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# Minimal/Measurable residual disease in AML
# European LeukemiaNet guidelines

**Arjan A. van de Loosdrecht**

Department of Hematology
Amsterdam UMC
VU University Medical Center
Cancer Center Amsterdam (CCA)
Amsterdam, The Netherlands

**London**
**8-9 November 2018**
**CDDF Multi-stakeholder workshop on MRD in AML and CLL**

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# Measurable Residual Disease (MRD) in AML

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- The persistence of leukemic cells after chemotherapy ***and*** which numbers are below the sensitivity detection level of routine morphology

- Represents the ***sum of the effect*** of all relevant cellular resistance mechanisms, pharmacokinetic resistance and other unknown factors affecting the effectiveness of the treatment

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**Hourigan CS, et al., Leukemia 2017;31:1482**
**Schuurhuis GJ, et al., Blood 2018;131:1275**

# 2016 European LeukemiaNet
## Response criteria in AML

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. §Response criteria in acute myeloid leukemia

| Category | Definition | Comment |
|---|---|---|
| **Response** | | |
| ➢ CR without minimal residual disease ($CR_{MRD-}$) | If studied pre-treatment, CR with negativity for a genetic marker by real-time quantitative polymerase chain reaction (RT-qPCR), or CR with negativity by multi-color flow cytometry | Sensitivities vary by marker tested, and by method used; therefore, test used and sensitivity of the assay should be reported; analyses should be done in experienced laboratories (centralized diagnostics) |
| 2 Complete remission (CR) | Bone marrow blasts <5%; absence of circulating blasts and blasts with Auer rods; absence of extramedullary disease; absolute neutrophil count ≥1.0 x $10^9$/L (1,000/μL); platelet count ≥100 x $10^9$/L (100,000/μL) | MRD positive or unknown |

**Diagnosis and Management of Acute Myeloid Leukemia in Adults: 2016 Recommendations from an International Expert Panel On behalf of the ELN. H. Dohner, et al. Blood 2017;129:424-447**

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# MRD Why?

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- To provide objective methodology to establish remission status
- To refine outcome prediction and inform post remission treatment
- To identify impending relapse to enable early intervention
- To allow more robust post-transplant surveillance
- To Use as surrogate endpoint to fasten drug approval

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Hourigan CS, et al., Leukemia 2017;31:1482
Schuurhuis GJ, et al., Blood 2018;131:1275

# MRD How?

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- Leukemia specific features at diagnosis distinguish residual leukemic cells from normal cells:
  - Aberrant immunophenotype (FCM)
  - Fusion transcripts (RNA PCR)/digital PC)
  - Gene mutations (DNA/RNA PCR, NGS)
  - Gene overexpression (RNA qPCR)



**Hourigan CS, et al., Leukemia 2017;31:1482**
**Schuurhuis GJ, et al., Blood 2018;131:1275**

# Limitation of morphological assessment to assess remission status in AML

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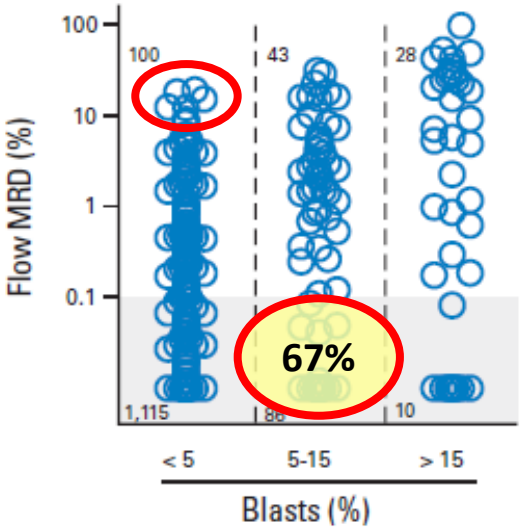
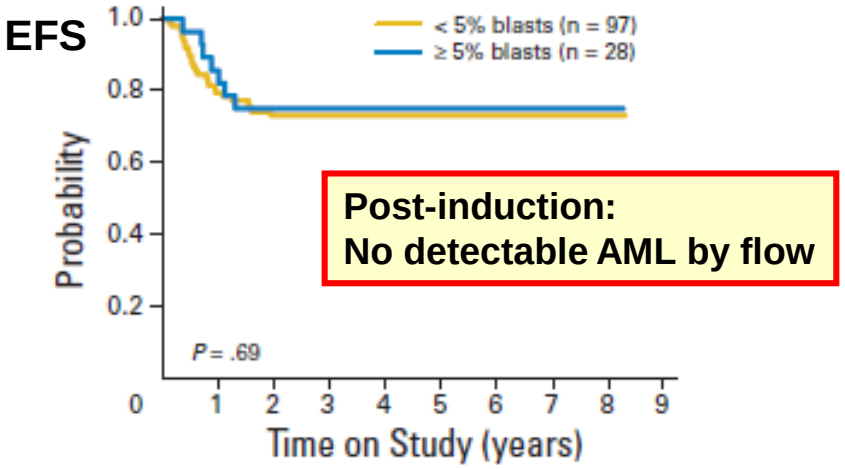
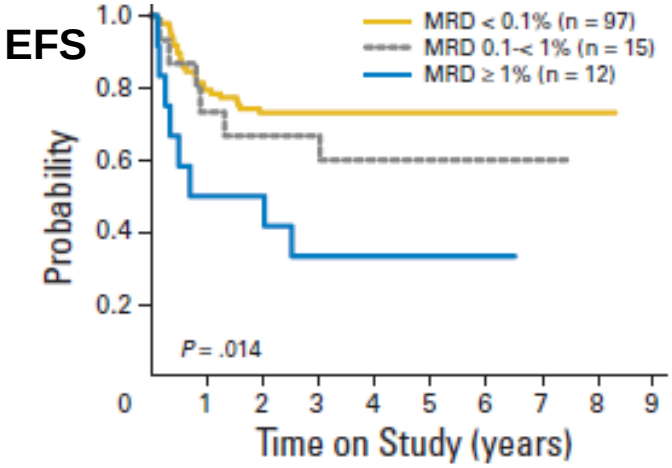


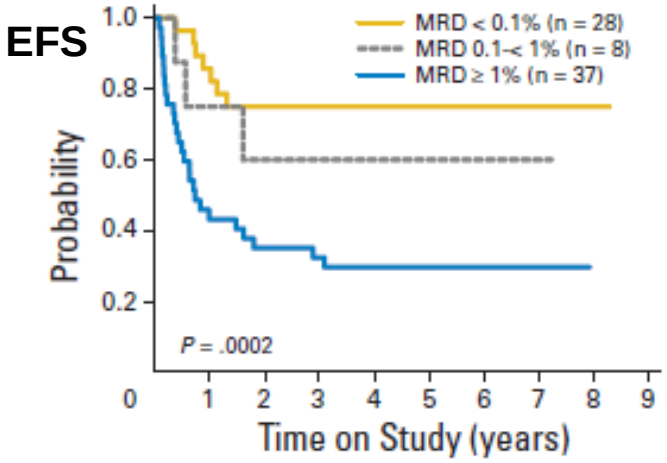




**Post-induction:**
**No detectable AML by flow**

**<5% blasts by morphology**




**≥5% blasts by morphology**

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Inaba H, *et al*, *J Clin Oncol* 2012;30:3625-32

# Mol-MRD status; the most powerful prognostic factor in NPM1 mutant AML

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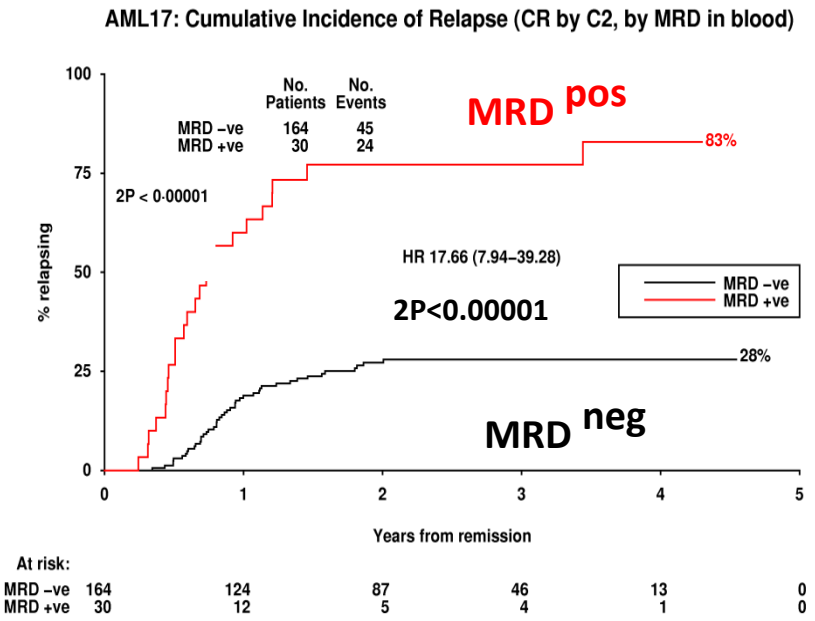
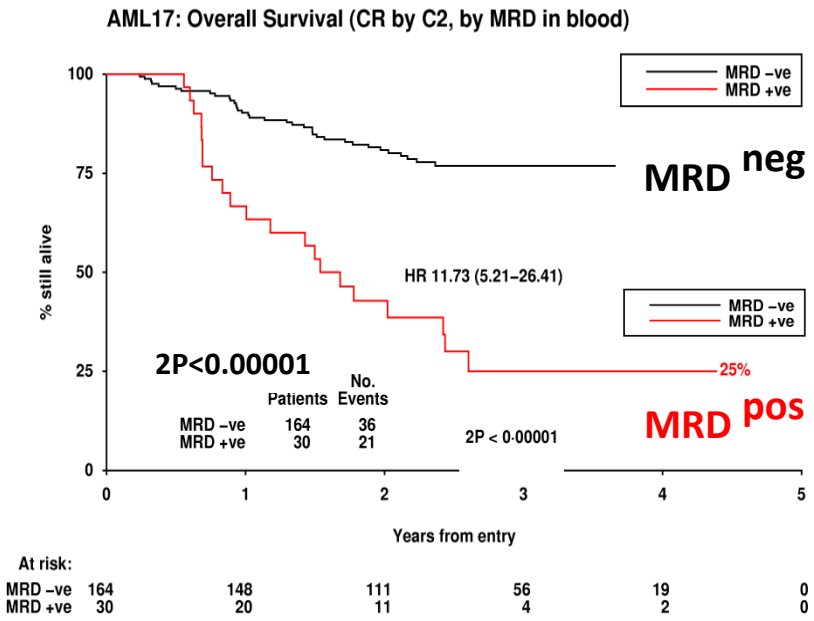
## NCRI AML17: PCR status in peripheral blood post-chemotherapy course 2

**CIR**




**OS**




**In multivariate analysis MRD status was only significant independent prognostic factor, considering:**

- **Age**
- **WBC**
- **Mutational profile (51 gene panel including *FLT3*-ITD, *DNMT3A*, *WT1*)**


**Ivey A, Hills RK *et al*. NEJM 2016;374:422-433**

**Hills RK, et al., NEJM 2016;375(6):e9**

# Application of next generation sequencing approaches for MRD detection

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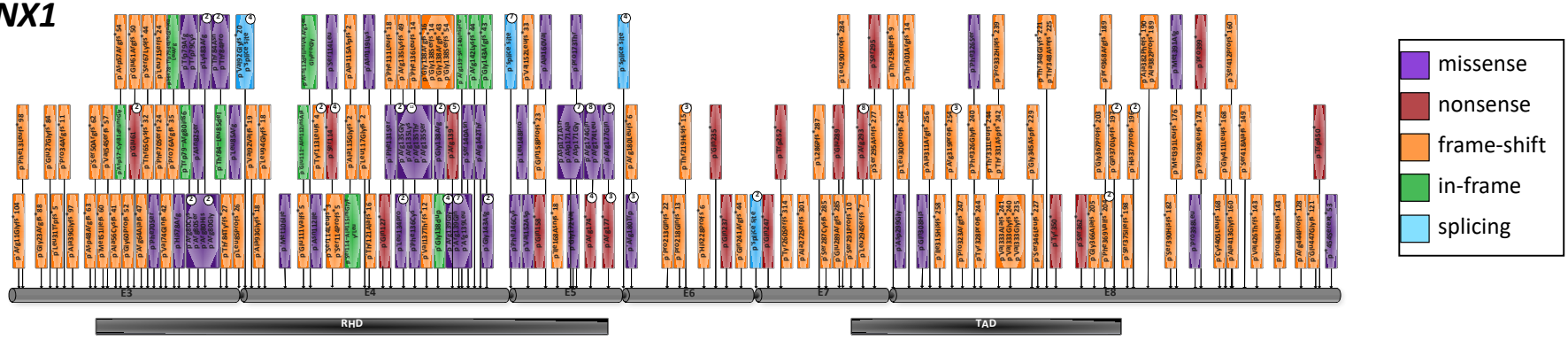
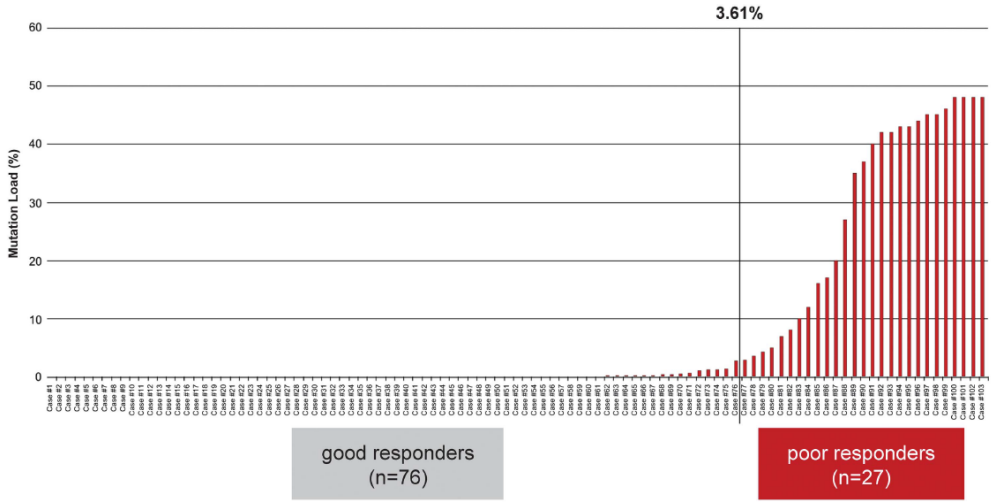
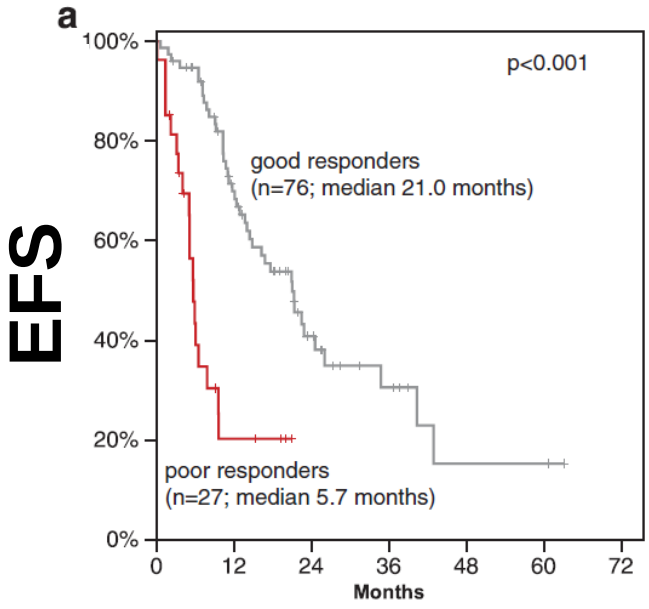
***RUNX1***




**RUNX1 residual mutation load at first follow-up(n=103)**

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**Kohlmann *et al*., *Leukemia* 2014;28:129-37**

# MRD in AML/MDS: NGS and FCM

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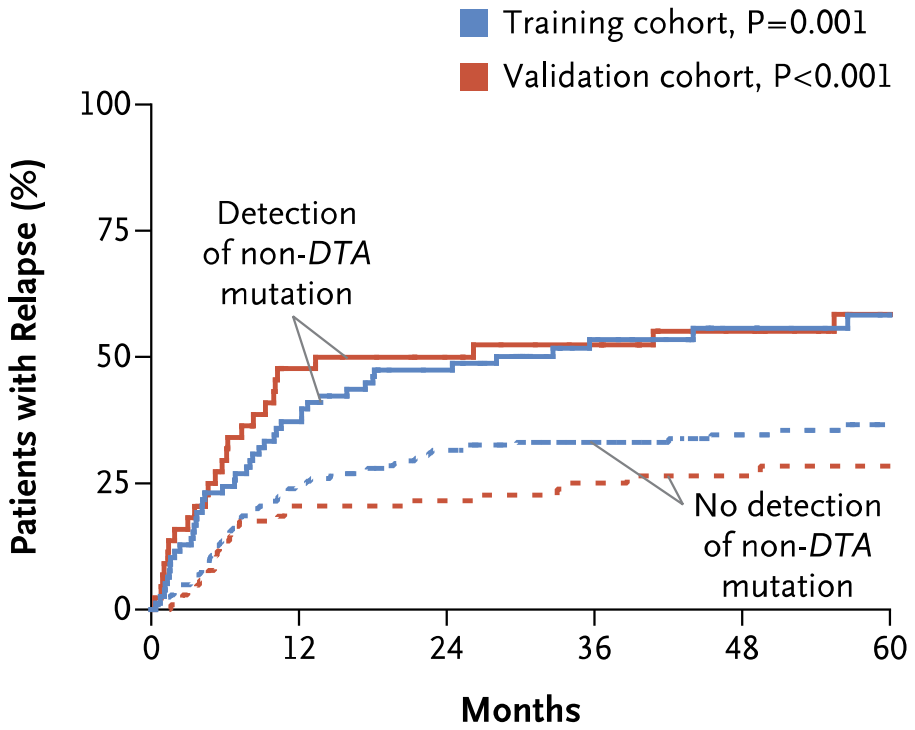


| No. at Risk | | | | | | |
|---|---|---|---|---|---|---|
| Training cohort | | | | | | |
| Detection | 78 | 47 | 37 | 23 | 15 | 13 |
| No detection | 205 | 143 | 118 | 99 | 71 | 43 |
| Validation cohort | | | | | | |
| Detection | 44 | 20 | 18 | 16 | 12 | 7 |
| No detection | 103 | 74 | 68 | 48 | 34 | 23 |

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Jongen-Lavrenic M, et al., N Engl J Med 2018:378;1189-99

# MRD in AML/MDS: NGS and FCM

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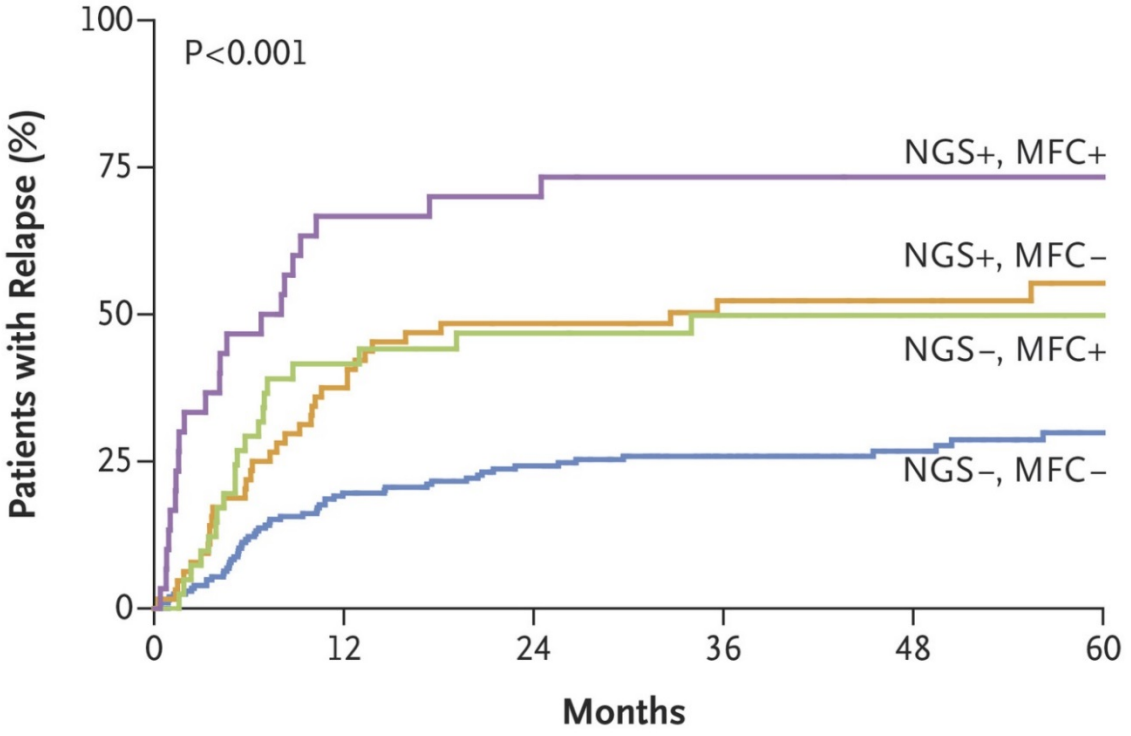


**No. at Risk**

| | | | | | | |
|---|---|---|---|---|---|---|
| NGS+, MFC+ | 30 | 8 | 7 | 5 | 4 | 4 |
| NGS–, MFC+ | 41 | 22 | 18 | 14 | 11 | 7 |
| NGS+, MFC– | 64 | 39 | 30 | 22 | 15 | 11 |
| NGS–, MFC– | 205 | 153 | 130 | 101 | 69 | 42 |

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Jongen-Lavrenic M, et al., N Engl J Med 2018:378;1189-99

# ELN: consensus document and strategies multiparameter flow cytometry

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- Goal: harmonize approaches, improve quality
  - Approach***: "LAIP-based different-from-normal (DfN)"***
  - Marker panels
  - Technical requirements for specimen collection/processing, flow cytometers
  - Calculation of MRD burden
  - Thresholds and time points for MRD assessments
  - Reporting



**Schuurhuis GJ, et al., Blood 2018;131:1275**

# Principles of Flowcytometric MRD in AML
# LAIP and DfN (different-from-normal)

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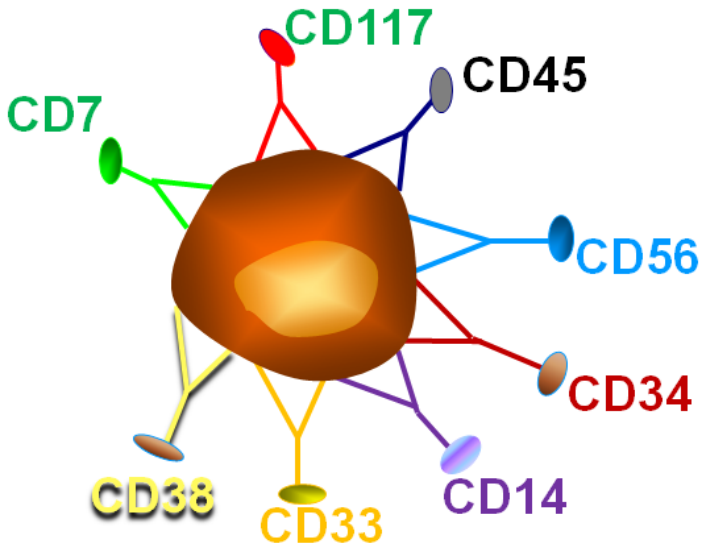
Leukemia Associated Immunophenotype (LAIP)

- CD45 WBC marker
- CD34 primitive marker
  CD117 primitive and myeloid marker
- CD33 myeloid marker
- CD38 activation and differentiation marker
- CD7 lymphoid marker
- CD14 monocyte marker
- CD56 NK cell marker




- Cross-lineage antigen expression
- Asynchronous antigen expression
- Antigen over-expression
- Antigen under-expression

**Note: in > 90% of the cases ≥ 1 LAIP can be defined**

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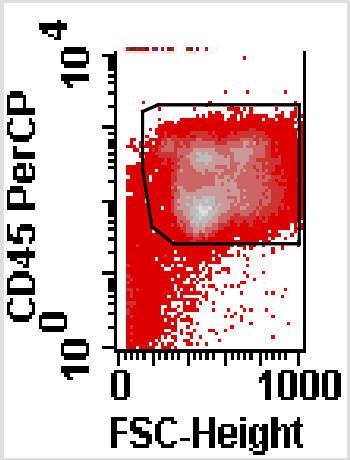
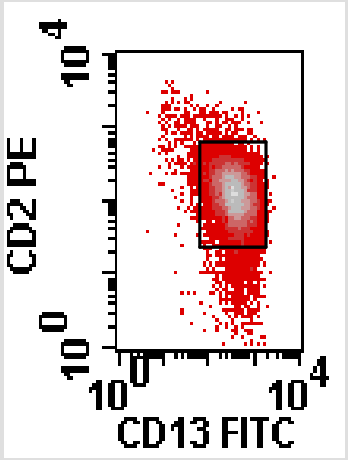
# MRD detection by first defining Leukemia Associated (Immuno)Phenotypes (LAIP) in diagnosis AML

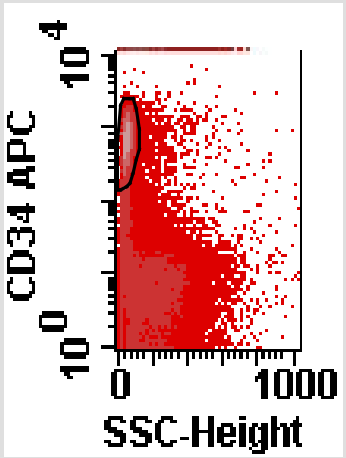


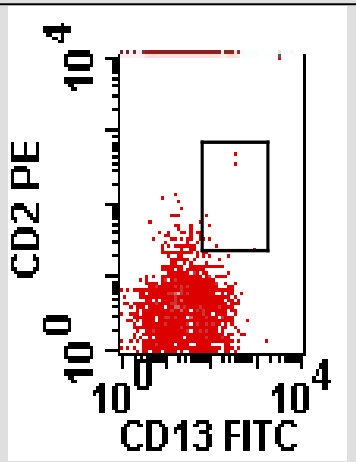




**AML**

***Combination of markers used for MRD***

*** primitive (CD34, CD133, CD117)**
*** myeloid (CD13, CD33)**
*** aberrancy defining (eg CD2)**
*** CD45 (pan-leukocyte)**




**CD2 is lymphoid marker**
**CD13 is myeloid marker**

**normal BM**

*See also:* Cloos J, et al., J Visualized Exp 2018;133:e56386

# LAIPs at AML diagnosis

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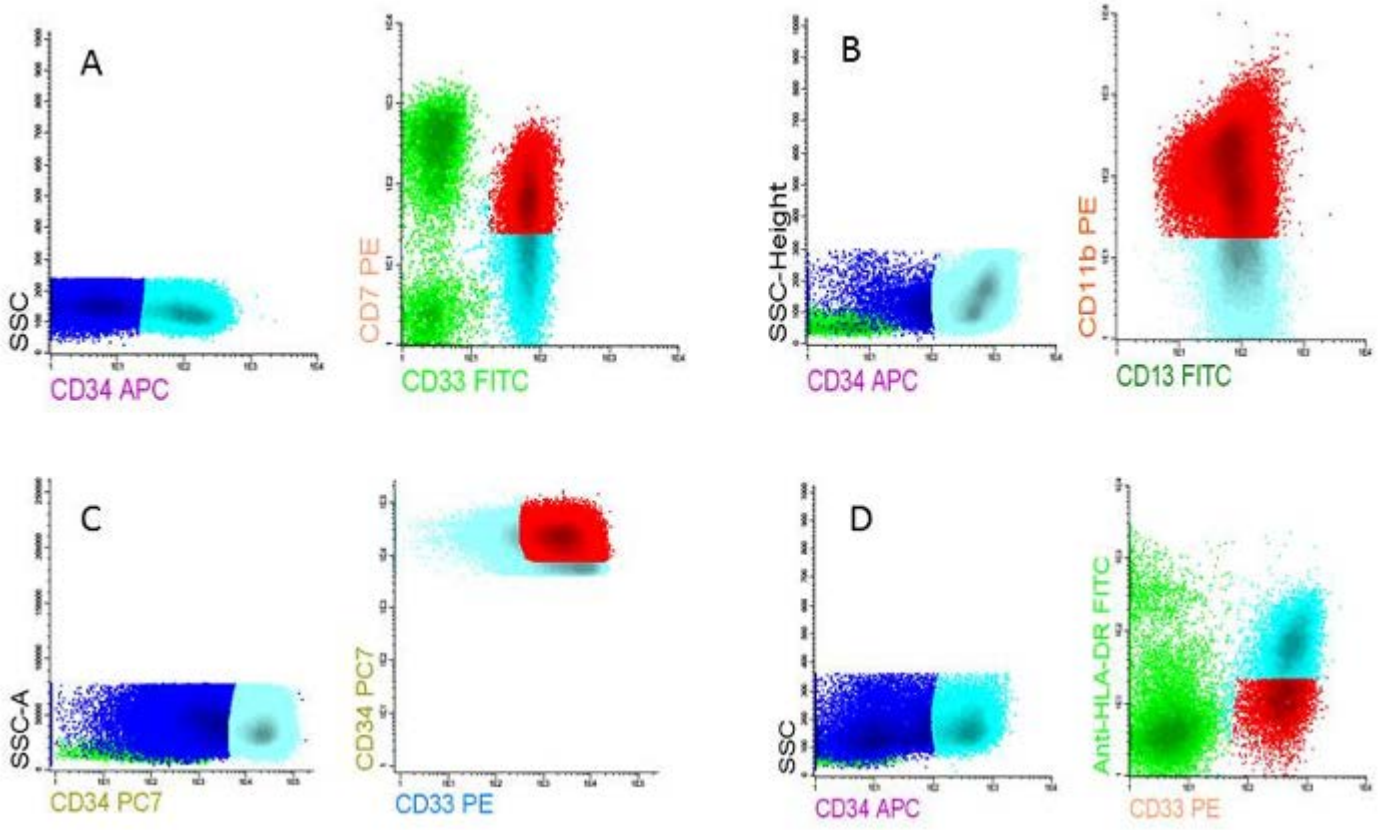


Cloos J, et al., J Visualized Exp 2018;133:e56386

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# Flow Cytometry Strategies for MRD detection

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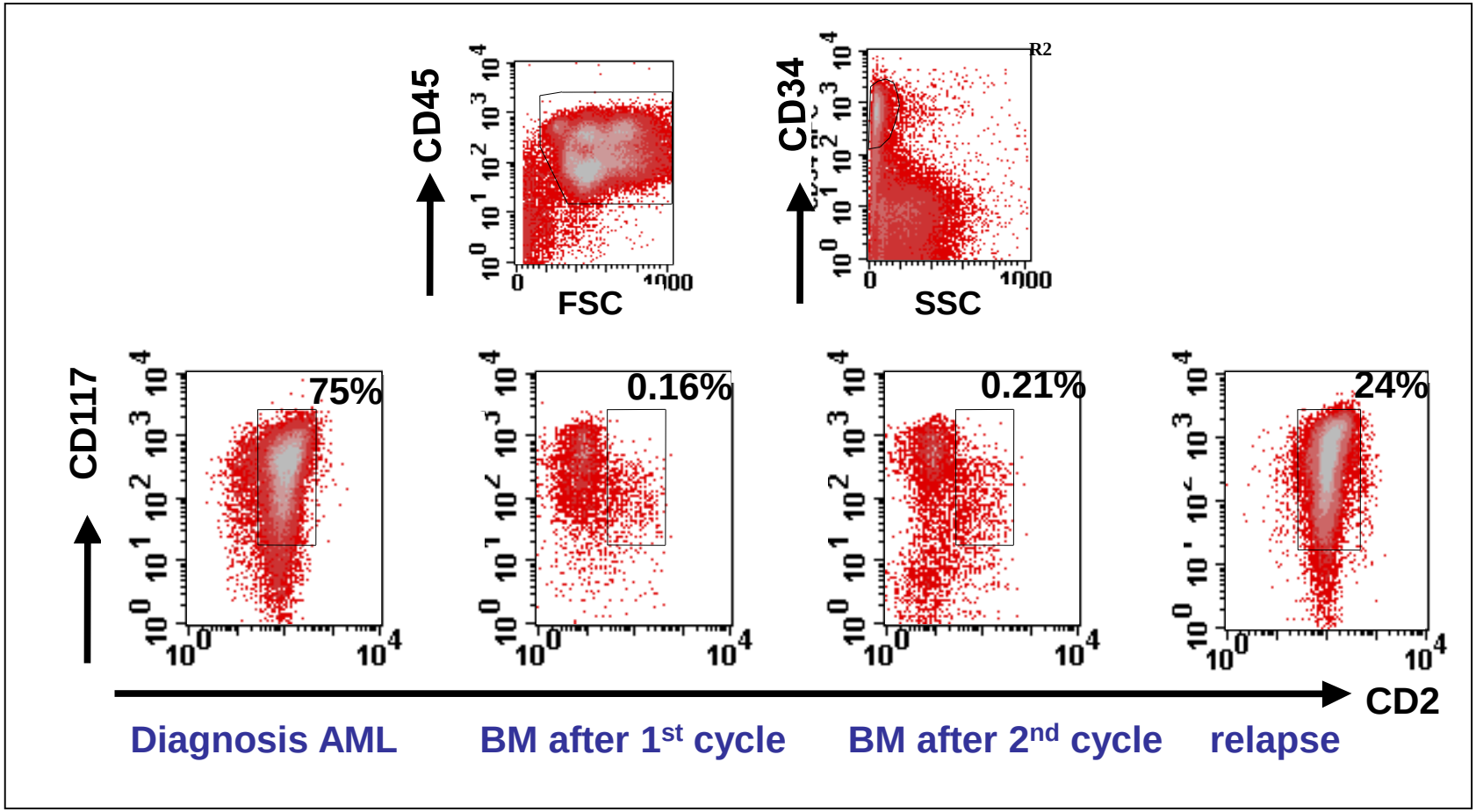


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*See also:* Cloos J, et al., J Visualized Exp 2018;133:e56386

# Sequential MRD monitoring in allo Tx

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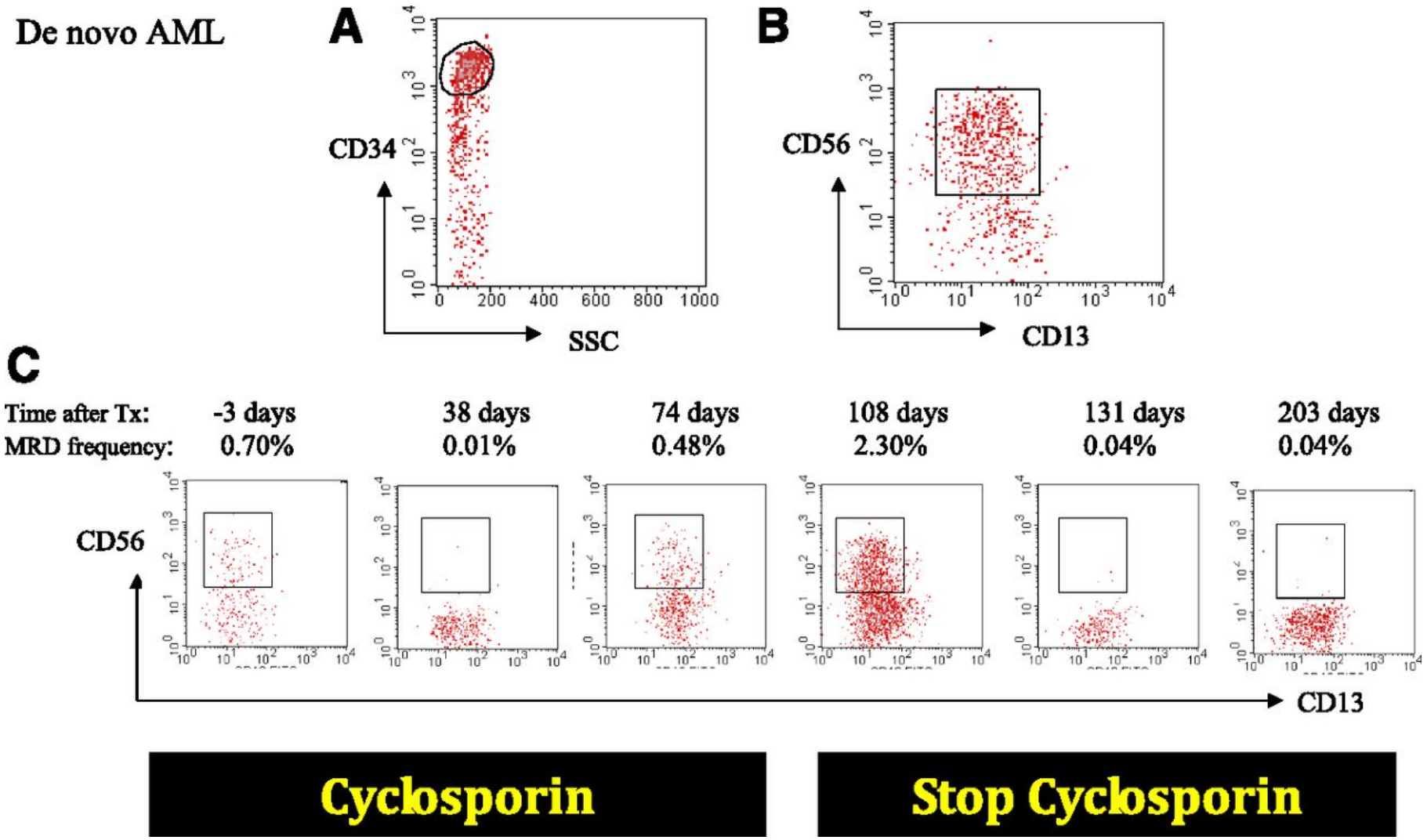


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Ossenkoppele GJ, Schuurhuis G. Hematology Am Soc Hematol Educ Program 2016;(1):356-365

# Technical requirements

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- EDTA vs heparin
- BM sampling first pool/hemodilution vs PB
- BM transport (< 72h)
- Instruments (settings/harmonisation [Harmonemia, Euro-flow consortium])
- Preparation of samples (stain/lyse/wash *vs* lyse/wash/stain/wash); fluorchromes

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Schuurhuis GJ, et al., Blood 2018;131:1275

# How to calculate LAIP?

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- Define LAIP (vs normal)
- Use the most specific and/or highest frequency LAIP
- Relate LAIP to $CD45^+$ cells (excl $CD45^-$ (erythroblasts)

- Use LAIP at diagnosis to optimally inform MRD gating
- Use DfN approach to identify any new LAIP

- Require between 0.5-1 $x10^6$ events (excluding; $CD45^-$ and debris) unless cluster of MRD is obvious)

- Perform in well experienced lab and trained operator

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Schuurhuis GJ, et al., Blood 2018;131:1275

# Tresholds and time points of MRD assessments and reporting

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- LAIP treshold: **0.1%** *(positive vs negative)*
- Sequential monitoring after induction and/or consolidation

- Reporting:
  - Absolute numbers of LAIP and % to CD45+ (WBC)
  - MRD pos vs neg vs **no** MFC MRD identified
  - Detection sensitivity
  - Comments on quality of the sample (i.e. hemodilution)
  - Clinical relevance i.e.
    - *MRD < 0.1%: MFC MRD detectable and quantifiable with uncertain significance*

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**Schuurhuis GJ, et al., Blood 2018;131:1275**

# Summary: Flow cytometry-based MRD detection in AML

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| Advantages | Disadvantages | Pitfalls |
|---|---|---|
| • Widely applicable (>90% of AML cases) | • Not as sensitive as PCR | • Not all AML cells have abnormal phenotype |
| • Quantification of abnormal cells | • Sensitivity not uniform across patients | • More than 1 phenotype possible |
| • Quantification of abnormal “stem/progenitor” cells | • Best results with fresh material | • Phenotype shift, loss |
| • Relatively sensitive | • Analysis, interpretation operator-dependent | • Abnormal cells may not have relapse potential |
| • Quick turn-around time | • Challenging to standardize/harmonize | |
| • Separation live/dead cells | | |
| • Assessment of hemodilution | | |
| • Identification of therapeutic target(s) | | |

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**Hourigan CS, et al., Leukemia 2017;31:1482**
**Schuurhuis GJ, et al., Blood 2018;131:1275**

# (1) Molecular MRD: markers

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- (validated) Markers for molecular MRD by qPCR
  - **NPM1** mutations
  - Fusion genes **CBF-AML**:
    - RUNX1-RUNX1T1 (t(8;21))
    - CBFB-MYH11 (inv(16))
  - Fusion genes:
    - PML-RARA (t15;17))
  - Rare: AML with BCR-ABL1 (p190 transcript)

- No role for CHIP related mutations: DNMT3A, ASXL1 and TET2
- No role for germ-line mutations: GATA2, RUNX1, CEBPA, DDX41, ANKRD26
- No role for WT1 (low sensitivity and specificity)



**Schuurhuis GJ, et al., Blood 2018;131:1275**

# (2) Molecular MRD: markers

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- In alloTx: role for chimerism as MRD marker
- Due to losses or gains of mutations at relapse
  - no role for mutations in FLT3-ITD, FLT3-TKD, NRAS, KRAS, IDH1/2, MLL-PTD
- For AML patients **not in the moleculair defined subgroups** (NPM1; CBF, APL) MRD should be assessed by FCM
- Failure to achieve MRD-neg CR or rising MRD levels are associated with relapse

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**Schuurhuis GJ, et al., Blood 2018;131:1275**

# Molecular assessments and definitions of molecular responses

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- Timepoints (note: for different markers; different interpretations
  - At diagnosis
  - > 2 cycles induction CT
  - > end of treatment

- Definitions:
  - CR MRD-
  - Molecular persistance at low copy numbers
  - Molecular progression
  - Molecular relapse



Schuurhuis GJ, et al., Blood 2018;131:1275

# Solutions to problem of "false-negative" MRD
# Experimental approaches on FCM MRD

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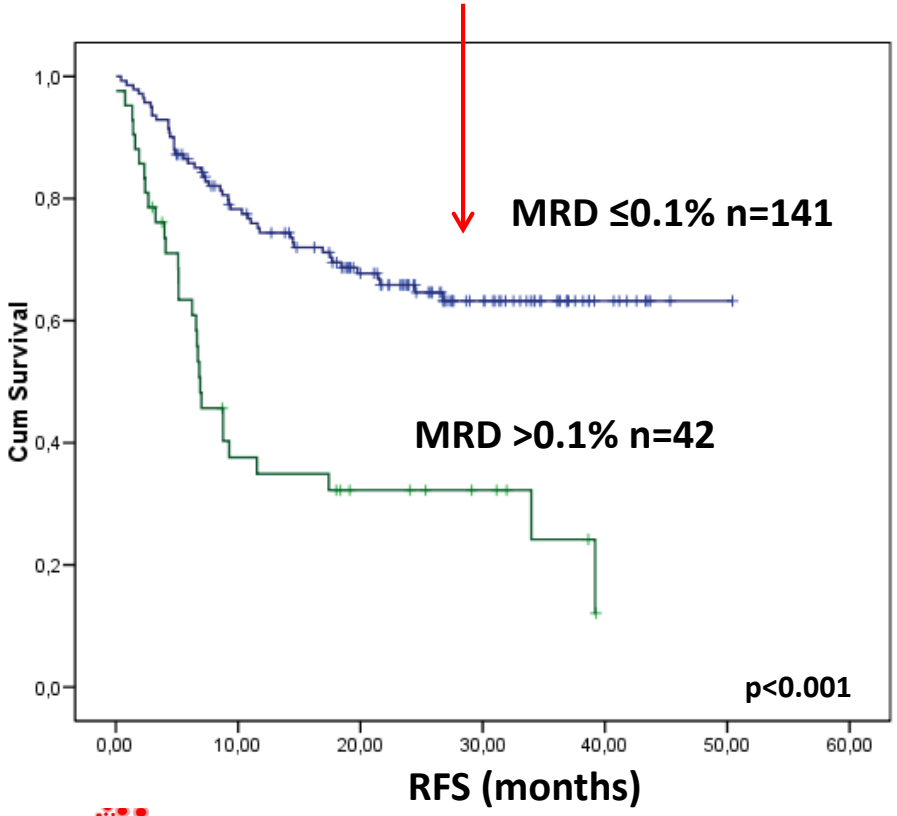
## 35%-40% of the MRD "low" patients nevertheless relapse




**How to identify these patients?**

**Increase sensitivity**

a. **of immuno-MRD**

b. **+/- molecular MRD (NPM1)**

**Differences in time of onset of relapses identifiable by sequential MRD**

**Contribution of leukemia stem cells**

**Take into account possible IF shifts**

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Schuurhuis GJ, et al., 2016 (with courtesy); see also Hanekamp D, et a., Int J Heamtol 2017:105;549

# Current known AML stem cell markers

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| | |
|---|---|
| • CD123 | Jordan et al, Leukemia 2000, 14: 1777 |
| • CD33 | Taussig et al, Blood 2005; 106: 4086 |
| • CD44 | Jin et al, Nat Med 2006, 12: 1167 |
| • CLL-1 (CLEC12A) | van Rhenen et al, Blood 2007, 110: 2659 |
| • Lineage markers | van Rhenen et al, Leukemia, 2007; 21: 1700 |
| • CD96 | Hosen et al, PNAS 2007, 104: 11008 |
| • CD47 | Majeti et al, Cell 2009; 138: 286 |
| • CD25 | Saito et al, Sci. Transl. Med. 2010; 2: 17ra9 |
| • CD31 | Saito et al, Sci. Transl. Med. 2010; 2: 17ra9 |
| • TIM-3 | Jan et al, PNAS 2011; 108: 5009 |
| • Hif1α | Wang et al. Cell Stem Cell 2011; 8: 399 |
| • CD99 | Chung et al, Blood 2013, 122: |
| • CD45RA | Goardon et al, Cell Stem Cell 2011, 19: 138-152<br>Kersten et al, Br J Haematol 2016 |
| • ALDH | Pearce 2005, Cheung 2007, Ran 2009, Gerber 2012, Schuurhuis et al, PlosONE 2013 |
| • Scatter aberrancies | Terwijn et al, PLoSOne 2014 Sep 22; 9(9):e107587 |

**Lineage aberrancies:**

- **CD2, CD7, CD11b,CD14, CD15, CD19, CD20, CD22, CD56,**
- **HLADR-CD15-, CD13-/+CD33+/-, CD13-CD33-**

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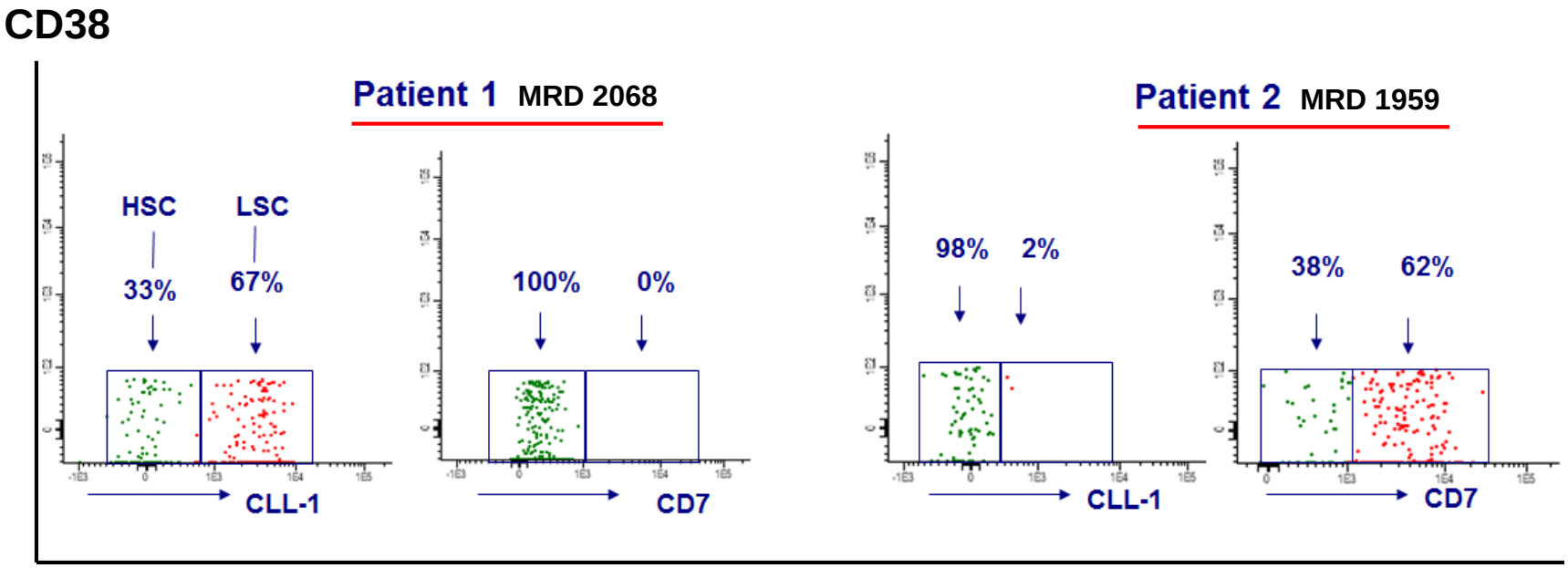
**Schuurhuis GJ, et al., 2016 (with courtesy); see also Hanekamp D, et a., Int J Hematol 2017:105;549**

# *Inter*-patient heterogeneity of marker expression in CD34+CD38- LSC forces use of >>1 stem cell marker (1)

## Aberrant marker expression




| marker | performance | |
|---|---|---|
| | patient 1 | patient 2 |
| CLL-1 | ++ | - |
| CD7 | - | ++ |

All within CD45$^{dim}$/ SSC$^{low}$ /CD34$^{+}$ gate

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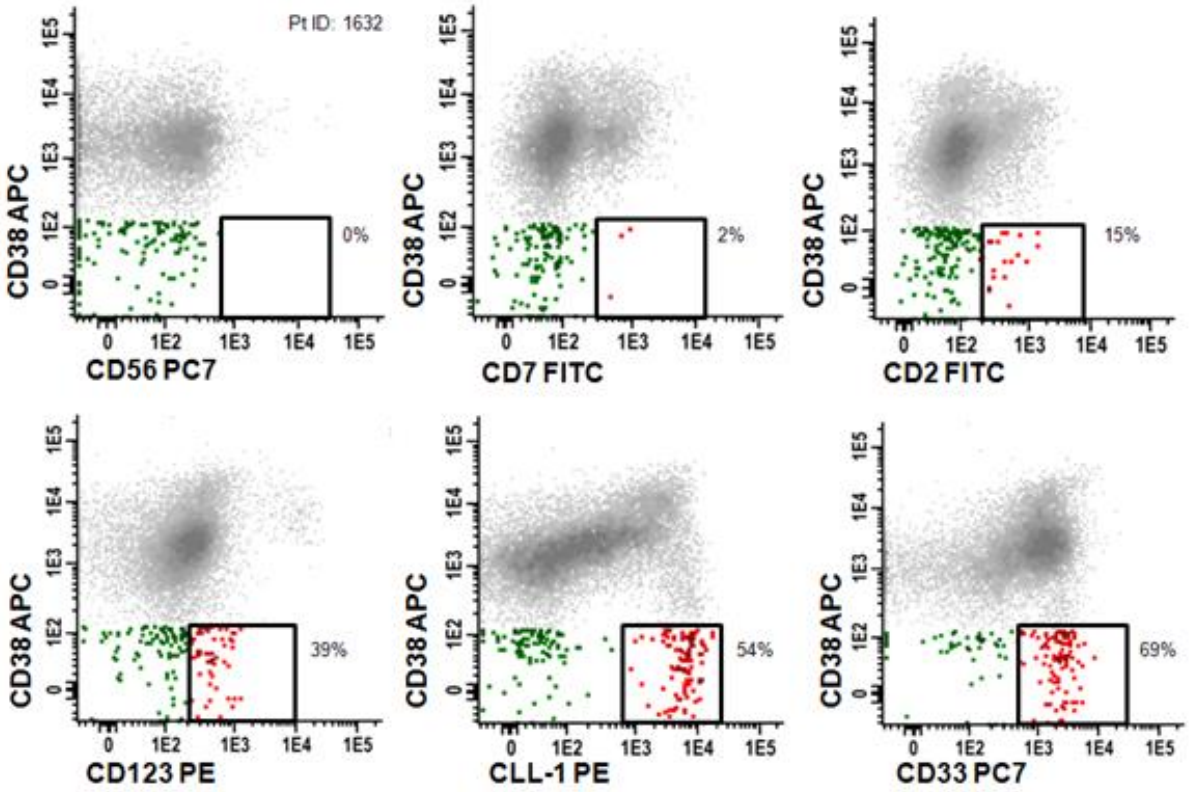
Zeijlemaker W, et al., Leukemia 2016;30:439

# *Intra*-patient heterogeneity of marker expression in CD34+CD38- LSC requires >>1 stem cell marker (2)




**CLL-1 and CD33 are good markers in *this* patient**

**All within CD45$^{dim}$/ SSC$^{low}$ /CD34$^{+}$ gate**

**Zeijlemaker W, et al., Leukemia 2016;30:439**

# Prognostic value CD34+CD38- LSC *at diagnosis*

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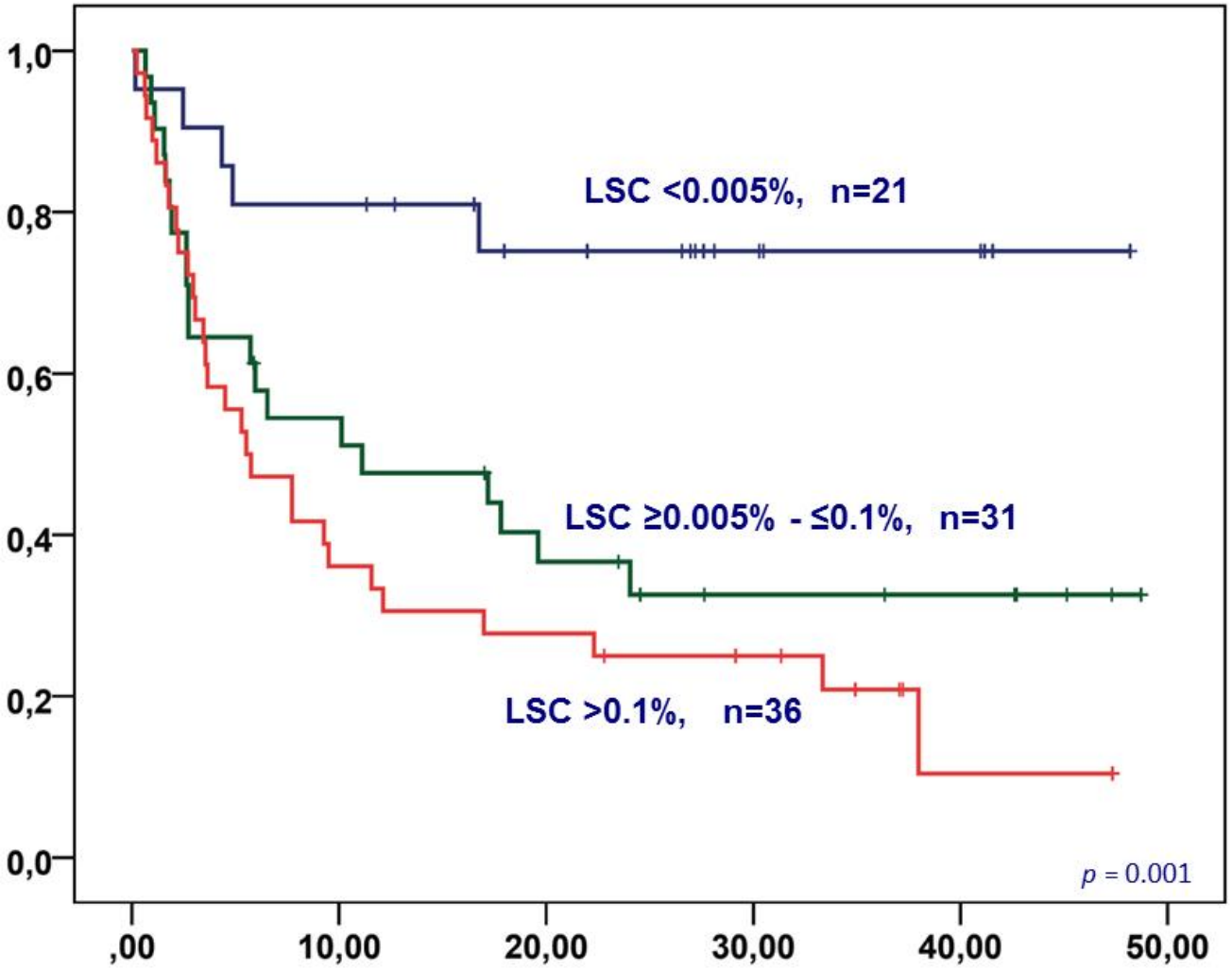


Terwijn M, et al. PloSOne 2014;9:e107587

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# LSC frequency : Prognostic value *after therapy*

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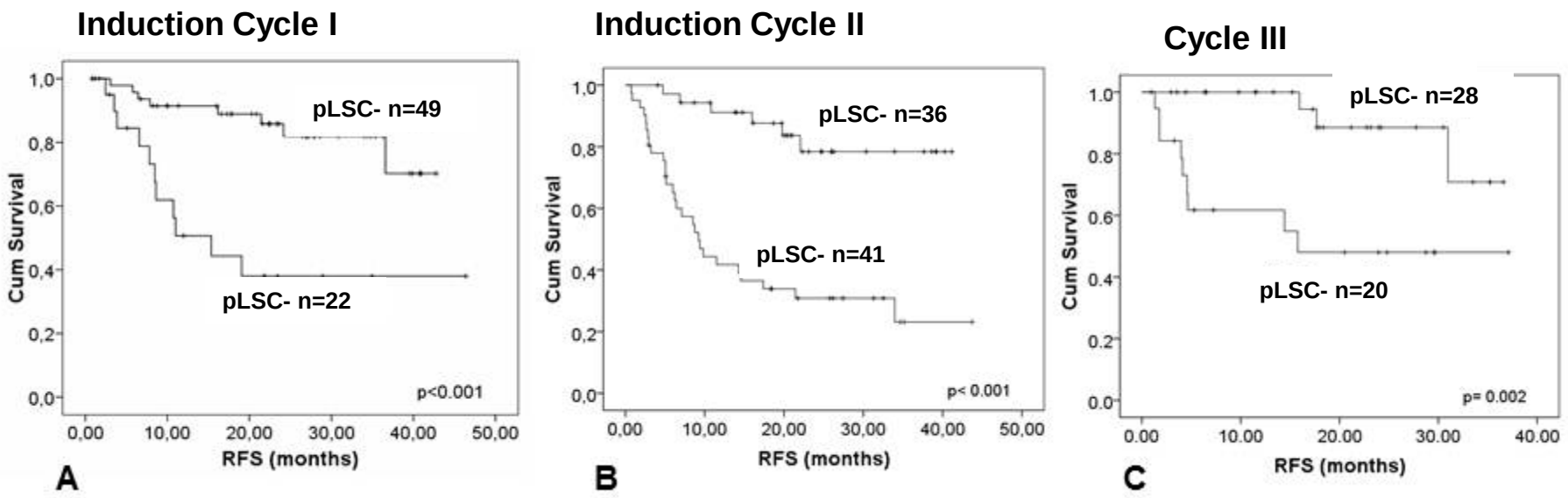


**pLSC= putative leukemic stem cell**

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Terwijn M, et al. PloSOne 2014;9:e107587

# Prognostic value MRD/LSC combination after induction therapy

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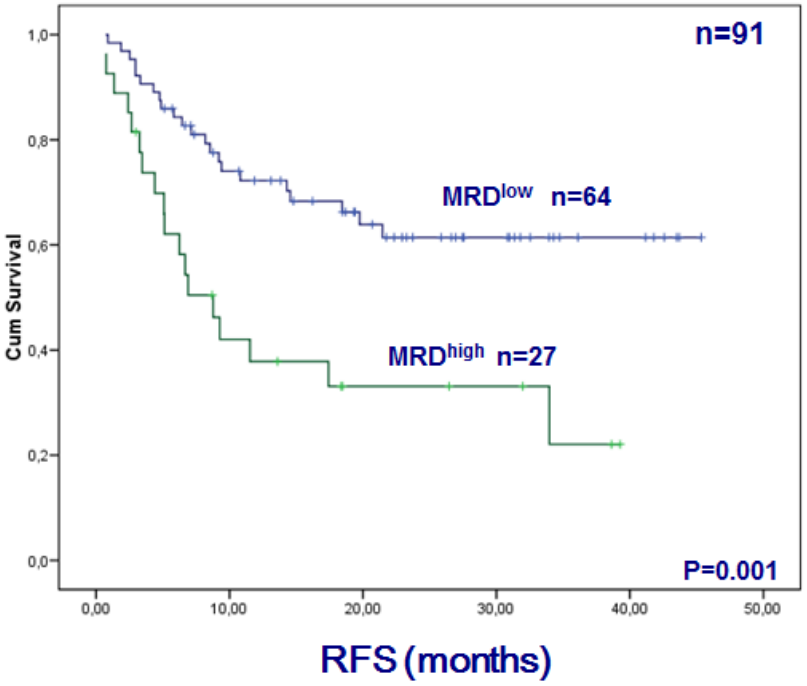
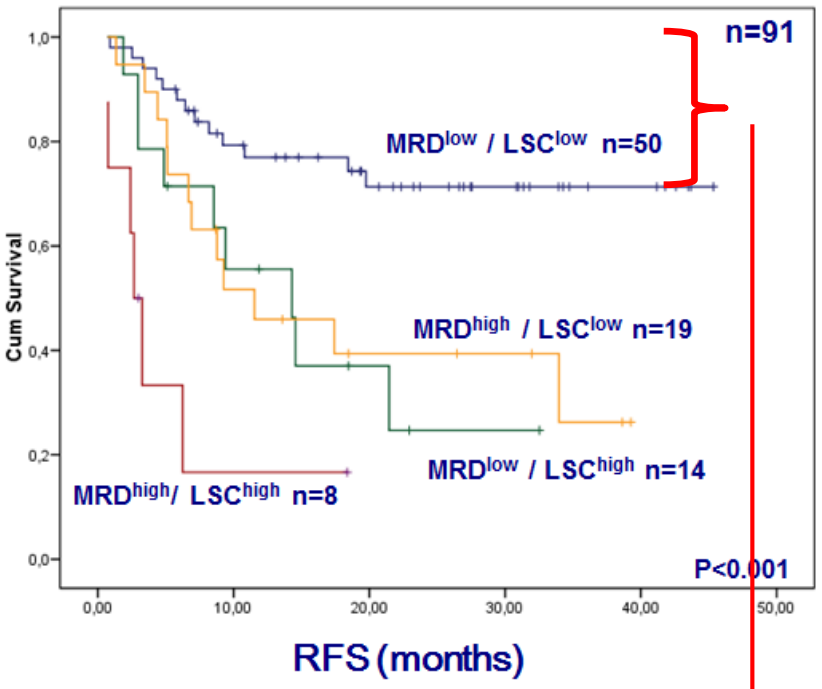
**H42a + H92**

After 2nd course




After 2nd course




**Still relapses in LSC "low"/MRD "low" patients**

**Solved by incorporating clonal/immunophenotype shifts?**

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# Present panel for LSC characterization

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| Tube | FITC | PE | PerCP-CY5.5 | PC7 | APC | APC-H7 | HV450 | KO |
|---|---|---|---|---|---|---|---|---|
| 1 | HLA-DR | CD33 | CD13 | PBS | CD38 | PBS | CD34 | CD45 |
| 2 | CD44 | Clec12a | CD13 | CD56 | CD38 | HLA-DR | CD34 | CD45 |
| 3 | CD7 | TIM-3 | CD13 | CD117 | CD38 | CD19 | CD34 | CD45 |
| 4 | CD2 | CD133 | CD13 | CD117 | CD38 | CD19 | CD34 | CD45 |
| 5 | CD36 | CD123 | CD13 | CD33 | CD38 | CD14 | CD34 | CD45 |
| 6 | CD11b | CD96 | CD13 | CD117 | CD38 | CD14 | CD34 | CD45 |
| 7 | CD15 | CD22 | CD13 | CD117 | CD38 | HLA-DR | CD34 | CD45 |

**Problem: availability of bone marrow**



Zeijlemaker W, et al., Leukemia 2016;30:439

# LSC detection kit for diagnostic purposes: assessment of total stem cell load (including vanishing and evolving populations) (1 tube, 8 colors, 13 markers)

VU medisch centrum

| Tube | FITC | PE | PerCP-CY5.5 | PeCy7 | APC | APC-H7 | BV421 | HV500C |
|---|---|---|---|---|---|---|---|---|
| 1 | CD45ra | CLL-1<br>TIM-3<br>CD7<br>CD11b<br>CD22<br>CD56 | CD123 | CD33 | CD38 | CD44 | CD34 | CD45 |

**PE channel contains antibodies negative on HSC**

- 1 tube for LSC detection at diagnosis and follow-up
- Identifies almost all CD34+CD38- LSC
- Needs no extensive experience in BM immunophenotyping
- Multi-institutional approach possible
- Takes into account immunophenotypic shifts
- Can be extended with other markers in the PE-channel

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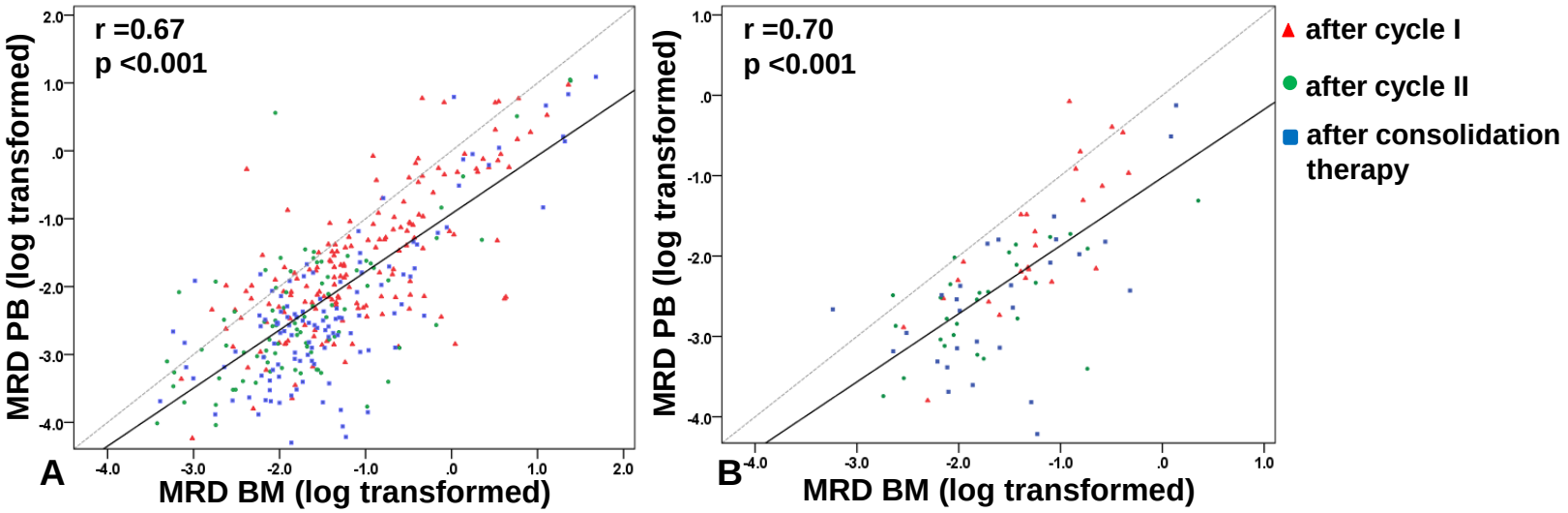
Zeijlemaker W, et al., Leukemia 2016;30:439

# Correlation between BM MRD and PB MRD in AML/MDS




**All LAIPs (1-5 per patient) considered**

**Only LAIPs used for correlations with clinical outcome considered**

Zeijlemaker W, et al., Leukemia 2016;30:708-715

# Conclusions: MRD/LSC in AML

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- A standard panel for MRD and LSC detection is available in AML
- MRD and LSC frequency is highly predictive for DFS/OS
- MRD in PB is a new tool and correlated with BM MRD: validation studies are needed
- MRD/LSC is not the only explanation for risk of relapse



Schuurhuis GJ, et al., Blood 2018;131:1275

# HOVON/SAKK 42a: MRD in AML< 60yrs treatment and sampling scheme

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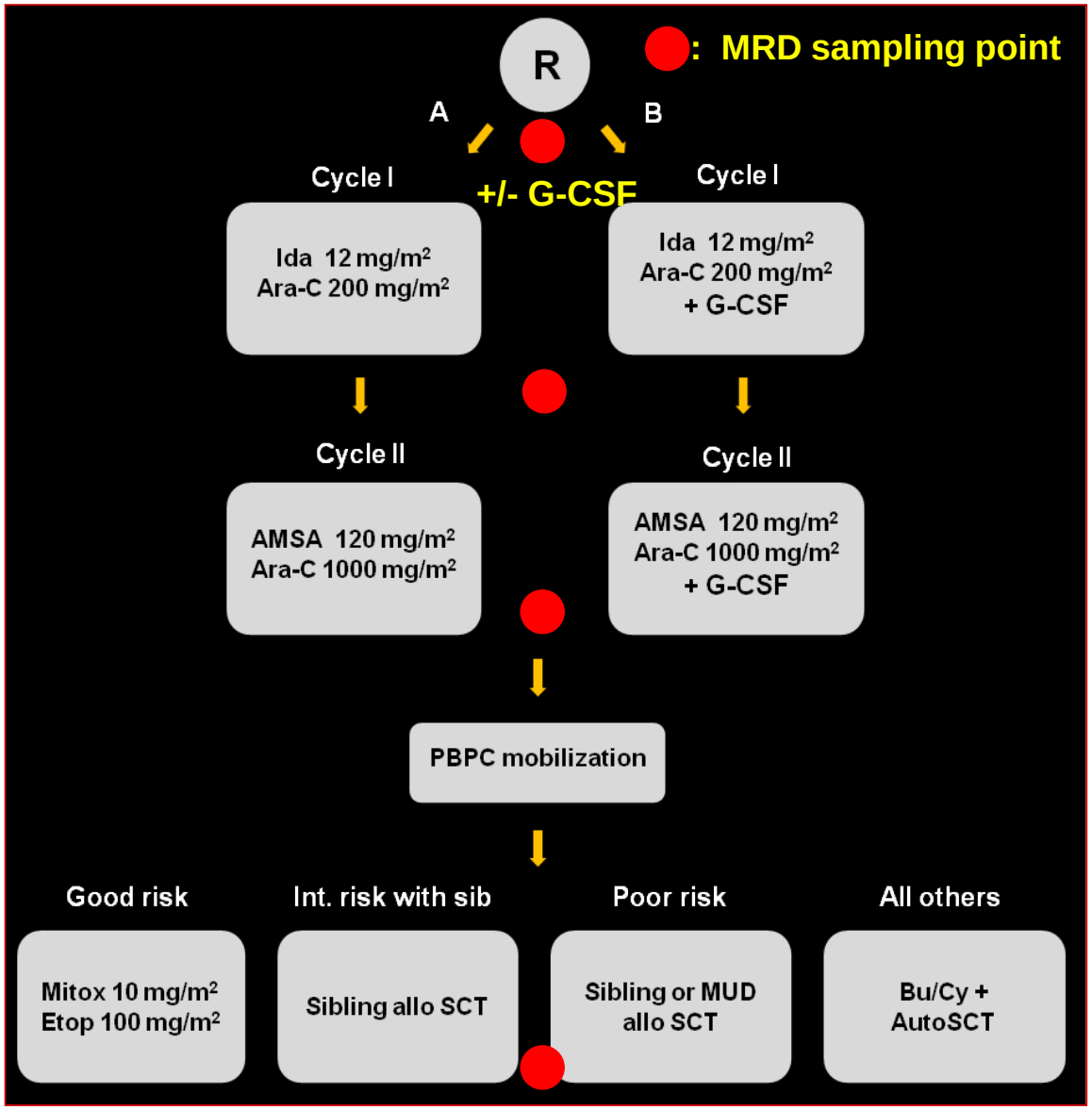
- International pro-spective multi-center study
- Patients 18- 60 years
- n = 517
- 31 Centers:
  - Netherlands
  - Belgium
  - Switzerland




M. Terwijn, et al.
J Clin Onco 2013;31:3889-97

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# Relapse incidence after different therapy cycles (A) and after cycle II in different risk groups(B)

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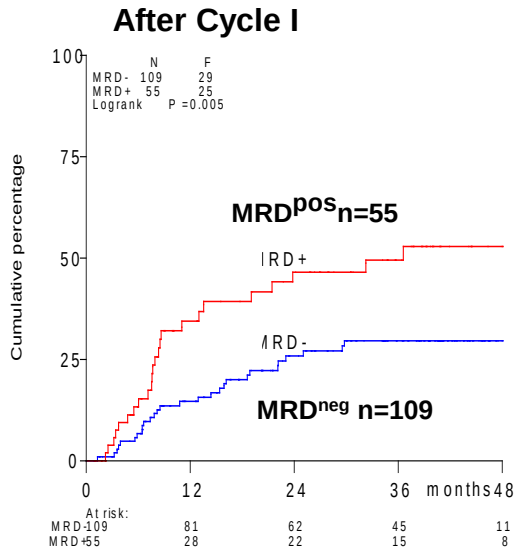
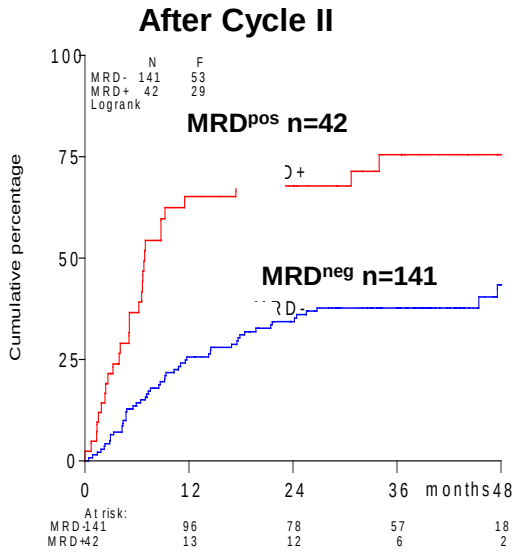
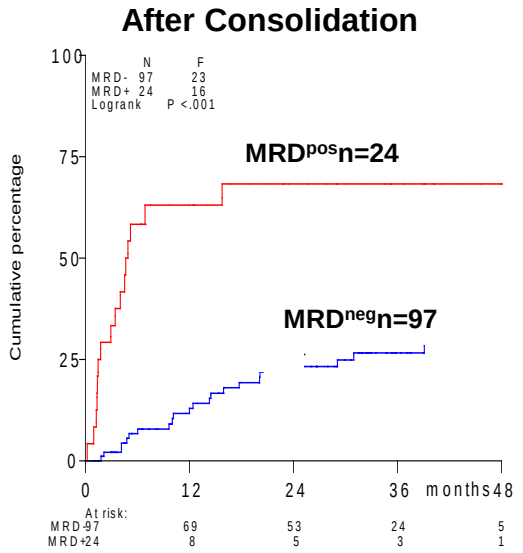
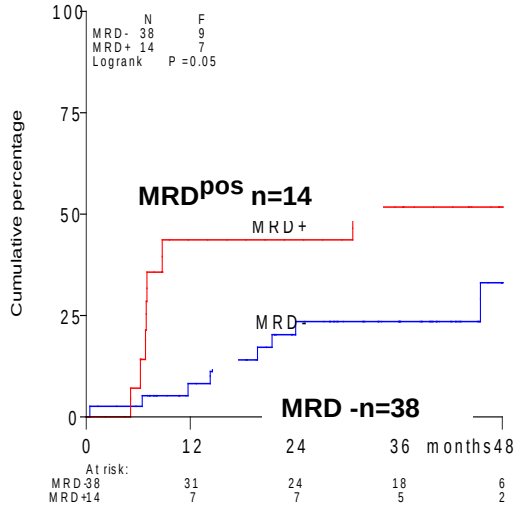
**A**

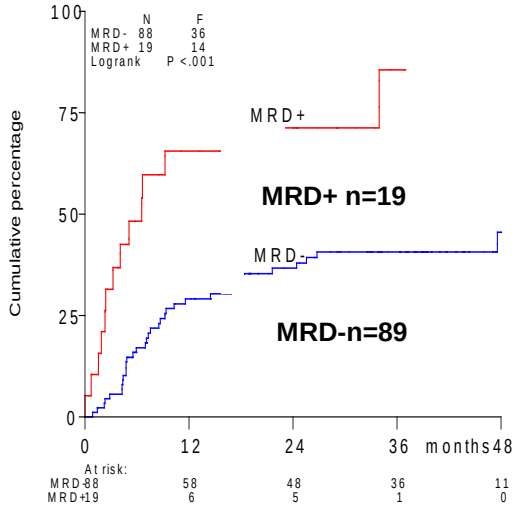


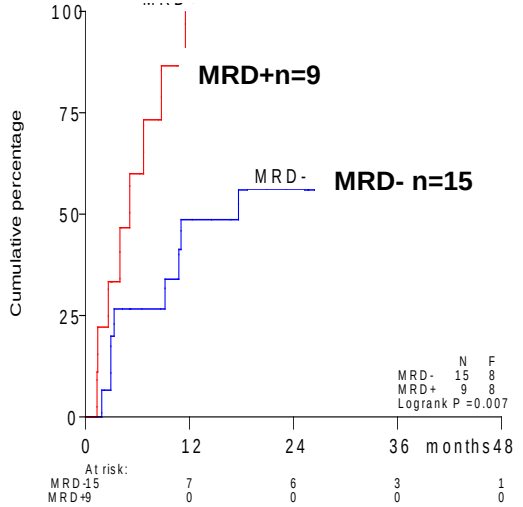




**B**

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# Implementation of flow-cytometric MRD detection in a multicenter clinical trial setting for older patients

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**AML16 (2006 – 2011)**

- 892 AML patients
- (median age 67 years)
- LAIP-MRD - prospectively assessed

(blind to clinical outcome)

- >2200 samples

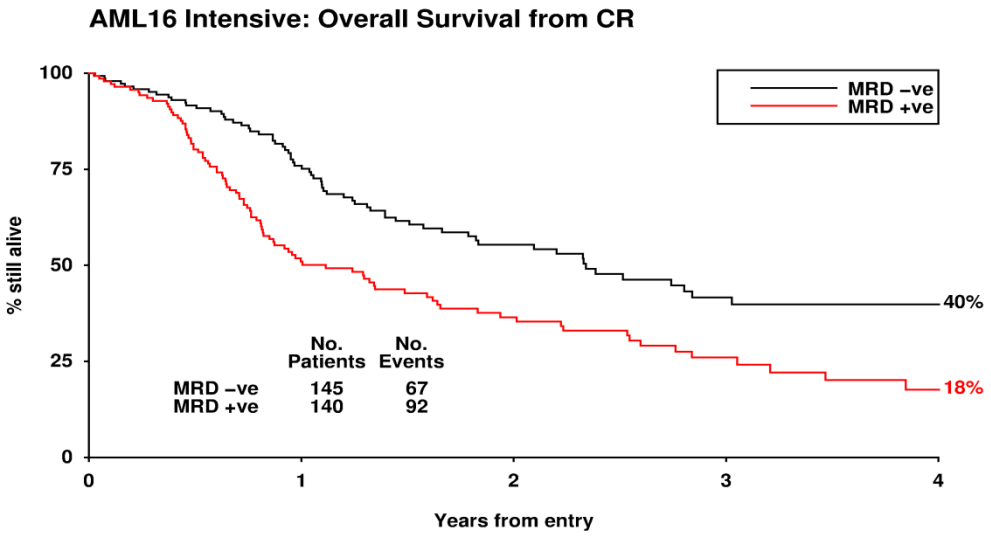
Pre = 833/ 892/ 1006 (~12% inadequate)

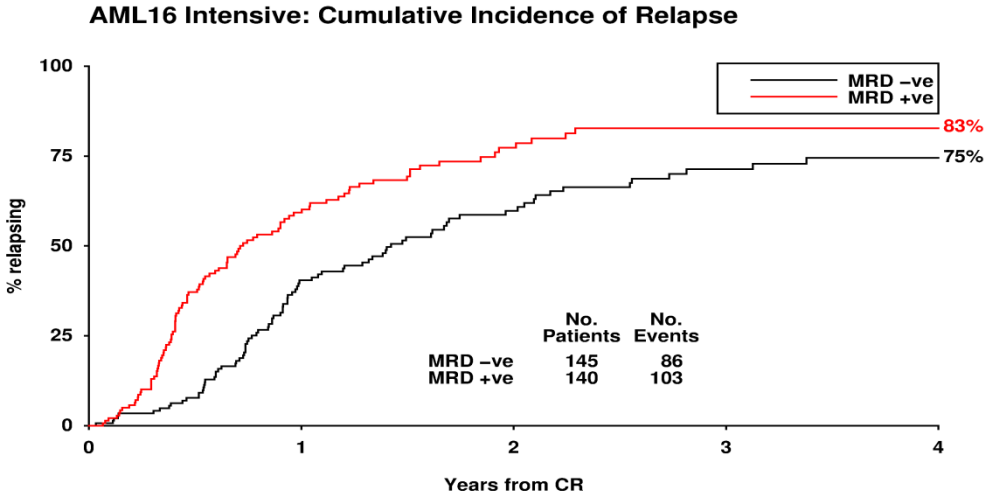
- C1 = 286 /739 (~10% inadequate)
- C2 = 279 /503 (~11% inadequate)

- >100 UK centers
- 2/3 labs centralised analysis

**Prognostic impact of flow MRD independent of:**

- Age
- Cytogenetics
- Wheatley index
- NPM1/FLT3-ITD status

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Freeman *et al, J Clin Oncol* 2013;31:4123-31

# OS and DSF with MRD Negative vs Positive Multi-Parameter Flow Cytometry

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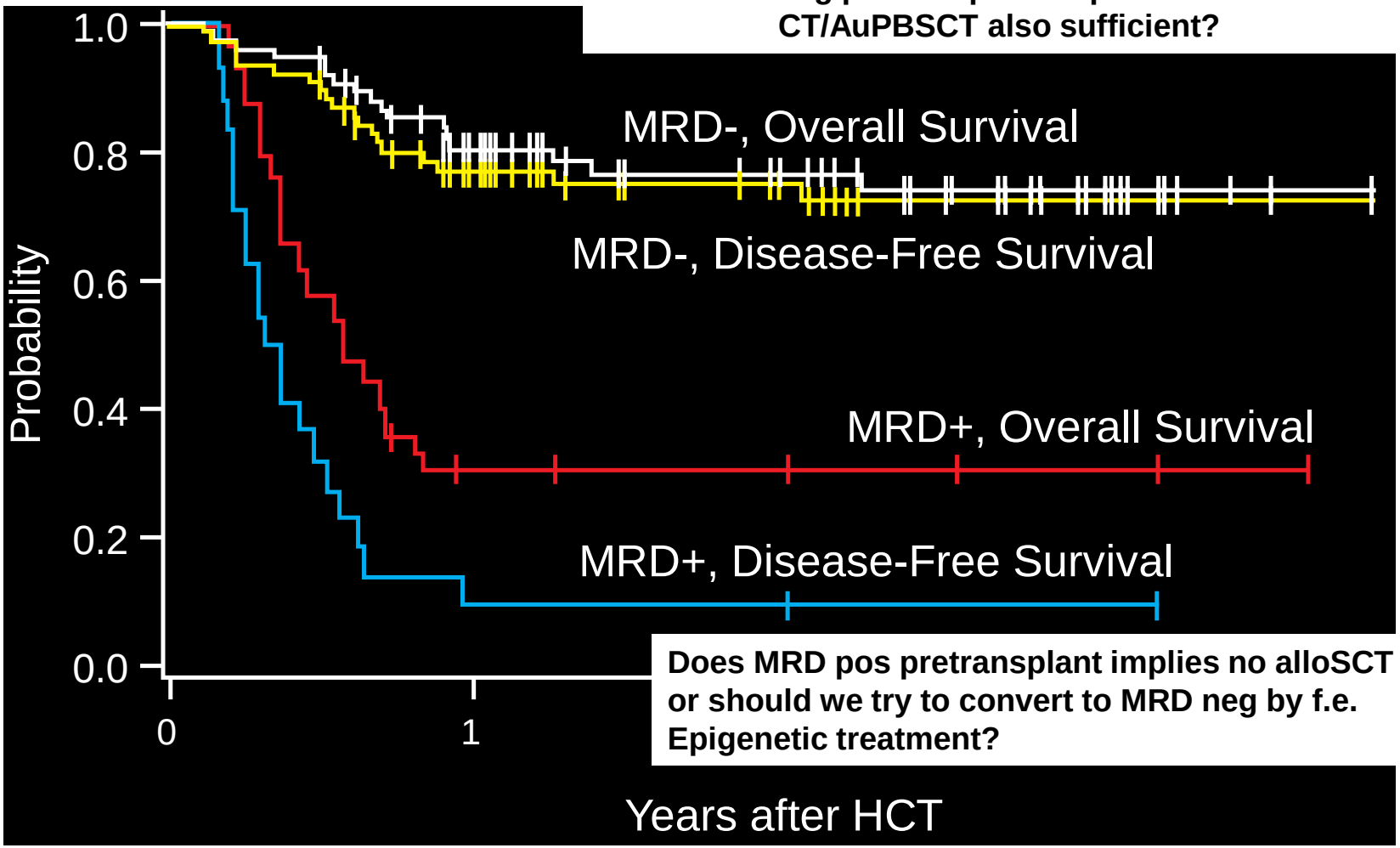
**Does MRD neg pretransplant implies alloSCT? Or is CT/AuPBSCT also sufficient?**




**Does MRD pos pretransplant implies no alloSCT or should we try to convert to MRD neg by f.e. Epigenetic treatment?**

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Walter RB, et al. *J Clin Oncol.* 2011 Mar 20;29(9):1190-7

# HO132 AML study: risk adapted consolidation based on MRD after induction cycle II

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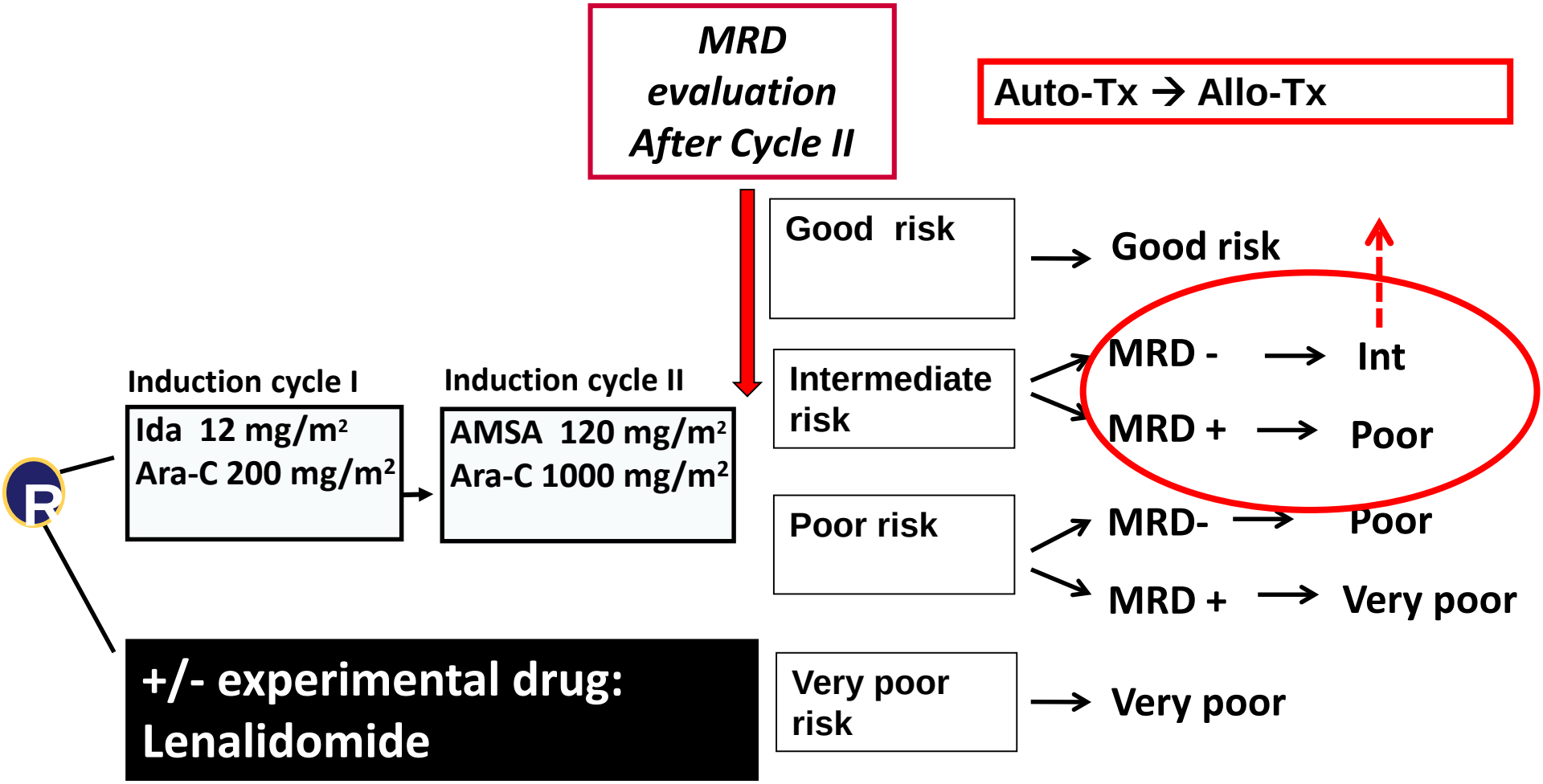

# MRD by NPM1 identifies distinct outcomes in AML and is influenced by gemtuzumab ozogamicin (ALFA-0701 trial).

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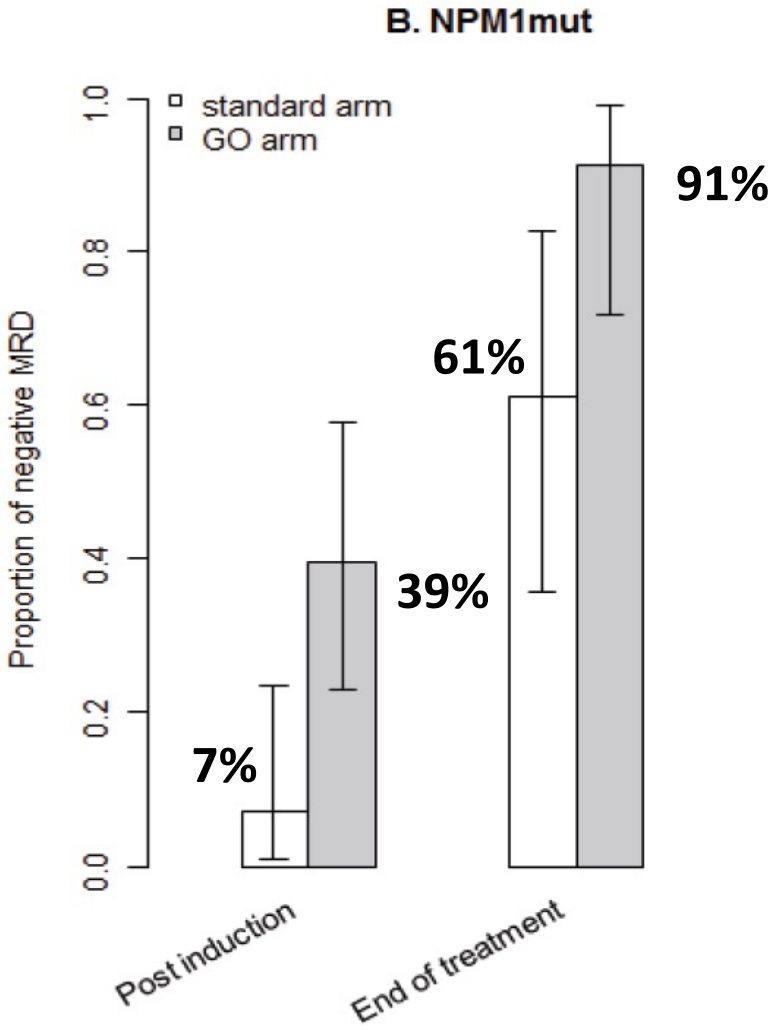
- Add. of GO no increase of CR
- Add. of GO improves 2y OS and EFS

- MRD might be useful as surrogate marker in new drug development

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Lambert J, et al, Oncotarget 2014;5:6280-6288

# MRD directed treatment in CBF AML: Trial design and patient accrual flowchart.

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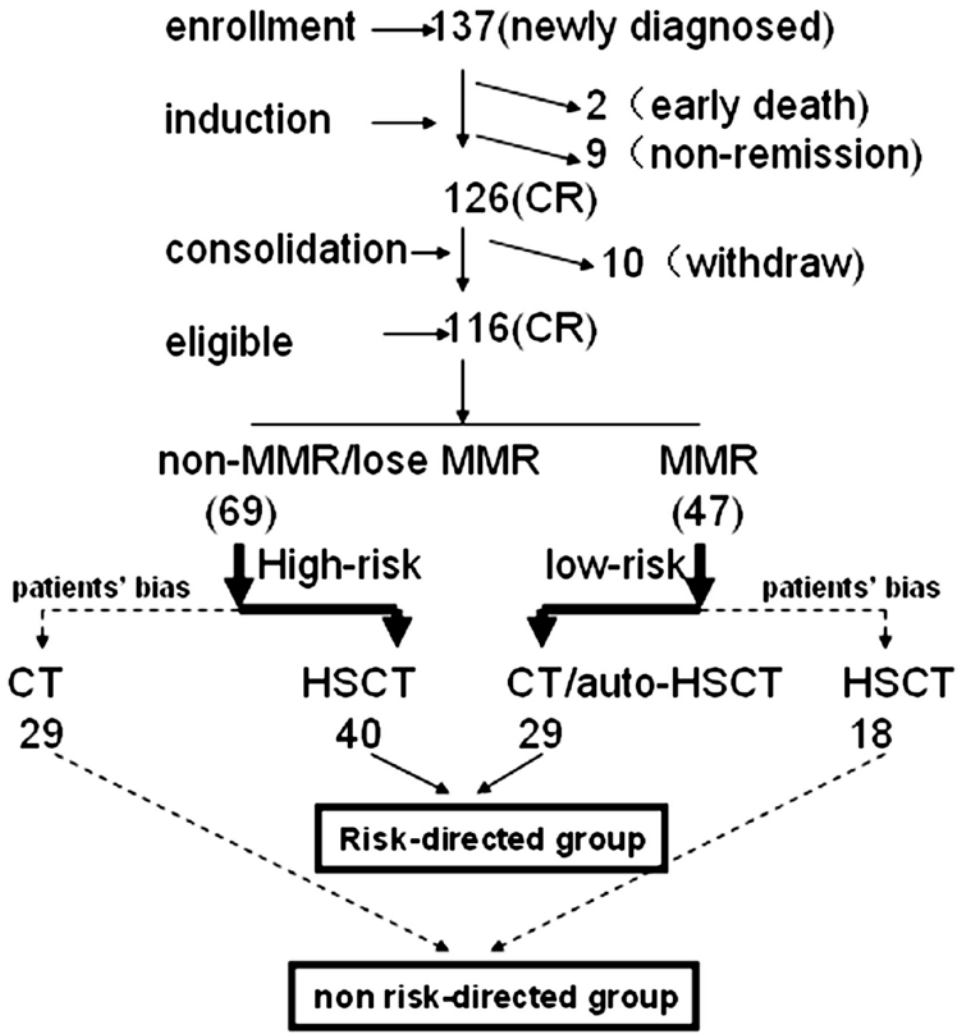
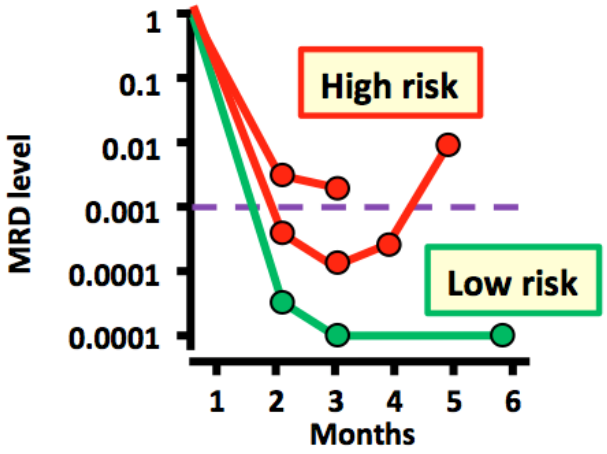


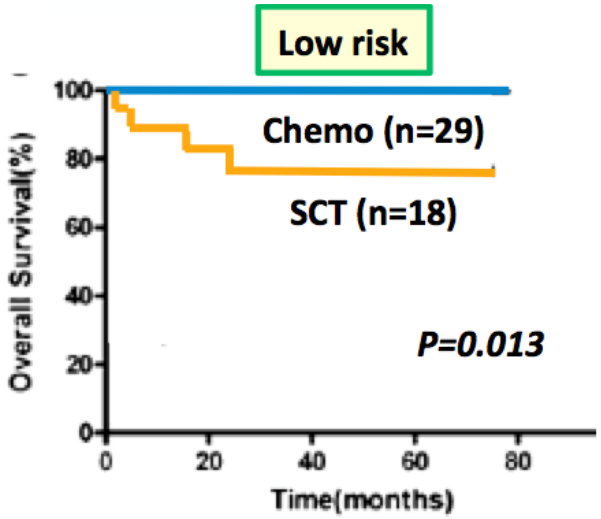

VU Hematologie

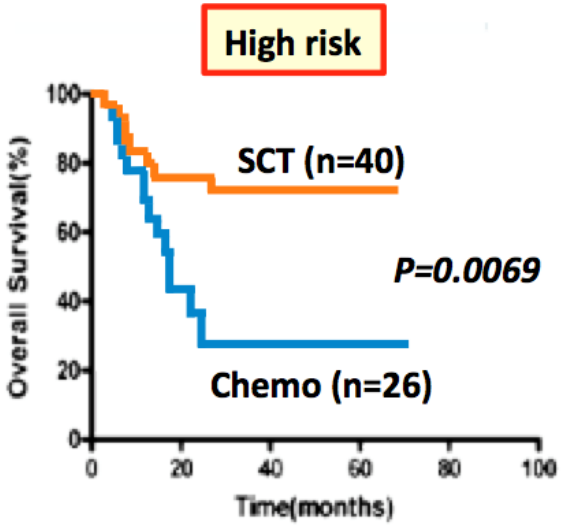
Hong-Hu Zhu et al. Blood 2013;121:4056-4062

# MRD directed treatment in CBF AML

VU medisch centrum

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Hong-Hu Zhu et al. Blood 2013;121:4056-4062

# HOVON-SAKK AML-102
# phase III study in AML/RAEB, 18-65 yrs

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R

Ara-C 200mg/m2 d1-7c.i.

Idarubicin 12 mg/m2 3-hr d1-3

Ara-C 200mg/m2 d1-7c.i.

Idarubicin 12 mg/m2 3-hr d1-3

Clofarabine 10 mg/m2 days 1-5

Ara-C 1000mg/m2 3-hr bid d1-6

Amsacrine 120 mg/m2 iv d4, 5, 6

Ara-C 1000mg/m2 3-hr bid d1-6

Amsacrine 120 mg/m2 iv d4, 5, 6

Clofarabine, 10 mg/m2
iv days 1-5

Cycle III, autoSCT, alloSCT

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# Clofarabine in AML (HOVON102)

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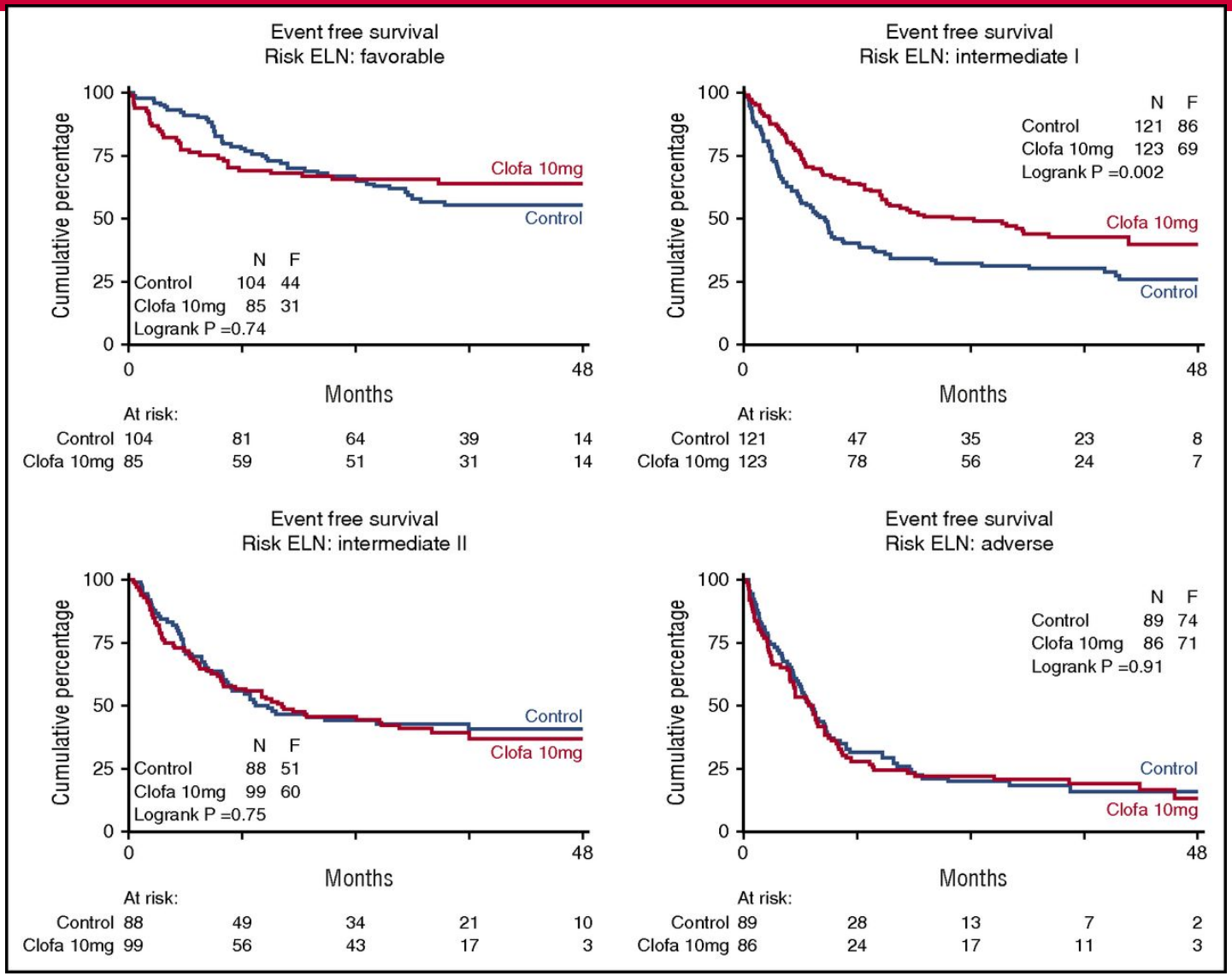


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Lowenberg B et al., Blood 2017;129:1636-1645

# MRD status correlates with clinical results of HOVON 102 trial

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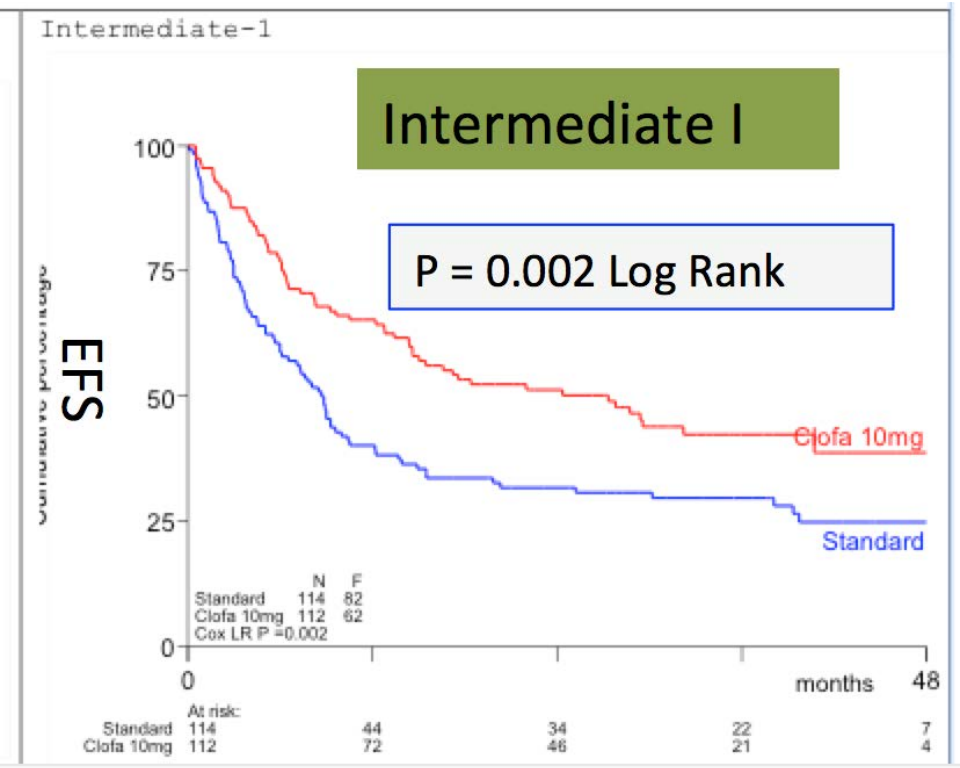


| MRD Status | Control arm | Clofarabine | Total |
|---|---|---|---|
| Negative | 24(53%) | 34(77%) | 58(65%) |
| Positive | 21(47%) | 10(23%) | 31(35%) |
| | | | P=0.018 |

VU Hematologie

Lowenberg B et al., Blood 2017;129:1636-1645

# Conclusions: MRD YES!

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- Provide objective methodology to establish remission status
- Refine outcome prediction and may inform post remission treatment: **randomized** studies should be performed
- Identify impending relapse to enable early intervention: **randomized studies** still to be performed
- Post-transplant surveillance with early intervention (IS/DLI)
- Surrogate endpoint to fasten drug approval

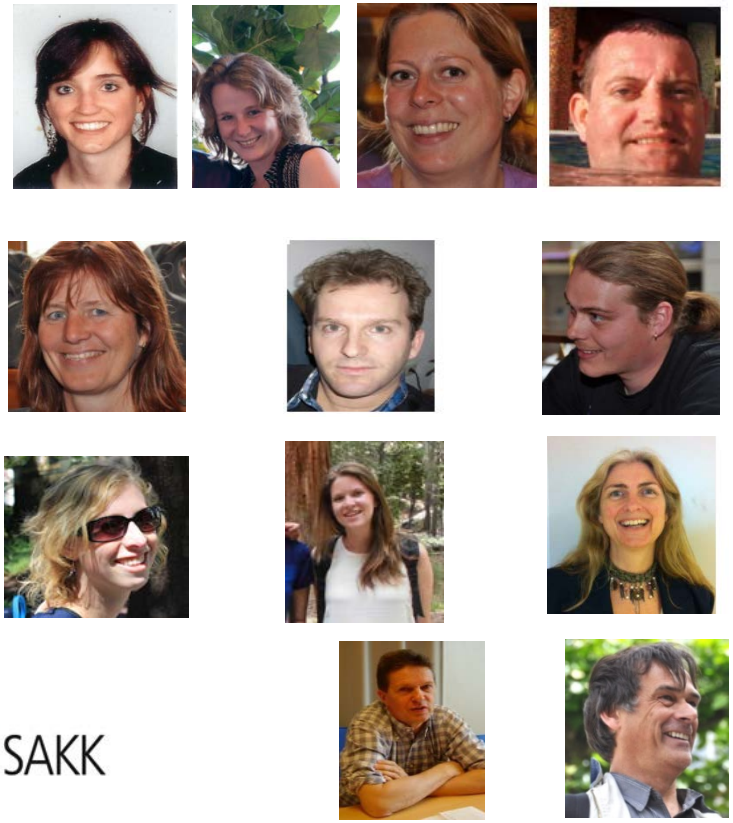
VU Hematologie

Schuurhuis GJ, et al., Blood 2018;131:1275

*Department of Hematology VU University Medical Center, CCA*
*Amsterdam, Netherlands*

**Wendelien Zeijlemaker**
**Angèle Kelder**
**Willemijn Scholten**
**Sander Snel**
**Monique Terwijn**
**Yvonne Oussoren-Brockhoff**

**Dennis Veldhuizen**
**Arjo Rutten**
**Jannemieke Ham**
**Diana Hanekamp**
**Gert Ossenkoppele**
**Jacqueline Cloos**
**Gerrit Jan Schuurhuis**



HOVON SAKK

KWF KANKER BESTRIJDING BD

**> 50 centers**
**All patients**

VUmc - Cancer Center Amsterdam (CCA)
VUmc - Institute for Cancer and Immunology (V-ICI)

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