

Drug Development for **Gene Therapy**

Translational Biomarkers, Bioanalysis,
and Companion Diagnostics

Edited by

Yanmei Lu

Boris Gorovits

WILEY

**Drug Development
for Gene Therapy**

Drug Development for Gene Therapy

Translational Biomarkers, Bioanalysis,
and Companion Diagnostics

Edited by

Yanmei Lu

*Sangamo Therapeutics
Richmond, California
USA*

Boris Gorovits

*Gorovits BioSolutions, LLC
Andover, Massachusetts
USA*

WILEY

Copyright © 2024 by John Wiley & Sons, Inc. All rights reserved.

Published by John Wiley & Sons, Inc., Hoboken, New Jersey.

Published simultaneously in Canada.

No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, scanning, or otherwise, except as permitted under Section 107 or 108 of the 1976 United States Copyright Act, without either the prior written permission of the Publisher, or authorization through payment of the appropriate per-copy fee to the Copyright Clearance Center, Inc., 222 Rosewood Drive, Danvers, MA 01923, (978) 750-8400, fax (978) 750-4470, or on the web at www.copyright.com. Requests to the Publisher for permission should be addressed to the Permissions Department, John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030, (201) 748-6011, fax (201) 748-6008, or online at <http://www.wiley.com/go/permission>.

Trademarks: Wiley and the Wiley logo are trademarks or registered trademarks of John Wiley & Sons, Inc. and/or its affiliates in the United States and other countries and may not be used without written permission. All other trademarks are the property of their respective owners. John Wiley & Sons, Inc. is not associated with any product or vendor mentioned in this book.

Limit of Liability/Disclaimer of Warranty: While the publisher and author have used their best efforts in preparing this book, they make no representations or warranties with respect to the accuracy or completeness of the contents of this book and specifically disclaim any implied warranties of merchantability or fitness for a particular purpose. No warranty may be created or extended by sales representatives or written sales materials. The advice and strategies contained herein may not be suitable for your situation. You should consult with a professional where appropriate. Further, readers should be aware that websites listed in this work may have changed or disappeared between when this work was written and when it is read. Neither the publisher nor authors shall be liable for any loss of profit or any other commercial damages, including but not limited to special, incidental, consequential, or other damages.

For general information on our other products and services or for technical support, please contact our Customer Care Department within the United States at (800) 762-2974, outside the United States at (317) 572-3993 or fax (317) 572-4002.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic formats. For more information about Wiley products, visit our web site at www.wiley.com.

Library of Congress Cataloging-in-Publication Data

Names: Lu, Yanmei, 1966- editor. | Gorovits, Boris, editor.

Title: Drug development for gene therapy : translational biomarkers, bioanalysis, and companion diagnostics / edited by Yanmei Lu, Boris Gorovits.

Description: Hoboken, New Jersey : John Wiley & Sons, Inc., [2024] | Includes bibliographical references and index.

Identifiers: LCCN 2023049872 (print) | LCCN 2023049873 (ebook) | ISBN 9781119852780 (cloth) | ISBN 9781119852797 (adobe pdf) | ISBN 9781119852803 (epub)

Subjects: MESH: Genetic Therapy--methods | Biomarkers, Pharmacological--analysis | Drug Development--methods

Classification: LCC RB155 (print) | LCC RB155 (ebook) | NLM QU 560 | DDC 616/.042--dc23/eng/20231214

LC record available at <https://lcn.loc.gov/2023049872>

LC ebook record available at <https://lcn.loc.gov/2023049873>

Cover Design: Wiley

Cover Image: © Jonathan Knowles/Getty Images

Contents

List of Contributors *xix*

Preface *xxiii*

Section I Introduction *1*

1	Introduction to AAV-based <i>in vivo</i> Gene Therapy	3
	<i>Oscar Segurado</i>	
1.1	Introduction	3
1.1.1	History of Gene Therapy	3
1.1.2	AAV-based <i>in vivo</i> Gene Therapy: A Revolution in Medicine	4
1.1.3	The AAV Vector Structure	11
1.1.4	Cell Entry and Transduction Pathway	12
1.2	Advantages and Disadvantages for AAV <i>in vivo</i>	13
1.2.1	Effectiveness and Advantages of AAV Vectors for <i>in vivo</i> Gene Therapy	13
1.2.2	Challenges of AAV Vectors for <i>in vivo</i> Gene Therapy	14
1.3	Technology Platforms of AAV-based <i>in vivo</i> Gene Therapy	14
1.3.1	cDNA Replacement	15
1.3.2	Genome Editing	15
1.3.2.1	ZFN	16
1.3.2.2	TALENs	16
1.3.2.3	CRISPR/Cas9	16
1.3.3	Base Editing and Prime Editing	17
1.3.4	RNAi Gene Silencing	17
1.3.5	Gene Addition	18
1.4	AAV Serotypes and Tissue Affinity	18
1.4.1	The Liver as a Biofactory	19
1.4.2	The CNS as a Biofactory	19

1.4.3	The Muscle as a Biofactory	19
1.5	Precision Medicine: Screening and Monitoring Biomarkers, Companion Diagnostics	19
1.5.1	Gene Therapy Clinical Trials: Spotlight on Hemophilia A	20
1.6	Predictions for Scientific and Medical Progress	22
1.6.1	Predictions for Challenges in the Field	22
1.6.2	Addressing Durability	23
1.6.3	Addressing Immunogenicity	24
1.6.4	Addressing Malignancy	24
1.7	Predictions for Market Adoption	24
1.7.1	Patients and Patient Advocacy Groups	25
1.7.2	Physicians, Clinical Guidelines, Regulatory Agencies	25
1.7.3	Payers	26
1.8	Final Thoughts	26
1.8.1	Can We Afford <i>in vivo</i> Gene Therapies?	26
1.8.2	Can <i>in vivo</i> Gene Editing Replace Gene Therapy?	27
	References	28

2 Recent Development in *in vivo* Clinical Gene Therapy Platforms 35

John Murphy and Jane Owens

2.1	Introduction	35
2.1.1	rAAV-cDNA Replacement Therapies	35
2.1.1.1	Introduction: Approved rAAV-cDNA Replacement Therapies	36
2.1.1.2	Glybera (alipogene tiparvovec), Marketed by uniQure	36
2.1.1.3	Luxturna (voretigene neparvovec-rzyl), Marketed by Spark Therapeutics	38
2.1.1.4	Zolgensma (onasemnogene abeparvovec), Marketed by Novartis	40
2.1.2	Introduction: rAAV-cDNA (gene) Therapy Candidates in Clinical Development	46
2.1.2.1	AAV-Gene Replacement Clinical Trials for the Eye	47
2.1.2.2	Clinical Trials for Heart Disease	47
2.1.2.3	Clinical Trials for Hematologic and Metabolic Disease (Targeting the Liver)	48
2.1.2.4	Clinical Trials for Skeletal Muscle	48
2.1.3	Introduction: rAAV-as a Vehicle for <i>in vivo</i> Gene Editing	48
2.1.3.1	Non-nuclease Mediated Methods	48
2.1.3.2	Nuclease-mediated Homology Directed Repair	52
2.1.4	Nuclease-mediated Gene Disruption following AAV Delivery	54
2.1.5	Challenges and Opportunities with AAV as a Delivery Vehicle for Nuclease-Mediated Gene Editing	56
	References	56

Section II Translational Biomarkers for Gene Therapy 61

3 Biomarker and Bioanalytical Readouts for the Development of AAV Gene Therapy 63

Yanmei Lu and Wibke Lembke

- 3.1 Introduction 63
 - 3.1.1 AAV-Mediated *in vivo* Gene Therapy 63
 - 3.1.2 Biomarker Category and Utility 65
- 3.2 Pharmacokinetic (PK) and Pharmacodynamic (PD) Biomarkers 66
 - 3.2.1 Viral Biodistribution and Shedding 66
 - 3.2.2 Transgene mRNA Expression 68
 - 3.2.3 Transgene and Target Protein Activity and Concentration 68
 - 3.2.4 Substrate and Other Distal PD Biomarkers 70
- 3.3 Safety and Monitoring Biomarkers and Readouts 71
 - 3.3.1 Assessment of genotoxicity 72
 - 3.3.1.1 AAV Integration/Insertional Mutagenesis Risk 72
 - 3.3.1.2 AAV Germline Transmission Risk 73
 - 3.3.1.3 Off-Target Gene Editing 73
 - 3.3.2 Biomarkers for Immune-Mediated Toxicity 74
 - 3.3.2.1 Hepatotoxicity 74
 - 3.3.2.2 Thrombotic Microangiopathy 76
 - 3.3.2.3 Muscle Toxicity 77
 - 3.3.2.4 Immunogenicity Assessment for rAAV Gene Therapy 77
 - 3.3.3 Safety Biomarkers for Nonimmune Organ-Specific Toxicity 78
 - 3.3.3.1 Dorsal Root Ganglia Toxicity 78
 - 3.3.3.2 Other Target Organ Toxicity Biomarkers 79
- 3.4 Predictive and Diagnostic Biomarkers for Study Enrollment and Patient Stratification 80
 - 3.4.1 Preexisting Anti-Capsid Antibody 80
 - 3.4.1.1 Companion Diagnostic 81
 - 3.4.2 Preexisting Anti-Transgene Protein Antibody 81
- 3.5 Summary 82
- References 82

4 Nonclinical and Clinical Study Considerations for Biodistribution, Shedding, and Pharmacokinetics/Pharmacodynamics 87

Manuela Braun and Kefeng Sun

- 4.1 Biodistribution and Viral Shedding 87
 - 4.1.1 Introduction to Biodistribution and Viral Shedding 87
 - 4.1.1.1 Definition and Terminology for Biodistribution and Shedding 88

4.1.1.2	Global Regulatory Guidance on Conducting Biodistribution and Shedding Studies	88
4.1.2	Nonclinical Biodistribution and Shedding Studies for AAV Vectors	89
4.1.2.1	Design, Execution, and Reporting	90
4.1.2.2	Examples	95
4.1.3	Clinical Biodistribution and Shedding Studies for AAV Vectors	96
4.1.3.1	General Considerations in Viral Shedding Studies in the Clinical Setting	97
4.1.3.2	Biodistribution Characterization in Human: Necessity and Concerns	98
4.1.3.3	Examples	98
4.1.4	Gaps and Challenges on Biodistribution and Shedding Characterization	99
4.2	Pharmacokinetic/Pharmacodynamic (PK/PD) Modeling and Clinical Dose Selection of Gene Therapy	100
4.2.1	Overview on PK/PD and Dose Selection Strategies for Gene Therapy	100
4.2.1.1	AAV Dosing Regimen – Safety Relationship and Safety-based Clinical Dose Projection	101
4.2.1.2	AAV Dose – Pharmacodynamics/Efficacy Relationship and Projection of Pharmacologically-Active Dose (PAD)	102
4.2.2	Dose Scaling Approaches: Allometric and Activity-Based Methods	102
4.2.3	Mechanistic Approaches to Modeling Gene Therapy	105
4.2.3.1	Modeling and Simulation of AAV Biodistribution	106
4.2.3.2	Modeling Transgene Product PK and PD of the Transgene Product	106
4.2.4	Clinical Pharmacology Considerations for Gene Therapy	106
4.2.4.1	Variability in Transgene Product Levels and/or Treatment Response	106
4.2.4.2	Durability of Transgene Expression and/or Treatment Response	107
4.2.5	Gaps and Challenges on PK/PD and Clinical Dose Selection	108
4.2.5.1	Interspecies difference in AAV Transduction and Immunogenicity	108
4.2.5.2	Availability of Clinical Samples and Bioanalytical Assays	109
4.2.5.3	Availability of Long-Term Follow-Up Data	109
4.3	Summary	109
	References	110

5 Immunogenicity of AAV Gene Therapy Products 117

Vibha Jawa and Bonnie Wu

5.1	Innate and Adaptive Immunity Induced by AAV-Based Gene Therapies	117
5.1.1	Innate Immune Response	117

5.1.2	Adaptive Immune Response	119
5.2	Preclinical Immunogenicity Risk Assessment	119
5.2.1	Product-related Risk Factors	120
5.2.2	Process and Manufacturing-Related Risk Factors	120
5.2.3	Patient-Related Risk Factors	121
5.2.4	Nonclinical Assessment of Immunogenicity	121
5.2.5	Animal Models for Assessing Innate Immunity	122
5.2.6	Animal Models for Assessing Adaptive Immunity	122
5.2.7	Impact of Immunogenicity on Animal Selection and Interpretation of Study Results	123
5.3	Clinical Manifestation Associated with Immunogenicity	123
5.3.1	Pre-existing Immunity Against AAV Vector May Compromise Therapeutic Efficacy and Patient Safety	124
5.3.2	Treatment Induced Anti-AAV Capsid Antibodies may Prevent Re-dosing	124
5.3.3	Antibody Specific to Transgene Protein could lead to Toxicity or Unwanted Immunity	125
5.3.4	Risk of Immunogenicity Associated with Different Administration Routes	125
5.3.4.1	Gene Delivery to the Eye or Central Nervous System	126
5.3.4.2	Gene Delivery to Liver	126
5.3.4.3	Gene Delivery to Muscle	126
5.3.5	Product- and Process-related Impurity Related Immunogenicity	127
5.4	Clinical Mitigation Strategy	127
	References	129

Section III Bioanalysis for Gene Therapy 135

6	Bioanalytical Methods to Detect Preexisting and Post-administration Humoral Immune Responses Against AAV Capsid Proteins	137
	<i>Christian Vettermann and Boris Gorovits</i>	
6.1	Introduction	137
6.2	Considerations for AAV Total Antibody Assays	138
6.2.1	Nature of AAV TAb Assay Analyte	138
6.2.2	Primary Analytical Methodologies applied for AAV TAb Detection	139
6.2.3	Tab Assay Critical Reagent Considerations	140
6.2.3.1	Positive and Negative Control Selection	140
6.2.3.2	Capture and Detection Reagents	141
6.2.3.3	Sample Testing Strategy	142
6.2.4	Key Assay Qualification/Validation Parameters	142

6.2.4.1	Assay Sensitivity	142
6.2.4.2	Serotype Specificity	142
6.2.4.3	Precision	143
6.2.4.4	Matrix Interference and Selectivity	143
6.2.4.5	Assay Cut-Point	143
6.2.5	TAb Assay Data Interpretation	144
6.3	Considerations for Cell-based Transduction Inhibition Assays	145
6.3.1	Principle and Methodology of Cell-based AAV TI Assays	145
6.3.2	AAV TI Assay Development: Designing for Clinical Relevance	146
6.3.3	Key Assay Validation Parameters	147
6.3.3.1	Screening and Titer Cut-Points	147
6.3.3.2	Limit of Detection	148
6.3.3.3	Precision	150
6.3.3.4	Specificity	150
6.3.3.5	Confirmatory Steps to Ensure Specific Detection of Neutralizing AAV Antibodies	150
6.3.3.6	Selectivity/Matrix Interference	151
6.3.3.7	Stability	151
6.3.4	Sample Testing Strategy and Monitoring Assay Performance	152
6.3.5	Data Interpretation: Preexisting TI Titer and Clinical Efficacy	152
6.3.6	Value and Challenges of Standardizing TAb and TI Assays	156
	References	157

7 Bioanalytical Methods to Study Biodistribution and Shedding of AAV-Based Gene Therapy Vectors 163

Christian Vettermann and Russell Soon

7.1	Introduction	163
7.2	Choice of Platform: qPCR vs. Digital PCR	164
7.3	Aspects of Method Development	168
7.4	Back-Calculation Formulas and Extraction Efficiency Assessments	172
7.5	Sensitivity Requirements	177
7.6	Specificity Requirements	179
7.7	Standard Curve Performance, Colinearity, Precision, and Accuracy	180
7.8	Selectivity Assessment and Matrix Interference	181
7.9	Sample Stability Considerations	182
7.10	Data Reporting Formats, Acceptance Criteria, and Trending	184
7.11	Immunocapture qPCR: An Ultra-Sensitive Method to Detect Intact AAV Capsids	187
	References	189

8	Transgene mRNA Expression Analysis	193
	<i>Venkata Vepachedu and Hsing-Yin Liu</i>	
8.1	Purpose of Measuring Transgene mRNA	193
8.1.1	Transgene Encodes Therapeutic Protein Entity	194
8.1.2	Transgene Encodes Other Entities	196
8.2	Technologies to Quantify Transgene Expression in Tissues	196
8.2.1	RT-qPCR or RT-dPCR	196
8.2.1.1	RNA Extraction (Separate vs. DNA/RNA Co-extraction), Quality Testing, and Quantification	197
8.2.1.2	Co-extraction of DNA and RNA from same Sample	199
8.2.1.3	Quantification and Quality Testing of total RNA in Purified Extracts	200
8.2.1.4	Quantification Using DNA vs. RNA Standards	201
8.2.1.5	Assay Qualification/Validation and Report	201
8.2.1.6	Reporting	205
8.2.2	In Situ Hybridization (ISH)	206
8.2.2.1	Values of ISH for Discovery Studies	207
8.2.2.2	Semi-quantitative, Tissue Fixation, Probe to Reference Classic Procedure	208
8.3	Summary	211
	References	211

9	Quantification of Transgene Protein Expression and Biochemical Function	215
	<i>Robert Dodge and Liching Cao</i>	
9.1	Introduction	215
9.2	Transgene Protein Concentration Determination	216
9.2.1	Human Transgene in Preclinical Species	216
9.2.2	Human Transgene Assessment for Intracellular Proteins	216
9.2.3	Human Transgene Protein Assessment for Non-secreted Proteins	218
9.2.4	Human Transgene Protein Assessment for Secreted Proteins	220
9.2.5	Human Transgene Protein Assessment for Expressed Therapeutics	221
9.2.6	Transgene Protein Assay Format Considerations	221
9.2.6.1	Immunoassays	222
9.2.6.2	Mass Spectrometry Assays	222
9.2.6.3	Semiquantitative Assay Formats	223
9.3	Transgene Protein Activity Determination	224
9.3.1	Method Development Considerations	224

- 9.3.1.1 Enzyme Kinetics, the Initial Rate of Reaction, and Substrate Concentration 224
- 9.3.1.2 Reference Standard 226
- 9.3.1.3 Sample Processing 228
- 9.3.1.4 Buffers and Incubation Temperature 230
- 9.3.1.5 Assay Dynamic Range, Minimum Required Dilution, Matrix Interference, and Parallelism 230
- 9.3.1.6 Specificity and Selectivity 231
- 9.3.1.7 Quality Controls (QCs) 232
- 9.3.2 Method Validation 234
- 9.4 Summary 234
- References 235

10 Substrate and Distal Pharmacodynamic Biomarker Measurements for Gene Therapy 239

Liching Cao, Kai Wang, John Lin, and Venkata Vepachedu

- 10.1 Introduction 239
- 10.2 Technologies to Quantify Substrate and Distal PD Biomarker 241
- 10.2.1 Liquid Chromatography/Tandem Mass Spectrometry (LC-MS/MS) 241
 - 10.2.1.1 Method Development Challenges and Resolutions 241
 - 10.2.1.2 Method Validation by LC-MS/MS 245
- 10.2.2 Histology 246
- 10.2.3 Functional Activity and Immunoassays 248
 - 10.2.3.1 Method Validation of Immunoassay 249
- 10.2.4 mRNA Detection of Downstream Target Expression as a PD Biomarker 253
 - 10.2.4.1 RT-qPCR for Relative Gene Expression Analysis 254
 - 10.2.4.2 RNA-seq 259
 - 10.2.4.3 Nanostring Technology 260
 - 10.2.4.4 Regulatory Considerations for RNA Quantitation in GLP Studies 261
- 10.2.5 Single-cell Analysis 263
- 10.3 Summary 265
- References 266

11 Detection of Cellular Immunity to Viral Capsids and Transgene Proteins 271

Maurus de la Rosa and Magdalena Tary-Lehmann

- 11.1 Introduction 271
- 11.1.1 Humoral and Cellular Immune Responses to Gene Therapy 271

11.1.2	Selected Clinical Observations Showing the Lack of Understanding About T-Cell-Mediated Immune Responses and the Need for Sensitive T-Cell Analytics	272
11.2	Methods for the Detection of Cellular Immune Responses	274
11.2.1	Methods to Detect T-Cell Responses in Clinical Trials	274
11.2.1.1	Enzyme-Linked Immunosorbent Spot Assay	274
11.2.1.2	Intracellular Cytokine Staining	276
11.2.1.3	Tetramer Staining	276
11.2.1.4	Proliferation Assays	276
11.2.1.5	Cytokine Bead Array	276
11.2.1.6	Gene Expression Profiling	277
11.2.1.7	Multiplexed Epitope Mapping	277
11.2.1.8	Conclusion	277
11.2.2	Technical Challenges of Detecting Cellular Immune Responses	277
11.3	Validation of Cellular Assays Using PBMC (Example ELISPOT)	278
11.3.1	Validation Strategies	278
11.3.1.1	Precision	279
11.3.1.2	Specificity	279
11.3.1.3	Limit of Detection and Range	280
11.3.1.4	Common Exceptions for ELISPOT Validation: Accuracy, Linearity, and Reproducibility	281
11.3.2	Parameters Affecting ELISPOT Assay Performance	282
11.3.2.1	PBMC Sample Handling: Temperature, Resting, and Serum	282
11.3.2.2	Antigen Concentration and Number of Replicates	285
	References	286

12 Detection of Humoral Response to Transgene Protein and Gene Editing Reagents 291

George Buchlis and Boris Gorovits

12.1	Pre- and Post-dose Humoral Immunity to Transgene-expressed Proteins	291
12.1.1	Risk-based Analysis of Response Probability and Impact	291
12.1.1.1	Route of Administration	291
12.1.1.2	Biodistribution of Vector, Vector Serotype, Dose, and Expression Level	293
12.1.1.3	Patient Immune Status: Age, Prior Exposure, No Endogenous Production, Immunosuppression, and Autoimmunity	293
12.1.1.4	Response Induction vs. Response Boosting	294
12.2	Relevance of Analytical Protocols Applied in Determining Immune Response to Protein Therapeutics to the Detection of Anti-Transgene Protein Responses	294

12.3	Analysis of Immune Response by Binding and Functional Antibody Assay Protocols	295
12.4	Comparative Analysis of the Immune Response Evaluation for Transgene Proteins that are Expressed Extracellularly vs. Intracellularly	297
12.5	Humoral Immune Response to Gene Editing Reagents	298
12.5.1	Diversity of Gene Editing Systems	298
12.5.2	Immunological Potential of CRISPR-Cas System	299
12.5.3	Detection of Anti-Cas9 Protein Immunity in Animal and Human Matrix	301
12.5.4	Strategies Proposed to Mitigate Anti-Cas9 Immunity	304
	References	304
13	rAAV Integration: Detection and Risk Assessment	317
	<i>Jing Yuan, Irene Gil-Farina, Raffaele Fronza, and Laurence O. Whiteley</i>	
13.1	Introduction	317
13.1.1	Biology of AAV Vectors as it Relates to Mechanisms of AAV Integration	318
13.1.2	Literature Review of AAV Studies in Relation to Neoplasia Development	318
13.2	Review of Regulatory Guidance and Discussion Points that Are Raised on AAV Carcinogenesis	324
13.2.1	Factors to Consider in the Design of Nonclinical Studies Evaluating AAV Integration	325
13.2.2	Methods for rAAV Integration Analysis	326
13.2.3	AAV Data Analysis Methods	328
13.2.3.1	AAV Primary Analysis	331
13.2.3.2	Impurity Analysis	332
13.2.3.3	AAV Genome Rearrangements	332
13.2.3.4	Integration Site Analysis	332
13.2.3.5	Clonality Analysis	333
13.2.3.6	Genotoxic Integrations	334
13.3	Assessing the Biologic Relevance of AAV Integration Profile	335
13.4	Conclusion and Future Direction	337
	References	338
14	Detection and Quantification of Genome Editing Events in Preclinical and Clinical Studies	347
	<i>Marina Falaleeva, Shengdar Tsai, Kathleen Meyer, and Yanmei Lu</i>	
14.1	Introduction	347
14.1.1	Genome Editing Modalities and Molecular Outcomes	348
14.1.2	Clinical Trials Using Genome Editing Technologies	350

14.2	Regulatory Guidance on Engineered Nuclease On- and Off-target Assessment	352
14.3	Strategies and Methodologies to Evaluate On-target and Off-target Activities	353
14.3.1	Strategies to Evaluate Off-target Sites in Preclinical and Clinical Studies	353
14.3.2	Techniques to Identify Genome Wide Off-target Sites	355
14.3.3	Targeted Approaches to Measure Short Insertions and Deletions	356
14.3.3.1	Droplet Digital™ PCR	365
14.3.3.2	Endonuclease Mismatch Cleavage Assays	366
14.3.3.3	Sanger Sequencing Combined with Sequence Trace Decomposition	368
14.3.3.4	Indel Detection by Amplicon Analysis (IDAA)	369
14.3.4	Technologies to Measure Large Genomic Rearrangements	369
14.3.5	Discussion	374
14.4	Concluding Remarks	376
	References	376

Section IV Companion Diagnostic Development for Gene Therapy 383

15 Introduction to Companion Diagnostics for Gene Therapy 385 *Paul Bartel and Jennifer Granger*

15.1	Introduction to Companion Diagnostics	385
15.2	Role in Gene Therapy	386
15.3	Overall Strategy	387
15.4	Development Process	387
15.5	Considerations for Commercialization	390
15.6	Conclusion	391
	References	391

16 Validation for Gene Therapy Companion Diagnostics 393 *Karen L. Richards and Kennon Daniels*

16.1	Introduction	393
16.1.1	Overview of FDA Oversight for the Use of Assays in Gene Therapy Clinical Trials and the Path to Commercialization with Corresponding Level of Validation	393
16.1.2	Summary of Validation Requirements for Gene Therapy Companion Diagnostics (GTx CDx)	395
16.1.3	Role of CDx in Therapeutic Development and Unique Challenges to Validating GTx CDx	395

16.1.4	Key Considerations for Developing GTx CDx	396
16.2	Development of CTAs for Use in GTx Clinical Trials	397
16.2.1	Stratification vs. Selection	397
16.2.2	Regulatory Risk Determination: Significant or Nonsignificant?	398
16.2.3	CTA Design Considerations	400
16.2.4	CTA Validation Requirements	401
16.3	Best Practices for Sample Banking and Consent of Subjects	401
16.3.1	Validation Strategies for CDxs for Commercial Use	401
16.4	Design Considerations	402
16.4.1	Single-site vs. Distributable Kit	402
16.4.2	Validation Requirements	402
16.5	Bridging Studies	404
16.6	Commensurate Regulatory Review and Approval of GTx CDx	406
16.7	Concluding Sections	406
16.7.1	Summary of Validation Considerations for CTAs/CDx in GTx Clinical Trials	406
16.7.2	Summary of Validation Considerations for CTAs/CDx to Enable GTx Marketing	407
	References	407

17 Regulatory Considerations for Gene Therapy Companion Diagnostics 409

Mica Elizalde and Paul Bartel

17.1	Introduction	409
17.2	US FDA	409
17.2.1	Clinical Trials for Investigational Device Exemption	410
17.2.2	US FDA Marketing Authorization Pathways	413
17.2.2.1	510(k) process	413
17.2.2.2	PMA Process	414
17.2.2.3	HDE Process	414
17.2.2.4	Differences Between 510(k) and PMA	415
17.2.3	US FDA Pre-submission Feedback	416
17.3	European Union	416
17.3.1	European Union Clinical Trials	416
17.3.2	European Union Marketing Authorization Pathways	418
17.4	Other Regulated Markets	420
17.4.1	Global Regulatory Strategy	421
17.5	Development Strategy with the Therapeutic	422
17.5.1	Considerations for Rare Disease Indications	423
17.6	Partner Relationship	424
17.6.1	Importance of the Partner Relationship	424

17.7	Commercial and Post-Approval Considerations	425
17.7.1	Future Proofing the Companion Diagnostic	425
17.7.2	Modifications of the Companion Diagnostic	426
17.8	Final Word	426
	References	426

Section V Regulatory Perspectives on Gene Therapy 429

18 Current Regulatory Landscape for Gene Therapy Product Development and the Role of Biomarkers 431

Laura I. Salazar-Fontana PhD and Mike Havert PhD

18.1	Introduction	431
18.2	What is Gene Therapy?	432
18.3	Biomarkers Defined	433
18.4	Early Gene Therapy Biomarkers	434
18.5	Current Expectations for Gene Therapy Biomarkers	437
18.6	Safety Biomarkers for Gene Therapy Products	438
18.6.1	Immune Toxicities to <i>in vivo</i> gene therapy	438
18.6.2	Immune Toxicities to Ex Vivo GT	441
18.6.3	Long-Term Risks	442
18.7	Concluding Remarks	442
	References	443

Index 449

List of Contributors

Editors

Yanmei Lu

Biomarker and BioAnalytical Sciences
Sangamo Therapeutics
Richmond, California
USA

Boris Gorovits

Translational Sciences, Bioanalysis &
Biomarkers
Gorovits BioSolutions, LLC
Andover, Massachusetts
USA

Authors

Paul Bartel

Companion Diagnostics
Myriad Genetics, Inc.
Salt Lake City, Utah
USA

Manuela Braun

Bayer AG
Berlin
Germany

George Buchlis

Department of Medicine
University of Pennsylvania

Philadelphia, Pennsylvania
USA

Liching Cao

Biomarker and Bioanalytical Sciences
Sangamo Therapeutics
Richmond, California
USA

Kennon Daniels

Precision for Medicine
Bethesda Metro Center
Maryland
USA

Maurus de la Rosa

Sangamo Therapeutics Allée de la
Nertière
Valbonne
France

Robert Dodge

Department of BioMedical Research
Novartis
East Hanover, New Jersey
USA

Mica Elizalde

Regulatory Digital Health
Merck Sharp & Dohme LLC
Rahway, New Jersey
USA

Marina Falaleeva

Preclinical Department
Sangamo Therapeutics
Richmond, California
USA

Raffaele Fronza

ProtaGene CGT GmbH
Heidelberg
Germany

Irene Gil-Farina

ProtaGene CGT GmbH
Heidelberg
Germany

Jennifer Granger

PharmaDx
ARUP Laboratories
Salt Lake City, Utah
USA

Michael Havert

Gene Therapy Partners, LLC
Arlington, Virginia
USA

Vibha Jawa

Clinical Pharmacology,
Pharmacometrics, Disposition and
Bioanalysis (CPPDB)
Bristol Myers Squibb
Princeton, New Jersey
USA

Wibke Lembke

Celerion Switzerland AG
Fehraltorf
Switzerland

John Lin

Frontage Laboratories
Exton, Pennsylvania
USA

Hsing-Yin Liu

Molecular Biology, Johnson and Johnson
Innovative Medicine
Janssen Pharmaceuticals
Spring House, Pennsylvania
USA

Kathleen Meyer

Preclinical Department
Sangamo Therapeutics
Richmond, California
USA

John E. Murphy

Arbor Biotechnologies
Cambridge, Massachusetts
USA

Jane Owens

Rare Disease Research Unit
Pfizer Inc.
Cambridge, Massachusetts
USA

Karen L. Richards

Precision for Medicine
Bethesda Metro Center
Maryland
USA

Laura I. Salazar-Fontana

LAIZ Reg Science Consulting
Lausanne
Switzerland

Oscar Segurado

ASC Therapeutics
Milpitas, California
USA

Russell K. Soon Jr.

BioMarin Pharmaceutical, Inc.
Novato, California
USA

Kefeng Sun

Quantitative Clinical Pharmacology,
Data Sciences Institute
Takeda Development Center Americas
Cambridge, Massachusetts
USA

Magdalena Tary-Lehmann

CTL-Contract Laboratory
Cellular Technology Limited
Shaker Heights, Ohio
USA

Shengdar Q. Tsai

Department of Hematology
St Jude Children's Research Hospital
Memphis, Tennessee
USA

Venkata Vepachedu

Molecular Biology, Johnson and Johnson
Innovative Medicine
Janssen Pharmaceuticals
Spring House, Pennsylvania
USA

Christian Vettermann

BioMarin Pharmaceutical, Inc.
Novato, California
USA

Kai Wang

GlaxoSmithKline
Collegeville, Pennsylvania
USA

Laurence O. Whiteley

Pfizer Inc. Drug Safety Research and
Development
Cambridge, Massachusetts
USA

Bonnie Wu

Biologics Development Sciences, Janssen
Research and Development
LLC Pharmaceutical Companies of
Johnson & Johnson Innovative Medicine
Spring House, Pennsylvania
USA

Jing Yuan

Department of Toxicology
Kymera Therapeutics
Watertown, Massachusetts
USA

Preface

Having dedicated more than a couple of decades to the development of biomarkers and bioanalysis in the realm of biologics, including monoclonal antibodies and recombinant protein therapies, we embarked on a career change with the anticipation that our wealth of experience could readily translate into the field of gene therapy drug development. However, what we hadn't fully grasped at the outset was the considerable complexity and formidable challenges associated with translational biomarkers, bioanalysis, and companion diagnostics when deploying adeno-associated virus (AAV) as a vector to introduce transgenes, encompassing cDNAs and gene editing tools, into human subjects.

The successful advancement of a gene therapy drug necessitates the meticulous collection of pharmacokinetic and biomarker data to underpin efficacy and safety assessments, as well as the selection of suitable patients. The multifaceted nature of gene therapy, coupled with the vast troves of data involved, encompasses a wide spectrum of methods and technology platforms. This repertoire includes polymerase chain reaction (PCR)-based techniques, such as quantitative PCR and digital PCR, for scrutinizing viral biodistribution and shedding patterns, reverse transcription-PCR for analyzing transgene expression, enzyme activity assays, mass spectrometry, immunohistochemistry/in situ hybridization, and immunoassays for evaluating target engagement, substrate interactions, and distal pharmacodynamic biomarkers.

Moreover, ligation-mediated (LM)-PCR and linear amplification-mediated (LAM)-PCR are indispensable for the in-depth analysis of recombinant AAV integration, while next-generation sequencing (NGS) is employed to assess off-target gene editing activity. The assessment of humoral antibody response and cellular immune response to AAV capsid and transgene products requires the application of anti-drug antibody and neutralizing antibody assays, as well as ELISpot technology.

In addition, the evolving landscape of companion diagnostic development, particularly in relation to the anti-AAV antibody screening assay supporting clinical

studies and drug approval, presents unique and rapidly evolving challenges. Furthermore, as clinical data continues to emerge from ongoing trials, the regulatory environment governing the evaluation of efficacy and safety in the gene therapy field is in a state of flux.

Over the past decade, the discovery and development of AAV gene therapy medicines have gained remarkable momentum. This surge in growth, marked by a proliferation of preclinical studies and clinical trials, has led to a shortage of qualified researchers in translational sciences. In this dynamic landscape, the adoption of best practices in biomarker and bioanalysis, combined with up-to-date knowledge of regulatory guidelines, is of paramount importance. Such information is invaluable for gene therapy developers, whether they are working in academia, industry, or government organizations, as it equips them with the timely insights required to navigate the constantly evolving challenges and opportunities in this dynamic field.

January 2024

Yanmei Lu
Sangamo Therapeutics

Boris Gorovits
Gorovits BioSolutions, LLC

Section I

Introduction

1

Introduction to AAV-based *in vivo* Gene Therapy

Oscar Segurado

ASC Therapeutics, Milpitas, CA, USA

1.1 Introduction

1.1.1 History of Gene Therapy

Watson and Crick first characterized the structure of DNA as a double helix in 1953 [1]. X-ray crystallography of DNA, performed by Franklin, confirmed this finding [2]. Knowing DNA's structure helped elucidate its functions, such as how it holds genetic information, can be copied, and gives rise to various proteins.

Although adeno-associated viruses (AAVs) were discovered in the 1960s [3], they would not be used as genetic vectors until the 1980s. The first attempt at genetic manipulation in humans is believed to be the work of Terheggen et al. in the 1970s. German scientists used the Shope papillomavirus in three children whose bodies were unable to produce arginase. Without arginase, arginine accumulates in the body, causing neurological and muscular defects. The virus, known to produce arginase, was injected intravenously (IV) in hopes that the genetic information from the virus could enter human cells, resulting in arginase production. Unfortunately, IV injections of the virus did not help any of the three sisters that had this rare disorder, and the youngest, who was given a larger dose as an infant, suffered a brief allergic reaction without any positive response to the treatment [4].

In the 1980s, retroviral gene therapy was in development [5–7], and the first recombinant AAV vectors were created [8]. Synthetic insulin was the first genetically engineered drug, reaching the market in 1982 [9]. Zinc fingers were discovered in 1985, later providing a method of targeted gene therapy through zinc

finger nucleases (ZFNs) [10]. The hepatitis B vaccine was the first recombinant vaccine available in 1986 [11], and the discussion of the human genome project began two years later [12]. Also in 1988, the first genetically modified crop was grown in US fields [13].

In 1990, research began in the United States, studying human gene therapy [14]. Dolly, the sheep, was cloned in 1996 [15]. By the year 2000, around 400 gene therapies had been tested in clinical trials [16]. The first gene therapy was approved in China in 2003, using a replication-incompetent adenovirus vector for treating advanced head and neck cancer [17]. Modified lentiviral vectors began emerging in clinical trials around this time as well [18]. In 2007, human-induced pluripotent stem cells (iPSCs) were first isolated, and this method is now quite common, using genetic reprogramming to compare patient-derived cells to isogenic control cells [19]. The first gene therapy was approved in Europe in 2012 using an adenovirus [16]. In 2013, CRISPR/Cas9 was developed, where it was first used as a research tool [20]; it was not until 2018 that the first clinical trial in humans utilizing this technology completed their enrollment. Patients with refractory non-small-cell lung cancer were treated with CRISPR-edited T cells [21]. This timeline can be viewed in Figure 1.1.

In 2020, over 400 gene and genetically modified cell therapies were in development, and today (2022), there are over 1000 in recruitment or active studies (clinicaltrials.gov). Gene therapies may replace inadequate and complex therapies in the near future. For some diseases, it may be able to reduce the amount and, eventually, the cost of treatments a person needs. Thus, it is likely to benefit those with poor quality of life due to an untreatable condition or an intense therapy regimen the most.

1.1.2 AAV-based *in vivo* Gene Therapy: A Revolution in Medicine

Despite gene therapies being developed and tested in the United States since the 1990s, only 26 cell and gene therapies have been Federal Drug Administration (FDA)-approved until February 2023, seven of which are cord blood treatments (Table 1.1). Of the other 19 therapies, 14 are *ex vivo* cell therapies and five are *in vivo* gene therapy treatments. Genetic diseases, those driven by mutations in the human genome, are ideal targets for treatments using gene therapy modalities. Gene therapy can address diseases driven by well-defined genetic abnormalities where the biological function of the altered or missing gene is well understood. In many cases, these are rare diseases with unmet medical needs, often requiring complex medical regimens with limited options for effective treatments. However, in recent years, gene therapies have been investigated for the treatment of non-monogenic diseases, for example, cancers and degenerative diseases of the visual and nervous systems.