Translational Oncology

Cancer Gene Therapy by Viral and Non-viral Vectors
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Provides expert, state-of-the-art insight into the current progress of viral and non-viral gene therapy
Translational medicine has opened the gateway to the era of personalized or precision medicine. No longer a one-size-fits-all approach, the treatment of cancer is now based on an understanding of underlying biologic mechanisms and is increasingly being tailored to the molecular specificity of a tumor.

This book provides a comprehensive overview of the pertinent molecular discoveries in the cancer field and explains how these are being used for gene-based cancer therapies. Designed as a volume in the Translational Oncology book series, Cancer Gene Therapy by Viral and Non-viral Vectors deals with the practice of gene therapy, with reference to vectors for gene expression and gene transfer as well as viral therapy. It covers the history and current and future applications of gene transfer in cancer, and provides expert insight on the progress of viral and non-viral gene therapy with regard to delivery system, vector design, potential therapeutic genes, and principles and regulations for cancer gene therapy. Presented in three parts, Cancer Gene Therapy by Viral and Non-viral Vectors covers:

Delivery Systems
- Translational Cancer Research: Gene Therapy by Viral and Non-viral Vectors
- Retroviruses for Cancer Therapy
- DNA Plasmids for Non-viral Gene Therapy of Cancer
- Cancer Therapy with RNA delivered by Non-viral Membrane/Cure Nanoparticles

Targeted Expression
- Cancer Gene Therapy by Tissue-specific and Cancer-targeting Promoters
- miRNAs as Drugs and Drug Targets in Cancer

Principles of Clinical Trials in Gene Therapy
- Regulatory Issues for Manufacturers of Viral Vectors and Vector-transduced Cells for Phase I/II Trials
- US Regulations Governing Clinical Trials in Gene Therapy
- Remaining Obstacles to the Success of Cancer Gene Therapy

Focusing on speeding the progress in clinical cancer care by bringing therapies as quickly as possible from bench to bedside, Cancer Gene Therapy by Viral and Non-viral Vectors is an absolutely vital book for physicians, clinicians, researchers, and students involved in this area of medicine.
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While our knowledge of cancer at a cellular and molecular level has increased exponentially over the last decades, progress in the clinic has been more gradual, largely depending upon empirical trials using combinations of individually active anticancer drugs to treat the average patient. The challenge for the immediate future is to accelerate the pace of progress in clinical cancer care by enhancing the bidirectional interaction between laboratory and clinic. Our new understanding of human cancer biology and the heterogeneity of cancers at a molecular level must be used to identify novel targets for therapy, prevention, and detection focused on each individual. Barriers must be removed to facilitate the flow of targeted agents and fresh approaches from the laboratory to the clinic, while returning relevant human specimens, images, and data from the clinic to the laboratory for further analysis.

In this title we will provide a brief overview of our current understanding of human cancer biology that is driving interests in targeted therapy and personalized management. Further development of molecular diagnostics should facilitate earlier detection, more precise prediction and prediction of response across the spectrum of cancer development. Targeted therapy has already had a dramatic impact on several forms of cancer and strategies are being developed to identify small groups of patients who would benefit from novel targeted drugs in combination with each other or with more conventional surgery, radiotherapy or chemotherapy. Development of personalized interventions – whether preventive or therapeutic in nature – will require multidisciplinary teams of investigators and the infrastructure to match patient samples and agents in real time.

To accelerate translational cancer research, greater alignment will be required between academic institutions, the US National Cancer Institute, the US Food and Drug Administration, foundations, pharma, and community oncologists. Ultimately, new approaches to prevention, detection, and therapy must be sustainable. In the long run, translational research and personalized management can reduce the cost of cancer care which has escalated in recent years. More accurate and specific identification of at-risk members and risk stratification will be helpful to minimize the risks of overdiagnosis and overtreatment, while maximizing the benefits of screening, early detection, and preventive intervention. Patients who would benefit most can be identified and funds saved by avoiding treatment in those whose cancers would not respond. Participation and education of community oncologists will be required, as will modification of practice patterns. For progress in the clinic to occur at an optimal pace, leaders of translational teams must envision a clear path to bring new concepts and new agents from the laboratory to the clinic, to complete pharmaceutical or biological development, to obtain regulatory approval, and to bring new strategies for detection, prevention, and treatment to patients in the community.

In a series of additional volumes regarding translational cancer research, several topics are explored in greater depth, including Biomarkers, Targeted Therapy, Immunotherapy, and this volume concerning Cancer Gene Therapy by Viral and Non-Viral Vectors. The purpose of these books has been not only to describe different strategies for particular forms of cancer, but also to identify some of the barriers to translation using different reagents or different strategies around common therapeutic or diagnostic modalities. Potential
barriers include not only the need for a deeper understanding of science, methods to overcome the challenge of tumor heterogeneity, the development of targeted therapies, the availability of patients with an appropriate phenotype and genotype within a research center with the investigators, research teams, and infrastructure required for clinical/translational research and the design of novel trials, but also adequate financial support, a viable connection to diagnostic and pharmaceutical development, and a strategy for regulatory approval, as well as for dissemination in the community.

*Cancer Gene Therapy by Viral and Non-Viral Vectors* considers many of these areas, including the strengths and limitations of the several types of viral and non-viral delivery systems, the potential importance of tumor-specific promoter systems, examples of where gene therapy has succeeded, the challenge of targeting all cancer cells, the advantages of targeting the tumor stroma and immunocytes, and the logistic barriers to preparation of materials required for clinical trials. The need for substantial antitumor activity and the importance of clinical responses in phase I–II trials are also highlighted. Overall, this volume provides substantial perspective regarding the translational potential of cancer gene therapy.

Robert C. Bast
Maurie Markman
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The idea of gene therapy was first proposed to correct errors associated with genetic disease by supplementing defective or missing genes. Advances in DNA technology and in understanding the basis of genetic diseases gave high hopes that gene therapy would be the next big breakthrough in medicine. However, the journey ahead was not without challenges and roadblocks. In 1999, a tragedy occurred when an 18-year-old gene therapy trial participant, Jessie Gelsinger, died 4 days after receiving adenoviral treatment for a genetic disorder from a massive immune response that led to multiple organ failure. This incident caused a major setback in the gene therapy field and the US Food and Drug Administration placed a hold on active gene therapy trials. Yet another event that followed brought more bad news to the field. In 2003, five patients who received CD34+ hematopoietic (bone marrow) stem cells transduced with a retrovirus carrying the interleukin-2 receptor γ chain gene to treat inherited X-linked severe combined immunodeficiency (SCID-XI) developed T-cell leukemia. One patient later died. Despite the dismissal of promising hopes of gene therapy in the early days as a result of these events, there is now optimism as more current research data have shown substantial progress in the clinical development of gene therapy after years of intense investigations to improve vector design and safety. Several successful gene therapy trials, including treatment of an inherited eye disease (Leber's congenital amaurosis), Parkinson's disease, blood disorders, SCID-XI, adenosine deaminase-deficient SCID, and Siemerling–Creutzfeldt disease (X-linked adrenoleukodystrophy), have been reported in the last few years. Most recently, two studies published in July 2013 in *Science* reported clinical efficacy in lentiviral-mediated gene therapy to treat metachromatic leukodystrophy and Wiskott–Aldrich syndrome (see Chapter 2 for more details). In addition to human trials, studies conducted in canines showed that achromatopsia (an inherited form of total color blindness), diabetes, and Duchenne muscular dystrophy were successfully treated by gene therapy; these encouraging findings will undoubtedly continue to pave the way for conducting human clinical trials to develop new drugs to treat these diseases.

While no gene therapy has yet been approved in the USA, two have been approved for use in other parts of the world. The Chinese State Federal Drug Administration approved the world's first gene therapy to treat head and neck cancer using Gencidine, an adenoviral vector expressing tumor suppressor p53. However, concerns about the therapeutic efficacy have been raised [1], and there are no further reported clinical outcomes after a decade of approval. In 2012, the European Commission approved the first gene therapy product (Glybera) in the Western world to treat lipoprotein lipase deficiency, a rare inherited disease of fat metabolism. The company uniQure is currently seeking regulatory approval in the USA, Canada, and other countries.

Cancer, cardiovascular, and infectious diseases, among many others, are also targets of gene therapy. Adenovirus remains the most popular type of vector used in gene therapy clinical trials worldwide, followed by retrovirus, naked/plasmid DNA, vaccinia virus, and lipofection in the top five. For viral vectors, the important parts of the virus required for gene delivery are kept and those that are not required are deleted, and the development of self-inactivating integrating viruses such as retrovirus and lentivirus eliminates the transactivation of neighboring genes after integration.
investigations also continue to broaden the viral vectors’ cell host range. For non-viral vectors, improvements have focused on the delivery system for therapeutic agents, including plasmid DNA, RNA interference (RNAi), and microRNAs, by increasing cellular uptake, protecting against microphage digestion, and optimizing nucleic acid payload release.

In the USA, clinical studies must be reviewed by regulatory committees such as the Institutional Review Board (IRB), Food and Drug Administration (FDA), Institutional Biosafety Committee (IBC), and Recombinant DNA Advisory Committee (RAC). Moreover, manufacture must also comply with Good Manufacturing Practice (GMP) guidelines set out by the FDA. The development of sufficient manufacturing capacity to meet the clinical demands after gene therapy attains approval is another concern.

The discovery of monoclonal antibodies brought much excitement as a new treatment modality in early 1980s. However, the lack of efficacy and the rapid clearance of murine monoclonal antibodies due to the development of human antimouse antibodies in patients led to the failure of many clinical trials. Nonetheless, perseverance allowed the development of technological improvements resulting in the eventual clinical success of monoclonal antibodies, which are now a standard approach for producing therapeutics targeting cell surface receptors. In a similar way, further improvements in gene therapy may allow this approach to follow the successful journey of monoclonal antibodies.

To ensure that gene therapy can be successfully developed into new drugs following the fate of monoclonal antibodies, there are several areas needing critical improvement, including efficient delivery, specificity, and well-designed clinical trials. In this book, we have invited experts to discuss the current updates on cancer gene therapy. The opening chapter by Cerullo et al. describes various types of viral therapy, particularly DNA viruses (adenovirus, vaccinia virus, herpes virus, parvovirus) and provides examples of their use in clinical studies. The following chapter by Zhou et al. focuses on the principal types and evolution of lentiviruses in cancer and HIV therapy with special interest in gene silencing by RNAi. The next two chapters describe non-viral delivery systems. First, in Chapter 3 Najjar et al. review various methods of plasmid DNA delivery, optimization of gene expression, and their application for therapy including cancer. Satterlee and Huang then explain in Chapter 4 the design and challenges of nanoparticles to deliver therapeutic RNAi. In the second part, starting with Chapter 5, Hsu et al. provide an introduction to the clinical applications of tissue-specific and cancer-targeting promoters in cancer gene therapy. As aberrant microRNA expression has been implicated in promoting and initiating carcinogenesis, in Chapter 6 Ling and Calin present an overview of the role of microRNAs in cancer and other diseases and discuss examples of anti-microRNA therapeutics.

The last part of the book provides some insight on the regulatory compliance of gene therapy clinical trials focusing on manufacturing regulations of viral vectors by Gee and Mei in Chapter 7 and review processes and requirements prior to obtaining FDA approval by Grilley in Chapter 8. In the closing chapter, Brenner discusses the tasks that must be accomplished to make gene therapy drugs more broadly applicable and the improvements in clinical trial design, as the development pathway of cancer gene therapy is distinct from and more complex than the traditional pharmaceutical model. It is our hope that this book can facilitate the maturation of gene therapy for its clinical application.

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Reference

PART I

Delivery Systems
CHAPTER 1

Translational Cancer Research: Gene Therapy by Viral and Non-viral Vectors

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Adenovirus

Adenovirus is among the most used vectors for gene therapy and gene transfer, and about 23% of all vector-based clinical trials have been performed with it (www.wiley.com/legacy/wileychi/genmed/clinical/). Adenovirus was first isolated in 1953 from human adenoids [1]. To date, 55 different human serotypes, subdivided into seven subgroups (A–G), have been characterized [2,3].

Adenovirus is a nonenveloped double-stranded DNA virus surrounded by an icosahedral protein capsid (Table 1.1). The capsid comprises penton and hexon proteins with knobbed fibers protruding from the vertices of the capsid [4]. Soon after its entry into the target cell viral DNA reaches the nucleus where starts its replication. Early genes, mainly involved in DNA replication, are transcribed first [5], followed by late genes mainly coding for structural proteins [4].

Adenoviruses tend to be species-specific with regard to permissivity to replication. However, there may be some exceptions to this general rule. It has been reported that adenovirus serotype 5 subgroup C (usually referred as Ad5, the most used gene therapy vector) can replicate to some degree in cotton rats [6,7], New Zealand rabbits [8], and Syrian hamsters [9]. This feature of Ad5 has been very important for scientists around the world because it has allowed them to use these animal models to develop new therapies for disease.

Historically adenovirus has been the most used vector for gene therapy and gene-transfer purposes. In 1970s F. Graham and colleagues discovered the importance of the E1 gene, that made possible the use of adenovirus as a viral vector for gene therapy [10]. In fact, as E1 gene products initiate the replication of the viral DNA, serotype 5 adenoviruses with E1 deleted are incapable of replicating and remain episomal. Taking advantage of this characteristic, scientists replaced E1 with different expression cassettes to avoid virus replication while promoting expression of the transgene inserted in place of E1. Later on, E1-deleted adenoviral vectors, also known as first-generation adenoviral vectors (FG-Ad), were developed into high-capacity adenoviral vectors or Helper-dependent adenoviral vectors (Hd-Ad). HD-Ad are devoid of all viral genes except the two inverted terminal repeats (ITRs) and the packaging signal (psi). They show a high cloning capacity (up to 36 kb) and reduced immunogenicity and toxicity [11] (Figure 1.1). Since then, it has been mainly used as vector for gene transfer for genetic diseases [12] or to treat cancer [13]. The immunogenicity of adenovirus may render it unsuitable for long-term...
gene expression but makes it attractive for treatment of cancer. Use of a replication-deficient adenovirus as a gene delivery vehicle is the classic approach, with some exciting clinical results [14,15,16,17], but no products have been approved outside of China. This approach has been reviewed recently [18]. In the past decade, many adenoviral gene therapists have focused on use of adenovirus as a replication-competent oncolytic virus and thus this will be focus of this chapter.

### Oncolytic Adenoviruses for Treatment of Cancer

Oncolytic adenoviruses are specifically modified to selectively replicate in and destroy cancer cells. This selectivity is achieved by modifications of the genes involved in viral replication so that the life cycle of the virus can occur only in cells than can transcomplement the defect, including cancer cells, while the replication of the virus is arrested in normal cells (transcriptional targeting) (Figure 1.2). An alternative approach is to use tumor-specific promoters to “drive” E1 expression to allow selective replication of the virus in cancer cells [19] (Figure 1.2).

Historically, the first adenoviruses used in patients were wild-type viruses [20]. The concept was revived with the first adenovirus proposed to have tumor selectivity, dl1520 (today known as ONYX-015) [21]. This adenovirus bears a naturally occurring variation that results in a nonfunctional E1B-55k product. E1B-55k is one of the proteins encoded by the early gene E1 and its normal

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### Table 1.1 The main characteristics of the viruses discussed in this chapter.

<table>
<thead>
<tr>
<th>Virus Type</th>
<th>Genome</th>
<th>Transgene Capacity</th>
<th>Genetic Targeting</th>
<th>Particle Retargeting</th>
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<tbody>
<tr>
<td>Adenovirus</td>
<td>Linear dsDNA, 36kb</td>
<td>≈3 kb</td>
<td>Deletion of genes essential for replication in normal cells (E1A, E1B)</td>
<td>scAb-binding domain in knob</td>
</tr>
<tr>
<td>Vaccinia virus</td>
<td>Linear dsDNA, 200kb</td>
<td>&gt; 25 kb</td>
<td>Tumor-specific promoters</td>
<td>Cell-specific peptides in fiber/knob</td>
</tr>
<tr>
<td>Herpes simplex virus</td>
<td>Linear dsDNA, 150kb</td>
<td>&gt;25kb?</td>
<td>MicroRNA targets in genome</td>
<td>Serotype fiber/knob exchange</td>
</tr>
<tr>
<td>Parvovirus</td>
<td>Linear ssDNA, 5.1 kb</td>
<td>≈40 kb?</td>
<td>Deletion of genes essential for replication in normal cells (VGF, TK)</td>
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