

22nd EWOG-MDS and 4th EWOG-SAA

Working meeting

Venue: Lindner Hotel & Residence MAIN Plaza,
Walther-von-Cronbergplatz 1
60594 Frankfurt/Main

April 16-17, 2010

Friday, April 16, 2010

SESSION I

Welcome and Opening of the meeting, Peter Bader.

Introduction into the afternoon program by Charlotte Niemeyer.

Charlotte Niemeyer stresses that the cooperation between the AML-SCT group and the MDS-SCT group is at the cornerstone of the afternoon session.

JMML

Charlotte Niemeyer presents data on the “role of CBL mutations in myeloproliferative disorders”.

Discussion:

1. Lynn Ball asks whether there were any hints to endothelial damage in patients with CBL mutations?

Charlotte Niemeyer replies that at least three patients revealed a bleeding tendency which might well be associated with endothelial damage.

2. Mary van den Heuvel-Eibrink asks whether there are Noonan-registries in the different countries and suggests to exchange the data collected.

Christian Flotho reports on aberrant DNA methylation characterizing a subtype of JMML with poor outcome

1. Gudrun Göhring proposes to correlate methylation/epigenetics to cytogenetics or telomerase activity.

2. Charlotte Niemeyer alludes to parallels between the work recently published in JCO by a group from Padua which has subdivided patients with JMML into an AML-like and a non-AML-like subgroup.

3. Marry van den Heuvel-Eibrink questioned how methylation status could specifically be correlated to prognosis.

4. Markus Schmutz addresses connections between globin regulation and methylation.

5. Charlotte Niemeyer speculated on the impact of hypermethylation detected in patients lacking mutation.

6. Mary van den Heuvel-Eibrink asks whether there are samples where methylation status changes over time and Christian Flotho replied that methylation appears elevated in relapse.

7. Christian Flotho proposed to include methylation status as a prospective study into the new JMML protocol.

8. Marry van den Heuvel-Eibrink discusses whether functional studies of the genes found to be hypermethylated should be performed. Christian Flotho replies that most of these genes have already been studied functionally and some of them have a role in the pathobiology of leukemia but not so apparently in MPD/MDS.

Arndt Borkhardt reflects on rearrangement and activation mechanisms of the ALK gene in patients with 2p23 aberration and unclear myeloid malignancies.

In all cases monosomy 7 was present and appears to be a change following ALK rearrangement. In addition, there was monocytosis and spontaneous colony growth. After all, a classification of these cases remains difficult.

Reference pathology

Irith Baumann provided an interim report from the pathology group

The discussion on unclear cases is helpful to reach a consensus among the different groups. Having finished the investigations on MDS and SAA another interesting field is myelofibrosis.

Cases of myelofibrosis have been examined preliminarily and could be divided into three subgroups:

- a. Myelofibrotic MDS without blast excess
- b. Myelofibrotic MDS with blast excess
- c. Immunologically induced myelofibrosis

These three subgroups are further characterized according to dysplastic findings, especially concerning megakaryopoiesis (micromegakaryocytes).

1. Marry van den Heuvel-Eibrink asks whether there was a threshold, a minimal amount of myelofibrosis considered normal.

2. Charlotte Niemeyer addresses the question of the study design for a possible future publication on myelofibrosis and suggests repeated rounds as a stepwise definition of the stage of myelofibrosis, its dynamics and dysplastic characteristics.

3. Stephan Schwarz points out that cases with myelofibrosis and monosomy 7 demonstrate specific features and could represent a distinct entity.

4. Lynn Ball questions whether myelofibrosis pattern is a reliable marker in childhood MDS or if the variability of myelofibrosis hampers classification and rather represents a reactive epiphenomenon.

Valerie de Haas reports on the DCOG pathology review

A web application, digital slides and video conferences are being established in order to share all pathology data from across the Netherlands.

1. Charlotte Niemeyer emphasizes that the pathologic data should be judged independently from for example cytogenetic data.

Stephan Schwarz reports on the results of the pathology review on hypocellular RC

The question of the pathology review is whether the pathologic differentiation between SAA and RC according to WHO criteria is reproducible among different pathologists. 100 cases of SAA and RC were distributed among participating pathologists and the consensus among the observers was determined. A high percentage of agreement was observed (kappa index 0.79).

Chromosomes, genes and cells in advanced MDS

Göhring

- presented cytogenetic subgroup findings in primary/secondary advanced MDS
- 5y-OS is similar for normal / -7 / other aberrations but significantly worse for "structural complex" (~20%), defined as ≥ 3 aberrations, of which 1 is structural
- when Haase Blood 2007 classifier is applied to EWOG samples: 3-4 aberrations do not have poor prognosis; >5 do have poor prognosis which is similar to "structural complex"
- Breems JCO 2008: "monosomal karyotype" proposed as better marker (def: ≥ 2 autosomal monosomies or 1 autosomal monosomy + 1 structural)
- when Breems classifier is applied: 11 children (monosomal but not "structural complex") have comparably good survival as others
- "structural complex" holds as prognostic marker after HSCT
- significant in multivariate analysis
- manuscript has been submitted

de Vries

- about 5% EWOG-MDS cases contain chr 6 aberration
- 30 pts based on conventional karyotyping
- 19 pts material available (10 primary, 9 secondary)
- Agilent 105k CGH: in 9/19 additional aberrations, 14/19 patients: breakpoints defined more precisely
- performed common breakpoint analysis
- 10 genes with particular interest
- IER3 gene: regulates death receptor-induced apoptosis
- IER3 expression (RT-PCR): unchanged
- "class I/II mutation search": 95 patients, 32 DCOG, 63 EWOG or Rotterdam local

- ~30% class I mutations
- ~40% class II mutations
- ~10-15% both
- ~20% TET2 mutations in adult MDS --> children?
- ASXL1 mutations in childhood MDS: to be investigated
- isocitrate dehydrogenase genes 1 and 2: of interest

Pfister

- case #1: 11yo girl, family hx uneventful, medulloblastoma with "complex" karyotype + abdominal rhabdoid; germline TP53 mutation
- case #2: medulloblastoma, apparently sporadic but strange karyotype; germline TP53 mutation
- both cases developed secondary MDS
- third case in the EWOG-MDS database with known Li-Fraumeni
- planned project: look for TP53 mutations in patients with secondary MDS after brain tumors (EWOG has ~20; 10 from Germany, 10 from other countries)
- logistics: regional coordinators send DNA from affected cases and try to procure brain tumor material
- Pfister group has looked at a total of ~300 medulloblastomas
- ethical committee approval issues need to be resolved
- legal implications for studies on germline material (new legislation in Germany)

van den Heuvel-Eibrink

- Vidaza study: rationale in children is based on some (but not abundant) literature
- Vidaza not EMEA-approved
- Study goal: pharmacokinetics
- JMML and advanced MDS (four strata, 6 pts. each)
- investigator-sponsored trial (drug provided by Celgene)
- to be opened in the course of 2010

Ball (non-member, Leiden)

- MSCs play important role in HSC support
- recent murine model for stroma-induced MDS
- Hypothesis: childhood MDS may result from functional dysregulation of stromal MSCs
- Material requirements: 12×10^6 BM MNC fresh, 20×10^6 BM MNC frozen
- MSCs expanded according to EBMT consortium protocol, then various studies (phenotype, diff. capacity, immune reg. capacity, genetics)
- current status: local Ethic Committee, financial support, PhD student, lab analyst
- 10 MDS samples (5 paired pre-/post-SCT); 5 JMML (4 paired)
- identified 1 JMML with aberrant osteoblast differentiation (first time in a pediatric hematologic malignancy)
- request additional samples from EWOG collaborators

HSCT in MDS and AML: Can we justify different preparative regimens?

Brigitte Strahm reports the results of the EWOG MDS 98 study applying Bu/Cy/Mel for patients with advanced MDS

In 97 patients receiving an allograft from either a MSD or an UD following a preparation with Bu/Cy/Mel the probability of OS and EFS was 63% and 59%, respectively. The relapse incidence and the cumulative incidence of TRM were 21 %, each. The main risk factors for relapse was a higher MDS subtype (MDR-AML vs. RAEB and RAEBt) and a complex karyotype. The main risk factor for TRM was age at HSCT and the presence of higher grade acute or chronic GVHD.

Discussion:

- The outcome of patients with complex karyotype is dismal (none of the 5 patients survived) and so far we have no adequate concept to offer.
- The data confirm that patients with RAEB and RAEBt have a similar outcome and therefore justify that RAEBt was not abandoned in the pediatric adaptation of the WHO classification.
- The lack of improvement over time – in particular the constantly high TRM rate - is astonishing and may be related to the special group of patients. A second aspect is the significant contribution of GVHD to TRM, which may only have been recognized after a period of reduced GVHD prophylaxis.
- Regarding the application of Busulfan: the majority (75%) of patients received Bu po, it is not documented how many patients received Bu adjusted to levels. There was no significant difference in relapse or TRM comparing Bu po to Bu iv. The data in the presented cohort do not allow firm conclusions on the necessity of TDM.
- A second HSCT after relapse is feasible in a selected group of patients. The retrospective analysis of patients that received a second HSCT for recurrent advanced MDS after HSCT resulted in a EFS of roughly 30 %.
- The choice of graft (PBSC vs BM) did not significantly influence the overall outcome.
- The role of endothelial damage induced by the use of the three alkylating agents in regards to the incidence of GVHD and TRM was discussed. Interestingly, AML patients conditioned with the same preparative regimen do not suffer from these high rates of GvHD and TRM. These observations rather argue for a “MDS specific” effect or a contribution of disease burden.
- There are some new single centre data that report a good outcome of haploidentical HSCT in patients with MDS. The results of MDS patients reported to the EWOG MDS study receiving haploidentical HSCT are not very convincing, but it is a very heterogenous group and therefore it is hard to draw any conclusions.

Brigitte Strahm reports the preliminary results of a Treosulfan based preparation for patients with MDS

There were 17 patients with a wide variety of diagnosis (primary as well as secondary MDS; 6 patients with RC, 11 with adMDS) included in the analysis. The conditioning was TT/Treo/Flu for all patients. 6 patients were transplanted from a haploidentical donor and received a CD34+ selected graft. Five patients relapsed and three died to transplant related

causes. The probability of EFS was 40%. The relapse incidence is remarkable in particular because there were 6 patients with RC included that have a very low risk of relapse.

Thomas Klingebiel reported the BFM strategy regarding HSCT in patients with AML.

The probability of OS in the AML BFM 2004 study is 63% and has been improving over time within the consecutive BFM trials. This is comparable to the NOPHO and MRC trials. The definition of high risk patients depends on cytogenetics and response to therapy. Within the AML BFM 98 trial SCT was restricted to patients with high risk features and the availability of a MSD. HSCT was performed with Bu/Cy. A risk benefit calculation within the AML BFM 98 trial comes to the conclusion that HSCT following Bu/Cy for patients with HR AML in CR1 is safe but not effective enough to improve the results over the chemotherapy only results.

Discussion

- The need for identification of groups of patients with a very high risk of relapse (i.e. cytogenetics, chimerism post HSCT) and the possible interventions (addition of myelotarg, DLI) was discussed.

Charlotte Niemeyer (replacing Franco Locatelli, who could not reach the meeting due to the volcano eruptions) presented Franco Locatelli's slides on the Italian experience of HSCT in patients with AML.

The retrospective analysis of 70 patients transplanted from a matched sibling donor following Bu/Cy/Mel resulted in a probability of OS and EFS of 77 % and 76%, respectively. Cumulative incidence of relapse and TRM was 17% and 7%, respectively. The time to reach CR1 significantly influenced the probability of EFS due to a higher chance of EFS in patients that reached CR1 within 42 days. There was a significant improvement of EFS over time due to a dramatic reduction of TRM.

Within the prospective Italian AML trial HR patients receive an induction with 2 cycles of ICE and a consolidation with AVE followed by HAM. Depending on the availability of a matched sibling donor they then go on to allo or auto HSCT. The outcome for the 25 patients that received an allo HSCT following Bu/Cy/Mel is as follows: EFS 77%, RI 20%, TRM 0%.

Peter Bader presents the Frankfurt experience regarding HSCT in AML.

The analysis includes 29 patients with AML transplanted in Frankfurt from 2005 to 2010. 16 patients were transplanted from a haploidentical donor, 4 from a MSD, 6 from a MUD and 3 from a MMUD. The majority of patients were in CR2 (18), whereas 7 patients did not reach remission prior to HSCT. The patients transplanted from haploidentical donors received Flu/TT/Mel as preparative regimen. Among the others 8 pts received Bu/Cy/Mel, 2 pts Treo/Flu/Mel, and some FLAMSA and Bu/Cy. Within the Bu/Cy/Mel group (n=8) 2 relapsed and 2 suffered from TRM, within the Flu/Mel/TT group (n=16) 4 patients relapsed and 1 suffered from TRM.

Discussion:

- The evidence for NK alloreactivity in MDS was questioned.

Martin Sauer presents the AML SCT BFM 2007 trial.

Looking at the trial design from a statisticians/epidemiologists view the problem is that one deals with very small numbers (i.e. about 20 pts/year that qualify for HSCT in CR2 and about 16 pts/year that qualify for HSCT from a MD with refractory AML). Therefore a decision has been made to choose Bu/Cy/Mel +/-ATG in order to be able to increase the numbers at least for some questions comparing AML and MDS.

Looking at the trial from physicians/patients point of view toxicity seems to be the main issue therefore clear stopping rules regarding TRM have been included in the protocol. If one hesitates to choose Bu/Cy/Mel due to toxicity possible alternatives could be Treo/Flu, Treo/Flu/TT, Treo/Cy, Bu/Flu, but none of the regimens is supported by sound data in the literature.

Looking at the trial from a biologists view the better understanding and definition of risk groups by cytogenetics will improve over time.

Discussion:

- Summarizing the results and discussions during the MDS/AML HSCT session it seems fair to conclude that there is an obvious difference between HSCT in AML and MDS. Patients with MDS are much more prone to TRM and the groups were not able to improve outcome in respect to TRM over time. In AML TRM is lower and has substantially improved over time. Therefore it seems reasonable to apply Bu/Cy/Mel for the AML group as long as there are clear stopping rules for the trials.
- Despite the fact that (as reported by Martin Sauer) the BFM group in 2005 very easily agreed on Bu/Cy/Mel as conditioning regimen it has never been applied in a uniform way (Peter Bader AML chimerism analysis: 84 pts. > 18 different conditioning regimens). This may be taken as indicator demonstrating the reluctance of some centers to apply a regimen which they consider to be too toxic.
- The goal must be the implementation of a SCT protocol to generate solid data.

Business Meeting EWOG-MDS
Business Meeting EWOG SAA

Saturday, April 17, 2010; 08:30

Participants: Peter Bader, Lynn Ball, Irith Baumann, Marc Bierings, Valerie de Haas, Barbare De Moerloose, Andrica De Vries, Alexandra Fischer, Christian Flotho, Monika Führer, Ingrid Furlan, Shinsuke Hirabayashi, Axel Karow, Mutlu Kartal, Elisabeth Korthoff, Rose Leguit, Charlotte Niemeyer, Peter Noellke, Ayami Noellke-Yoshimi, Markus Schmutge, Stefan Schwarz, Brigitte Strahm, Wilfried Truckenmüller, Marry Van Den Heuvel-Eibrink, Marcin Wlodarski

1. Item: Welcome and farewell of joining and departing

Markus Schmutge has replaced Eva Bergsträsser as regional coordinator of Switzerland. Susanne Matthes-Martin + Michael Dworzak have substituted Monika Trebo as regional coordinators in Austria.

Dominik Turkiewicz is the new regional coordinator for Sweden and follows Albert Bekassy, who resigned last year.

Jakub Musial replaces Dorota Wojcik as Polish regional coordinator since Dorota moved to Bergen permanently.

Spain and Ireland are both applying members of EWOG-MDS. Spain may reach member status soon.

Since there is a growing amount of JMML samples coming in from Turkey (especially Ankara) a more intense cooperation in the future may be fruitful.

2. Election of the new chair:

Marry van den Heuvel was suggested to follow Franco Locatelli as chair of EWOG-MDS. Markus Schmutge and Barbara de Moerloose agreed on a phone conference to be held soon in order to confirm Marry in her new position.

3. Peter Noellke presents news from the EWOG MDS database

The change from paper-based to electronic flow of patient data is being promoted and MARVIN is being set up as a common SCT-platform. Peter demonstrates some possibilities of this new database, which will be established in June this year and is going to comprise data from all pediatric SCT patients in Germany.

The question of how to deal with the former database has yet to be negotiated.

Data can be transferred between different programs, i. e. PROMISE, although the programs cannot be directly connected.

Marry van den Heuvel addressed the practical approach in terms of implementing MARVIN in each country/region. According to Charlotte Niemeyer there are already such efforts on the way in other fields like the EWING group and people are trained on how to handle MARVIN.

4. Upcoming meeting

There will be a meeting of the MDS Foundation in May 2011 and our group has to decide on how we could contribute.

Franco Locatelli offered to hold the next EWOG-MDS-meeting in Rome, where he has now moved to.

A suitable date for a meeting could be late January 2011.

Irith Baumann pointed out there is going to be a pathology meeting in late autumn this year.

Marry van den Heuvel:

Marry reminded the group that also her term as chair of the scientific board of EWOG MDS is also coming to an end.

Proposals:

Onel Kenan from the University of Chicago suggested joint projects on advanced MDS and offered collaborations. EWOG-MDS regional coordinators will decide on a collaboration only after a defined proposal is submitted.

The clinical paper on secondary MDS (started by Dr. Amman from Bern), which had already been presented during the ASH 2009 should be finished and published. Andrika de Vries is going to take initiative and could be first author.

Papers pending:

The cytogenetic paper by Gudrun Goehring is discussed as a tremendous, interesting and important piece of work. The data had been presented at this meeting and has just been submitted as a short report to Blood.

The paper on transplant data has been submitted to blood by Brigitte Strahm.

CBL paper is about to be resubmitted to Nature genetic and chances for publication appear to be good.

The pathology group is about to publish the instrumental paper on RC-SAA based on the latest WHO classification.

The paper on NPM1 by Marco Zecca is also pending, especially since he is currently occupied by clinical work.

An update on the JMML transplant paper is planned by Charlotte Niemeyer and Franco Locatelli.

EWOG-SAA (Monika Fuehrer):

The regional coordinators for SAA are:

Nils Clausen (Denmark)

Jan Sary (Czech Republic)

Markus Schmugge (Switzerland)

Michal Matysiak (Poland)

Michael Dworzak (Austria)

Marco Zecca (Italy)

Michael Zwaan (Netherlands)

Barbara de Moerloose (Belgium)?

Monika suggested electing a chair for EWOG-SAA as well.

MARVIN is going to be employed as data platform in SAA like in MDS.

Saturday, April 17, 2010

SESSION III

Refractory Cytopenia

Numbers and mutational analysis (Charlotte Niemeyer)

Charlotte provides an update from the EWOG-MDS database on clinical course and survival of RC patients according to different criteria like cellularity.

Charlotte stresses the importance of pathology-based diagnoses and classification.

No standard recommendation a priori concerning therapy is possible for hypocellular RC, the results of different therapy strategies comparable.

Axel Karow provides an update on the mutation analyses for inherited bone marrow failures in RC patients.

More than 100 patients have been screened revealing the following results:

SBDS: only 1 patient with heterozygous mutation (probably a carrier)

TINF2: 2 patients with heterozygous mutation

TERT: 1 patient with heterozygous mutation

TERC: 2 patients with heterozygous mutation

NOP10/ NOLA3: no mutations

NHP2/ NOLA2: no mutations

RUNX1/ AML1: no mutations

The data demonstrate that the cohort of MDS patients is rather “pure” and the vast majority of patients has been thoroughly examined by clinicians.

Some patients with failure to thrive or kidney involvement were investigated in the cohort, but there was no correlation to mutation status.

Charlotte Niemeyer remarked that thorough clinical examination allows recognition of IBMF a priori, and exclusion of acquired RC.

Christian Flotho remarked that it would be important to discriminate between germline vs. somatic mutation in patients with mutations.

Marry van den Heuvel provides insight into the interim analysis of the RC 06-IST study.

PNH clones are present in ~ 20% of analysed RC patients (10 of 55 pts.)

Auto-immunity, Vbeta repertoire along with immunophenotype are also going to be investigated

The project is still in the middle of the analysis.

Peter Bader gives an interim report on chimerism studies in EWOG-MDS SCT RIC-06

Only 9 pts had been included in the study during the last almost 5 years since the study is conducted regionally. Brigitte and Peter have planned to meet again over the question whether more patients are eligible.

Brigitte Strahm reports on selected SCT data from RC patents.

Myeloablative regimen:

The majority of patient was transplanted after BU Cy Mel and received BM as stem cell source. Children transplanted after 2003 showed disease free survival of 83% due to significantly low TRM. Conditioning with Bu CY only appears to even improve these results.

Patients who received RIC revealed excellent EFS, OS. Lower graft ($< 3 \times 10^6$ cells / kg) was associated with complication. Engraftment was significantly prolonged. There was a significant number of graft failure (5 out of 59 patients), which could be rescued in 2nd or 3rd transplant. Viral infections were predominant complication.

It was discussed how one could deal with blood group incompatibility (reduced cell number after depletion?). The use of PDGF for slow or non-platelet engraftment was also discussed.

Ayami Yoshimi reports on the current practice of source selection for allogeneic HSCT in bone marrow failure:

PSC employed in a considerable number of cases with bone marrow failure shows a higher rate of chronic GvHD compared to BM.

Cord blood is mentioned as an alternative source associated with lower risk of GvHD.

Ayami provided a regional overview over transplant numbers in bone marrow failure all across the world. The choice of source appears to be highly variable among the different countries.

SESSION IV

EWOG-SAA 2010

ATG Fresenius Study (C. Niemeyer)

Due to changes in the management of the Fresenius company the study had no priority any more, the study was cancelled. The company expected an investigator initiated study instead; which is not going to be planned.

Genzyme is willing to support a trial either.

ATGAM is not available either.

SAA-Registry (M. Führer)

The patients of the SAA94 study are included in the SAA-registry. A follow-up is therefore possible. New patients are also registered in the SAA-registry.

The SAA cohort comprises 369 patients. There are around 23 new patients per year, the median age is 9 years, there are more male than female. 32 patients died.

The long term survival is 86%. With IST the long term survival is 84%, with BMT 92%.

The strongest predictor of survival is the response to IST after 1 year.

The neutrophil response at day 112 ($> 500/\mu\text{l}$) is a strong predictor for OS (n= 8 patients with neutrophil non-response). Charlotte Niemeyer advises that it would be reasonable to plan to look again at these 8 patients - maybe the histopathological diagnosis is not SAA.

The probability of clonal disease before review is around 40%, after review it dropped to 3%.

Clonal disease was defined as aberrant karyotype or blasts, not PNH.

Charlotte Niemeyer suggests that the pathologists should plan to look again at the diagnostic biopsies of all patients who developed clonal disease.

EWOG-SAA Clinical protocol (M. Führer)

This protocol includes a standardized diagnostic approach and guidelines for therapy.

NSAA patients are only about 7-10% of the cohort.

The guidelines for SCT are presented (conditioning, stem cell source, dose, GVHD prophylaxis). The guidelines for IST are shown (ATG dose, CSA, G-CSF, CSA-tapering). Until now the new ATG-regimen (rabbit Genzyme) results in no increased toxicity and no increased EBV-lymphoproliferation.

The definitions of response are presented (CR, GPR, PPR, NR).

In contrast to adults, the second line therapy in children with SAA is SCT as with second IST the FFS is < 20%.

Most patients respond between 3 and 6 months. If there is NR at 4 months, SCT from a compatible donor is advised. NR at 6 months qualifies for SCT from alternative donor, as do granulocyte non-responder at 4 months.

Peter Bader asked to include a flow-chart concerning therapy decision in the protocol (i.e. IST/ SCT, NR/ SCT..).

The latest time for donor search is at 4 months, but many centers already start at diagnosis.

The general problem of low cell number in a bone marrow transplant from MSD is addressed; until now no engraftment problems have been described, even with low cell numbers.

It is asked why the SCT regimen in early non-responders is not like in MDS? It is stated that according to the diagnostic criteria of the prospective SAA-protocol, all patients in this trial should be SAA. The question remains nevertheless open.

Scientific questions (Wlodarski)

M. Wlodarski presents the scientific projects associated with the SAA-protocol.

1) Telomere length measurement: SAA patients with short telomeres have a higher risk of clonal disease (literature data). Telomere length of SAA patients is generally not shorter than in healthy control.

2) SNP-arrays: This method allows the detection of cryptic genomic lesions. This method also allows the detection of LOH/ copy number neutral lesions. It is planned to analyze somatic and germline DNA.

Charlotte Niemeyer states that the primary objectives (telomere length + SNP-array) can be done on DNA. It will be important to have a standardized lab manual/ protocol for handling/ processing material. All labs should use the same method. The content of manual will be: methods to be used, material (i.e. DNA), priority of research projects. The manual should be finished in the next 2 months. M. Wlodarski already prepared a first version of a laboratory manual.

Marry van den Heuvel remarks that often there are very few cells, and it is difficult to have extra material.

3) TCR Vb-analysis: There is preliminary work of A. Borkhardt, Düsseldorf, on TCR expansion in SAA. He showed an increase of clonality and a decrease of complexity. It is planned to correlate the TCR skewing to IST response.

4) PNH-analysis: PNH cells may serve as trigger of T-cell expansion. Another hypothesis is that PNH cells may immunologically escape T-cell autoreactivity. In the SAA-study PNH will be correlated with IST response, survival and TCR expansion. PNH clones will be detected in RBC and granulocytes. The experimental FLAER-method will additionally be added for granulocyte PNH detection.

5) In vitro response to ATG: This will be analyzed by phospho-FACS of PBMC. The baseline status will be measured, after exposition to ATG the activation status will be measured. Maybe a prediction of response to ATG will be possible.

The material needed for research is hep. PB, hep. BM and germline DNA (fibroblasts, nails).

Charlotte Niemeyer sees the need for a discussion on how to share material and research. The first step is to create a lab manual. DNA-studies can be shared by all; it is therefore planned to perform telomere studies and SNP-arrays in 100% of patients. Around 50% of patients will participate in PNH-studies, for the other research studies there will be less patients.

Monika Führer asks if there is a difference between studies on PB vs. BM. Bone marrow is important for TCR studies. When bone marrow of SAA patients is separated by Ficoll, very few granulocytes are left. IT is nevertheless important to separate granulocytes from bone marrow. The rest of the material is often only activated lymphocytes.

Markus Schmugge asks if his center should send pellet, or fresh material. Fresh material is always preferred in Freiburg.

Monika Führer states that if there is material left, further projects should be decided in a group. At least 1 ng of DNA of all patients should be asserved.

The presentation of TCR data by A. Borkhardt was skipped.

SAA-Protocol – to do:

The Regional Coordinators should be defined.

The second bone marrow aspiration should be done after 2 weeks.

A phone conference should be planned concerning the agreement on the protocol.

A decision on who will share which research part is needed.