

Contents

Preface xv

Introduction: Successes and Challenges in Antiviral

Drug Development 1

Zhengqiang Wang and Michael J. Sofia

Introduction 1

Antiviral Drugs Targeting Human Herpesviruses 2

Antiviral Drugs Targeting Human Immunodeficiency Virus 4

Drugs Targeting Reverse Transcriptase 4

Drugs Targeting Protease 6

Drugs Targeting Integrase Strand Transfer 7

Drugs Targeting Viral Entry 7

Drugs Targeting Viral Capsid 8

Antiviral Drugs Targeting Hepatitis B Virus 9

Antiviral Drugs Targeting Hepatitis Delta Virus 10

Antiviral Drugs Targeting Hepatitis C Virus 11

Antiviral Drugs Targeting Acute RNA Respiratory Viruses 11

Drugs Targeting Human Influenza Viruses 11

Drugs Targeting SARS-CoV-2 14

New Trends and Challenges in Antiviral Drug Development 15

References 16

Part I Human Herpes Viruses 31

1 Letemovir for the Prevention of CMV Infection in Transplant Recipients 33

Peter Lischka and Holger Zimmermann

1.1 Background 33

1.1.1 Cytomegalovirus 33

1.1.2 Immunocompromised Patients 34

1.1.3 Patient Groups with a High Risk for CMV Complications 34

1.1.4 Available CMV Treatments Before Letemovir 35

1.1.4.1 Antiviral Drugs 35

1.1.4.2	Dominant Treatment Strategies	37
1.1.4.3	Unmet Medical Need	38
1.2	Discovery Phase	38
1.3	Preclinical Characterization	39
1.3.1	Antiviral Potency/Selectivity/Inhibitory Profile	39
1.3.1.1	<i>In Vitro</i> Potency Versus Laboratory CMV Strains	39
1.3.1.2	<i>In Vitro</i> Potency Versus Clinical CMV Isolates and Resistance-breaking Profile	40
1.3.1.3	<i>In Vivo</i> Efficacy (Xenotransplant Model)	41
1.3.1.4	<i>In Vitro</i> Antiviral Specificity	42
1.3.1.5	Other Characteristics of Letemovir's Inhibitory Profile	43
1.3.1.6	Summary	43
1.4	Mechanism of Action Studies	43
1.4.1	Target Identification	43
1.5	Terminase Inhibitors	46
1.5.1	Previous and Contemporary Drug Candidates Targeting the Terminase Complex	46
1.5.2	Letemovir: Same Target, Different Interaction	49
1.5.3	Advantages of Terminase Inhibitors	49
1.6	Preclinical Safety Evaluation	50
1.7	Clinical Development and MAA/NDA Submission	51
1.7.1	Regulatory Support for Clinical Development	51
1.7.2	Phase 1	51
1.7.2.1	Drug–Drug Interaction Studies	51
1.7.2.2	Special Populations	51
1.7.2.3	IV Formulation	52
1.7.3	Clinical Proof-of-concept	52
1.7.3.1	Phase 2a Clinical Trial (AIC001-2-001)	52
1.7.3.2	Emergency IND Treatment of a Lung Transplant Patient with Multiresistant CMV Disease	53
1.7.3.3	Credentials Established	54
1.7.4	Letemovir for CMV Prophylaxis in HSCT Patients	55
1.7.4.1	Phase 2b: First Prophylaxis Trial in HSCT Patients	55
1.7.4.2	Phase 3 CMV Prophylaxis Trial in HSCT Patients	55
1.7.4.3	Marketing Approval in HSCT Recipients	57
1.7.4.4	Further Clinical Development and Real-world Data	57
1.7.4.4.1	Phase 3 Trial for Extension of the Prophylaxis Period	59
1.7.4.4.2	Follow-up Trials in Specific Populations	59
1.7.5	Letemovir for CMV Prophylaxis in KT Patients	60
1.7.5.1	Phase 3 Noninferiority Trial in KT Recipients	61
1.7.5.2	Marketing Approval in KT Recipients	61
1.7.5.3	Further Clinical Development and Real-world Data	62
1.8	Drug Resistance	62
1.8.1	Genetic Characterization of Letemovir Resistance	62
1.9	Letemovir Resistance in Clinical Trials	64
1.10	Real-world Resistance	65

1.11	Outlook for Letermovir	65
	Acknowledgments	66
	References	66
2	Discovery and Development of the Helicase–Primase Inhibitor Pritelivir for the Treatment of Immunocompromised Patients with Resistant HSV Infection	75
	<i>Alexander Birkmann</i>	
2.1	HSV Virology and Disease	75
2.2	Treatment	76
2.3	Resistant Infections	79
2.4	Pritelivir Discovery and Target Identification	80
2.5	Nonclinical Data	82
2.5.1	<i>In Vitro</i> Studies	82
2.5.2	<i>In Vivo</i> Studies	83
2.5.2.1	Guinea Pig Model for Genital HSV-2 Infection	83
2.5.2.2	Mouse Model for HSE	84
2.5.2.3	Pritelivir in Immunocompromised Mouse Model	85
2.6	Clinical Data	85
2.6.1	Phase 1 Program	85
2.6.2	Genital HSV	86
2.6.3	Resistant Infections	88
2.7	Pritelivir Resistance	92
2.8	Conclusion and Outlook	92
	References	93
	Part II Immunodeficiency Virus	101
3	The Discovery and Early Development of the HIV-1 Integrase Strand Transfer Inhibitor Dolutegravir (S/GSK1349572)	103
	<i>Sherene Min, Soong Hoon Kim, Mark Underwood, Brian Wynne, William Spreen</i>	
3.1	Introduction	103
3.2	Medicinal Chemistry	105
3.2.1	Discovery of S/GSK1349572 (DTG)	105
3.2.1.1	Evolution of the Chemical Structure Leading to DTG	105
3.3	Virology	111
3.3.1	<i>In Vitro</i> Studies Indicated Robust Efficacy and High Barrier to Resistance of DTG	111
3.4	Clinical Development	115
3.4.1	PK Studies Supported Once-daily 50 mg Dosing of DTG	115
3.4.2	Clinical Studies in People Living with HIV-1	116
3.4.3	Clinical Studies in Individuals with Prior Treatment Failure	119
	Acknowledgments	120
	References	120

4	The Discovery and Development of Islatravir (4'-Ethynyl-2-fluoro-2'-deoxyadenosine [EFdA], MK-8591)	127
	<i>Alexa A. Snyder, Xin Wen, Eleftherios Michailidis, Karen A. Kirby, Hiroaki Mitsuya, Stefan G. Sarafianos</i>	
4.1	Introduction	127
4.2	HIV Replication Cycle	128
4.3	Structure and Function of HIV RT	128
4.4	DNA Synthesis by RT	130
4.5	RT Inhibitor Classes	132
4.6	EFdA Development	133
4.6.1	4'-Ethynyl Ribose Modifications	134
4.6.2	2-Fluoro Adenosine Modifications	135
4.6.3	3'-Hydroxy Ribose Modification	136
4.7	EFdA: A Compound with 4'-Ethynyl, 2-Fluoro, and 3'-Hydroxy Modifications	137
4.7.1	Effects of the 4'-Ethynyl Modification on EFdA	137
4.7.2	Effects of the 2'-Fluoro Modification on EFdA	140
4.7.3	Effects of the 3'-Hydroxy Modification on EFdA	141
4.7.3.1	Mechanisms of Inhibition: EFdA	141
4.7.3.1.1	Biochemical Mechanisms of Inhibition	141
4.7.3.1.2	Structural Mechanisms of Inhibition	142
4.8	Mechanism of Resistance/Hypersusceptibility to EFdA	144
4.8.1	Mechanisms of Viral Resistance to EFdA	144
4.8.2	Mechanisms of Viral Hypersusceptibility to EFdA	146
4.8.3	Combination Therapies with EFdA	146
4.8.4	Synthesis Advances	148
4.9	Animal Studies	149
4.9.1	EFdA Pharmacokinetic Studies in Rodents	149
4.9.2	EFdA Antiviral Activity in Humanized Mice	150
4.9.3	EFdA Antiviral Activity in Rhesus Macaques	151
4.9.4	EFdA Long-acting Activity in Various Animals	152
4.10	Clinical Trials	160
4.11	Long-acting Methods of Delivery for EFdA Treatment	160
4.12	NRTTIs as a Drug Class	166
	References	166
	Part III Hepatitis Viruses	185
5	Discovery of the RNA Interference Therapeutic Indusiran, a GalNAc-conjugated siRNA	187
	<i>Emily Thi</i>	
5.1	Introduction	187
5.2	Comparison of Preclinical Anti-HBV Efficacy Between Lipid Nanoparticle-encapsulated and GalNAc-conjugated siRNA	188
5.3	Comparison of Tetravalent Versus Trivalent GalNAc Ligands	190

5.4	siRNA Selection: Comparison of siRNA Activity Against HBsAg and HBx Targets	191
5.5	Imdusiran Design and Preclinical Characterization	192
5.5.1	Abrogation of siRNA Immunostimulatory Potential	192
5.5.2	Imdusiran siRNA Target Site Sequence	193
5.6	Imdusiran Antiviral Activity	197
5.6.1	Imdusiran Antiviral Activity in Primary Mouse and Human Hepatocyte HBV Models	197
5.6.2	Imdusiran <i>In Vivo</i> Antiviral Activity	199
5.7	Imdusiran Combination Treatment	200
5.7.1	Imdusiran Combination with Standard of Care and Investigational Agents	200
5.8	Perspectives	203
	References	206
6	Discovery and Development of ARO-HBV/JNJ-3989	211
	<i>Christine I. Wooddell, Oliver Lenz, Thomas Schlupe, Man-Fung Yuen, Michael Biermer</i>	
6.1	Introduction	211
6.1.1	Chronic Hepatitis B Virus Infection	211
6.1.1.1	HBV Structure and Associated Molecular and Cellular Biology	212
6.1.1.1.1	Clinical Course of HBV Infection	215
6.2	Development of RNAi Therapeutic ARO-HBV/JNJ-73763989 (JNJ-3989)	216
6.2.1	RNAi as a Therapeutic Modality	216
6.2.2	Use of siRNA to Treat HBV Infection	216
6.2.2.1	JNJ-3989 siRNAs Have Broad Cross-reactivity to HBV Genotypes and HBV Transcripts	217
6.2.2.1.1	Reduction of Serum HBsAg, HBeAg, and HBV DNA in Mouse Model of Chronic HBV Infection	218
6.3	Pharmacokinetics and Safety of JNJ-3989 in Humans	218
6.4	Clinical Studies in Chronically HBV-infected Patients	220
6.4.1	Direct Antiviral Treatment Approach with JNJ-3989-based Regimens	220
6.4.1.1	Pharmacological Response to JNJ-3989 with Short-term Treatment	220
6.4.1.2	REEF-1 Clinical Study	225
6.4.1.3	REEF-2 Clinical Study	232
6.4.2	Combination Approaches of JNJ-3989 with Immune Modulation	233
6.4.2.1	PENGUIN Clinical Study	234
6.4.2.2	REEF-IT Clinical Study	234
6.4.3	HBsAg Targeting siRNA JNJ-3989 in Chronic Hepatitis D	236
6.4.3.1	REEF-D Clinical Study	236
6.5	Discussion and Perspectives	238
	References	239

7	The Discovery and Development of Sofosbuvir as the Backbone of HCV Curative Therapies 247 <i>Michael J. Sofia</i>
7.1	Introduction 247
7.2	Identification of the 2'- α -F, 2'- β -C-Methyl Cytidine Nucleoside PSI-6130 248
7.3	Identification of the 3',5'-Diisobutyrate Ester Prodrug RG7128 and Clinical Proof-of-concept 250
7.4	Development of the 5'-Phosphoramidate Uridine Nucleotide Prodrug for Liver Targeting and Clinical Proof-of-concept 251
7.5	Sofosbuvir: The Backbone of HCV Curative Therapies 255
7.6	Conclusion 261 References 261
8	The Discovery and Development of Harvoni® and Epclusa®: Ending the Interferon Era; Curing All Hepatitis C Genotypes 267 <i>John O. Link</i>
8.1	Cure 267
8.2	Introduction 267
8.3	The Discovery of Ledipasvir [13–15] 270
8.4	Early Development of Ledipasvir 278
8.5	Ledipasvir/Sofosbuvir STR Clinical Trial Results and Real-world Effectiveness 280
8.6	Initiation of a Pangenotypic NS5A Inhibitor Program 283
8.7	Discovery of the Pangenotypic NS5A Inhibitor Velpatasvir [40–42] 284
8.8	The Development of Velpatasvir 293
8.9	Clinical Trial Efficacy and Real-world Effectiveness with the Pangenotypic Sofosbuvir/Velpatasvir STR 297
8.10	Conclusion 300 Acknowledgment 300 Compliance with Ethical Standards 301 References 301
	 Part IV Respiratory Viruses 307
9	Presatovir: A Once-daily Oral Respiratory Syncytial Virus Fusion Inhibitor for the Treatment of Respiratory Syncytial Virus Infection in Adults and Infants 309 <i>Richard L. Mackman, Jason Chien, Dustin S. Siegel</i>
9.1	Introduction to Respiratory Syncytial Virus 309
9.2	Discovery of Presatovir 311
9.2.1	Lead Identification 311

9.2.2	Lead Optimization	313
9.3	Pharmacological Profiling	317
9.3.1	Mechanism of Action Studies	317
9.3.2	RSV Animal Models	319
9.3.3	Spectrum of Activity	320
9.4	Pharmacokinetics	320
9.4.1	Preclinical Intravenous and Oral Pharmacokinetics	320
9.4.2	Lung Distribution Properties	322
9.4.3	Human Dose Prediction	323
9.5	Clinical Development	323
9.5.1	Regulatory Considerations	323
9.5.2	Phase 1 Program	324
9.5.3	Phase 2 Program	325
9.5.4	Treatment Emergent Mutations	327
9.6	Conclusion	328
	References	328
10	Remdesivir for the Treatment of COVID-19 and Other Viral Infections with Pandemic Potential	333
	<i>Tomas Cihlar, Meghan S. Vermillion, Joe Llewellyn, Charlotte Hedskog, Richard L. Mackman</i>	
10.1	Introduction	333
10.2	Discovery of Remdesivir	335
10.2.1	Discovery of 1'-Modified C-Nucleosides	335
10.2.2	Phosphoramidate Prodrug Optimization for RSV	336
10.2.3	Synthesis	338
10.3	Pharmacokinetics	339
10.3.1	<i>In Vitro</i> Metabolism	339
10.3.2	<i>In Vivo</i> Pharmacokinetics	341
10.3.3	<i>In Vivo</i> Lung Metabolism	342
10.3.4	Oral Delivery and the Discovery of Obeldesivir	343
10.4	Antiviral Pharmacology	343
10.4.1	Mechanism of Action	343
10.4.2	Spectrum of Antiviral Activity	344
10.4.3	Preclinical Antiviral Efficacy in Animal Models	346
10.5	Clinical Studies	348
10.5.1	Phase 1	348
10.5.2	Ebola Clinical Development	349
10.5.3	COVID-19 Clinical Development	349
10.5.4	Obeldesivir Clinical Development	351
10.6	Antiviral Resistance	351
10.7	Summary	353
	Acknowledgments	355
	References	355

11	Discovery and Clinical Development of Ensitrelvir: An Oral SARS-CoV-2 3CL Protease Inhibitor for the Treatment of COVID-19 365
	<i>Yuki Tachibana, Ryosuke Shimizu, Haruaki Nobori, Yuko Tsuge, Masaharu Shinkai</i>
11.1	Introduction 365
11.2	Discovery of Ensitrelvir 369
11.3	Antiviral Activity of Ensitrelvir 374
11.3.1	<i>In Vivo</i> Antiviral Efficacy of Ensitrelvir 376
11.3.1.1	Delayed-treatment Model 376
11.3.1.2	Pharmacokinetics and Pharmacodynamics 378
11.3.2	Pharmacokinetics 380
11.3.2.1	Pharmacokinetic Profiles 380
11.3.2.2	Effect of Food 381
11.3.2.3	Drug–Drug Interactions 382
11.3.3	Clinical Trial of Ensitrelvir 383
11.4	Outlook 384
11.5	Conclusions 384
	References 385
12	Discovery and Development of VV116: A Novel Oral Nucleoside Anti-SARS-CoV-2 Drug 389
	<i>Huilong Wang, Yuanchao Xie, Tianwen Hu, Jingshan Shen</i>
12.1	Introduction 389
12.2	Discovery 390
12.3	Synthesis 394
12.4	Preclinical Study 396
12.4.1	Virology 396
12.4.2	Mechanism of Antiviral Activity of VV116 398
12.4.3	Preclinical Pharmacokinetics 398
12.4.4	Toxicology 400
12.5	Clinical Study 400
12.5.1	Pharmacokinetics 400
12.5.2	Safety and Tolerability 402
12.5.3	Phase III Studies 402
12.6	Future Direction 403
12.7	Conclusions 404
	References 405
13	Molnupiravir 411
	<i>Richard K. Plemper</i>
13.1	Introduction 411
13.2	Mechanism of Antiviral Activity 412
13.3	Potential for NHC-TP Incorporation into the Host Genome 413
13.4	Risk of Molnupiravir-enhanced Viral Evolution 415
13.5	Therapeutic Benefit of Molnupiravir 416
13.6	Conclusions 418

Acknowledgments	419
Conflict of Interest	419
References	419

Part V Tropical Disease Viruses 425

14	Development of a Novel Class of Highly Potent Dengue Virus Inhibitors: From Hit to the Clinical Candidate Mosnodenvir	427
	<i>Suzanne J. F. Kaptein, Bart Kesteley, Olivia Goethals, Oliver Ackaert, Dorothée Bardiot, Dominik Kiemel, Guillermo Herrera-Taracena, Ralf Bartenschlager, Marnix Van Loock, Tim H. M. Jonckers, Arnaud Marchand, Patrick Chaltin, Johan Neyts</i>	
14.1	Introduction	427
14.2	Discovery and Optimization of New Dengue Drugs for Prophylaxis	429
14.2.1	Identification of the Hit CIM020928	429
14.2.2	Early SAR Exploration	431
14.2.3	Early Evaluation of <i>In Vitro</i> ADME-Tox and <i>In Vivo</i> Pharmacokinetic Properties	433
14.2.4	Improving Oral Bioavailability	433
14.2.5	Identification of Preclinical Candidates	434
14.2.6	Identification of the Clinical Candidate	435
14.3	Biological Characterization	436
14.4	Efficacy of Mosnodenvir in Preclinical DENV Infection Models	438
14.5	Safety Profile and Pharmacokinetics of Mosnodenvir in Humans	440
14.6	Conclusions	441
	Acknowledgments	441
	References	441

Part VI Orthopoxvirus 445

15	Discovery and Development of Brincidofovir	447
	<i>E. Randall Lanier, Scott Foster, Graciela Andrei</i>	
15.1	Discovery	447
15.1.1	Key Points	448
15.2	Summary of Current and Potential Indications for BCV	449
15.3	Antiviral Activity	450
15.3.1	Antiviral Activity Against Double-stranded DNA Viruses	450
15.3.1.1	Antiviral MOA	451
15.3.1.1.1	Viruses with Viral DNA Polymerases	451
15.3.1.1.2	Viruses Without Viral DNA Polymerases	452
15.3.2	Antiviral Activity Against RNA Viruses	453
15.3.2.1	Ebola	453
15.3.2.2	SARS-CoV-2	454

15.4	Key Clinical Pharmacology and Safety Considerations	454
15.4.1	Metabolism and Animal Models	456
15.5	Clinical Development	457
15.5.1	Poxviruses	457
15.5.1.1	Introduction to BCV Animal Models	459
15.5.1.2	Rabbitpox Model	460
15.5.1.3	Mousepox Model	461
15.5.1.4	Scaling to Humans	461
15.5.1.5	Vaccine Interaction Studies	462
15.5.1.6	Resistance	462
15.5.1.7	Other Poxviruses (VACV, Mpox)	462
15.5.1.7.1	Vaccinia Virus	462
15.5.1.7.2	Monkeypox Virus	463
15.5.2	Adenovirus	463
15.5.2.1	Clinical Experience with Oral BCV for AdV	464
15.5.2.2	Adenovirus Type and Response to BCV	466
15.5.2.3	AdV Resistance to BCV	467
15.5.2.4	ATHENA (IV BCV for AdV)	468
15.5.3	Cytomegalovirus	469
15.5.3.1	Clinical Experience with Oral BCV for CMV	469
15.5.3.2	CMV Resistance to BCV	471
15.5.4	Polyomaviruses	474
15.5.4.1	BKV Clinical Data with CDV/BCV	474
15.5.4.2	JCV Clinical Data with CDV/BCV	475
15.6	Neurodegenerative Diseases	476
15.6.1	EBV and Multiple Sclerosis	476
15.6.2	HSV and Alzheimer's Disease	477
15.6.3	HHV-6	478
15.7	Antiproliferative Activity	478
15.7.1	Papillomavirus-associated Tumors	479
15.7.1.1	<i>In Vitro</i> Activity Against Papillomavirus-associated Tumors and MOA	479
15.7.1.2	Animal Studies	480
15.7.1.3	Clinical Studies	480
15.7.2	EBV-associated Tumors	481
15.7.3	Glioblastoma	482
15.7.4	Antiproliferative MOA	483
15.8	Summary and Future Directions	483
	Acknowledgments	485
	References	485
	Index	501