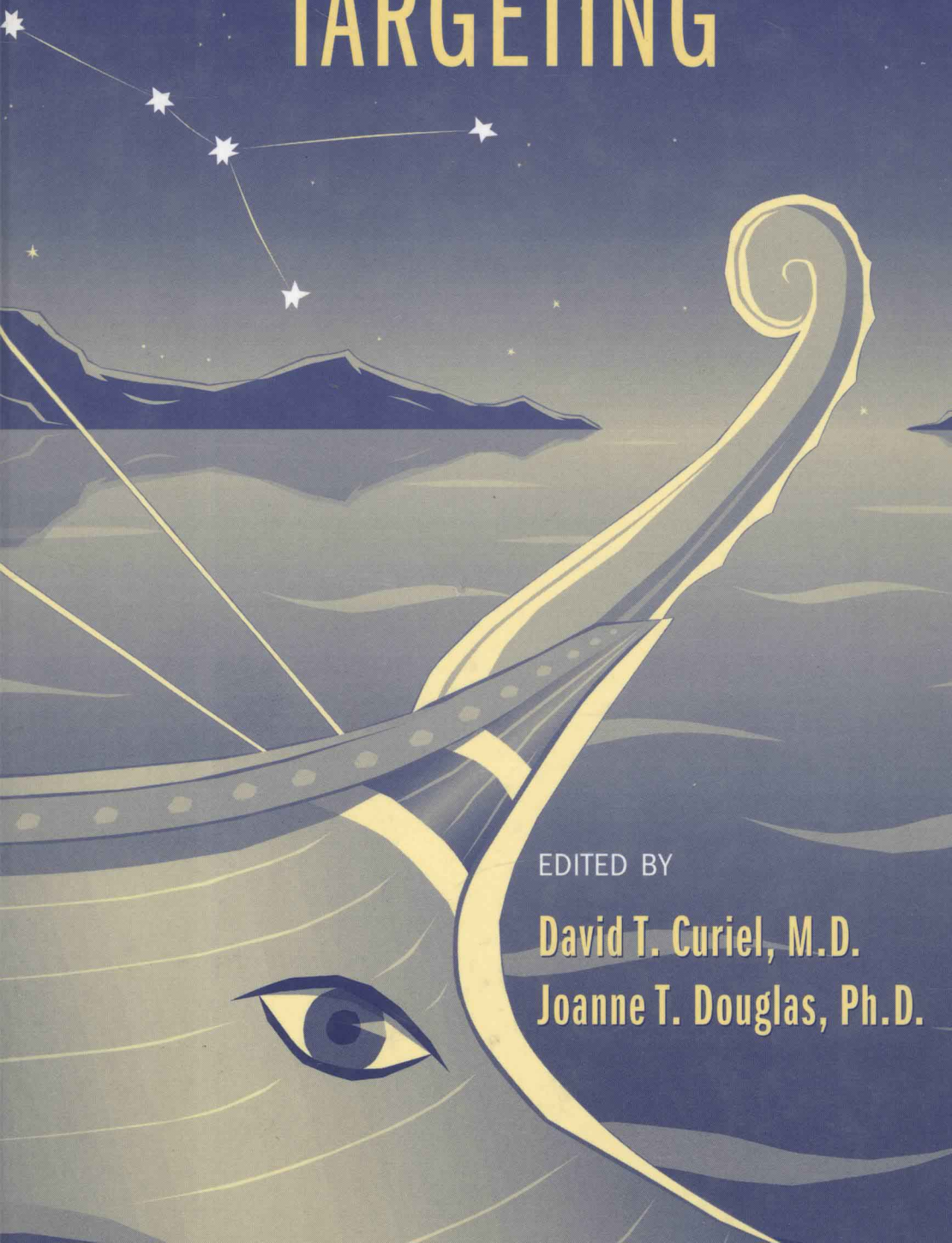


VECTOR for Therapeutic Gene Delivery TARGETING



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Vector Targeting for Therapeutic Gene Delivery

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PREFACE

The basic mandates for gene therapy were formulated before the existence of any practical basis for the application of the concept. In this regard, early proponents of genetic therapy defined the basic criteria to be met prior to actual implementation of any human gene therapy approach. Specifically, it was required that the therapeutic gene be efficiently delivered to the relevant target cells, that the gene be expressed at an appropriate level, and that both of these ends be achieved with an acceptable margin of safety. It was thus implicit even at the earliest stage of field conceptualization that targeted gene delivery would need to be accomplished.

Despite this recognition, the earliest human gene therapy approaches proceeded without explicit attention to targeting *per se*. From the vector standpoint, the earliest issues addressed were related to achieving basic efficacy parameters needed to make practical gene therapy feasible. In the context of nonviral vectors, exploitation of nonspecific cellular transport processes provided the basis of genetic transduction. These methods were inefficient and, by their very design, nontargeted in intent. As an alternative, viral vectors were developed that incorporate a heterologous transgene within the viral genome and exploit the relatively more efficient processes of viral infection of a target cell. Such virus-mediated delivery, however, was restricted by the native tropism of the parent virus.

Early generations of vector systems thus prioritized the development of efficient gene delivery. By virtue of the design logic for both nonviral and viral approaches, specific targeting capacities were not embodied. In fact, the situation predicated vectors having tropism capacities frequently at odds with cell-specific targeting goals. Recognition of these limits led to initial gene therapy approaches whereby target cells could be modified *ex vivo*. In this schema, cell-specific transduction was achieved by a priori selection and isolation of target cells, followed by vector-mediated transduction. This schema implicitly recognized the limitations of available vector systems for achieving targeting goals. Unfortunately, only a few diseases are amenable to *ex vivo* gene therapy interventions, reflecting the limited repertoire of parenchymal cells that can be manipulated via

extracorporeal methods. These considerations thus highlight the degree to which vector limitations, specifically limitations in vector targeting, restricted the practical implementation of the range of candidate gene therapies.

The goal of extending the range of gene therapy disease targets was fostered by the development of vector systems capable of *in vivo* gene delivery. In this regard, the capacity of various viral vector systems to achieve *in situ* gene transfer allowed the conceptualization of gene therapy approaches unconstrained by extracorporeal modifications. On this basis, gene therapy approaches for a variety of inherited and acquired disorders advanced to animal model systems and human clinical trials. Consideration of the results of these various *in vivo* gene therapy approaches defined the limitations of current vector systems and thereby established the clear rationale for targeting strategies.

In many respects, cancer gene therapy has illustrated key issues with respect to targeting, reflecting the fact that antitumor strategies frequently involve the delivery of toxin genes. In this scenario, the consequences of ectopic, nontargeted delivery would be manifested most prominently as therapy-related toxicity. Thus, the early clinical trials for cancer provided key insights as to the requirements for targeting and potential gains therein. In this regard, the obvious lack of targeting capacity of available vectors mitigated against approaches for disseminated neoplastic disease. Such diseases would have required tumor-selective gene delivery following systemic vector administration. The lack of any vector with such attributes meant it was necessary to address disease contexts in which targeting stringency was not such a paramount consideration. To this end, tumors localized to natural body compartments (central nervous system, thoracic cavity, peritoneal cavity) appeared to offer the ideal scenario—a space-confined tumor allowing vector concentration and containment. Thus, initial *in vivo* anticancer gene therapy approaches were endeavored for glioma, pleural mesothelioma, and peritoneal carcinomatosis.

The results of these trials were highly disappointing. First, extremely low levels of tumor cell transduction were achieved. Thus, despite apparent vector efficacy in model system studies, target cell transduction rates following *in vivo* gene delivery were limited. Secondly, ectopic gene delivery occurred in various *in vivo* delivery schemas, irrespective of theoretical vector containment based on anatomic aspects. Further, ectopic gene deliveries were associated with vector-related toxicities. Thus, whereas the overall profile of anticancer gene therapy has suggested an acceptable safety/toxicity profile, the occurrence of suboptimal target transduction rates might logically predicate advanced dosing schemas, a strategy countermanded by the observed phenomenon of ectopic gene delivery. *In vivo* gene delivery, even in the optimized scenario of compartment-based models, therefore exhibits all of the limitations invoked as countervailing systemic delivery schemas—limited tumor cell transduction and ectopic gene delivery. These considerations thus clearly establish the universal applicabilities that might derive from targeting, irrespective of delivery route.

The very recent aspect of these findings explains the only recent development, and application, of targeting for gene therapy applications. In this regard, the basic ideas of retargeting vectors for selective gene delivery have been previously studied as a means to improve vector efficiency per se, as in the context of receptor-mediated conjugate vectors. In other words, the idea of targeting to improve gene therapy outcomes has been most generally recognized as a consequence of these disappointing results in human clinical trials. However, basic field paradigms have only been recently established. Further, the actual translation of targeting paradigms into the clinical context has awaited these key proof-of-principle studies whereby direct gene therapy gain via targeting has been established.

For selected viral and nonviral vector systems it has now been demonstrated that targeting strategies can allow targeted, cell-specific gene delivery. However, this has largely been demonstrated only in in vitro proof-of-principle studies. A much smaller subset of studies has demonstrated the capacity to alter vectors in the context of in vivo gene delivery schemas. Such studies have also allowed demonstration of valid functional gene therapy endpoints including increased target cell transduction for enhancement of phenotype correction and mitigation of vector-mediated toxicities. Such powerful results have predicated consideration of translating such approaches into the clinical context to validate the human therapeutic uses embodied in these targeting approaches.

The unfortunate demise of a young man in a human clinical gene therapy trial represented a field landmark. In addition to the obvious field setback provoked, basic limits inherent in current vector systems became apparent. Although a temporary retrenchment in clinical activities could have been predicted, in fact, the longer term effects have been to positively radicalize the field and regulatory agencies with respect to considering vector redesign as of paramount importance to the field. Further, the positive findings in three recent human trials (for X-linked severe combined immunodeficiency, hemophilia B, and ischemic heart disease) have been generally recognized to have been gained via vector improvements. Thus, the formula that vector gain equals gene therapy gain has clearly been established. On this basis, recognition of the need for vector targeting strategies has allowed recent approval by the National Institutes of Health Recombinant DNA Advisory Committee of a variety of human clinical trials that embody targeting principles. The use of tropism-modified viral vectors represents a fundamental paradigm shift of the basic concept of exploitation of viruses for gene therapy applications. The realization of direct gene therapy gains to these trials—that is, an improved therapeutic outcome and/or a reduction of treatment-associated toxicities—will constitute a critical validation of the targeting principle with wide implications for the field.

It is against this historical background that this book has been conceptualized. The first and second sections focus on transductional targeting strategies designed to achieve the selective delivery of the therapeutic gene by both non-viral and viral vectors, respectively. The third section discusses the alternative, but complementary, approach of transcriptional targeting, in which the thera-

peutic gene is placed under the control of transcriptional regulatory sequences activated in the disease cells but not in normal cells and therefore target expression selectively to the tumor cell. Any transductional targeting approach mandates ligands that can be exploited to achieve cell-specific gene delivery. Therefore, the fourth section is dedicated to the consideration of a variety of strategies that can be employed to define appropriate cellular targeting moieties. Finally, it is becoming increasingly recognized that therapeutic gene delivery in the clinical setting could greatly benefit from strategies to monitor the extent of gene expression. Accordingly, the last section of the book is dedicated to this topic. Whereas the gains of targeting have begun to become apparent in model systems, it is clear that additional, and profound, gains may yet be realized by further endeavors of this type. However, the true gains have yet to be defined in the ultimate context—human clinical gene therapies.

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