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

Graham Molineux
MaryAnn Foote
Tara Arvedson *Editors*

Twenty Years of G-CSF

Clinical and Nonclinical Discoveries

Springer

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Preface

On April 25, 1953, 58 years ago, JD Watson and FHC Crick published their article entitled "A Structure for Deoxyribose Nucleic Acid" in the journal *Nature*. This article has been cited for its brevity, only 1 page and 1 diagram. The impact of this article cannot be fully measured, but it is safe to suggest that recombinant DNA biopharmaceuticals, such as recombinant human granulocyte colony-stimulating factor (rmet-HuG-CSF), would not be available today without the basic knowledge of DNA structure.

A quick search of PubMed suggests that no articles had been published on the topic of rmet-HuG-CSF or even G-CSF as of 1953. Forward to April 2011 and a quick search of PubMed cites 31,965 articles tagged to "G-CSF," 1,753 tagged to "filgrastim," 350 tagged to "pegfilgrastim," 295 tagged to "lenograstim," and 13 tagged to "biosimilar filgrastim."

We have come a long way in 58 years since the publication of the proposed structure of DNA and further since the first approval of filgrastim by the US Food and Drug Administration in 1991 for the treatment of patients with chemotherapy-induced neutropenia. In the intervening 20 years since this first marketing approval, countless patients worldwide have been treated with a recombinant form of G-CSF for the treatment of chemotherapy-induced neutropenia; severe chronic neutropenia; neutropenia due to disease; to mobilize peripheral blood stem cells for transplantation, either autologous or allogenic; and for bone marrow recovery after bone marrow or stem cell transplantation, to name a few. rmet-HuG-CSF has been tried in the treatment of infections, diabetic foot ulcers, neonatal sepsis, and community-acquired pneumonia.

In almost all settings, it can be said that rmet-HuG-CSF ameliorated neutropenia, increased neutrophil counts, reduced the need for intravenous antibiotics, and/or reduced the need or duration for hospitalization. Thus, it is appropriate to celebrate 20 years of research and therapy with rmet-HuG-CSF.

The authors of several chapters are some of the early clinical investigators of rmet-HuG-CSF and staff of Amgen, which manufactures filgrastim and pegfilgrastim. The editors have allowed information in chapters to provide various

perspectives on topics. We are hopeful that readers will find the presentations varied but balanced.

The editors have tried to obtain the necessary permissions and authorizations before publication, and great care has been exercised in the preparation of this volume. Nevertheless, errors cannot always be avoided. The editors, their employers or companies, and the publisher cannot accept responsibility for any errors or omissions that inadvertently occurred. The views and opinions expressed in the book are those of the participating individuals and do not reflect the views of the editors, the publisher, Amgen Inc., or any other manufacturer of pharmaceutical products named herein. The current package insert should be consulted before any pharmaceutical product is administered.

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Glossary and Abbreviations

A

ACE	Angiotensin-converting enzyme
ADCC	Antibody-dependent cell-mediated cytotoxicity
ADSCN	Autosomal dominant severe congenital neutropenia
aGVHD	Acute graft-versus-host disease
AIDS	Acquired immunodeficiency syndrome
ALDH	Aldehyde dehydrogenase
ALL	Acute lymphocytic leukemia/acute lymphoblastic leukemia
alloHCT	Allogeneic hematopoietic cell transplantation
AML	Acute myeloid leukemia
ANC	Absolute neutrophil count
Ang	Angiopoietin
APP	Amyloid precursor protein
Ara-C	Chemotherapy with idarubicin/daunorubicin and cytosine arabinocide
ARDS	Acute respiratory distress syndrome
ASCO	American Society of Clinical Oncology
ASH	American Society of Hematology
AUC	Area under the curve
AuSCT	Autologous stem cell transplantation

B

B-CLL	B-cell chronic lymphocytic leukemia
BFU-E	Erthyroid blast-forming units

C

CAE	Chemotherapy regimen of cyclophosphamide, doxorubicin, and etoposide
CAFC	Cobblestone area forming cells
CALGB	Cancer and Leukemia Group B
CDC	Centers for Disease Control and Prevention complement-dependent cytotoxicity
CFC	Colony-forming cell
CFU-C	Cell colony-forming unit
CFU-G	Granulocyte progenitor cell
CFU-GEMM	Granulocyte–erythrocyte–monocyte–macrophage progenitor cell
CFU-GM	Granulocyte–macrophage progenitor cell
cGVHD	Chronic GVHD
CHOEP	Chemotherapy regimen of cyclophosphamide, doxorubicin, vincristine, etoposide, and prednisone
CHOP	Chemotherapy regimen of cyclophosphamide, doxorubicin, vincristine, and prednisone
CHR	Cytokine-binding homology region
CIN	Chronic idiopathic neutropenia
CLL	Chronic lymphocytic leukemia
CML	Chronic myeloid leukemia
CMML	Chronic myelomonocytic leukemia
CMV	Cytomegalovirus
CNOP	Chemotherapy with cyclophosphamide, mitoxantrone, vincristine, and prednisone
CNTF	Ciliary neurotrophic factor
COR	Circulating opsonin receptor
CRH	Cytokine receptor homologous
CSF	Colony-stimulating factor
CSF-1	Another name for M-CSF
CSF-2	Another name for GM-CSF
CSF-3	Another name for G-CSF
CT	Computed tomography

E

ECOG	Eastern Cooperative Oncology Group
EBMT	European Group for Blood and Marrow Transplantation
ECOG	Eastern Cooperative Oncology Group
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EORTC	European Organization for Research and Treatment of Cancer

eNOS	Endothelial nitric oxide synthase
EPO	Erythropoietin
EPOR	Erythropoietin receptor
ESA	Erythropoiesis-stimulating agent

F

5-FU	5-flurouracil
FAB	French-American-British
FACS	Fluorescence-activated cell sorting
FDA	Food and Drug Administration
FEC	Chemotherapy with flurouracil, epirubicin, and cyclophosphamide
FGF	Fibroblast growth factor
FIV	Feline leukemia virus
FL	Flt3 ligand
fMLP	*N*-formyl-methionyl-leucyl-phenylalanine
FNIII	Fibronectin type III-like

G

GALT	Gut-associated lymphoid tissue
G-CSF	Granulocyte colony-stimulating factor
G-CSFR	Granulocyte colony-stimulating factor receptor
GFP	Green fluorescent protein
Gfi-1	Growth factor independence-1
GH	Growth hormone
GHR	Growth hormone receptor
GIST	Gastrointestinal stromal tumor
GM-CSF	Granulocyte–macrophage colony-stimulating factor
GMP	Granulocyte–monocyte committed progenitors
GSD 1b	Glycogen storage disease 1b
GVHD	Graft-versus-host disease

H

HAART	Highly active antiretroviral therapy
H-ARS	Hematopoietic subsyndrome of acute radiation syndrome
HCP	Hematopoietic cell phosphatase
HGF	Hepatocyte growth factor
HIES	Hyperimmunoglobulin E syndrome

HIF-1α Hypoxia inducible factor-1α
HIV Human immunodeficiency virus
HPC Hematopoietic progenitor cell
HPLC High-pressure liquid chromatography
HR Hazard ratio
HSC Hematopoietic stem cell
HSPC Hematopoietic stem/progenitor cells

I

ICAM Intercellular adhesion molecule
ICER Incremental cost effectiveness ratio
IFN Interferon
Ig Immunoglobulin
IL Interleukin
IL-1ra IL-1 receptor antagonist
iNOS Inducible nitric oxide synthase
IPSS International Prognostic Scoring System
IST Immunosuppressive therapy

J

Jak Janus protein tyrosine kinases/Janus kinase

K

kDA Kilodalton

L

LD Lethal dose
LIF Leukemia inhibitory factor
LIFR Leukemia inhibitory factor receptor
LPS Lipopolysaccharide
LSC Leukemia stem cell
LTC-IC Long-term culture-initiating cells
LVEDV Left ventricular end-diastolic volume
LVESV Left ventricular end-systolic volume

LVEF Left ventricular ejection fraction
LYG Life year gained

M

MAI *Mycobacteria avium* infection
MAPK Mitogen-activated protein kinase
MCM Medical countermeasure
M-CSF Macrophage colony-stimulating factor
MDS myeLodysplastic syndromes
MDSC Myeloid-derived suppressor cell
MGDF Megakaryocyte growth and development factor
MIP Macrophage inflammatory protein
MMP Matrix metalloproteinase
MOR Maximum opsonin receptor
MRD Minimal residual disease
MT-1 MMP Membrane type-1 MMP
mTOR Mammalian target of rapamyicin
M-VAC Chemotherapy with methotrexate, vinblastine, doxorubicin, and cisplatin

N

NCCN National Comprehensive Cancer Network
NHL Non-Hodgkin's lymphoma
NIH National Institutes of Health
NK Natural killer
NMR Nuclear magnetic resonance
NSAA Nonsevere aplastic anemia
NSCLC Nonsmall-cell lung cancer

O

OSM Oncostatin M

P

PBCT Peripheral blood cell transplantation
PBMC Peripheral blood mononuclear cell

PBPC	Peripheral blood progenitor cell
PBSC	Peripheral blood stem cell
PCR	Polymerase chain reaction
PCI	Percutaneous coronary intervention
PD	Pharmacodynamics
PDGF	Platelet-derived growth factor
PDGFR	Platelet-derived growth factor receptor
PECAM-1	Platelet/endothelial cell adhesion molecule-1
PEG	Polyethylene glycol
PET	Positron emission tomography
PlGF	Placenta growth factor
PK	Pharmacokinetics
PMNL	Polymorphonuclear leukocyte
PPO	Pluripotent colony-stimulating factor
PRL	Prolactin
PRLR	Prolactin receptor
PS1	Presinilin 1

Q

QALD	Quality-adjusted life day
QALY	Quality-adjusted life year

R

RA	Refractory anemia
RAEB	Refractory anemia with excess blasts
RAEB-T	Refractory anemia with excess blasts in transformation
RARS	Refractory anemia with ringed sideroblasts
RES	Reticuloendothelial system
rHuEPO	Recombinant human erythropoietin
rHuCSF	Recombinant human colony-stimulating factor
rHuG-CSF	Recombinant human granulocyte colony-stimulating factor
rHuGM-CSF	Recombinant human granulocyte–macrophage colony-stimulating factor
rHuIL-3	Recombinant human interleukin-3
RIT	Radioimmunotherapy
RR	Risk ratio; relative risk
RTKI	Receptor tyrosine kinase inhibitors
RT-PCR	Reverse transcription polymerase chain reaction

S

S1P	Sphingosine-1-phosphate
SAA	Severe aplastic anemia
SCF	Stem cell factor
SCLC	Small-cell lung cancer
SCN	Severe chronic neutropenia
SCNIR	Severe Chronic Neutropenia International Registry
SCT	Stem cell transplantation
SDF	Stromal cell-derived factor
SDS	Shwachman Diamond syndrome
SIV	Simian immunodeficiency virus
SLE	Systemic lupus erythematosus
SNS	Strategic National Stockpile
SOCS	Suppressor of cytokine signaling
SoS	Son of sevenless
STAT	Signal transducers and activators of transcription
SWOG	SWOG

T

TAF	Tumor-associated fibroblast
TA-GVHD	Transfusion-associated graft-versus-host disease
TAM	Tumor-associated macrophage
TAN	Tumor-associated neutrophil
TBI	Total body irradiation
TEM	Tie2-expressing monocyte
TGF	Transforming growth factor
TKI	Tyrosine kinase inhibitor
TNF	Tumor necrosis factor
tPA	Tissue plasminogen activator
TPO	Thromobopoietin
TRAIL	TNF-related apoptosis-inducing ligand

V

VAPEC-B	Chemotherapy regimen of vincristine, doxorubicin, prednisolone, etoposide, cyclophosphamide, and bleomycin
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor

VICE	Chemotherapy regimen of vincristine, ifosfamide, carboplatin, and etoposide
vIL	Viral interleukin
VLA-4	Very late antigen-4
VSAA	Very SAA

W

WBC	White blood cell
WHO	World Health Organization
WT	Wildtype

Z

ZDV	Zudovidine

Part I
Basic Science

Hematopoiesis in 2010

George Morstyn

1 Brief History of Hematopoietic Growth Factors

In 1987, the first clinical results of the use of hematopoietic growth factors were presented at a small meeting in Garmish-Partenkirchen [1]. It is timely, 23 years later, to review what we have learned since that first report.

Donald Metcalf reviewed for the 50th Anniversary of the American Society of Hematology (ASH) our knowledge of the regulation of hematopoiesis by specific growth factors [2], and we have previously reviewed the important features of hematopoiesis: the cell hierarchy, the movement of cells from multipotential progenitors to mature, committed cells with specific functions, and the many cytokines that regulate the process [3]. It was possible to purify the regulators and obtain protein-sequence data for cloning of the hematopoietic growth factors because of the development of various biologic assays in the preceding 50 years and the development of recombinant DNA technology in the 1980s [2].

The regulator we knew most about was erythropoietin (EPO), initially as an activity detectable in the urine of patients with aplastic anemia. Until the cloning and expression of EPO and the development of an immunoassay, monitoring of red cell-stimulating activity was cumbersome, and radioactive iron incorporation into red blood cells was used. The assays that were used to measure granulocyte–macrophage progenitor cells were carried out on semisolid cultures that allowed the counting of colonies of mature cells produced from myeloid precursors [4]. The assays were later adapted to identify red cells, megakaryocytes, and even earlier precursors.

Early work with fluorescent-activated cell sorting (FACS) allowed the identification, morphologically and functionally, of these precursors, and it became

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apparent that the production of mature cells in the blood, the red cells and granulocytes, was dependent on the presence of specific regulators such as EPO, granulocyte colony-stimulating factor (G-CSF), and granulocyte–macrophage colony-stimulating factor (GM-CSF). It was not until the 1990s that the megakaryocyte regulator was identified. The role of these factors was slightly different in murine models compared with their role in humans, but many of the biologic findings were directly comparable between the species.

GM-CSF (also known as CSF2), macrophage colony-stimulating factor (M-CSF) (also known as CSF1), and G-CSF (also known as CSF3) were identified as growth factors for myeloid progenitor cells (reviewed in [5]). The cytokines stimulate the proliferation, differentiation, maturation, and survival of granulocytes and macrophages. The CSF acts through specific receptors. The G-CSF receptor (G-CSFR) is a member of the type-1 cytokine receptor family; the GM-CSF receptor consists of a unique α chain and a common β chain through which signaling occurs.

The control of platelet production is different to that of granulocytes and macrophages. Platelets form by the fragmentation of mature megakaryocytes. The production of megakaryocytes is under the control of the *c*-Mpl receptor, and its ligand was identified as thrombopoietin (TPO). TPO is the primary regulator of platelet production and elimination of either TPO or the *c*-Mpl receptor results in severe thrombocytopenia. Importantly, TPO does not seem to accelerate platelet shedding and so its actions are slower than that of G-CSF that acts on increasing not only the production of myeloid precursors but also their maturation. Both G-CSF and TPO blood concentrations appear to be reduced by the mass of mature cells; granulocytes, megakaryocytes, and platelets increase, respectively, and this provides a feedback loop for control. G-CSF, TPO, and EPO are critical to the maintenance of hematopoiesis, and knock-outs of the genes for the ligand or receptor lead to profound neutropenia, thrombocytopenia, or anemia [6].

EPO is a 34.4-KD glycoprotein hormone and was cloned in 1985. EPO is regulated by hypoxia. It acts on erythroid precursors to enhance red blood cell production and thus the oxygen-carrying capacity of the blood. EPO, which is produced predominantly in the kidney, is required for the production and terminal differentiation of red blood cells. Like G-CSF and GM-CSF, EPO controls proliferation, maturation, and survival of red blood cells. The receptor exists as a dimer and when the ligand binds, a conformational change and a cascade of activation occur through transphosphorylation of JAK2.

Controversy exists about where the EPO receptor (EpoR) is expressed and on what cell types it is functional. This controversy has become important in evaluating reported nonclinical and clinical effects on the central nervous system and the cardiovascular system, and explaining adverse outcomes in the cancer setting.

The actual regulation of hematopoiesis, the feedback loops, the role of a plethora of cytokines in maintaining homeostasis in the hematopoietic system, and then creating an appropriate response to perturbations, such as sepsis, requires a broad approach. The complexity that could be investigated was reviewed [7] in the context of a systems biology approach.

In the clinic, beginning in the late 1980s, we generally did not exploit the complexity of multiple overlapping activities of some of the factors, other regulators such as stem cell factor (*c*-kit ligand), M-CSF, interleukin (IL)-11, multicolony-stimulating factor (IL-3), and IL-6. These cytokines also entered clinical development but have not found broad utility.

In this chapter, I focus on the trials and tribulations of the development of 3 families of regulation: the erythropoiesis-stimulating agents (ESA), the G-CSF, and the thrombopoietic agents.

Don Metcalf pointed out the value of 50 years of laboratory research before the initiation of the clinical development of each of these factors. It is apparent, however, that despite an extensive knowledge of murine biology and in vitro human studies, there were many surprises in the clinic and, in some cases, issues not strictly scientific, such as economic and legal issues, also impacted on the development and use of these agents.

The theoretical challenges encountered during the development of the ESA, G-CSF (filgrastim and lenograstim), and thrombopoietic agents had both common and unique features. Each was a critical regulator of an important cell lineage. Therefore, questions were raised whether accelerated depletion of the bone marrow would occur with prolonged use. This situation did not occur. There was concern that the receptors for each factor would be present on malignant cells either of the hematopoietic systems, such as the myeloid leukemias or on other cancers, and that this situation could have had an adverse outcome due to undesirable tumor cell stimulation. There was also concern that neutralizing antibodies to the recombinantly produced proteins would cross-react with the normal endogenous regulators and result in single lineage or multi-lineage aplasia. There were also concerns that the rate of rise in mature cells such as neutrophils, red blood cells, or platelets would cause harm or that the absolute high numbers of these cells could be harmful. During the development of these agents, some of these potential adverse events did become apparent, however, sometimes only after the agents entered clinical practice, and their doses and target populations were greatly expanded.

In general, millions of patients have received the hematopoietic agents with significant reductions in morbidity and mortality, and improvements in quality of life. The first study that identified the theoretical concerns that could occur was a randomized study of recombinant human EPO (rHuEPO) in patients who were receiving dialysis and who had heart disease in whom the concept of achieving high hemoglobin concentration to improve cardiac function resulted in significant adverse events [8]. It was reported that targeting a normal hematocrit significantly increased the incidence of thromboses and that there were more deaths in patients treated to obtain a normal hematocrit target than in patients treated to obtain a lower hematocrit target.

A second concern was realized during the development of a TPO (megakaryocyte growth and development factor, MGDF) when normal volunteers developed neutralizing antibodies after two or more doses that cross-reacted with endogenous TPO to produce prolonged thrombocytopenia. Another example of the potential harm of neutralizing antibodies was the development in a small number of patients

receiving rHuEPO of pure red cell aplasia due to the development of cross-reactive neutralizing antibodies to endogenous EPO [9].

The concern about off-target stimulation of malignancies took longer to emerge. Large randomized studies in patients with cancer did appear to show in some studies poorer cancer outcomes – but the studies were not always well designed and were not stratified.

At the same time as the therapeutic window was narrowed, positive developments occurred including more convenient forms of rHuG-CSF (pegfilgrastim) and an ESA (darbepoetin alfa), and a new agent was developed that stimulated the TPO receptor but did not induce cross-reacting antibodies.

A new treatment paradigm, the use of peripheral blood progenitor cells (PBPC), was established and the risk of leukemia development did not appear to be significantly increased, although studies in severe chronic neutropenia and the myelodysplastic syndromes are still investigating the issue [10, 11]. In parallel to these developments, some of the clinical indications were expanded.

Not only did we learn the limits of the therapeutic agents, but the clinical settings also evolved. In oncology, the paradigm of using chemotherapeutic drugs to maximum tolerability thus causing the neutropenic complications reduced by rHuG-CSF was challenged. Guidelines appeared, although initially on the appropriate use of growth factors rather than the chemotherapy regimes (reviewed in chapter "Practice Guidelines for the Use of rHuG-CSF in an Oncology Setting" by Saraf and Ozer). The issue of cost benefits, cost offsets, and reimbursement dominated the development of the granulocyte-stimulating factors. Reimbursement also became important in determining the use of ESA and iron-replacement therapy, and this issue again led to guidelines that were modified as data emerged.

More recently, the cytokine area has attracted the development of biosimilars and discussion about whether given the challenges that have been identified during the development of cytokines, can other agents be approved without substantive clinical experience. I briefly discuss what we have learned about each of these agents.

2 Erythropoiesis-Stimulating Agents

Administration of rHuEPO is effective in increasing red blood cell counts. Anemic patients develop high concentrations of measurable endogenous EPO if they do not have renal disease, but in patients with renal failure or with malignancies, there can be inappropriately low amounts of endogenous EPO.

The first clinical use of rHuEPO was in patients with anemia who were relatively deficient in endogenous EPO due to renal disease. In early clinical trials of rHuEPO in patients with renal disease, there was a rapid reversal of the anemia, and although formal quality-of-life measurements were often not incorporated into the earliest studies, it seemed clear that patients developed improved states of well-being when their red blood cell counts recovered.

The increase in hemoglobin was observed in the first patients treated, and the agent was rapidly incorporated into therapy. Issues that arose included adverse effects such as thrombosis and hypertension in early studies, but were not perceived to be at a higher frequency than in control patients. It was also noted that patients needed to be replete with iron before the full effects of ESA were manifest.

After incorporation into therapy for renal disease, the anemia of cancer became a target for therapy. Initially, there was focus on patients who were receiving nephrotoxic chemotherapy such as cisplatin, but subsequently it was thought that patients with cancer who were receiving chemotherapy might have inappropriately low amounts of endogenous EPO for the degree of their anemia, and therapy with ESA was initiated to obviate the need for blood transfusions and also to improve quality of life.

The use of ESA became more complicated. There was much effort in trying to define optimal hemoglobin targets in both anemia of renal failure and anemia associated with cancer and cancer chemotherapy. It was suggested that higher hemoglobin concentrations could lead to a reduction in complications in the cardiovascular system of patients with chronic renal failure and in pre-dialysis patients. In addition, in oncology, the aim moved from preventing the need for red cell transfusions to improving the well-being of patients.

These studies led to an increase in the expenditure on ESA, particularly in the USA. An unexpected finding of the larger randomized studies, however, was that targeting a higher hemoglobin concentration seemed to lead to excess deaths. The phenomenon did not seem to depend on the level of hemoglobin reached but the increased dosing of ESA to reach the target. The basis for this remains unclear. There may also be a relationship between toxicity and the rate of rise in hemoglobin. Treatment guidelines and label warnings were adjusted for these findings [12]. In parallel, a new form of ESA which had additional glycosylation (darbepoetin alfa) was developed to reduce the frequency of dosing needed with rHuEPO and to improve convenience.

What began as a relatively clear benefit to anemic patients became much more complicated, and our assumptions about risk benefit had to be reviewed [12, 13]. It now seems that we have found the edges of the therapeutic window with attempts to normalize hemoglobin concentrations in pre-dialysis and dialysis patients, leading to increased adverse events and even mortality [8]. In the oncology setting, sometimes non-stratified randomized clinical trials have led to data suggesting reduced survival and loss of local cancer control. These findings were unexpected and have led to controversy about whether EPO receptors are present and functional on cancer cells and endothelium, and whether EPO acts directly on these cells to stimulate cancer growth [14, 15]. Others have suggested that while mRNA for the EpoR can be identified, the receptors are not functional [16].

Another unexpected aspect of the EPO story was its use in blood doping by cyclists to increase their red cell concentrations and endurance. In an episode in Europe, certain vials appeared to lead to immunogenicity due to the development of neutralizing antibodies and pure red cell aplasia in patients who received rHuEPO from this batch [9, 17]. This episode is often thought of in the context of quality

control for biosimilar drug [18] development, particularly for agents that are glycosylated. A new agent has been developed that can stimulate the receptor but does not cross-react with neutralizing antibodies [19, 20].

The development of rHuEPO and ESA has taught us a great deal about how an agent that has been studied extensively non-clinically and for which there is a direct pharmacodynamic marker can lead to surprises when adopted broadly in clinical practice, and the need for appropriately designed phase 4 trials [21–23].

3 Granulocyte Colony-Stimulating Factors

The story of G-CSF has some similarities. The human molecule was first purified and cloned by a group in the USA (reviewed in chapter "Discovery of G-CSF and Early Clinical Studies" by Welte). It was not clear whether rHuG-CSF or rHuGM-CSF would prove more useful. In the mouse, rHuGM-CSF appeared to produce higher peripheral blood counts than rHuG-CSF; however, from the earliest clinical studies of rHuG-CSF [24–27], it was clear that rHuG-CSF produced significant increase in neutrophil counts and was well tolerated. Nonclinical studies suggested that rHuG-CSF could be used in patients including those with severe congenital neutropenia and those who had chemotherapy-induced neutropenia. Another application that was considered was in patients with normal neutrophil values who had sepsis and who might benefit from improved neutrophil function or higher neutrophil counts. A special setting that was also investigated was HIV-related infection and therapy that often led to neutropenia. In parallel to rHuG-CSF development, rHuGM-CSF was cloned and tested in the clinic, but will not be further discussed. Both agents were approved and incorporated into practice.

The early studies with rHuG-CSF produced some surprises [28]. The findings included that rHuG-CSF produced a transient decrease in circulating neutrophils in the first few minutes after injection, presumably due to tissue entry, and that the neutrophils were available to the tissue [26, 29]. The neutrophils were "left shifted" and rHuG-CSF not only stimulated production but also accelerated maturation. Studies also showed no change in frequency of progenitor cells in the bone marrow, but very rapid mobilization into the periphery [30]. The latter observation led to the practical widespread application of PBPC transplantation [31, 32; reviewed in chapter "Use of rHuG-CSF in Peripheral Blood Progenitor Cell Transplantation" by Beligaswatte et al.]. The basis for the mobilization is now better understood as disruption of the interactions between adhesion molecules and their ligands [33, 34].

The next set of agents to enter the clinic in 1986 was rHuG-CSF, rHuGM-CSF, and more recently, a pegylated form of rHuG-CSF (pegfilgrastim). The first indications that were approved were in the reduction of the infection complication of chemotherapy and as a consequence, the use of rHuG-CSF to intensify the doses of chemotherapy. These studies are reviewed extensively elsewhere. It was clear that in every setting, rHuG-CSF reduced the duration of neutropenia and the risk of febrile neutropenia by 40–50% [28].