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Cystic Fibrosis

Diagnosis and Protocols, Volume I: Approaches to Study and Correct CFTR Defects

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Foreword

This book represents a milestone in the worldwide cystic fibrosis (CF) community's efforts to continue to pave the way toward the development of new and innovative therapies that address the basic defect in CF. But no book on this subject would be possible without the invaluable contributions of the many patients, families, and disease-related organizations that played a key role in creating the science outlined in these chapters. As an orphan disease, CF does not receive sufficient funding from traditional supporting agencies but depends instead on a vast network of people who selflessly give their time and energy to raise the dollars to support the research that will lead to new treatments and a cure. Much of the science described in the pages that follow is the result of funds raised by the CF community, as well as the willingness of patients to provide tissue specimens, share their data in patient registries, and participate in clinical studies. These contributions have been critical to the success that CF research is experiencing worldwide.

The global cystic fibrosis community is clearly unique and has often been described as a "culture of research." Shared among the many patient groups that represent about 70,000 people with CF worldwide is the belief that we will ultimately cure this disease through research. The constancy of this shared mission to cure CF by focusing on research is part of what sets the CF community apart from other rare diseases. The clear promise of small molecules and the excitement over gene therapy have kindled a sense of optimism that is critical to sustaining the momentum toward finding a cure.

In addition to the consistent focus on research, there are a number of other unique traits that the CF community around the world possesses that make it one of a kind. Some of the distinguishing qualities include the following:

- *Willingness to share*: Because of the insidious nature of CF, there is a rare sense of cooperative spirit among scientists, physicians, caregivers, patients, and families all over the world who are dedicated to a cure. CF research data know no borders, and waiting until data are published is not part of the CF research culture. The advancement of science is an open book, and the rapid exchange of new ideas and approaches is a mainstay of CF conferences and workshops in North America and overseas.
- *Willingness to take risks*: The search for the gene in the 1980s is an example of the risks and rewards of the pioneering work of the global CF populace. In the early 1980s, CF communities began to collect blood and tissue samples from families with multiply-affected individuals with CF. With newly evolved technologies (such as chromosome jumping), which could be quickly applied to these samples, and the rapid exchange of information, numerous efforts to find the gene were launched. The discovery of the CF gene in 1989 was the result of an intricate and highly successful international collaboration and is hailed today as one of the major milestones in modern medical research. Because of the involvement of CF families, patients, and the US Cystic Fibrosis Foundation, this groundbreaking discovery occurred over 14 years prior to the publication of the human genome. Importantly, it gave scientists an opportunity to explore the relationship of the genetic defect with the pathogenesis associated with CF. This discovery was a prelude to the effort to move from a knowledge acquisition

mode of research to the current CF research efforts, whereby scientists are using acquired knowledge about CF to develop new approaches to treat the disease.

- *Willingness to take responsibility for its own destiny*: Because there are only 70,000 CF patients throughout the world, there has been reluctance in the biopharmaceutical arena to enter the field of CF. Without their involvement, the ability to develop novel therapeutics is limited. In the late 1990s, frustrated by this fact, the US CF Foundation dramatically changed its business model and created a program to reduce risk of industry involvement in CF research by providing early funding and access to scientific and clinical expertise. This successful array of alliances with industry has led to an exciting clinical pipeline of products, including small molecules that are now being tested in centers worldwide, any of which could have a profound impact on individuals with CF.

Similarly, upon realizing industry's dwindling interest in gene therapy for CF, the British Trust launched a Gene Therapy Consortium that has painstakingly worked through some of the critical issues and problems associated with applying this pioneering mode of therapy for CF. As a result, and with a significant financial investment by the British Trust, the most comprehensive gene therapy clinical trial process is underway in the British Isles.

More recently, clinical trial networks have been established throughout North America and Europe to facilitate the evaluation of new clinical entities in order to hasten the regulatory process leading to drug approval. These clinical trial networks are linked through the sharing of data, expertise, and experience to contribute to the worldwide clinical trial efforts.

These are just a few examples of the willingness of the CF community to make strategic investments, some of which, in the case of other diseases, would be taken by industry or the government to bring us closer to accomplishing our mission.

- *Willingness to accept responsibility for the coordinating role in the areas of care, teaching, and research*: In many countries, scientists and clinicians look to the government for funding – agencies like the US National Institutes of Health and other equivalent organizations. However, not only is the science of CF frequently funded by CF organizations, but its direction is often defined by these entities as well. Similarly, the outstanding care that is provided all over the world is driven by the standards and guidelines set by these universal CF organizations. These guidelines are commonly established in international forums sponsored by CF groups. Once again, the community looks to CF organizations for leadership.

These volumes are the result of a distinct and worldwide undertaking. The environment, funding, and culture that have been put in place by patient organizations, coupled with the ability to bring the best minds to the field of CF, have made the science described in this book possible. This publication will be a useful tool as we continue to translate the knowledge acquired during decades of basic research to the development of new therapies that will modify and change the course of the disease in CF patients in the years ahead. *Cystic Fibrosis: Diagnosis and Protocols* is the fulfillment of decades of hard work by the volunteers and staff of the patient groups and organizations that have helped to pave the way toward our ultimate goal: a cure and control for cystic fibrosis.

President and Chief Executive Officer
Cystic Fibrosis Foundation

Robert J. Beall

Preface

More than 20 years have passed since the identification of the gene responsible for cystic fibrosis (CF) and undoubtedly many milestones have highlighted this area of research. But we have to admit it, progress towards finding a way of curing the disease has been slower than we initially expected and wished.

Apparently, this is not due to a lack of research efforts in the field, since in recent years the CF research community has been producing on average ~1,500 papers annually. So, probably we still need to dig deeper and with better tools to understand further the basic biological mechanisms underlying this complex disease. Nevertheless, it is increasingly difficult to grasp and use the already wide and still growing range of diverse methods currently employed to study CF so as to understand it in its multidisciplinary nature.

The aim of these *Cystic Fibrosis: Diagnosis and Protocols* volumes is thus to provide the CF research community (and that in related fields) with a very wide range of high-quality experimental tools, as an easy way to grasp and use classical and novel methods applied to CF. Hence, it is expected that it will contribute to accelerate the advancement of knowledge in this area. The purpose is thus to offer selected "good practice protocols" with a level of technical detail which is rarely published in peer-reviewed journals. Moreover, it is expected that this information will also enable researchers to identify subtle differences regarding techniques in their own laboratories, which often account for apparently "contradictory" data in the literature. Co-authorship from both sides of the Atlantic was particularly encouraged.

In the 2002 edition of this volume and in another previous comprehensive compilation of *Methods for Cystic Fibrosis and CFTR Research*[1], a large set of classical techniques used for CF research were already covered. So, here the focus is placed on innovative methodologies (some revolutionizing our way of doing science) by describing in detail how to perform and exploit these emergent techniques applied to CF. Moreover, a complete section has been devoted to available resources such as useful software and databases, as well as cell lines and animal models, reviewed for their usefulness towards multiple purposes. Notwithstanding, the more "classical" methods can also undergo improvements and thus their most up-to-date and revised versions are also recapped here by the leading experts. All book sections are introduced by an overview discussing the applicability and practicality of the protocols with examples.

It is hoped that the methods presented and revised here will provide users with optimal working tools to address their pressing questions in the best technical way while helping all of us, as a research and clinical community, to move faster hand-in-hand towards unravelling the secrets of this (and possibly other) challenging disorder(s) and cure it.

Finally, we wish to thank all authors for their enthusiasm in joining us in this project by contributing with their best protocols to this book and also for their patience with

[1] Journal of Cystic Fibrosis (2004), volume 3 (Supplement 2), a special issue focused on "Methods for Cystic Fibrosis and CFTR Research" and The online "Virtual Repository of the Cystic Fibrosis European Network" at http://central.igc.gulbenkian.pt/cftr/vr/index.htm

our multiple requests. Special thanks to Renata Vincent for her help in dealing with the manuscripts. Moreover, we would like to express our gratitude to the whole CF community in general, researchers, clinicians and all caregivers and other professionals, not forgetting CF patients and their families, for their continuous efforts towards finding a way out of this still devastating disease. We believe that we will be there soon and we hope this book somehow contributes to getting there sooner. Then, when our goals are met, all efforts will have been worthwhile, or as the Portuguese poet Fernando Pessoa has put it, "All is worthwhile if the soul is not small".

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