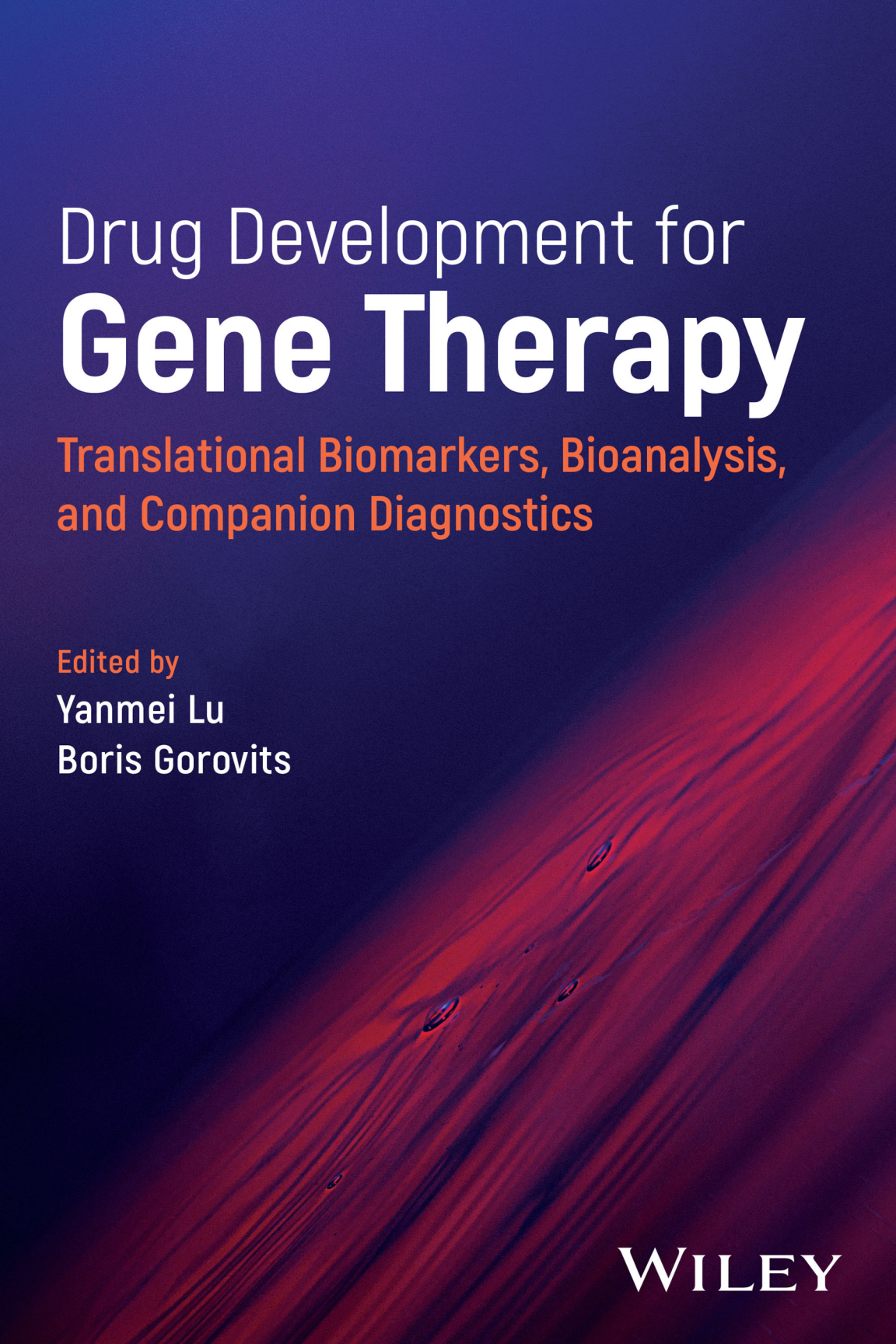
Drug Development for Gene Therapy

Translational Biomarkers, Bioanalysis, and Companion Diagnostics

Edited by
Yanmei Lu
Boris Gorovits



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Contents

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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.

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Section I

Introduction

1

Introduction to AAV-based *in vivo* Gene Therapy

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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

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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.