Minimal Residual Disease After Stem Cell Transplantation. The current status

Sergio Giralt, MD
Professor of Medicine
Weill Cornell College of Medicine
Chief, Adult BMT Service
Memorial Sloan Kettering Cancer Center
New York, New York

Learning Objectives

- Identify different assays for minimal residual disease (MRD) detection
- List diseases in which MRD monitoring is considered the standard of care
- Define potential uses of MRD monitoring

Topics

- Minimal Residual Disease
  - Definition
  - Assays
  - Relevance
- MRD in CML
- MRD in Myeloma
- MRD in AML
- MRD in ALL
- Summary

Lets go back in time…

- The year is 1995
- 32-year-old female
- Has recently been experiencing increasing fatigue and weight loss
- WBC 48,000 with 10% myeloblasts in peripheral blood
  and 5% basophils
- Bone marrow 10% blasts; 5% basophils
- Cytogenetic studies show t(9;22) with an additional 20q- abnormality
- Has a 39-year-old sister who is a 6/6 HLA match

Case Presentation

- 3 months post allograft she is in a complete cytogenetic remission.
- 18 months later a new test called PCR is reported as positive
- She continues to be followed.
- At 24 months the tests is negative.
- At 30 months post BMT the test is positive again
- What should be done (remember it is 1998 now)

CML: ARS Question #1

In patients with CML a QUALITATIVE PCR (pos or neg) predicts relapse.

1. True
2. False

CML: ARS Question #2

In this patient what would the appropriate next step be with this positive test for minimal residual disease (MRD)?

1. Request donor lymphocyte infusion (DLI) as soon as possible
2. Start interferon
3. Wait until 1999 and get her on a protocol with the new STI571
4. Perform bone marrow aspiration and determine whether there was cytogenetic evidence of disease

ARS Question #3: What is MRD anyway?

Which is the correct definition for MRD?

1.Minimal residual disease refers to disease that is left over after treatment that only can be seen by an expert pathologist.

2. Minimal residual disease only relates to CML and represents presence of disease at a 1 in 100000 level.

3.Minimal residual disease is the name given to small numbers of leukemic or other tumor cells detected by very sensitive methods that remain in the patient during treatment, or after treatment when the patient is in remission. It is the major cause of relapse in cancer and leukemia.

MRD-Definition
When in doubt ask WIKIPEDIA

- "Minimal residual disease is the name given to small numbers of leukaemic cells that remain in the patient during treatment, or after treatment when the patient is in remission. It is the major cause of relapse in cancer and leukaemia."

Minimal Residual Disease

- Not totally true
- MRD has usually referred to disease detected by non-traditional methods (xray or pathology). The two most commonly used methods are flow cytometry and polymerase chain reaction.
- MRD by flow cytometry or PCR predicts for a higher risk of relapse after chemotherapy and also after transplantation in SOME but NOT ALL diseases.

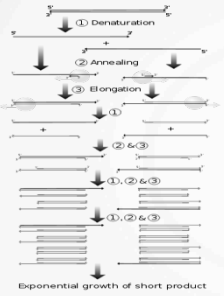
MRD Detection

- Cytogenetic methods, including FISH
  - Generally not sensitive enough to be real minimal residual disease measure
- Flow cytometry
  - Based on aberrant antigen expression ("Leukemia-associated immunophenotype")
- PCR
  - Adaptable to different targets
  - Can measure clonal abnormality or abnormal expression

Polymerase Chain Reaction

- Developed in 1983 by Kary Mullis, PCR is now a common and indispensable technique for DNA cloning and sequencing which has ubiquitous applications in diagnosis of hereditary diseases, forensic sciences, minimal residual disease detection and infectious diseases.
- In 1993, Mullis and Michael Smith were awarded the Nobel Prize for their work on PCR.
- The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers (short DNA fragments) containing sequences complementary to the target region along with a DNA Polymerase
- Almost all PCR applications employ a heat-stable DNA polymerase, such as Taq polymerase (an enzyme originally isolated from the bacterium Thermus aquaticus

PCR



DNA-based tests

Detect tumor specific DNA sequences using the polymerase chain reaction (PCR), a highly sensitive technique.
Useful for chromosomal translocation, microsatellites (chimerism), immunoglobulin and T cell receptor rearrangements.

RNA-based tests

Detect tumor specific RNA sequence. Uses reverse transcription of the RNA followed by polymerase chain reaction. RNA-based tests are normally utilized when a DNA test is impractical.
BCR-ABL most commonly used
The markers used for RNA-based testing are almost exclusively chromosomal translocations such as t(9;22) BCR-ABL, t(15;17) PML-RARA and t(12;21) ETV-RUNX1 (TEL-AML1).

Types of PCR Methods

- Antigen receptor PCR
  - Most suited to lymphoid malignancies
- Fusion transcript PCR
  - Several tumor types but only limited subsets of most tumors (CML excepted)
- PCR for gene mutations
  - AML subsets, e.g. FLT3 or NPM1
- mRNA PCR
  - Suitable for upregulated genes, e.g. WT1

- **Immunological tests**
- Flow cytometry is an immunological-based testing of leukemias or other cancers utilizes proteins on the surface of the cells. Leukemic and other cancer cells often show quite unusual and unique combinations (leukemic phenotype) of these cell surface proteins. These proteins can be stained with fluorescent dye labeled antibodies and detected using flow cytometry. The limit of detection of immunological tests is generally about 1 in 10,000 cells and cannot be used on cancers that don't have an identifiable and stable phenotype

Flow Cytometry-WIKIPEDIA

- Flow **cytometry** is a laser-based, biophysical technology employed in cell counting, sorting, biomarker detection and protein engineering.
- Principle: suspend cells in a stream of fluid and passing them by an electronic detection apparatus. It allows simultaneous multi-parametric analysis of the physical and chemical characteristics of up to thousands of particles per second.
- Flow cytometry is routinely used in the diagnosis of health disorders, especially blood cancers.
- **History**
- Mack Fulwyler was the inventor of the forerunner to today's flow cytometers
- Wolfgang Gohde developed in 1968 fluorescent based flow cytometry

- **Patient-specific testing**
- Patient-specific MRD detection using immunoglobulin (IG) or T cell receptors (TCR).
- Measures MRD in tumors that do not contain a chromosomal translocation or other specific marker.
- These tests are very specific, and detect leukaemic cells at levels down to one cell in a million, though the limit typically achieved is 1 in 10,000 to 1 in 100,000 cells. For comparison, the limit of what one can detect using traditional morphologic examinations using a microscope is about 1 cell in 100.

Methods For Detecting Chimerism

- XY FISH
  - Easy, but not very sensitive; only applicable to a subset of patients
- PCR methods
  - Microsatellite markers(short tandem repeats (STRs) or variable number tandem repeats (VNTR))
    - Informative in nearly all cases; sensitivity around 1-5%
    - Most widely used
  - TaqMan qPCR against single nucleotide polymorphisms
    - Sensitivity 0.1% or better, and quantitation better but perhaps not informative as often; more limited data
  - Y chromosome PCR even more sensitive ($1/10^5$)
- Lineage-specific chimerism more specific

General Considerations

- Two broad approaches
  - Chimerism
    - Not a direct measure of disease
    - Applicable to all patients
    - Extent of chimerism not necessarily related to disease burden
  - Minimal residual disease detection
    - Genetic approaches to detect actual clone (PCR, FISH)
    - Phenotypic approaches to detect abnormal expression (flow cytometry, mRNA)
  - Imaging and other clinical monitoring discussed in manuscript

CML-Audience Response Questions

- False
  - Patients with CML may have low level QUALITATIVE PCR without ever relapsing
- In this patient what would the appropriate next step be with this positive test for minimal residual disease (MRD)?
- Perform bone marrow aspiration and determine whether there was cytogenetic evidence of disease.

Comparison of Flow Cytometry and PCR for MRD Detection

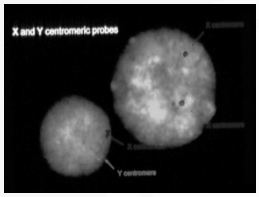
PCR

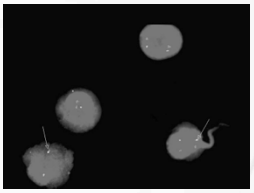
- Advantages
  - Highly sensitive and reproducible
  - Clone specific
  - Most data in many diseases
- Disadvantages
  - Not applicable to all diseases
  - Ag receptor PCR requires allele specific oligos and is expensive and time consuming
  - Clonal evolution a potential pitfall

FLOW

- Advantages
  - Rapid and relatively inexpensive, allowing early intervention
  - Widely applicable in many diseases (not CML)
- Disadvantages
  - Not as sensitive as PCR
  - Not well standardized

FISH to Detect Chimerism or MRD





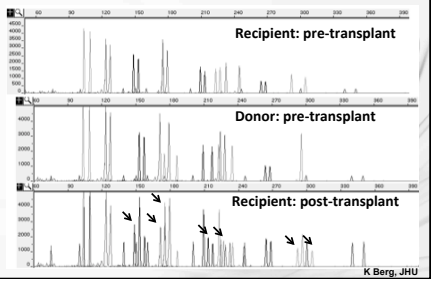
XY FISH BCR-ABL FUSION

Microsatellite DNA: Chimerism analysis

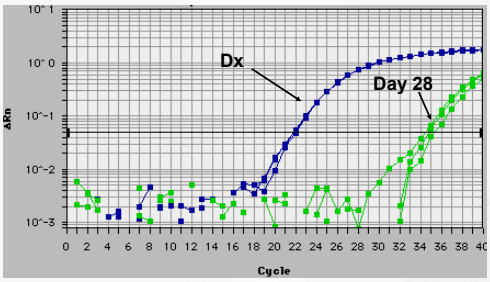
Recip DNA only

Donor DNA only

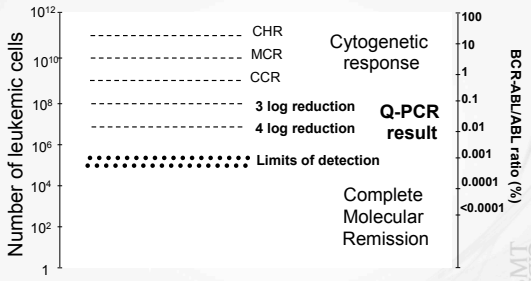
Donor + Recip DNA



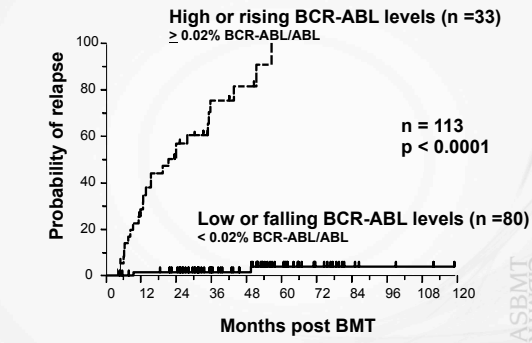
Real-Time PCR Detection of MRD t(12;21)



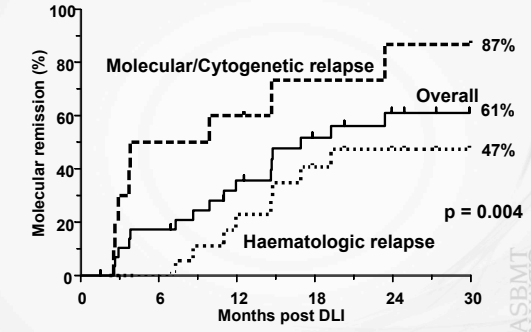
Quantitating Leukaemic Cell Load in CML



Quantitative RT-PCR and relapse post BMT



Complete molecular response to DLI



Probability of death after CML relapse

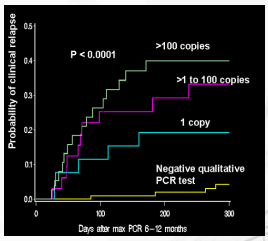
Hazard ratio from Haem/Non-Haem relapse to death

		Hem.Rel→Death	Non-Hem.Rel → Death
EBMT risk score	Low	1.00	1.00
	Intermediate	1.42 (0.62-3.26)	1.14 (0.58-2.21)
	High	2.99 (0.92-5.72)	4.35 (1.86-10.20)
Occurrence of relapse in	1993–1996	7.42 (3.07-17.97)	3.50 (1.79-6.81)
	1997-1999	8.14 (3.50-18.94)	2.34 (1.15-4.76)
	2000-2003	6.54 (2.46-17.37)	0.60 (0.22-1.64)

Risk of relapse and Q-PCR bcr-abl transcript level after allogeneic SCT

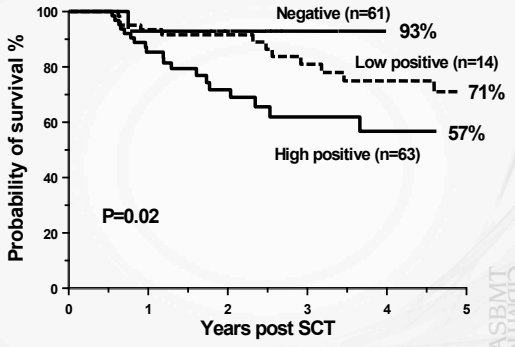
Hazard ratio associated with attaining a maximum PCR value within a specific interval

Copy number	3-6 mo.	6-12 mo.	12-18 mo.	>18 mo.
Negative	1.0	1.0	1.0	1.0
1	1.0	2.5 *	----	3.2
>1 to 100	0.7	3.6 *	1.9	3.4
>100	1.9 *	5.1 *	3.2 *	3.9 *



Days after max PCR achieved at 6-12 months

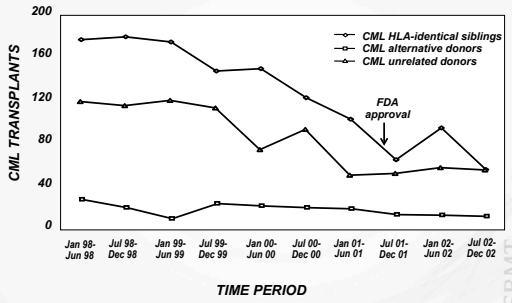
Overall Survival for CML in CP by Early PCR result



CML Summary

- MRD monitoring is well established with Q-PCR for BCR-ABL
- PCR positivity predicts for relapse (all types) and disease progression
- PCR monitoring can be used for assessing response to treatment of relapse (DLI +/- Imatinib)
- Treatment of early relapse (molecular-cytogenetic) results in superior response rates and survival
- There is a need for standardization of PCR methodology
- Future clinical trials should focus on MRD monitoring after treatment with TKIs post allogeneic SCT

Transplants for CML in North America Registered with the CIBMTR 1998-2002



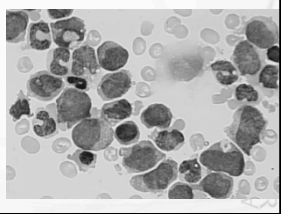
MRD Methods in Different Diseases

DISEASE	PCR	FLOW
ALL	Yes	Yes
AML	Subgroups only	Yes
CML	Yes	No
CLL	Yes	Yes, probably best
NHL	Yes	Limited data
Myeloma*	Yes	Yes
Hodgkin lymphoma	No	No
MPN	Limited data (JAK2)	No

*free light chain assay may play a role as well

NCI SCT Relapse Monitoring Subcommittee - Monitoring strategies in Acute Myeloid Leukemia and Myelodysplastic Syndromes

Ulrike Bacher, MD
Clinic for Stem Cell Transplantation
University Cancer Center Hamburg
Germany



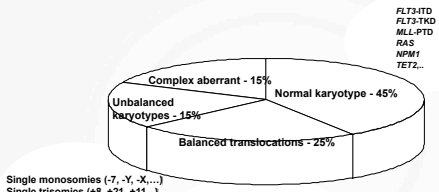
Case Presentation

- 55 year old male with relapsed acute leukemia
- Has an t(8;21) and initial remission lasted 24 months
- Undergoes an allogeneic SCT.
- 18 months post SCT in in a hematologic and cytogenetic remission but PCR is still reported as positive
- What should be done (remember it is 2014 now)

ARS #4: What to do?

1. Proceed to 2nd allo SCT
2. Proceed to DLI
3. Proceed to chemo with cytarabine
4. Continue to monitor with PCR and treat only if increasing levels of disease or evidence of hematologic relapse develops

I. Genetic Heterogeneity of AML



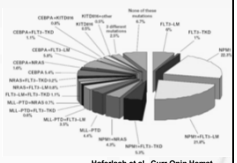
FLT3-ITD
FLT3-TKD
MLL-PTD
RAS
NPM1
TET2,..

Single monosomies (-7, -Y, -X,…)
Single trisomies (+8, +21, +11,..)
Structural alterations
Unbalanced translocations

t(8;21)
inv(16)
t(15;17)
11q23 rearrangements

Parameters for post-transplant molecular MRD monitoring in AML?

Reciprocal rearrangements:
MRD diagnostic with RQ-PCR well established
"favorable" rearrangements >> minor role for allo-SCT
Other suitable markers for the SCT setting?



Haferlach et al., Curr Opin Hemat, 2006

Normal karyotype:
45% of all cases

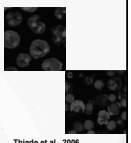
NPM1: 55%?
FLT-ITD: 35%?
NRAS: 10%
FLT3-TKD: 55%
MLL-PTD: 10%
RUNX1: 10%

Post-transplant MRD monitoring?

Nucleophosmin1 mutations for post-transplant monitoring in AML

NPM1 mutation:
- 55% in normal karyotype
- association with *FLT3*-ITD

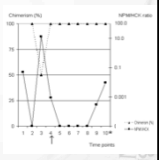
13 stem cell recipients with *NPM1* mutated AML



Thiede et al., 2006

Post Transplant:
NPM1A negative > indicator for remission
NPM1A positive > always relapse post-SCT

Morphological relapse: n=9
Preceded by increase of *NPM1A* mutation: 100%
Interval: ø 24 days (12-38)



Bacher et al., Exp Hematol, 2009

NPM1A-mutation> suitable for post-transplant MRD monitoring in AML (although underrepresented in SCT patients)
Short interval between increase of mutation load and relapse!

FLT3 mutations for post-transplant MRD monitoring in AML?

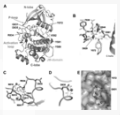
***FLT3* mut:**
~40% in normal karyotype AML
Adverse prognosis

Scholl *et al.*, 2005:
4 AML patients + *FLT3*-ITD/TKD-mutation
>> RQ-PCR in the post-transplant period

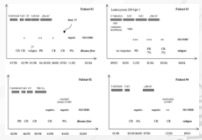
Strong correlation of the mutation load with post-transplant outcomes

?

- Instability of *FLT3* mutations at relapse?
- *FLT3*-ITD: RQ-PCR requires design of specific primers



Frohling et al., Cancer Cell, 2007



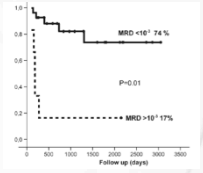
Scholl et al., Clin Cancer Res, 2005

Flow cytometry for post-transplant monitoring in AML?

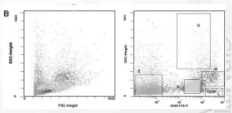
Leukemia associated immunophenotypes in AML:
- **"Cross-lineage" expression: CD7**
- **Loss of antigens: HLA-DR**
- **Aberrant levels of expression**

Diez-Campelo *et al.*, 2008
- **Flow cytometry in 41 stem cell recipients with AML/MDS**
- **≥10^{-3} leukemia cells at 3 months: 4-yr EFS < 20%**
- **< 10^{-3} leukemia cells: >70%**

Flow cytometry might contribute to MRD in the post-transplant period of AML, but very few studies have so far been performed.

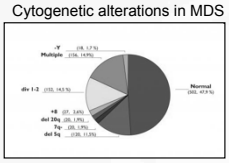


Diez-Campelo et al., Am J H, 2008



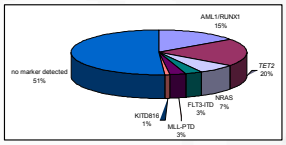
II. Genetic heterogeneity of MDS

Cytogenetic alterations in MDS



Duesseldorf Registry, 2000

Molecular mutations in advanced MDS

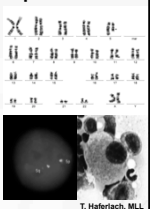


Suitable markers for post-transplant follow-up in MDS?

So far, no MRD strategy for the follow-up of MDS patients is available
Only exception: *WT1* monitoring
(Tamura et al., 2006; Cilloni et al., 2003)

MDS: Cytogenetic alterations in 55% of cases
>> Interphase FISH as post-transplant strategy?

Fuehrer *et al.*, Int J Mol Med, 2005:
23 pediatric patients (of those, 8 AML, 2 MDS)
> Interphase FISH
> Stable remission: n=19 >> no aberrant interphase nuclei
> relapse: n=4 >> aberrant interphase nuclei



T. Haferlach, MLL

Further evaluation in the post-transplant setting of MDS?

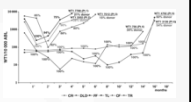
Measurement of *WT1*-expression in AML and MDS

Wilms tumor gene (*WT1*): RQ-PCR >> overexpression in AML/MDS and other malignancies

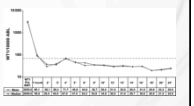
Candoni *et al.*, 2009:
- Relapse in 6/38 AML patients post SCT
- *WT1* expression ↑ in all 6 patients
- Progression: n=5/6
- Successful DLI/chemotherapy: n=1/6

WT1- expression:
Irrespective of subgroups in AML/MDS

Less specific than molecular mutations
Background expression as well in healthy individuals

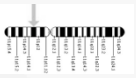


WT1 expression in patients with post-transplant relapse of the AML



WT1 expression in AML patients with stable post-transplant remission

Candoni et al., Eur J Haemat, 2009



Chimerism in AML and MDS

Bader *et al.*, 2004:
STR-PCR chimerism in 81 pediatric patients with AML

Increasing mixed chimerism (MC): > Relapse: 47%

Complete DC/decreasing MC > Relapse: 13% (p<0.05)

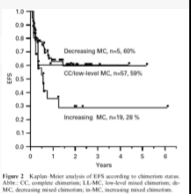


Figure 2 Kaplan Meier analysis of EFS according to chimerism status. Abbr.: CC, complete chimerism; LL-MC, low-level mixed chimerism; de-MC, decreasing mixed chimerism; in-MC, increasing mixed chimerism.

Bader et al., BMT, 2004

Zeiser *et al.*, 2005:
CD34+ chim. in 168 AML/MDS
> mixed chim.: relapses 89%
> full donor chim.: relapses 6%

The kinetics of mixed chimerism is highly relevant in AML/MDS

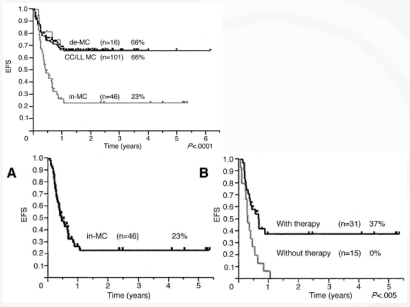
Disease-Specific Methods and Strategies for Monitoring Relapse Following Allogeneic Stem Cell Transplantation

Pediatric Acute Lymphoblastic Leukemia

on behalf of the Sub-Committee

Peter Bader, Wendy Stock, Andre Willasch, Alan Wayne

Hematopoietic Chimerism in Children with ALL



Bader et al. J Clin Oncol 2004;33:1696.

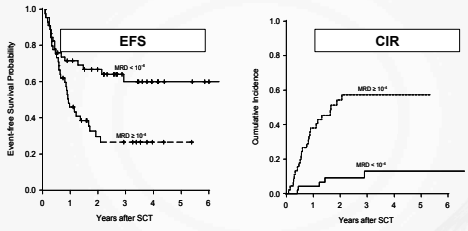
Studies on Chimerism and Intervention

Author	Number of patients	Diagnosis	Interval of investigations	Methods	Relapses
Formakova Haematologica 2003	54	AL, CML and MDS children	weekly to +100; monthly	STR	MC associated with rejection and relapse Immunotherapy was possible
Gorczynska BMT 2004	14	ALL, AML children	weekly to +100; monthly	STR	In-MC could be converted by immunotherapy to CC
Bader JCO 2004	163	ALL children	weekly to +100; monthly	STR	MC associated with rejection and relapse Immunotherapy was possible
Horn BMT 2008	20	AL children	1,3,6,12 months; In MC bi-weekly	STR	MC associated with relapse IT was not possible

Retrospective Studies - MRD prior to SCT Literature

Author	Number of patients	Diagnosis	Time of investigation	Methods	Survival according to MRD status
Knechtli Blood 1998	64	ALL	prior to conditioning	Ig / TCR PCR	high level pos. – 0% low level pos. – 36% negative – 73%
Bader Leukemia 2002	41	ALL	prior to conditioning	Ig / TCR PCR	high level pos. – 23% low level pos. – 48% negative – 78%
Uzunel Blood 2001	30	ALL	prior to conditioning	Ig / TCR PCR	high level pos. – 47% low level pos. – 50% negative – 100%
Sramkova Ped Blood Cancer 2007	25	ALL	prior to conditioning	Ig / TCR PCR	positive – 0% negative – 94%

Prospective Study: MRD Prior SCT ALL REZ BFM Group: CR2



MRD
< 10^{-4}: n = 46; cens.= 29; pEFS = .60 ± .08 CI (relapse) = .13 ± .06
≥ 10^{-4}: n = 45; cens.= 14; pEFS = .27 ± .07 CI (relapse) = .57 ± .08
p = .0004 p < .001

Bader et al. J Clin Oncol 2009;27:377-84.

Conclusions II

- MRD prior to stem cell transplantation has a profound impact on post transplant outcome!

- What adds MRD post transplant?

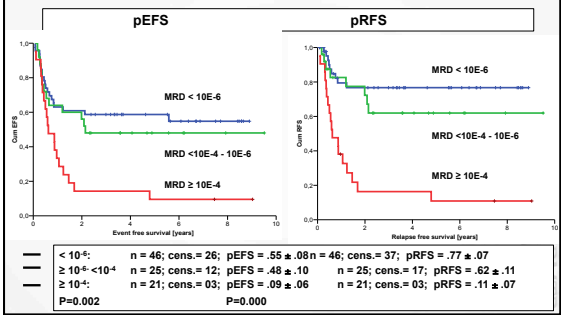
Retrospective Studies -MRD Post SCT Literature

Author	Number of patients	Diagnosis	Time of investigation	Methods	Survival and MRD status
Knechtli BJH 1998	68	ALL	up to 24 months post SCT	Ig / TCR PCR	relapse – 88% pos. remission – 22% pos.
Uzuel BJH 2003	23	ALL	24 months	Ig / TCR PCR	MRD pos. associated with relapse
Sanchez BJH 2002	40	ALL	d30, 60, 90, every 2-3 months	Flow cytometry	positive – 33% negative – 74%

Prospective Study BFM Group

N	92		
Diagnosis	ALL		
Remission	≥ CR2		
Transplant Period	Jan 1999		May 2006
Evaluation	January 15th 2009		
Follow up	Median	Min	Max
[Years]	5.13	3.44	6.48

MRD - Highest Level post SCT All Patients



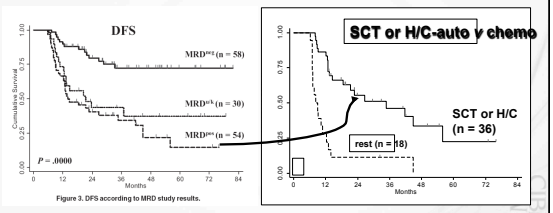
Conclusions III and Summary

- MRD assessment in BM post transplant is predictive for relapse
  - Serial BM investigations are warranted.
  - Current working recommendations of the BFM: days 30, 60, 100, 200, 300, 365, at 18 months and 24 months.
- Summary:
  - Patients with mixed chimerism have a high risk for relapse
  - Patients, who become/remain MRD positive $\geq 10^{-4}$, have a very high risk to develop relapse
    - Additional treatment in these patients is warranted

MRD in adults with ALL

- Shown to be useful predictor of DFS in many studies (non-transplant)
  - Independent prognostic feature
  - Mostly using PCR techniques – IgH/TCR, fusion genes
    - "Informative" assay available in 60-90% of patients
- Early CR time-points predictive of outcome: from 4-22 weeks following initiation of treatment
- Fewer studies evaluating role of MRD in setting of alloSCT

AlloSCT improves outcome of MRD^{pos} in CR1 but much room for improvement

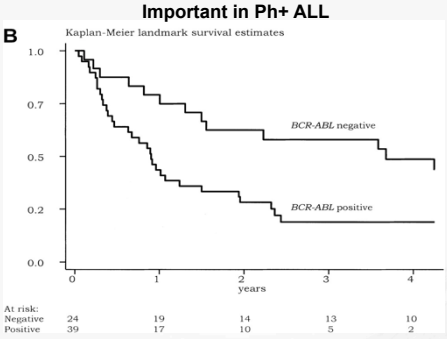


Bassan, R. et al. Blood 2009;113:4153-4162.

MRD following alloSCT in Adults with ALL

Author	Number of patients	Diagnosis	Time of investigation	Methods	DFS and MRD status
Mortuza JCO 2002	19	ALL (B-lineage)	From 1-20 mos.	Ig / TCR PCR Semi-quant.	positive – 0% negative – 100% CCR
Spinelli Haematologica 2007	37	ALL	Day +100	Ig/TCR or fusion gene PCR Quantitative	positive $>10^{-4}$: 20% negative: 93%
Bassan* Blood 2009	18	ALL *All were PCR+ prior to transplant	Not defined	Ig / TCR PCR	positive $>10^{-4}$: 0 negative: 50%

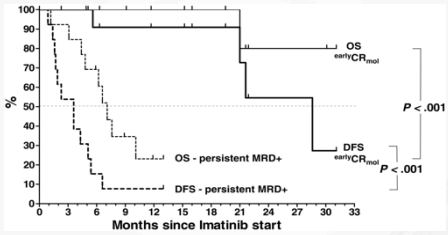
Achievement of Molecular Remission Prior to AlloSCT is Important in Ph+ ALL



MRD status prior to transplant predicts DFS

Dombret, H et al. Blood 2002;100:2357-66.

Imatinib Treatment of Molecular Relapse with Following Allo-SCT for Ph+ ALL



Wassmann, B. et al. Blood 2005;106:458-463.

Summary

- MRD detection both prior to and following alloSCT for adults with ALL is associated with poor DFS
- Clinical interventions based on MRD measurements suggest utility but data are very limited:
  - Allocation to alloSCT in CR1
  - Post-transplant intervention to prevent relapse
    - Targeted therapy (e.g. imatinib) following transplant
- Challenge: implementation of standardized MRD assays that can be done in "real-time"
  - IgH/TCR qPCR assays are laborious
  - Data on flow cytometric measurements of MRD in adults with ALL are lacking

Disease specific Monitoring of Relapse after Allogeneic Hematopoietic Cell Transplantation

Multiple Myeloma
NCI Workshop 1./2.-11.2009

Nicolaus Kröger

Conventional techniques for monitoring

- **Bone marrow aspiration: infiltration often underestimated**
- **Serum/24h urine electrophoresis (agarose gel or capillary zone): lowest detectable level of M-component: 0.2 - 0.6 g/L**
- **Immunofixation (serum/urine): lowest detectable level of M-component: 0.12 - 0.25 g/L**
- **Free light chain assay (κ/λ ratio) : useful in light chain disease and non-secretory, necessary to determine sCR, early response assessment due to short half time (6h)**

Imaging monitoring

- More than 80% of the pts develop osteolytic bone lesions
- The hallmark of myeloma bone disease is an increased osteoclastic bone resorption and an exhausted osteoblast function resulting in a reduced bone formation even in patients in complete remission
- Standard: conventional radiology as skeletal survey involving cervical, thoracic and lumbar spine, skull, chest, pelvis, humeri and femora
- Disadvantage: low sensitivity, no exact response assessment
- CT: high sensitivity, but higher radiation dose
- MRI: high sensitivity, no radiation dose, detect extramedullary disease
- PET-CT: highest sensitivity for extramedullary disease

Flow-cytometry

- Flow cytometry has become an easy applicable method to detect residual myeloma cells The European Myeloma Network recommends a minimal panel including
- CD19, CD56, CD20, CD117, CD28 and CD27.
- Plasma cell gating should be based on CD38 vs. CD138 expression
- This method is less sensitive (10^{-4}) than allele-specific oligonucleotides PCR (ASO-PCR)

Rawston A.C., et al. Haematologica 2008;93:431-438.

Allele-specific oligonucleotides PCR (ASO-PCR)

- Patient-specific primers (IgH rearrangement)
- High sensitivity of (10^{-5} - 10^{-6}) and highly specific (100%)
- Time-consuming (for each patients), does not detect extramedullary disease

Rate of molecular remission based on rearranged immunoglobulin heavy chain genes

In CR:	after allograft:	50% molecular CR
	after autograft:	7% molecular CR
In CR:	after allograft:	50% molecular CR
	after autograft:	16% molecular CR

Corradini, P et al. J Clin Oncol 1999;17:208-15.
Martinelli G, et al. J Clin Oncol 2000;18:2273-81.

Minimal residual disease after allogeneic stem cell transplantation

Multiple Myeloma (EBMT-Studie): Pat with CR

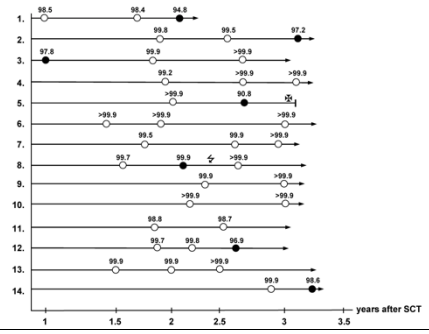
	PCR neg	PCR mixed	PCR pos
No. of pts	16	19	13
5 year cumulativ risk of relapse	0%	33%	100%

Corradini et al. Blood 2003;102:1927-9.

Chimerisms

- Not specific for relapse, in majority of relapse donor cell chimerism persisted
- Lineage specific chimerism (plasma cell-chimerism: CD138+ BM cells)
- By using real-time PCR the sensitivity of the method is 10^{-4} to 10^{-5}. The disadvantage of the methods is the lack of specificity.

Quantitative donor plasma-cell chimerism in patients with negative immunofixation



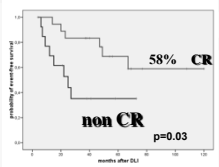
Predictive value of donor-plasma-cell chimerism for relapse

- **93% with stable or increasing donor-plasma-cell chimerism remained immunofixation-negative.**
- **83% with a decrease of donor-plasma-cell chimerism was associated with relapse in the sense of becoming immunofixation-positivity (in 2: 3 and 6 months prior than immunofixation becomes positive)**

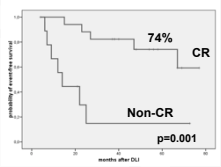
Kröger, N et al. Exp Hematol 2006;34:688-94.

Depths of remission and survival post allografting

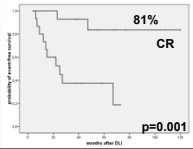
According EBMT criteria



According Flow cytometry



According to molecular methods



Kröger, N et al. Exp Hematol 2009;37:791-8.

Summary

- MRD assessment is now routinely performed in the setting of hematologic malignancies.
- MRD presence can predict disease recurrence in some but not all instances.
- Although frequently done the impact of early intervention based on MRD assessment has only been shown to be effective in CML.
- Both patients and physicians should be encouraged to participate in clinical trials.