Experimental Hematology Today-1989

N.C. Gorin L. Douay Editors

Experimental Hematology. Today—1989

Selected Papers from the 18th Annual Meeting of the International Society for Experimental Hematology, July 16-20, 1989, Paris, France

With 57 Illustrations



the major developing on the constitution of

Springer-Verlag New York Berlin Heidelberg London Paris Tokyo Hong Kong

plike the New Set oneside, prints

this notice for A NEO making a lighter black of the Set of the S

nd it has a state of the state

Norbert C. Gorin, M.D.

Department of Hematology, Saint-Antoine Hospital, 184, rue du Faubourg Saint-Antoine, 75571 Paris, France

Luc Douay, M.D.

Department of Hematology, Saint-Antoine Hospital, 184, rue du Faubourg Saint-Antoine, 75571 Paris, France

国对学规的特殊

* AC min

Library of Congress number 79-641222.

Printed on acid-free paper.

©1990 Springer-Verlag New York Inc.

All rights reserved. This work may not be translated or copied in whole or in part without the written permission of the publisher (Springer-Verlag New York, Inc., 175 Fifth Avenue, New York, NY 10010, USA), except for brief excerpts in connection with reviews or scholarly analysis. Use in connection with any form of information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed is forbidden.

The use of general descriptive names, trade names, trademarks, etc., in this publication, even if the former are not especially identified, is not to be taken as a sign that such names, as understood by the

Trade Marks and Merchandise Act, may accordingly be used freely by anyone.

While the advice and information in this book is believed to be true and accurate at the date of going to press, neither the authors nor the editors nor the publisher can accept any legal responsibility for any errors or omissions that may be made. The publisher makes no warranty, express or implied, with respect to the material contained herein.

Permission to photocopy for internal or personal use, or the internal or personal use of specific clients, is granted by Springer-Verlag New York, Inc. for libraries registered with the Copyright Clearance Center (CCC), provided that the base fee of \$0.00 per copy, plus \$0.20 per page is paid directly to CCC, 21 Congress St., Salem, MA 01970, USA. Special requests should be addressed directly to Springer-Verlag New York, 175 Fifth Avenue, New York, NY 10010, USA.

to the fire Tubus or Paris, the

Apparational NUTS adds

Camera-ready copy provided by the editors.

Printed and bound by Edwards Brothers, Ann Arbor, Michigan.

Printed in the United States of America.

9 8 7 6 5 4 3 2 1

ISBN 0-387-97246-3 Springer-Verlag New York Berlin Heidelberg ISBN 3-540-97246-3 Springer-Verlag Berlin Heidelberg New York

Preface

What they had bligg worder. ties, engr. samuel

A. O. Mark --

or supprove and have the beauty

The State Stores and the ing ground can avoid with the nest revine a repuil a rough requi The property of the state of

ne stor out on with the six and streeting eraning the option on the decision round producthe account , enoughly the menutement-bility of new motor designs. Prototypes rindurgo riguroria efectività i nete cere provinci memo evitto lunos bellet, then al anno you the day in the flat steep of meadling

material come, and ording the beg 8 O State was on first homero, protes nest continue of tricker the acognized insended sestion rempetable with it the

not dome deviceing configurati. We are given and the configuration of an important with a configuration of the con

the contract of name of any configurations and a volugios such re-retary, erripressating successor is an enretage Smith for the

to the train the second to the first 14.17 + 123.00 hopers

The 18th annual meeting of the International Society for Experimental Hematology took place in Paris on July 16-20, 1989. A thousand participants attended and 589 papers were presented, a record over the previous years. Experimental Hematology Today - 1989 cannot be considered as fully representative either of the meeting or even of the status of research in our field. It only represents a subjective selection of a few papers taken from a large mass of outstanding contributions.

Manuscripts in this book have been chosen from the group that received the highest score from the scientific committee and finally selected by the editors of the book. The book is divided in 4 parts:

Present aspects of transplantation of stem cells Control of hemopoiesis Hemopoiesis in malignancies From molecular biology to gene transfer.

The present yearbook of experimental hematology somehow is a testimony of the activity of our society, and I believe that it has a place of value in all scientific libraries.

> N.C. Gorin Chairman of the ISEH '89 Meeting

to their board part on the offer, and feeling are as a seems enoting, apportunity of that

to whe be adapted to me what time other pants. The PMS represents another step into the 21 concerns a subsent to pre-

and designation of the samp eyeter. the state of the state of the state of

district the factors of the factors

Francisey Compa decision and an information of the control of the

12/81/02/

whereberg the fant a

THE REAL PROPERTY.

pre

Sant Age

Mibular Design:

Afterna Tar To Cost . Locates Hole West ! Allers San Service Violation Williams Design Range

hece has intraduced a total fee once assurance project

For Intermation Please and

this billing matter ty to the fire the wife of a color

Strate at the second

Contributors

Maria-Alesandra Allieri, M.D., Unit for Research on Hematopoietic Stem Cell Grafts, Saint-Antoine University Hospital Center, Paris, France.

Thomas Course M.D. Instanta for Molecular Denetics. Normal Hughers.

the way of the control of the contro

to add the second of the secon

The company of the state of the

the state of the s

Marie and Louis Court build and account work

cal lostique Perior Conserve Medition Propins Torse

des l'argange, of longe, standy ou longe, lagant en

ABLE ORDEDS INT TO E PRODUCTION

- Frank D. Andrews III, M.D., University of Washington, Seattle, Washington, 98195, USA.
- James O. Armitage, M.D., Department of Internal Medicine, University of Nebraska Medical Center, Omaha, Nebraska 68105, USA.
- T.A. Bakx, Department of Gene Therapy, Radiobiological Institute TNO, Rijswijk, The Netherlands.
- Glenn C. Begley, M.D., Metabolism Branch, National Cancer Institute (NCI), National Institutes of Health (NIH), Bethesda, Maryland 20892, USA.
- John W. Belmont, M.D., Ph.D., Institute for Molecular Genetics and Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas 77030, USA.
- Rolande Berthier, M.D., DRF/Hematology Laboratory, INSERM, Center for Nuclear Studies, Grenoble, France.
- Matthias Bickel, Dr. med. dent., Division of Cytokine Biology, Center for Biologics Evaluation and Research (CBER), Food and Drug Administration (FDA), Bethesda, Maryland 20892, USA.
- E. Bouffet, M.D., Bone Marrow Transplant Unit, Centre Léon Bérard, Lyon, France.
- Marco Bregni, M.D., National Tumor Institute, Cristina Gandini Bone Marrow Transplant Unit, Milan, Italy.
- Rebecca H. Buckley, M.D., Department of Pediatrics, Duke University School of Medicine, Durham, South Carolina 77030, USA.

- Chele Son o

- C. Thomas Caskey, M.D., Institute for Molecular Genetics, Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas 77030, USA.
- Shigeru Chiba, M.D., The Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo, Japan.
- V. Combaret, M.D., Bone Marrow Transplant Unit, Centre Léon Bérard, Lyon, France.
- Denis Cournoyer, M.D., Institute for Molecular Genetics, Baylor College of Medicine, Houston, Texas 77030, USA.
- Karel A. Dicke, M.D., Ph.D., Department of Internal Medicine, University of Nebraska Medical Center, Omaha, Nebraska 68105, USA.
- Mary Jean Dicke-Evinger, Ph.D., Department of Pathology/Microbiology, University of Nebraska Medical Center, Omaha, Nebraska 68105, USA.
- Luc Douay, M.D., Associate Professor of Hematology, Formation Associée Claude Bernard, Unit for Research on Hematopoietic Stem Cell Grafts, Saint Antoine Hospital and Hematology Laboratory, Trousseau Hospital, Paris, France.
- Alain Duperray, Ph.D., DRF/Hematology Laboratory, INSERM, Center for Nuclear Studies, Grenoble, France.
- Marie Favrot, M.D., Bone Marrow Transplant Unit, Centre Léon Bérard, Lyon, France.
- Dario Ferrero, M.D., Department of Medicine and Experimental Oncology, Division of Hematology, University of Turin, Turin, Italy.
- D. Floret, M.D., Edouard Herriot Hospital, Lyons, France.
- C.R. Franks, M.D., Eurocetus, Amsterdam, The Netherlands.
- Eugenio Gallo, M.D., Department of Medicine and Experimental Oncology, Division of Hematology, University of Turin, Turin, Italy.
- Alessandro M. Gianni, M.D., National Tumor Institute, Cristina Gandini Bone Marrow Transplant Unit, Milan, Italy.
- Norbert C. Gorin, M.D., Professor of Hematology, Saint-Antoine Hospital; Department of Hematology, Bone Marrow Transplantation Unit, Saint-Antoine University Hospital Center and National Blood Transfusion Institute; President, Working Party on Autologous Bone Marrow Transplantation of the European Bone Marrow Transplantation Group (EBMT), Paris, France.
- Masayuki Hino, M.D., The Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo, Japan.

Contributors

Toshio Kitamura, M.D., The Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo, Japan.

- Gerald Krystal, Ph.D., Department of Pathology, University of British Columbia, Vancouver, British Columbia, V5Z 1L3, Canada.
- A. Kukler, Department of Gene Therapy, Radiobiological Institute TNO, Rijswijk, The Netherlands.
- Pierre M. Lehn, M.D., Bone Marrow Transplantation Unit, Saint-Louis Hospital, Paris, France.
- Manuel Lopez, Ph.D., INSERM, U76 and National Blood Transfusion Institute, Saint-Antoine Hospital, Paris, France.
- Gerard Marguerie, Ph.D., DRF/Hematology Laboratory, INSERM, Center for Nuclear Studies, Grenoble, France.
- Yasusada Miura, M.D., Division of Hematology, Department of Medicine, Jichi Medical School, Tochigi-ken, Japan.
- Kohei Miyazono, M.D., The Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo, Japan.
- Kateri Moore, D.V.M., Institute for Molecular Genetics, Baylor College of Medicine, Houston, Texas 77030.
- Alice L.-F. Mui, Terry Fox Laboratory, B.C. Cancer Research Centre, Vancouver, British Columbia, V5Z 1L3, Canada.
- Sudish C. Murthy, Ph.D., Terry Fox Laboratory, B.C. Cancer Research Centre, Vancouver, British Columbia, V5Z 1L3, Canada.
- Albert Najman, M.D., Laboratory for the Study of Hematopoiesis, Saint-Antoine University Hospital Center, Paris, France.
- Hiromitsu Nakauchi, M.D., Laboratory of Molecular Regulation of Aging, Frontier Research Program, The Institute of Physical and Chemical Research (RIKEN), Tsukuba, Japan.
- S. Negrier, M.D., Edouard Herriot Hospital, Lyon, France.
- John J. Nemunaitis, M.D., Department of Oncology, Fred Hutchinson Cancer Research Center, Seattle, Washington 98105, USA.
- Lan Nguyen, INSERM, U76 and National Blood Transfusion Institute, Saint-Antoine Hospital, Paris, France.
- Shin-ichi Nishikawa, M.D., Department of Immunopathology, Kumamoto University Medical School, Kumamoto, Japan.

xii Contributors

Jill M. O'Brien, B.S., Department of Pediatrics, Medical College of Wisconsin, Milwaukee, Wisconsin 53226, USA.

- Akira Ohara, M.D., Department of Pediatrics, Toho University School of Medicine, Tokyo, Japan.
- Seiji Okada, Division of Hematology, Department of Medicine, Jichi Medical School, Tochgi-ken, Japan.
- W.R. Paukovits, Department of Growth Regulation, Institute for Tumor Biology-Cancer Research, University of Vienna, Vienna, Austria.
- T. Philip, M.D., Bone Marrow Transplant Unit, Centre Léon Bérard, Lyon, France.
- Alessandro Pileri, M.D., Department of Medicine and Experimental Oncology, Division of Hematology, University of Turin, Turin, Italy.
- Dov Herbert Pluznik, Ph.D., Division of Cytokine Biology, Center for Biologics Evaluation and Research (CBER), Food and Drug Administration (FDA), Bethesda, Maryland 20892, USA.
- Stefan Ruhl, Dr. med. dent., Division of Cytokine Biology, Center for Biologics Evaluation and Research (CBER), Food and Drug Admir.is ration (FDA), Bethesda, Maryland 20892, USA.
- AND SHIP SHIP SHIP SHIP SHIPS SHIP Mie Rullian, M.D., Veterans Administration Medical Center, Seattle, Washington 98108, USA.
- Maurizio Scarpa, M.D., Institute for Molecular Genetics, Baylor College of Medicine, Houston, Texas 77030, USA.
- Kyoichi Shibuya, M.D., The Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo, Japan.
- Fritz Sieber, Ph.D., Department of Pediatrics, Medical College of Wisconsin, Milwaukee, Wisconsin 53226, USA.
- Salvatore Siena, M.D., National Tumor Institute, Cristina Gandini Bone Marrow Transplant Unit, Milan, Italy.
- Jack W. Singer, M.D., University of Washington, Seattle, Washington 98195; Veterans Administration Medical Center, Seattle, Washington 98108, USA.
- Nicole Smadja, M.D., Hematocancerologic Cytogenetics Laboratory, Saint-Antoine Hospital, Paris, France.
- Poul H.B. Sorensen, M.D., Ph.D., Terry Fox Laboratory, B.C. Cancer Research Centre, Vancouver, British Columbia, V5Z 1L3, Canada.
- Verneeda Spencer, M.D., Bone Marrow Transplantation Department, M.D. Anderson Cancer Center, Houston, Texas 77030, USA.

a shirth codar from the s

Halto of the Swelley and the

Contributors xiii

Jorge A. Spinolo, M.D., Bone Marrow Transplantation Department, M.D. Anderson Cancer Center, Houston, Texas 77030, USA.

Merchanic Charries

April Control Assembly

- Junko Suda, M.D., Department of Pediatrics, Josai Hospital, Yuki, Ibaraki-ken, Japan.
- Toshio Suda, M.D., Division of Hematology, Department of Medicine, Jichi Medical School, Tochigi-ken, Japan.
- Tetsuo Sudo, Biomaterial Research Institute Company, Ltd., Yokohama, Japan.
- Fumimaro Takaku, M.D., The Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo, Japan.
- Corrado Tarella, M.D., Department of Medicine and Experimental Oncology, Division of Hematology, University of Turin, Turin, Italy.
- Naomi Tokuyama, Division of Hematology, Department of Medicine, Jichi Medical School, Tochigi-ken, Japan.
- Alain Troesch, M.D., Hematology Laboratory, INSERM, Grenoble, France.
- Chris Tompkins, Veterans Administration Medical Center, Seattle, Washington 98108, USA.
- D. Valerio, Ph.D., Department of Gene Therapy, Radiobiological Institute TNO, Rijswijk, The Netherlands.
- D.W. van Bekkum, M.D., Ph.D., Radiobiological Institute TNO, Rijswijk, The Netherlands.
- V.W. Van Beusechem, Department of Gene Therapy, Radiobiological Institute TNO, Rijswijk, The Netherlands.
- A.J. Van der Eb, Ph.D., Department of Medical Biochemistry, State University of Leiden, Leiden, The Netherlands.

Contents of mel distribution from Hematopoleric Stem Cell Differentiation of the Bush of the Contents of the C

Contents

| 12 130 | The recommendation of the breakt remarks at the printed and a wall | 100 |
|--------------------------------------|--|---------|
| | 10 Biotynthesis and Processing of the Cytoudhesine, GPIIb/IIId - and Vitrongeren/Retrigion | |
| Preface. Contrib | A. Doesch, A. Deperrey, R. Bertster, and D. Marenerte. Services and D. Marenerte. story of the contract of t | v ix |
| Part I. | Present Aspects of Transplantation of Stem Cells | |
| n. J | The Role of Autologous Bone Marrow Transplantation in Acute Leukemia | |
| Garage Transport | Karel A. Dicke, M.J. Dicke-Evinger, J. Spinolo, and V. Spencer | 3 |
| Franck - 12 - 12/8 / 1974 - 14 | Photosensitizing Agents for Marrow Purging in Autologous Bone Marrow Transplantation | |
| Silmes O. | F. Sieber. | 10 |
| A. Bul | Autologous Bone Marrow Transplantation in Hodgkin Lymphoma James O. Armitage | 16 |
| 18 | 4 Circulating Hemopoietic Precursors after High-Dose Cyclophosphamide: Efficacy of In Vivo GM-CSF Administration D. Ferrero, C. Tarella, E. Gallo, M. Bregni, S. Siena, A. Pileri, and A.M. Gianni | 20 |
| | Merocyanine 540 Sensitized Photoinactivation of Enveloped Viruses and Its Application in the Sterilization of Blood Products J.M. O'Brien and F. Sieber | 26 |
| Part II. | Control of Hemopolesis | |
| E.A.Bost | 6 Interleukin-2 Receptors on Monocytes and Their Relation to the Process of Monocytic Cell Differentiation | 22 |
| | S. Ruhl, G.C.Begley, M. Bickel, and H. Pluznik | 33 |
| Tigan | 7 Properties of the Murine Interleukin-3 Receptor A.LF. Mui, P.H.B. Sorensen, S.C. Murthy, and G. Krystal | 38 |

Presence of Strongst Cells and Interlegisin-7 (IL-72) at the difference

errors Suda, A. Ohara, J. Suda, S. Okada, N. Tokuyama, Y. Misria, et al. 600, S. I. Visinkowa, curi H. Nakauchi

9 - Mairow Sciencel Cells, Hematopoietic Growth Forters and

Extracellular Matrix Proteins

| | Presence of Stromal Cells and Interleukin-7 (IL-7) | |
|--|--|------------|
| | T. Suda, A. Ohara, J. Suda, S. Okada, N. Tokuyama, Y. Miura, | |
| | T. Sudo, S.I. Nishikawa, and H. Nakauchi | 45 |
| Local Street | The second secon | |
| 9 | Marrow Stromal Cells, Hematopoietic Growth Factors and | \$ 5, TE 1 |
| inbular D.C. | Extracellular Matrix Proteins | Link |
| AND AND THE RESERVE | J. Nemunaitis, C. Tompkins, F. Andrews, M. Rullian, and J. Singer. | 53 |
| | | |
| 10 | Biosynthesis and Processing of the Cytoadhesins, GPIIb/IIIa and Vitronectin Receptor | |
| | A. Troesch, A. Duperray, R. Berthier, and G. Marguerie | 58 |
| Part III. | Hemopolesis in Malignancies | |
| | | |
| 11 | Establishment of an AML-CFU Colony Assay to Evaluate the Sensitivity of Leukemic Progenitors to Cryopreservation: | 187 |
| | Incidence for Bone Marrow Transplantation | |
| | M.A. Allieri, L. Douay, M. Lopez, N. Smadja, L. Nguyen, | |
| | A. Najman, and N.C. Gorin | 67 |
| £ 10 | Con Call (CELLS) Inhibition Provides Pichelal Provides | |
| 12 | Stem Cell (CFU-S) Inhibitory Peptides: Biological Properties | |
| a francisco de la companione de la compa | and Application of pEEDCK as Hemoprotector in Cytostatic Tumor Therapy | |
| n relate a con | W.R. Paukovits. | 70 |
| n or frakting | W.R. Paukovits | 72 |
| 12 | Effects of Ubenimex on Proliferation and Differentiation of Human | |
| n differen | Bone Marrow Cells and Leukemic Cell Lines, and the Possible Mechanisms of Its Action | |
| die geen | K. Shibuya, S. Chiba, M. Hino, T. Kitamura, K. Miyazono, | |
| | and F. Takaku | 81 |
| | * Or gristing Hemopoletic Properties and High Digitary | N. |
| 14 | Stimulation of the Immune System for the Therapy of Advanced Stage Neuroblastoma | |
| -05 | M.C. Favrot, V. Combaret, E. Bouffet, S. Negrier, C.R. Franks, | |
| | D. Floret, and T. Philip | 88 |
| 26.00 | 5 Memorystine 548 Sensitional Photology, fivelight of Payeloyed Vi | |
| | From Molecular Biology to Gene Transfer | |
| 20 | LM. O'Brien and F. Sleber | |
| 15 | Progress in the Development of Somatic Cell Gene Therapy for | |
| | Adenosine Deaminase Deficiency | |
| | V.W. Van Beusechem, T.A. Bakx, A. Kukler, A.J. Van der Eb, | - PE |
| Self-Here | D.W. van Bekkum, and D. Valerio | 95 |
| | | 100 |
| 16 | Retroviral Gene Transfer of Human Adenosine Deaminase into Hematopoietic Cells | |
| TE | D. Cournoyer, M. Scarpa, K. Moore, R.H. Buckley, J.W. Belmont, | |
| | and C.T. Caskey | 100 |
| 17 | Present Status of Retroviral Mediated Gene Transfer into the | |
| 44.64 | Hematopoietic Tissue In Vivo: Possible Applications in Man | |
| | P.M. Lehn | 107 |

Licie 8 . header Service Com

he Role of Autologous Hone Marrow Transplantation

Part I. Present Aspects of Transplantation of Stem Cells

and the state of t

Tiguesia is trees to succeed the control of the con

otroduck very "to room The mander to gips been only and ductions in access to the adding conference to as helps as some contained wood for ring stress. The place is the contribution of coding velocities of creation build or, its ofer your cleanings, it is improved to burning, and higgly reastons to the cond-shock and making.

European range manufastituers, going to self-close overselor the first time, than and the colo in splies in the first over position for colors the come expensive constitues class basis in the past

the too a interestion and a sample, on-tact, attack the interior of Company 2007 South Hall and Van to Silvery In 10008. This plant of 20 March 15 or at

The News Commons Transfectures on a call the there is a rical proving can extract the transfer of the meet not are the call the c and meet a variety of application for the state of application for the state a particular of a second state of a second

verlety of figure will be easily and the contract of the contr

have also restatant to the month, including the Nativar products for Nativar products to the control of the con ureven motesta A for man automass. cable a crabbing an Those har a highest a products with race

then 10 gates of experience and a strong commitment to quality out service. The receipting told to take inspectation in fire discould assume that in the contract of the contr visit ist unid distretifice and are recognized by Underwelters Infraratacy and are CSA

Entropy a apprehently occaning 190 of 05

enting player with a floor floor of such a bigs. Loudsond through as Tusul Quality language here progress Toll Conter the street production employees in the out calcula to fix coppliers under the Perhand in Oholiky program.

the factors while one official above

there is no 500 has possible at their common

Allaichta. Cuirtean 1860 - Campaige peachada A CONTROL OF THE STATE OF THE S

1 The Role of Autologous Bone Marrow Transplantation in Acute Leukemia ceclarions, o continuidam with the processing and

Karel A. Dicke, M.J. Dicke-Evinger, J. Spinolo, and V. Spencer see refuelts to witheling communities on the parties of the contract of the co

TREATMENT OF ACUTE LEUKEMIA With conventional dose chemotherapy, the promotional dose chemotherapy, the promotion of adult acute leukemia varies with the morphological type from 25% long-term disease-free survival. (DFS) in acute myelogenous leukemia (AML) to 40% in acute lymphocytic leukemia (ALL). For this reason, we would like to discuss the role of autologous bone marrow transplantation (ABMT) separately for each disease modality. separately for each disease modality.

The to service to a very relative to the land of the property of the property

ACUTE MYELOGENOUS LEUKEMIA (AML) From published data there is evidence in support of AML as a clonal disease. The leukemic cell population appears to escape the normal growth regulatory mechanism at either the stem cell level or at a more differentiated level such as the myeloblast or promyelocyte and begin proliferating in an uncontrolled manner. Such unresponsiveness to normal growth regulatory mechanisms is intrinsic to the leukemic cell and is most likely not due simply to a change in the feedback regulatory system of the cell. Numerous chromosomal abnormalities have been described for AML many of which involve specific gene rearrangements or deletions. Based on such chromosomal markers, prognostic factors have been identified to predict the rate as well as the durability of response to rate as well as the durability of response to therapy. One such model is that described by Keating (1). This model predicts the probability of achieving remission (PR) and the probability of staying in remission for one year (PCRI). Using this model we are able to predict that the remission durability in patients with the -5, -7 chromosome deletion is short (PCR1 <0.6 or 60%) whereas inversion 16 or translocation 15,17 have a high probability of staying in remission for 1 year (PCR1 >0.6 or 60%). The DFS in patients with an inversion 16 or a 15,17 translocation in an inversion to or a 15,17 translocation in the leukemic cell clone is approximately 40% whereas the majority of the patients with the -5, -7 leukemic cell population survive less than a year. Variability in prognosis is a pitfall for any treatment modality to be

MAY • 190 shoplance

Petrological Property Control

tested on its effectiveness since patient selection may heavily influence the outcome of the study. To avoid the influence of patient selection, such studies must either be performed separately for each leukemic subpopulation or be performed under strict randomization.

tions and resistant in the same of August and designation of the land of the

only to the contract of the co

with problems against Mitsessia groups in 22 Legal Domain Cost TCT; y 150 Et sp. Legalitation (ext@Elfa) & minimumi and the energy America y Deligas and for most classication, estimate and literature

AUTO-ENT IN AML CR1 For the reasons mentioned above, the European ABMT data in AML collected by Gorin (2) have restricted value. The patients described were transplanted in numerous institutions and the criteria for patient entry at these various centers are not known. Gorin has reported a 32% long-term survival in AML patients transplanted in CRI with unpurged matrow harvested within six months after achieving CR. Patients treated with cyclophosphamide and total body irradiation (CRI) rescued with matrow cleansed in vitro (TBI) rescued with marrow cleansed in vitro with mafosfamide had a 56% three year DFS. When comparing the treatment of these two patient groups, purging may appear the difference in the ability to achieve increased DFS, however, the time of marrow harvest may also influence the prognosis. As a result, multivariate analysis of the data is necessary to evaluate purging.

Lowenberg reported the first randomized study in CR1 AML comparing conventional dose therapy to remission intensification with cyclophosphamide + TBI and unfractionated marrow, shortly after consolidation treatment (3). Randomization was done immediately after achieving complete remission. Statistically the outcome of these two groups were not significantly different. Time of marrow harvest was early after remission induction (within 2-3 months) and the intensity of the consolidation program was moderate — most likely leaving significant numbers of leukemic cells in the marrow at the time of harvest.

ing the same with them to an an ing the control of

Caracter on the account of Walks with the last

cells. Due to minimal cell loss with this technique, a combination with chemopurge is possible without significantly compromising the hematopoietic restoration potential of the sample. Whereas patients with good prognosis in AML may only need in vivo purging, our recommendations in AML are a double in vitro purge. Effective intensification and consolidation programs for in vivo purging do not exist yet. It is likely that a different mode of treatment is necessary for this disease. Not only are residual cells in the harvest marrow biologically significant, but also leukemic cells escaping the conditioning regimen. Escape may not be due to lack of sensitivity but to the sanctuary sites of ALL cells.

Results in CR2, CR3 are poor. The best data are published by the Minnesota group in 22 children treated with Cy + TRI and monoclonal antibody purged marrow (8). The inversion rate was 31%. Since this has not been compared with conventional chemotherapy results, interpretation is limited.

In conclusion, the role of ABMT in ALL is unclear. The results seem less favorable than in AML. A continuing relapse rate is noted after BMT so that more intensive and longer therapy might be necessary. In vitro purging with a combination of antibody purge and chemopurge might be effective. Studies for conclusive evidence need to be undertaken.

EFFICACY OF PURGING

As mentioned earlier it is unclear if the purging of bone marrow from leukemic cells is beneficial to the long term survival of AML patients undergoing ABMT. The biological significance of such procedures will depend in part on the role these re-infused leukemic cells play in the recurrence of disease after transplantation. The fewer leukemic cells to escape the transplantation conditioning regimen, the more significant the leukemic cell population in the graft will be. It is likely, then, that the biological significance of purging will be greater in first rather than in second or subsequent remissions. A significant drawback in the answering of this question has been the inability to monitor the assumed removal of these residual leukemic cells. There are two major constraints in the detection of minimal numbers (<1%) of tumor cells: 1) the lack of technology available with sufficient sensitivity and 2) identification of specific tumor markers.

When testing leukemic cell separation techniques on untreated or relapsed leukemic patients, it is possible to monitor the separations with little difficulty using attendard techniques such as morphology, attochamistry, cytogenetics and the in vitro colony formation assays. None of these assays are specific and sensitive enough to detect the small numbers of leukemic cells which may be present in remission marrow. Since the purpose of the above mentioned separation techniques is to be used for remission marrow, such detection methods leave us to do these

separations blindly. For the past decade considerable effort has been made to use molecular technology to resolve these problems.

Since these tumor cells are part of a heterogeneous population of cells, the use of Southern and/or Northern blotting techniques have not enabled us to detect fewer than 1% contaminating tumor cells unless preselection is used through which our sensitivity increases to 0.1% at best. From estimates of the leukemic cell population in remission marrow, the frequency of the leukemic cell population is one leukemic cell in 20,000 cells, or 0.005%, which is far below the detection limits of the techniques discussed above. Therefore, these assays cannot be used to monitor tumor cell separation techniques in remission marrow.

ABERRANT GENE EXPRESSION IN LEUREMIA
There are numerous reports of aberrant protooncogene expression in tumor cells and more
specifically in AMI (9-17). The importance
of these genes in normal cellular
proliferation and differentiation has become
well accepted; many of these genes appear to
be related to either growth factors and their
receptors, or involved in the signal pathway
(18). We have found that several of the
proto-oncogenes analyzed by Northern blot
hybridization were expressed in
hematologically normal bone marrow (15),
supporting the concept that these genes do
play a role in adult hematopoietic
metabolism. There is growing evidence that
qualitative and quantitative changes in
proto-oncogene expression may be associated
with neoplasia (19).

The hematopoietic system has proven attractive for these studies since several proto-oncogenes are located at the breakpoint of chromosomal translocations or inversions specifically associated with various leukemias (20). Perhaps this leads to the deregulated expression of these genes. Furthermore, the enhanced expression of cellular oncogenes have been consistently observed in neoplastic hematopoietic cells. Several groups in addition to ourselves have identified two genes, MYC and SIS, to be present at abnormally high levels in the peripheral blood and bone marrow cells of untreated and/or relapsed AME patients (21-26). By Northern and dot blotting analyses we were unable to detect this abnormality in the bone marrow cells of any AML patient clinically classified as in remission. Since the limitations of these techniques are in the range of 1-5% contamination of leukemic cells in the samples tested, any abnormality present at lower levels would remain undetectable.

DETECTION OF LEUKEMIC CELLS BY RNA-IN SITU HYBRIDIZATION

Our approach to this problem has been the identification of an abnormality which is detectable at the single cell level. We have reported the development of an extremely

Our study of ABMT in CR1 AML was originally designed as a randomized study comparing intensification of remission with ABMT versus conventional dose therapy (4). beginning of the study, however, the patients refused randomization so that patients were entered in the ABMT arm on a voluntary basis. The treatment schedule is outlined in Table 1. The time interval between onset of CR and marrow harvest was -3 months, and between CR and ABMT, six months. Remission was induced by amsacrine, ARA-C, vincristine and prednisone, intensification occurred by HD ARA-C 12-15 gm/m² and amsacrine, 250-350 ug/m². To harvest sufficient numbers of marrow cells, patients were treated after intensification with three courses of AD-OAP followed by Cytoxan; 6 g/m², BCNU: 300 ug/m², and VP-16: 750 mg/m² (CBV). The CBV treatment schema is depicted in Table 2. Two days after the last dose of VP-16, unpurged marrow was infused. After hematopoietic recovery, 3-6 courses of AD-OAP/AMSA-OAP were administered. The results of 18 patients are documented in Table 3. Ten out of 18 patients (56%) are alive and in CCR; the shortest remission is three years, the longest six years. Eight patients relapsed with a median remission time of 19 months (range 7-22 months). It appeared that of the 12 patients with favorable prognostic factors (PCR1 \geq 0.6) eight are still in CCR (67%) whereas only two out of six patients (33%) with unfavorable factors (PCRl ≤ 0.6) are still in CCR.

Our data are comparable to the European data published by Gorin (2) reporting a 56% DFS in patients treated with Cy +TBI and purged marrow. Although we have not used any in vito manipulation of the marrow, the intensification with ARA-C and AMSA prior to harvest may have acted as an in vivo purge rendering the leukemic cell burden to a minimal level. We do not expect CBV alone to be more anti-leukemic than Cy + TBI; however, with the addition of CBV alone two months after treatment with HD ARA-C and AMSA, CBV might be strongly anti-leukemic. AMSA, ARA-C combination might be very effective as observed in the BAVC regimen (BCNU, ARA-C, VP-16,). A 75% DFS (median follow-up two years) has been reported with this regimen in CR2 by the Rome group (5).

Like the European data, the interpretation of our results is limited due to possible selection of patients. Promising as they may be, a randomized study to prove the biological significance is necessary.

AUTO-EMT IN AML CR2

The interpretation of transplantation results in second remission, as in first remission, is open to question. The biological effect of a procedure can be measured by comparing the length of remission after ABMT with the duration of remission preceding those remissions in which BMT occurred. When the transplantation remission is longer than the previous CR this procedure may have changed the natural history of the disease. The inversion rate with conventional dose

chemotherapy is 10-20% as published by Keating et al (1). Wiernick et al (6) reported a 30% DFS and inversion rate in second remission with multiple pulses of HD ARA-C and mitoxantrone. The transplantation data reported by us, Gorin and Santos are no different. Santos reported a 25-30% inversion rate and contributed this to in vitro purging with 4-hydroperoxy-cyclophosphamide (4HC). However, in the light of the data published by Keating and Wiernick, those data are not significantly better so that no conclusions concerning the efficacy of purging can be drawn. In addition, the transplantation data in CR2 reported by our group are not significantly better than conventional chemotherapy. The only transplantation data more favorable than the conventional chemotherapy data are those reported by the Rome group using the EAVC regimen with an inversion rate of 50%.

The question of whether or not purging has a biological effect still remains unanswered. It is likely that a biological effect can only be expected when the leukemic cell population escaping the conditioning regimen is low. Primarily, this occurs in CRI where the leukemic cell population is least resistant to cytoreductive the apy. Systematic studies to solve this question have not been done.

In conclusion, in AML the question of ABMT still remains unresolved. Studies in CRl are underway to prove its efficacy. The role of purging is controversial; especially in CR2 with potentially high leukemic burden after cytoreductive therapy the significance of purging may remain questionable. We recommend a randomized study in CRl with in vivo purged marrow in the good and intermediate prognostic patients, and a one arm study with in vitro and in vivo purged marrow in bad prognostic patients.

ACUTE LYMPHOCYTIC LEUKEMIA

The European ABMT data in adult ALL in CR1 reported by Gorin (2) are equivalent to the conventional chemotherapy data of the German Cooperative Group published by Hoelzer et al., reporting close to a 40%, five year DFS (7). As in AML, prognostic factors need to be taken into account before any interpretation of data can be done. In our studies the ALL data look less favorable to draw definite conclusions than the AML CR1 data although the median follow up of 31 months is relatively short. Fifteen of the 28 patients are still in CCR, however, it looks as if a plateau of the curve has not been reached. A continuous relapse rate is noted in contrast with AMI.

It may well be that in ATL more than one intensification is necessary in addition to longer post transplant chemotherapy. Potential elimination of ALL cells from the graft without loss of hematopoietic stem cells might be possible based on antibody, separation technology, since ALL cells differ in phenotype from early progenitor.

sensitive and rapid RNA-in situ technology which permits the detection of specific mRNAs within individual cells (27). This technology enables us to identify cells with the abnormal expression of any marker gene selected at a level of 1/50,000 cells. Using this RNA-in situ hybridization methodology, we have identified the abnormally high expression of two genes, MYC and SIS, which occur in approximately 90% of untreated and relapsed AML patients (26). MYC, a nuclear oncogene, has a major role in the regulatory pathway of normal cellular proliferation. In AML, it has been reported to be over-expressed at both the mRNA and protein levels (26,28,29,30). The second gene, SIS, encodes for a protein homologous to the beta-chain of platelet-derived growth factor (31,32). PDGF has been reported to stimulate expression of genes including MYC in fibroblasts (33,34).

Since the expression of these genes is associated with cellular proliferation, one could postulate that the elevated expression of MYC in AML could be a reflection of an increased proliferative potential no different than what would be present in normal hematopoietic cells during rapid proliferation. In a recent study in our laboratory (35) we have focused on the expression of MYC and SIS in normal bone marrow cells at a time of rapid proliferation to determine if their expression levels approach the high level found in the leukemic cells.

Bone marrow cells of 18 breast cancer patients were examined in a longitudinal study with several time points before and after BMT. In all cases, bone marrow was determined to be free of tumor involvement by morphology on aspiration. For each of these patients, both pre and post ABMT samples were obtained. The pre-transplant sample was used as the baseline standard against which post-transplant specimens were compared (i.e., each patient was his own control). In addition, bone marrow samples from normal donors were utilized as independent controls. Of the 18 patients examined, 15 expressed MYC and SIS within normal limits and three had a small population of cells present which had an elevated expression of either MYC or SIS.

After high dose chemotherapy with CVP or MVT and ABMT, samples were aspirated when the patient's peripheral white blood count indicated bone marrow regeneration with increasing monocytes and a total white blood count >800. In not one case did the immature hematopoietic cells proliferating in the bone marrow after transplantation express MYC or SIS at the high Levels found in AML. We did find an 'increase in the number of cells present expressing normal levels of MYC implying, perhaps, that a greater proportion of the population was actively proliferating during bone marrow regeneration.

The high levels of MYC and SIS mRNA found in these cells cannot simply be attributed to the proliferative capacity of these cells or to the presence of normal, immature hematopoietic cells. As one line of evidence that the cells we examined actually belong to the leukemic cell compartment, comparisons were made with the percentage of blast cells determined morphologically in these AML patients. The presence of such abnormal cells, as defined by gene expression, in these AML patients correlated well with the percentage of blast cells determined morphologically. As shown in Table 4, the percentage of cells over-expressing either MYC or SIS at least equals, and often exceeds, the number of blast cells present in the marrow.

In addition, we have identified the presence of such abnormal cells in AML patients studied shortly after remission induction, but these cells are often present at a much lower frequency than that found in either untreated or relapse AML (Table 5). Several, but not every patient, in which we found this abnormal group of cells have relapsed; in contrast, none of the patients whose bone marrow cells were found to be normal in their expression of MYC and/or SIS have relapsed since this study was completed. These results led us to question whether the presence of such an abnormal population of cells could be predictive of early relapse in acute leukemia.

To help us determine the significance of this abnormal cell population in the eventual clinical stability of the AML patient, we have also examined bone marrow cells of 10 AML patients who are long term survivors after BMT. The median CR duration at the time of examination for this group was 38 months with the individual remissions ranging from 14-78 months.

The presence of an abnormal cell population expressing MYC at high levels similar to that found in AML short term remission patients, does not occur in this patient group. However, in 3/10 patients a high level of SIS expression alone was present in a variable percentage of cells, occasionally as high as 80%. At this time, none of the three patients identified with this abnormality at the RNA level have been classified as having a recurrence of leukemic cells in the bone marrow by conventional morphological criteria.

MONITORING OF PURGING PROCEDURES

It is unclear if the purging of bone marrow of leukemic cells is beneficial to the long term survival of AML patients undergoing ABMT. As discussed earlier, a significant drawback in the answering of this question has been the inability to monitor the assumed removal of these residual leukemic cells. We have begun to apply our RNA-in situ hybridization technique to this problem. Since we find such high levels of MYC to be present in AML patient bone marrow, we were interested to see if these cells are removed during a purging procedure using monoclonal

七为试读,需要完整PDF请访问: www.ertongbook.com