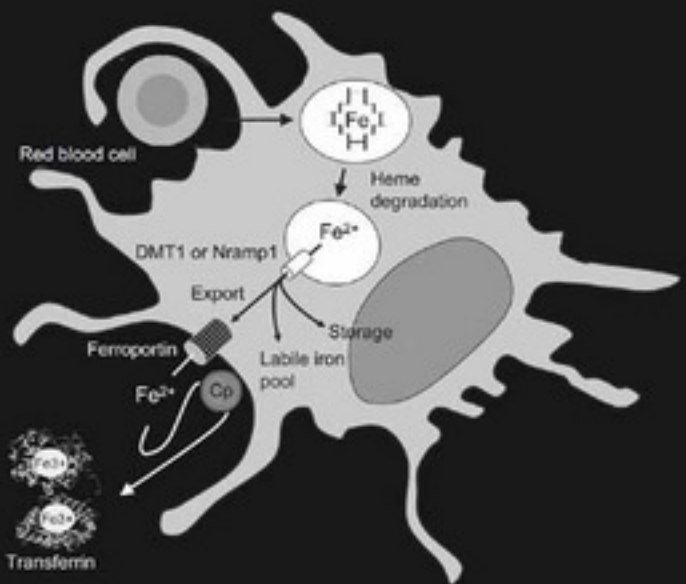
Milestones in Drug Therapy
Michael J. Parnham
Jacques Bruinvels
Series Editors

Erythropoietins, Erythropoietic Factors, and Erythropoiesis

Molecular, Cellular, Preclinical, and Clinical Biology

2nd Revised and Extended Edition

Steven G. Elliott
MaryAnn Foote
Graham Molineux
Editors



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MDT

Series Editors

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2nd Revised and Extended Edition

Edited by
Steven G. Elliott, MaryAnn Foote, and Graham Molineux

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Preface to the first edition

Research on, and interest in, red blood cell formation spans several centuries and was thought to have peaked in the 1980s with the cloning of the erythropoietin (EPO) gene. In the years subsequent to the cloning of EPO and its expression as a recombinant protein, much was written about EPO. Although much has been learned and published, new, exciting data are becoming available on almost a daily basis. *Erythropoietins and Erythropoiesis: Molecular, Cellular, Preclinical, and Clinical Biology* compiles both pertinent historical and very recent research on this molecule and its clinical utility.

The book is divided into two sections: Background and Basic Science and Clinical Uses of Recombinant Erythropoietins. To begin, Israels and Israels describe the biology of red cells, the hierarchy of erythropoietic progenitor cells, their development to mature cells, and the effects of endogenous EPO on their development. Foote summarizes the historical interest in, and search for, an erythropoietic factor. Once EPO was identified, cloned, and expressed, the path was set for the study of other aspects of EPO biology both within erythropoiesis and other cellular systems.

The structures of recombinant human erythropoietin (rHuEPO) and its receptor (EPOR) have been studied and modeled using X-ray crystallography and other techniques, and the chapter by Osslund provides three-dimensional structural information. Activation of EPOR by EPO is essential for the survival, proliferation, and differentiation of red blood cells. EPOR is also expressed in many organs, including the brain, heart, endothelium, and ovaries, and may have physiological roles in these organs. Although studies are underway to establish the role of EPOR signaling in various organs, it is becoming increasingly apparent that red cells are not the only targets of EPO. Dame provides further data on the effects of both endogenous EPO and rHuEPO on hematologic and non-hematologic tissues. EPO has long been known to have a direct effect on the formation of red blood cells, and more recent work suggests that it may have a myriad of diverse effects that may allow the use of rHuEPO in clinical settings of neurological, cardiac, neonatal care, and as well as in other settings. Heatherington presents detailed information on the pharmacokinetics of EPO and rHuEPO in various patient populations. The pharmacokinetic properties of rHuEPO are some of the major factors that determine dosing regimens and mode of administration, and this literature review offers extensive information. Molineux reviews the basic biology of EPO and rHuEPO. The effects of treatment with rHuEPO are wide-ranging, especially in patients with degenerating kidney performance, suggesting effects beyond mere replacement of the missing endogenous EPO.

This section ends with a description by Chuck et al. of the production techniques for rHuEPO, from establishment of a cell bank to purification of the final clinical product.

Several clinical settings are discussed in detail, including treatment of the anemias of nephrology (Macdougall), oncology (Glaspy), and chronic diseases (Means) and also use in surgery (Cushner). Glaspy discusses the design of clinical trials using rHuEPO and offers insight into some published clinical data with rHuEPO and darbepoetin alfa, an erythropoiesis-stimulating protein that persists in the circulation three times longer than rHuEPO. The anemias of chronic diseases are among the most common syndromes in clinical medicine, but they often are under-recognized and under-treated. Clinical studies have demonstrated the efficacy of rHuEPO in the management of anemias of chronic diseases and have established a role for iron therapy as an adjunct to rHuEPO in this syndrome. Blood loss is inherent to the surgical setting. Using the orthopedic surgery model, Cushner focuses on the use of rHuEPO not only to decrease allogeneic transfusions but also to maximize blood parameters, such as hematocrit and hemoglobin concentration, during the peri-operative period.

Because rHuEPO is so effective in stimulating production of red blood cells, it has the potential for abuse by athletes, particularly Olympic athletes in endurance sports, such as running, cycling, and cross-country skiing. Catlin et al. describe how laboratories test for the illegal use of rHuEPO and how athletes are monitored and charged.

In 2001 and 2002, numerous cases of pure red cell aplasia, a rare but potentially life-threatening condition, were suddenly reported to health authorities worldwide. These cases of pure red cell aplasia were first noted in patients with kidney disease who were receiving rHuEPO. Mayeux and Casadevall describe this adverse event and how they test patients for the presence of anti-EPO antibodies, and they provide a possible explanation for the occurrence of pure red cell aplasia.

Finally, Elliott discusses current approaches in construction of EPO analogs to stabilize or increase activity, chemical modification of rHuEPO, gene delivery, and development of slow-release formulations. One successful strategy discussed is glycoengineering of rHuEPO, which involves construction of glycosylation analogs with increased content of sialic acid-containing carbohydrate. One such glycoengineered molecule, darbepoetin alfa, has been approved for marketing in the United States, the European Union, Australia, and Canada. Clearly, more than 20 years after the initial identification, isolation, cloning, and expression of the gene for EPO, research continues.

This book contains much information on erythropoiesis and red blood cell production, its regulation, and areas of continued or possible research, and is a resource for new and veteran researchers. Since different perspectives allow readers to arrive at their own conclusions and serve to stimulate scientific thought, we have not removed areas of controversy or overlap among chapters. We hope that this book proves useful and we invite your comments.

We have tried to acquire the necessary permissions and authorizations before publication, and great care has been taken in the preparation of the chapters. Nevertheless, errors cannot always be avoided. The editors and publishers, therefore, cannot accept responsibility for any errors or omissions that have inadvertently occurred. The views and opinions expressed in the book are those of the participating individuals and do not necessarily reflect the views of the editors, the publisher, Amgen Inc., or any other manufacturer of pharmaceutical products named herein. The package insert should be consulted before administration of any pharmaceutical product.

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Steven G. Elliott, PhD

Thousand Oaks, California
February 2003

Preface to the second edition

Cloning of the erythropoietin (EPO) gene was accomplished in the 1980s, allowing a breakthrough in the treatment of anemia. Now more than 2 decades later, important new understandings on erythropoiesis and EPO are revealed as a consequence of continued research in this area. The first edition of this book provided a review of the field in 2003. This new edition continues the examination of this branch of learning with new information, updates, and changes.

To begin, Torbett and Friedman provide an overview of both normal and abnormal erythropoiesis as a basis for understanding various disease states and the role and action of recombinant erythropoiesis-stimulating agents (ESA). Mole and Ratcliffe discuss the production of endogenous EPO by defining *cis*-acting regulatory sequences that control *EPO* gene expression. The biology of EPO is covered in the chapter by Molineux and Sinclair. Prchal and Gregg have provided a new chapter for this edition concerning genetic abnormalities in erythropoiesis, and Constantinescu has written a new chapter on the mechanism of EPO receptor activation. Another new chapter was provided by Doshi, Perez-Ruixo, Jang, and Chow that contains updated information on the pharmacokinetics and clearance of ESA.

In the clinical section of the book, the use and abuse of ESA is described. Catlin and Hatton provide an update on the abuse of ESA by athletes. Goodkin has written a new chapter on the use of ESA in the setting of renal disease. Arvedson and Sasu have contributed a new chapter that discusses the roles iron and regulation of iron metabolism in both normal erythropoiesis and disease states. Another new chapter is by Jelkmann, Depping, and Metzen on the non-hematopoietic effects of ESA.

As with the first edition, we have allowed different perspectives and some overlap of material to allow readers to arrive at their own conclusions and to stimulate scientific thought and discussion. The views and opinions expressed in the book are those of the participating individuals and do not necessarily reflect the views of the editors, the publisher, Amgen Inc., or any other manufacturer of pharmaceutical products named herein. We have tried to acquire the necessary permissions and authorizations before publication, and care has been exercised in the preparation of the chapters. Nevertheless, errors cannot always be avoided. The editors and publishers, therefore, cannot accept responsibility for any errors or omissions that have inadvertently occurred.

The book is a review of ESA which are widely used in the treatment of anemia. The views and information contained herein are not meant to be a guide in the use of these agents. Instead, the package insert should be consulted before administration of ESA or any other pharmaceutical products.

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Graham Molineux, PhD

Thousand Oaks, California
November 2008

Abbreviations

ADM	adrenalmedulin
AGM	aorta-gonad-mesonephros
AIDS	acquired immunodeficiency syndrome
Akt	protein kinase B
AMPK	AMP kinase
ANP	atrial naturetic peptide
ARNT	aryl hydrocarbon nuclear receptor translocator
ASGR	asialoglycoprotein receptor
ATM	ataxia telangiectasia mutant
2,3-BPG	2, 3 biphosphoglycerate
B-CLL	B-cell chronic lymphocytic leukemia
bHLH	basic-helix-loop-helix
BFU–E	burst-forming unit–erythroid
BPA	burst-promoting activities
BTG-1	B-cell translocation gene 1
CAD	*C*-terminal activation domain
CAIX	carbonic anhydrase-9
CAS	Court of Arbitration for Sport
CEPO	carbamoylated EPO
CFU–E	colony-forming unit–erythroid
CFU–GEMM	colony-forming unit–granulocyte, erythroid, macrophage, megakaryocte
CHMP	Committee for Medicinal Products for Human Use
CHO	Chinese hamster ovary
CHr	mean corpuscular hemoglobin
CKD	chronic kidney disease
CP	Chuvash polycythemia
CR1	complement receptor 1
CSF	cerebrospinal fluid
DFO	desferrioximine
DHFR	dihydrofolate reductase
DOPPS	Dialysis Outcomes and Practice Pattern Study
DMT1	divalent metal transporter 1
DcytB	duodenal cytochrome b
eEPO	endogenous EPO
EMEA	European Medicines Evaluation Agency
EMP	EPO mimetic peptide
eNOS	endothelial nitric-oxide synthase

EPAR	European Public Assessment Report
EPO	erythropoietin
EPOR	erythropoietin receptor
ERK	extracellular signal-regulated kinase
EryP-CFC	primitive erythroid colony-forming cell
ESA	erythropoiesis-stimulating agent
ET	essential thrombocythemia
EU	European Union
Fab	fragment of antibody
FGF	fibroblast growth factor
FIH-1	factor-inhibiting HIF-1
FIS	International Ski Federation
FOB	follow-on biologics
FRET	fluorescence resonance energy transfer
Gas6 protein	growth arrest-specific 6 protein
GC-MS	gas chromatography-mass spectrometry
G-CSF	granulocyte colony-stimulating factor
GFR	glomerular filtration rate
GLUT	glucose transporter
GMP	Good Manufacturing Processes
HCP	hematopoietic cell phosphatase
HCP-1	heme carrier protein-1
HIF	hypoxia inducible factor
HNF	hepatic nuclear factor
HO-1	hemoxygenase-1
HRE	hypoxia-responsive element
HUVEC	human umbilical vein endothelial cell
IEF	isoelectric focusing
IFN	interferon
IL	interleukin
INN	International Nonproperity Name
IOC	International Olympic Committee
iPAS	inhibitory PAS
IRS	insulin receptor substrate
IU	international unit
JAK2	Janus-type tyrosine kinase
JAK-STAT	Janus kinase/signal transducers and activators of transcription
JH	JAK homology
kDa	kilodalton
KDOQI	Kidney Disease Outcomes Quality Initiative
LC-MS-MS	liquid chromatography coupled to tandem mass spectrometry
LDHA	lactate dehydrogenase A
LPS	lipopolysacharride
MALDI	matrix-assisted laser desorption/ionisation
MAPK	Ras/mitogen-activated kinase