European Protocol
of the
Severe Chronic Neutropenia International Registry

Reviewed and approved by the Ethics Committee of the Medizinische Hochschule Hannover
on June 25th, 2002

INTRODUCTION

The Severe Chronic Neutropenia International Registry

The Severe Chronic Neutropenia International Registry is an organization dedicated to the elucidation of the pathophysiology and continuing improvement of the therapy of severe chronic neutropenia. The registry was initially established under the sponsorship of Amgen Inc. in 1994 to monitor the clinical course and long-term treatment of SCN patients. In July 2000, Amgen Inc. withdrew sponsorship of the SCNIR but provided a major donation which enabled the Registry to become an independent foundation based at the University of Washington, Seattle, USA. The SCNIR is maintaining two data coordinating centers located in Seattle, WA, USA, and Hannover, Germany.

Objectives

The objectives of the SCNIR have considerably changed during the last eight years. Whereas in the beginning of the Registry, the long-term safety of G-CSF therapy represented a major focus, the observation and follow-up of serious late sequelae became more and more important. The current objectives of the SCNIR are:

1. Document the clinical course of SCN and monitor clinically significant changes
2. Evaluate the outcome of pregnancies and hematological parameters
3. Study the incidence and/or outcome of the following previously identified adverse events:
    a. osteoporosis
    b. vasculitis
    c. splenomegaly
    d. cytogenetic abnormalities
    e. myelodysplastic syndrome and leukemia
4. Follow-up of former SCN patients after bone marrow transplant
5. Establish a cell bank for the SCNIR for future SCN related research
6. Provide a comprehensive information system to educate physicians and patients
7. Promote research

PATIENT ELIGIBILITY

Inclusion Criteria

1. Patients with severe chronic neutropenia (ANC of less than 0.5 x 10^9/L)
2. Patients with severe chronic neutropenia (ANC of less than 1.0 x 10^9/L) **and** recurrent severe infections
3. Independent of hematological parameters, all patients with:
    - Shwachman-Diamond syndrome (SDS)
    - Glycogen storage disease type 1b (GSD1b)
    - Barth's syndrome
    - other inherited or metabolic diseases associated with neutropenia
4. Immune neutropenia with positive anti-neutrophil antibodies (meeting criterion 1. or 2.)
5. Secondary MDS/Leukemia with documented history of severe chronic neutropenia (meeting criterion 1. or 2.)
6. All SCN patients previously enrolled in Amgen sponsored SCN studies

Exclusion Criteria

1. Neutropenia known to be drug-induced
2. Primary MDS/Leukemia
3. Aplastic Anemia
4. Known HIV positive
5. Known immune diseases, such as rheumatoid arthritis and systemic lupus
6. Chemotherapy-induced neutropenia

   Thrombocytopenia (platelets <50.000/mm3) or Anemia (hemoglobin < 8g/dl) – with the exception of patients with SDS, GSD1b, Barth's syndrome or secondary MDS/leukemia

Diagnostic Evaluation

For the differential diagnosis of the different types of neutropenia please see the flow charts shown in figures 1 and 2.

DIAGNOSTIC EVALUATION OF NEUTROPENIA PATIENTS

Figure 1: Differential Diagnosis of Severe Chronic Neutropenia

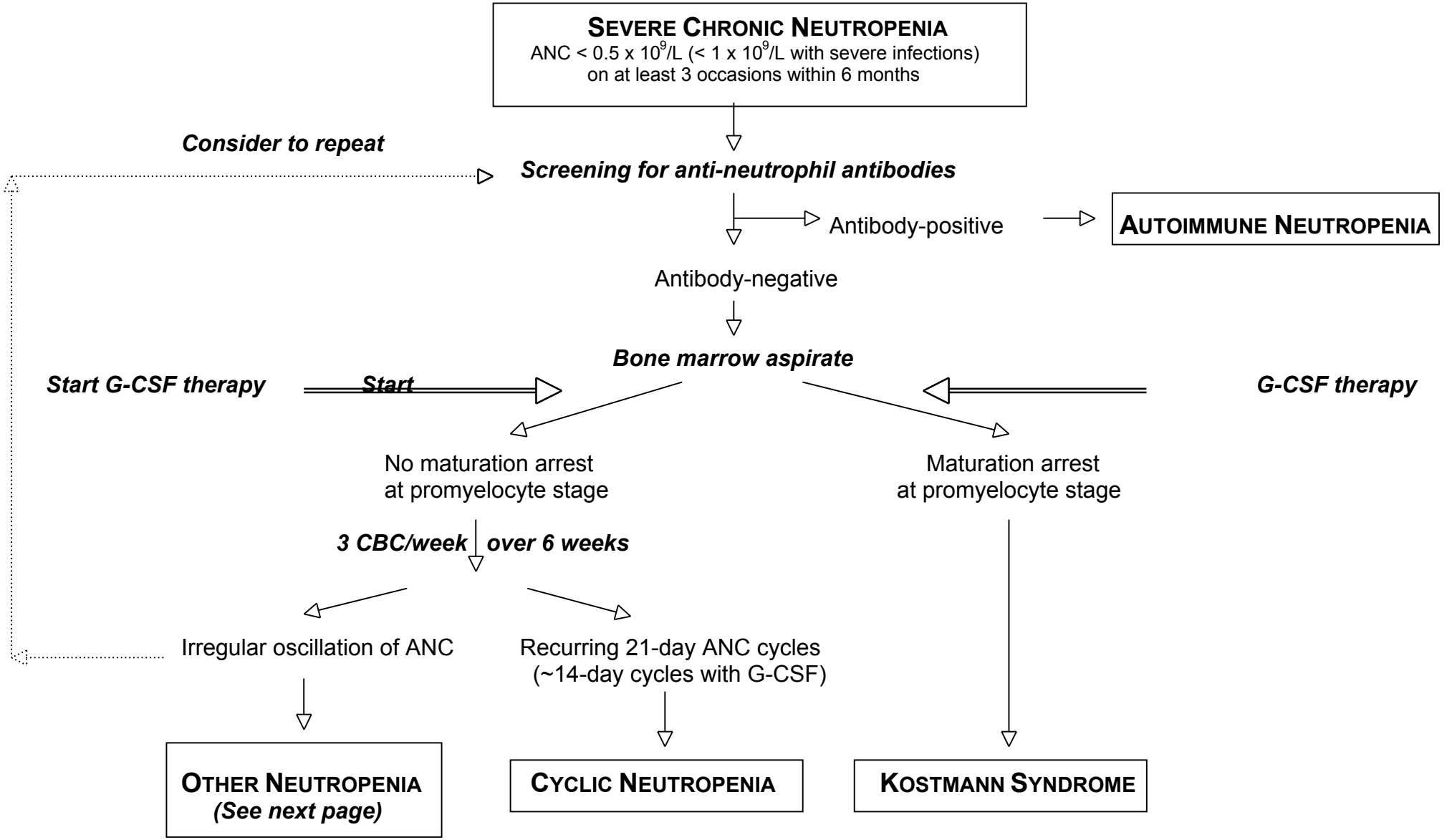
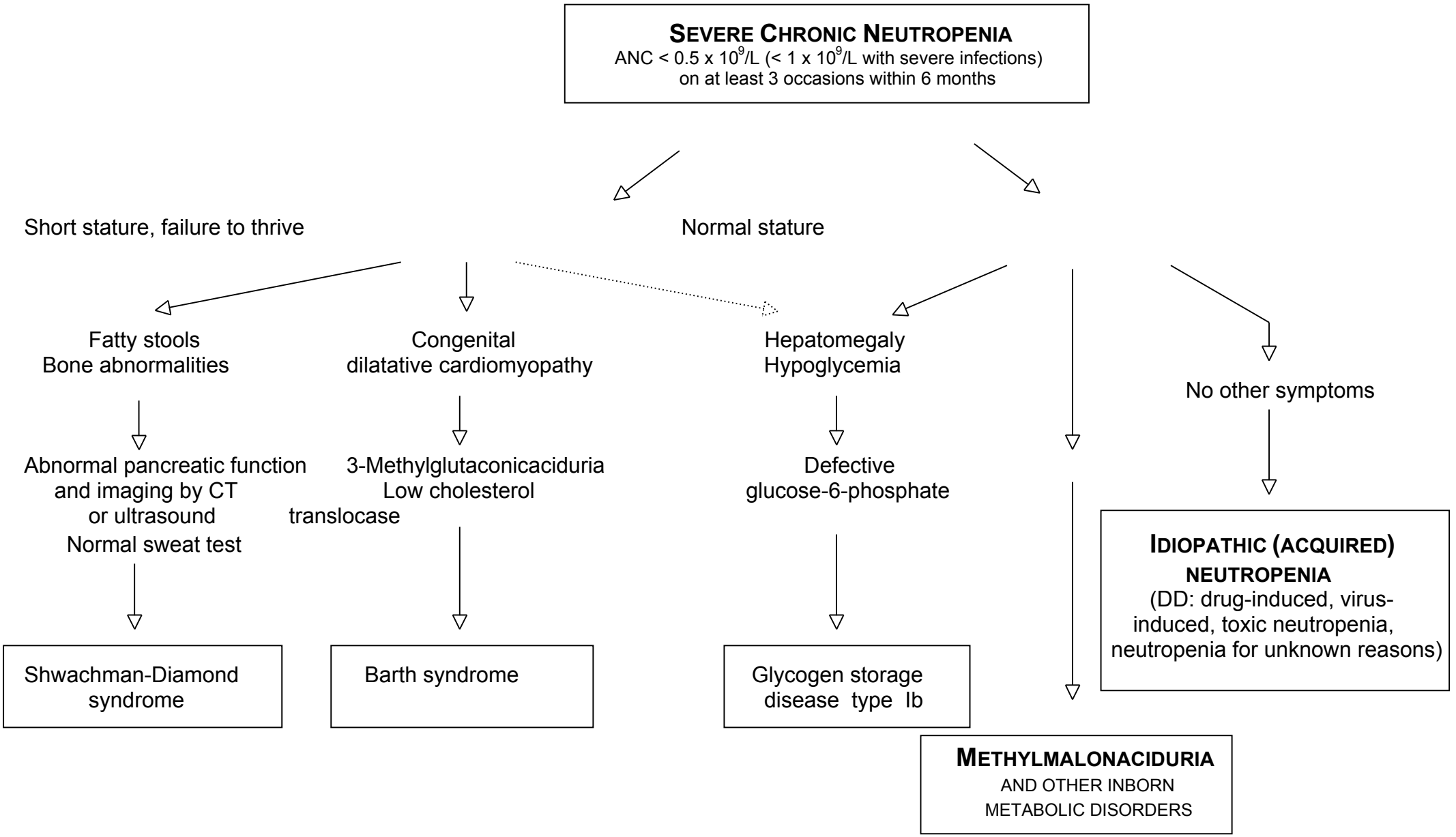


Figure 2: Differential Diagnosis of Other Types of Severe Chronic Neutropenia



ENROLMENT

Requested Medical Information

The following medical information is requested prior to enrolment in the SCNIR (see also table 1 below):

1. Complete blood counts (CBC):
    a. Congenital, immune, and idiopathic neutropenia: At least 3 CBC taken within 6 months prior to submission (for patients without G-CSF treatment) or within 6 months prior to the onset of G-CSF therapy (for patients receiving G-CSF treatment) documenting
        i. absolute neutrophil counts of less than 0.5 x 10^9/L or
        ii. absolute neutrophil counts of less than 1.0 x 10^9/L for patients suffering from recurrent severe infections or
        iii. any absolute neutrophil count for patients with SDS, GSD Ib, and Barth syndrome.
    b. Cyclic neutropenia: At least 18 CBC (taken 3 times a week for 6 consecutive weeks) showing repetitive 21-day cycles with ANC of less than 0.2 x 10^9/L during neutropenic phases without G-CSF treatment, or, showing consistent repetitive cycles of less than 21 days under G-CSF therapy, respectively.
2. Bone marrow aspirate and cytogenetic evaluation:
   for G-CSF treated congenital, cyclic and idiopathic neutropenia patients (not required for immune neutropenia of infancy).
3. Positive test for anti-neutrophil antibodies:
   for immune neutropenia patients.
4. Written informed consent signed by patient or patient's parent: for all patients and written assent for minor patients. Appendix A-1 represents a basic written consent form. However, this consent form may have to be translated and modified according to the individual national data protection laws.

Enrolment Procedure

1. Submission of Patients

Patients meeting the inclusion criteria stated above are submitted for enrolment in the Severe Chronic Neutropenia International Registry by utilizing the official registration form (see Appendix A-2). The completed registration form, the signed consent form (see Appendix A-1) or a copy of it, and copies of all reports indicated in the registration form (e.g. cytogenetics report, bone marrow report etc.) are to be sent to the responsible Data Coordinating Center (see table 2 for country responsibilities).

Table 1: Requested Medical Information (required: ■ recommended: □)

Neutropenia Patients	**CBC**	**BM**	**Cyto**	**α-N Ab[1]**	**Consent**	**Physical Exam[2]**	**Clinical History[3]**
No G-CSF therapy	within 6 mo prior to submission						
congenital – isolated neutropenia	3 (ANC < 500)[4]	■	□	□	■	■	■
congenital – other[5]	(any ANC)	□	□	□	■	■	■
cyclic	18 (21-day cycle)[6]	□	□	□	■	■	■
idiopathic	3 (ANC < 500)[4]	□	□	□	■	■	■
immune	3 (ANC < 500)[4]			■	■	■	■
With G-CSF therapy	within 6 mo prior to G-CSF						
congenital – isolated neutropenia	3 (ANC < 500)[4]	■	■	□	■	■	■
congenital – other[3]	(any ANC)	■	■	□	■	■	■
cyclic	18 (cycling)[7]	■	■	□	■	■	■
idiopathic	3 (ANC < 500)[4]	■	■	□	■	■	■
immune	3 (ANC < 500)[4]			■	■	■	■

[1]test for anti-neutrophil antibodies
[2]height/weight, liver and spleen measurements
[3]infectious and non-infectious episodes , previous and current treatment
[4]pts. w/ recurrent infections: ANC< 1000
[5]SDS, GSD1b, Barth syndrome and other metabolic disorders
[6] taken within 6 consecutive weeks and nadir ANC < 200
[7]taken within 6 consecutive weeks and showing consistent cycles of ≤ 21 days

Table 2: Areas of Responsibility of the DCC of the SCNIR

COUNTRY/ GEOGRAPHICAL REGION	RESPONSIBLE DATA COORDINATING CENTER
Australia Canada Middle America South America U.S.	SCN International Registry DCC Seattle 600 Stewart Street, Suite 1503 Seattle, WA 98101-2509 USA
Asia Europe Middle East North Africa	Internationales SCN Register DCC Europe Medizinische Hochschule Hannover Kinderklinik D-30623 Hannover Germany

2. Review of Registration

Submitted Registration Forms are reviewed by the responsible Clinical Manager and potential queries and requests for still missing reports etc. will be returned to the referring physician. Subsequently in-coming replies will be incorporated and the Registration Form will be completed and approved by the responsible Advisory Board Member.

Patients who do not exactly meet distinctive requirements of the inclusion criteria of the SCNIR (e.g. ANC between 1000 and 1500 despite recurring severe infections) or registration forms lacking parts of medical information (e.g. less than three CBC prior to onset of G-CSF) may be submitted nevertheless and are checked for a possible exceptional enrolment.

The SCNIR reserves the right to categorize patients according to internal classification criteria independently of the actual diagnosis of the referring physician. Therefore, patients submitted as cyclic neutropenia patients, e.g., are enrolled in the group of idiopathics if there is no clear-cut proof of repetitive 21-day cycles (or periodic cycles of less than 21 days in case of patients treated with G-CSF).

Patients, who do not meet several inclusion criteria cannot be enrolled in the SCN International Registry and are excluded from enrolment.

Notice of enrolment (or exclusion) is given to the referring physician after approval (or rejection).

Follow-up of Patients

1. Annual Follow-up

Patients enrolled in the SCNIR are followed on an annual basis. The three-page follow-up form was designed to gather information on clinical events, physical examinations, CBC, bone marrow examination, current therapy, and special in-vitro investigations (see Appendix A-3). Copies of all medical reports are to be provided as supplements to the follow-up form.

Severe clinical events like the transformation to malignancy or death of any enrolled patient should be reported to the SCNIR immediately as the SCNIR is forwarding the information on to the concerning pharmaceutical companies for the patients' safety.

2. Follow-up of patients with secondary MDS/leukemia

Neutropenia patients who transform to MDS/leukemia should be reported to the SCNIR immediately. In turn, the referring physician will receive a form requesting specific information on the clinical course and therapy (see Appendix A-4). The patients will be followed with a specific annual follow-up form.

The death of any enrolled patient is to be reported to the SCNIR immediately. The referring physician will be provided with a specific death form requesting the cause of death and other death-related information (see Appendix A-6).

3. Follow-up on BMT patients

Patients who underwent bone marrow transplant either for non-response to treatment or for secondary MDS/leukemia are followed on an annual basis starting from the actual date of BMT. A specific so-called "Initial BMT form" is sent to the referring physician upon notification of BMT requesting information on the specific type of malignancy, conditioning regimen, stem cell source, complications etc.. Subsequently, annual follow-up forms will be provided to observe the clinical course of the patients (see Appendix A-5).

Specific Medical Information

Frequently, medical issues of special interest, e.g. newly observed late sequelae, are raised through the periodic analyses of the data collected by the SCNIR. A closer investigation of these issues is then initiated by requesting more detailed information on the respective phenomena. The information is requested by specifically developed questionnaires that are sent to referring physician of any patient reported with these symptoms/phenomena. So far, specific surveys are conducted on vasculitis (see Appendix A-7), glomerulonephritis and pregnancy (see Appendix A-8) in neutropenic patients.

In addition to the information collected on special clinical events also information on in-vitro research investigations performed with samples from registered patients is collected in a separate research database. So far, these investigations include analyses of the G-CSF-receptor and the neutrophil-elastase gene. Again, a specific form is provided to the referring physician as soon as a patient is reported with 'in-vitro investigations'.

Database and Data Analysis

Database

All personal data on any clinical record form will be anonymized and stored under a specific 9-digit medical record number. The signed consent including the personal data of the patients will be stored separated from the database. If medically indicated or requested by the patient him/herself, tracking back of clinical information to a certain patient is possible only at the site where the consent of the patient is kept.

The database system used by the SCNIR within Europe is an internet-based system called ProMISe (Project manager internet server). The server for the SCNIR is located and maintained at the University of Leiden, The Netherlands. Access to the database is enabled by combinations of individual user names and passwords, only. ProMISe has been proven to be a safe and sufficient internet-based system within Europe by the EBMT (European Bone Marrow Transplant Working group).

Periodical Data Analyses of the SCNIR

All data collected by the SCN International Registry are analyzed periodically (usually annually) under specific consideration of the following aspects:

- Patient Demographics
- Clinical Characteristics
  - Type and frequency of infectious episodes
  - Type and frequency of non-infectious clinical events
  - Analysis of physical assessments
- Treatment
  - Applied therapies
  - Side-effects of treatment
  - Treatment safety
  - Non-Responsiveness
- Secondary Events
  - MDS/leukemia
  - Bone marrow transplant
- Deaths

The SCNIR Reports are available to all physicians referring patients to the SCNIR. In-between regular SCNIR Reports synopses of the most relevant issues are summarized in the 'Updates' of the SCNIR which, again, are distributed among all collaborating physicians and may also be provided to the interested public upon request.

Cell Bank

The SCNIR maintains a bank of samples from registered patients. The collection includes frozen blood and bone marrow cell samples, DNA and RNA samples and blood and bone marrow slides.

1. Consent

By signing the Consent Form of the SCN International Registry (see Appendix A-1) each patient enrolled in the SCNIR agrees that biological samples arising from diagnostic or routine blood samples, bone marrow punctures or other investigations may be sent to the SCNIR by the referring physician. The SCNIR reserves the right to initiate processing and/or storage of these samples in adjacent laboratories at either one of the responsible locations (see table 2 on page 6). Samples will be stored indefinitely and may be used for neutropenia-related research approved by the SCNIR. The SCN International Registry is obliged to return non-used samples to the patient on request at any time.

2. Samples

a. Bone marrow slides

With each new registration the SCNIR requests at least one bone marrow slide preferentially from the diagnostic bone marrow aspirate or any other bone marrow sample prior to the onset of any G-CSF therapy.

b. Blood or bone marrow samples

The SCNIR collects blood samples and bone marrow aspirates from all registered patients. The referring physician is requested to reserve a sample of the patient's bone marrow each time a bone marrow biopsy is performed. Either 20 ml heparinized blood and/or 5 ml heparinized bone marrow aspirate should be send by express or overnight mail to the reference center (see Appendix C) together with an accompanying form reporting the corresponding data (see Appendix C-1). Material arriving in bad condition is discarded.

3. Storage of samples

Blood and bone marrow samples from registered patients are processed according to the most current laboratory procedures. Sera, RNA, DNA and/or isolated mononuclear cells (lymphocytes and monocytes) are frozen and stored in liquid nitrogen in the cell bank (see Appendix C) indefinitely. By declaring her/his consent, the patient irrevocably transfers all rights on her/his biological samples to the SCNIR. Mutually, the SCNIR returns non-used samples to a patient upon request.

4. Usage of samples

Biological material from registered patients is used exclusively for neutropenia-related research projects initiated or supported and approved by the SCNIR. Investigators from outside the SCN International Registry may submit proposals on neutropenia-related research projects to the registry which may also include a request for biological materials from the SCNIR Cell Bank. Any investigator requesting Cell Bank materials for a project approved and supported by the SCNIR must sign an agreement that the material from the Cell Bank is used exclusively as indicated in the proposal.

Research Projects

It is one of the major goals of the SCN International Registry to facilitate the progress in basic and clinical research of neutropenia by supporting neutropenia-related research projects. This support not only includes financial but also logistical support by providing biological material from the Cell Bank of the SCNIR. Investigators working in the field of neutropenia are explicitly encouraged to submit proposals on their research projects to the SCNIR.

1. Submitting Proposals

Any research proposal submitted to the SCNIR is to be structured as follows

Project Description
- Title
- Aims of the study
- Previous project-related research of the applicant
- Timelines

CV of applicant (including project-related publications)
List of requested support.

Three complete sets of documents are to be submitted to the responsible DCC of the SCNIR (for the respective area of responsibility and mailing address see table 2 on page 6).

2. Review of Submitted Proposals

Any research project submitted to the SCNIR for support/funding will be reviewed by both chairmen and the Research Committee of the SCNIR. The applicant will be informed of any decision usually within three months after submission or after providing additional information which may be requested by the SCNIR for the matter of completeness.

3. Publications

The SCNIR is entitled to review any manuscript arising from research projects supported/funded by the SCNIR and reserves the right to raise objections to certain issues of the manuscript prior to submission for publication. The author is notified of any concerns or objections of the SCNIR within two weeks after providing the manuscript. The contribution of the SCNIR to the project is to be acknowledged in the manuscript. Potential co-authorships of single members of the SCNIR are to be negotiated individually between the respective principle investigator and the SCNIR.

APPENDIX A: Forms

A-1 Consent Form

A-2 Registration Form

A-3 Follow-up Form

A-4 BMT Forms

A-5 Death Summary Form

A-6 Vasculitis Form

A-7 Pregnancy Form

APPENDIX B: Ethical Guidelines

ETHICAL GUIDELINES: THE DECLARATION OF HELSINKI

Adopted by the 18th WMA General Assembly Helsinki, Finland, June 1964 and amended by the 29th WMA General Assembly, Tokyo, Japan, October 1975 35th WMA General Assembly, Venice, Italy, October 1983 41st WMA General Assembly, Hong Kong, September 1989 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996 and the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000

A. INTRODUCTION

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.
2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.
3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."
4. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.
5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.
6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.
7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.
8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.
9. Research Investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well

as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

10. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.
11. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.
12. Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.
13. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.
14. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.
15. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.
16. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.
17. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.
18. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.

19. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.
20. The subjects must be volunteers and informed participants in the research project.
21. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.
22. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.
23. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.
24. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.
25. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.
26. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.
27. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in the publication.

Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

28. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.
29. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists.
30. At the conclusion of the study, every patient entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study.
31. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.
32. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

APPENDIX C: Mailing Address for Biological Materials

Prof. Dr. J. Skokowa / Prof. Dr. K. Welte
Med Klinik II
Universitätsklinikum Tübingen
Bettenbau West (501), Eb. 02, Raum 532
Otfried-Müller-Str. 10
72076 Tübingen
Germany

Appendix C-1 Accompanying form for biological materials