic A $\beta$ Aggregates 341				
	10.9.2	The Role of Microglial Cells in Aβ Brain Clearance 342				
10.10 Conclusion <i>343</i>						
		List of Abbreviations 344				
		Acknowledgments 347				
		References 347				
	11	Carbohydrate-Based Antithrombotics 353				
		Antonella Bisio, Marco Guerrini, and Annamaria Naggi				
	11.1	Introduction 353				
	11.2	Antithrombotic Drugs 354				
	11.3	Heparin 354				
	11.4	Mechanism of Interaction with Coagulation Factors 357				
	11.4.1	Antithrombin-Mediated Activity 357				
	11.4.2	Heparin Cofactor II Mediated Activity 360				
		Additional Factors 360				
	11.4.4	Adverse Effects of Heparin 360				
	11.4.4.1	Heparin-Induced Thrombocytopenia 361				
	11.4.4.2	Osteoporosis 361				

11.5.1	Ultralow Molecular Weight Heparins 363				
11.6	Drugs Based on Natural GAG Mixtures 363				
11.6.1	The Role of Dermatan Sulfate 364				
11.6.2	Sulodexide 364				
11.6.3	Danaparoid 365				
11.6.4	Mesoglycan 365				
11.7	Defibrotide 366				
11.8	Pentosan Polysulfate 367				
11.9	Fondaparinux and Related Synthetic Oligosaccharides 367				
11.10	Chemoenzymatic Synthesis of Oligosaccharides 369				
11.11	Conclusions and Perspectives 369				
	Acknowledgment 369				
	References 370				

11.5 Low Molecular Weight Heparins 361

Index 381