

Contents

Chapter 1	Introduction to Antibody–Drug Conjugates	1
	<i>John M. Lambert and F. L. van Delft</i>	
1.1	ADCs – A Historical Perspective	1
1.2	Structure and Mode of Action of ADCs	7
1.3	Marketed and Clinical ADCs	9
1.4	Targets and Antibodies	16
1.5	Payloads	24
1.6	Conjugation Chemistries	26
1.7	Linkers in ADCs – The Crucial “Part in the Middle”	27
	References	28
Chapter 2	Antibody Conjugation Technologies	32
	<i>G. T. Hermanson and F. L. van Delft</i>	
2.1	Introduction	32
2.2	The Art of mAb-to-ADC Conversion	34
2.3	Conjugation Technologies in Marketed and Clinical-stage ADCs	37
2.3.1	Mercapto Group (–SH)	37
2.3.2	Amino Group (–NH ₂)	48
2.3.3	Carbonyl Group (–C(O)R)	54
2.3.4	Amido Group (–C(O)NHR)	55
2.3.5	Azido Group (–N ₃)	59
2.4	Miscellaneous Functional Groups for Antibody Conjugation	60
2.4.1	Phenol (Tyrosine Sidechain)	60

Drug Discovery Series No. 81

Chemical Linkers in Antibody–Drug Conjugates (ADCs)

Edited by Floris L. van Delft and John M. Lambert

© The Royal Society of Chemistry 2022

Published by the Royal Society of Chemistry, www.rsc.org

2.4.2	Methyl Thioether (Methionine Sidechain)	62
2.4.3	Imidazole (Histidine Sidechain)	64
2.4.4	N-terminal Aminogroup	64
2.5	Discussion	65
	References	65
Chapter 3	Linker Design and Impact on ADC Properties	71
	<i>M. Frigerio and N. Camper</i>	
3.1	Key Mechanistic Requirements of an ADC	71
3.2	ADC Developability	74
3.3	Functionality – Decreasing the Minimum Effective Dose	78
3.3.1	ADC Processing and Drug Release Mechanism	78
3.3.2	Linker Types and Drug Release Mechanisms	78
3.3.3	Linker Design and ADC Efficacy	96
3.4	Safety – Increasing the Maximum Tolerated Dose	102
3.4.1	Stability Evaluation and Contribution of ADC Linkers	105
3.4.2	Effect of Linker Type on the Catabolism of ADC	106
3.4.3	PK Considerations and Hydrophobicity	109
3.5	Manufacturability – Accelerating Investigational New Drug Filing	114
3.6	Summary – How Optimal Linker Designs Drive Successful ADC Programs	118
3.6.1	Future ADC Linker Designs and Further Impact on ADC Properties	119
	Abbreviations	120
	Acknowledgements	121
	References	121
Chapter 4	Non-cleavable Linkers: Permanently Linked, for Better or for Worse	136
	<i>Julien Dugal-Tessier and Nareshkumar Jain</i>	
4.1	Introduction	136
4.1.1	History of Non-cleavable Linkers	137
4.2	Design of Non-cleavable Linkers	144
4.2.1	Metabolism and Trafficking	145
4.2.2	Modulation of Activity	147

4.3	Clinical data for ADCs Made with Non-cleavable Linkers	149
4.3.1	Maytansinoid-based ADCs	149
4.3.2	Auristatin-based ADCs	155
4.3.3	Other Molecules	160
4.4	Pre-clinical Data for Novel Non-cleavable Drug-linkers: Beyond First-generation Maytansinoid and Auristatin Non-cleavable Drug-linkers	160
4.5	Concluding Thoughts	165
	References	166
Chapter 5	Protease-sensitive Linkers	173
	<i>S. Johannes, A. Sommer and H.-G. Lerchen</i>	
5.1	Introduction and Scope of the Chapter	173
5.2	Cathepsins – Lysosomal Enzymes Suitable for ADC Activation	174
5.3	Cathepsin B-cleavable Linker	176
5.3.1	Evolution of Cathepsin B-cleavable Val-Cit Linker	176
5.3.2	Case Studies of Approved ADCs with Val-Cit Cleavable Linkers and ADCs Undergoing Pivotal Clinical Trials	178
5.3.3	Premature Payload Release of Val-Cit-PABC-MMAE ADCs	179
5.3.4	The Hidden Cleavage Force Behind Cathepsin B	184
5.3.5	ADC Design for Specific Cleavage by Cathepsin B	185
5.3.6	Less Hydrophobic Val-Ala- and Ala-Ala-linker in PBD Dimer and Indolinobenzodiazepine Pseudodimer ADCs	186
5.4	Legumain-cleavable ADCs	191
5.4.1	Legumain, an Interesting Lysosomal Cysteine Protease	191
5.4.2	Legumain-cleavable ADCs with KSP Inhibitor Payloads	191
5.5	Protease-cleavable Linkers with Other Substrate Sequences	192
5.5.1	The Tetrapeptide Linker Gly-Gly-Phe-Gly	194
5.5.2	The Triglycine Linker	195
5.5.3	Epimeric Ala-Ala Linker in Maytansinoids	196

5.5.4	Ala-Ala-Ala Linker	198
5.5.5	Val-Lys-Gly Linker	200
5.6	Extracellular Activation of ADCs	201
5.6.1	Extracellularly Cathepsin-cleavable ADCs	201
5.6.2	Matrix Metalloproteinases	202
5.7	Summary and Conclusion	204
	References	205
Chapter 6	Acid-labile Linkers	213
	<i>E. A. Savoy, F. P. Olatunji, H. Yoon, N. Mesbahi, J. R. Knight and C. E. Berkman</i>	
6.1	Why Acid-labile Linkers?	213
6.2	From Extracellular to Intracellular Space	213
6.3	Desirable Characteristics of Acid-labile Linkers for ADCs	214
6.4	Clinically Relevant Acid-labile Linkers	214
6.5	Next-generation Acid-labile Linkers	220
6.5.1	Dialkyl Silyl Ether-based Linkers	220
6.5.2	Spiro Diorthoester Linkers (SpiDo)	223
6.5.3	Iminoboronate Linkers	223
6.5.4	<i>Cis</i> -aconitic Acid and Maleic Acid-based Linkers	224
6.5.5	Phosphoramidate-based Linkers	226
6.6	Conclusions and Future Prospects for Acid-cleavable Linkers	227
	References	228
Chapter 7	ADC Linkers Strategies for the Release of Alcohol-containing Payloads	232
	<i>Jared T. Miller and L. Nathan Tumey</i>	
7.1	Introduction	232
7.2	Carbonates and Esters	234
7.3	Carbamates	238
7.4	Hemiaminals	242
7.5	Phosphates	245
7.6	Aryl Sulfonates	248
7.7	Silyl Ethers	251
7.8	Release by Exogenous Triggers	252
7.9	Summary and Conclusions	254
	References	254

Chapter 8	Click-cleavable ADC Linkers	263
	<i>R. Rossin and M. S. Robillard</i>	
8.1	Introduction	263
8.2	The Inverse Electron-demand Diels–Alder Reaction	265
8.3	The IEDDA Pyridazine Elimination Reaction	266
8.4	Other Click-to-release Reactions	270
8.5	Click-cleavable ADCs	274
8.6	Conclusion	280
	References	281
Chapter 9	The Use of Uniform PEG Compounds in the Design of ADCs	286
	<i>M. W. Giese, R. H. Woodman, G. T. Hermanson and P. D. Davis</i>	
9.1	Introduction	286
9.2	Bioconjugation with Polyethylene Glycol Spacers	294
9.2.1	Introduction to Polyethylene Glycol	294
9.2.2	Uses of PEG in Bioconjugation	297
9.2.3	Potential Issues with PEG	299
9.3	The Use of PEG Spacers in ADCs	300
9.3.1	Physicochemical Properties	300
9.3.2	Pharmacodynamic Properties	328
9.3.3	Pharmacokinetic Properties and <i>In Vivo</i> Pharmacology	340
9.4	Conclusion	365
	Abbreviations	365
	References	366
Chapter 10	Enhancing the Polarity of the Linker-drug in ADCs	377
	<i>Jorin Hoogenboom and Sander S. van Berkel</i>	
10.1	Introduction	377
10.2	Polar Payloads	383
10.3	Polar Linkers	385
10.3.1	Polyethylene Glycol Linkers	387
10.3.2	Peptide-based Linkers	390
10.3.3	Saccharide-based Linkers	392
10.3.4	Sulfur/Phosphorus-based Linkers	393
10.3.5	Amine-based Linkers	398

10.4	Polar Capping	401
10.4.1	Polar Capping of Linkers	401
10.4.2	Polar Capping of Payloads	409
10.5	Emerging Developments	410
	References	414
Chapter 11	Trastuzumab Deruxtecan Targeting HER2-expressing Cancers with a DXd-ADC System Consisting of a Novel Protease-sensitive Linker and DNA Topoisomerase I Inhibitor with a Hydroxyl Group	422
	<i>Takashi Nakada, Yuki Abe and Toshinori Agatsuma</i>	
11.1	Introduction	422
11.2	Linker Chemistry	423
11.2.1	Strategy for Creation of DXd Linker-payload System	425
11.2.2	Discovery and Optimization of Linker	427
11.2.3	Key Attributes of the Linker-payload System	429
11.3	Nonclinical Studies	431
11.3.1	Antitumor Efficacy in Nonclinical Studies	432
11.3.2	Contribution of Bystander Antitumor Effect	433
11.3.3	Nonclinical Safety Assessments	435
11.3.4	Nonclinical Pharmacokinetic Evaluations	436
11.3.5	Trastuzumab Deruxtecan-enhanced Antitumor Immunity	437
11.4	Clinical Studies	440
11.4.1	Dose-escalation Portion of the Phase I Study	440
11.4.2	Pivotal Clinical Study for HER2-positive Breast Cancer	442
11.4.3	Pivotal Clinical Study for HER2-positive Gastric Cancer	443
11.5	Summary and Further Progress	446
	Abbreviations	447
	References	447
	Subject Index	451