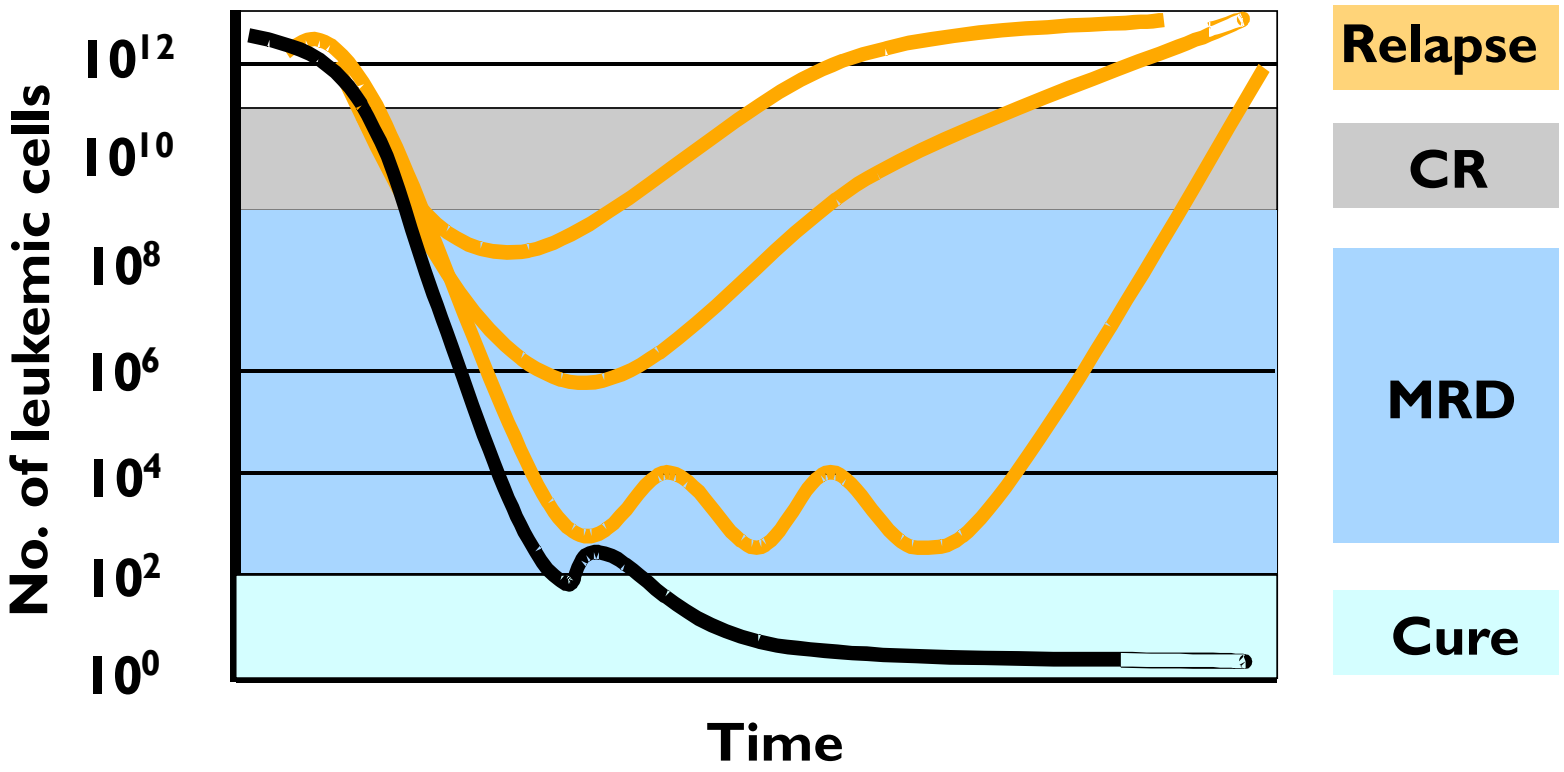
ACUTE MYELOID LEUKEMIA MEETING

Ravenna - October 27, 2017

Tools for MRD in AML: flow cytometry

Francesco Buccisano

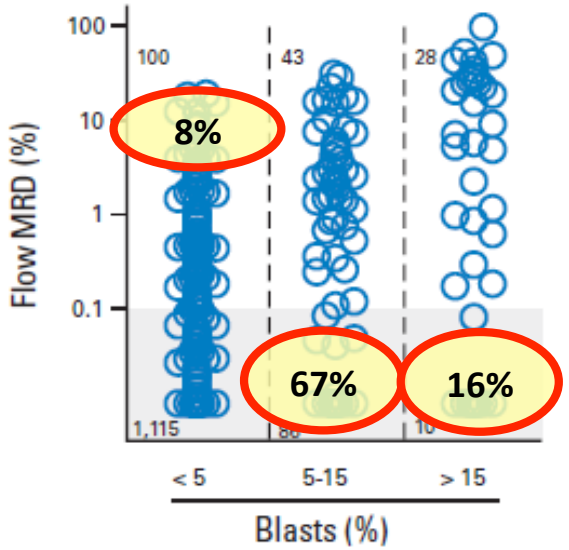
Can MRD improve outcome determination?

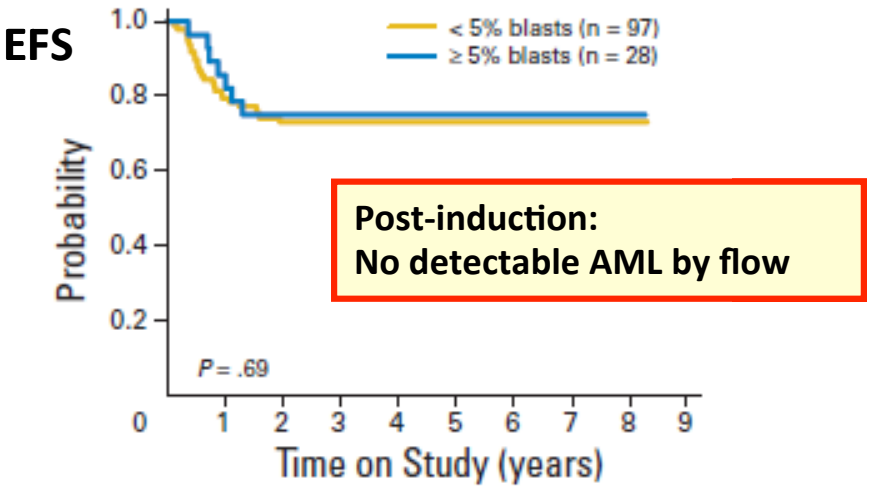


This modality may not only capture differences in treatment response that reflect the underlying molecular heterogeneity, but also inter-patient variability in drug availability and metabolism, which may also significantly influence outcome

Grimwade, Best Practice 2012

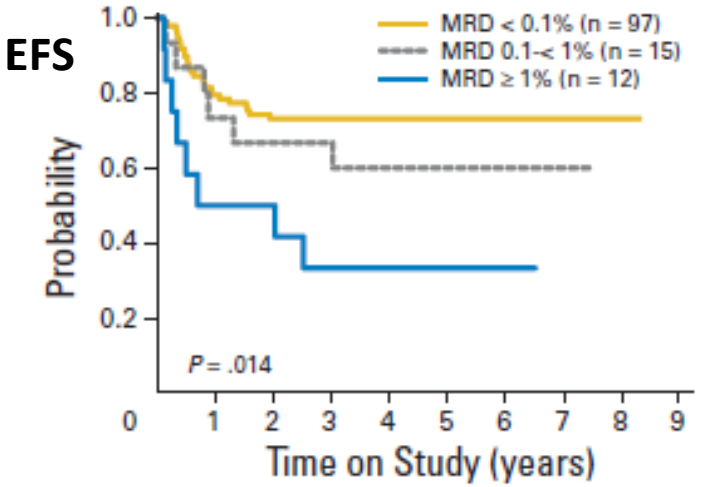
Redefining induction failure



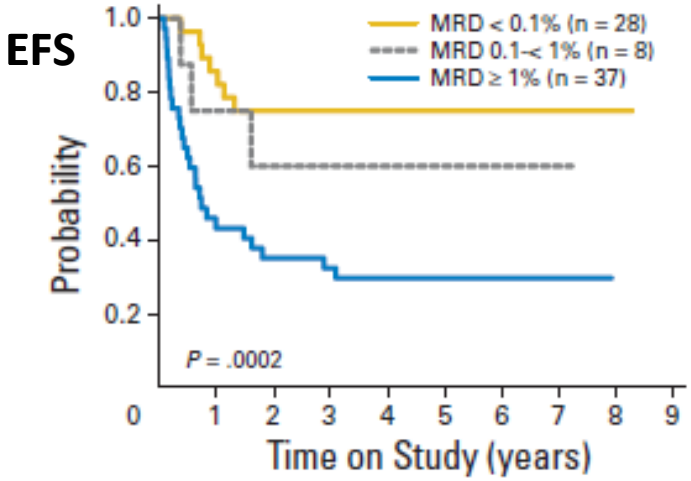


Post-induction:
No detectable AML by flow

<5% blasts by morphology

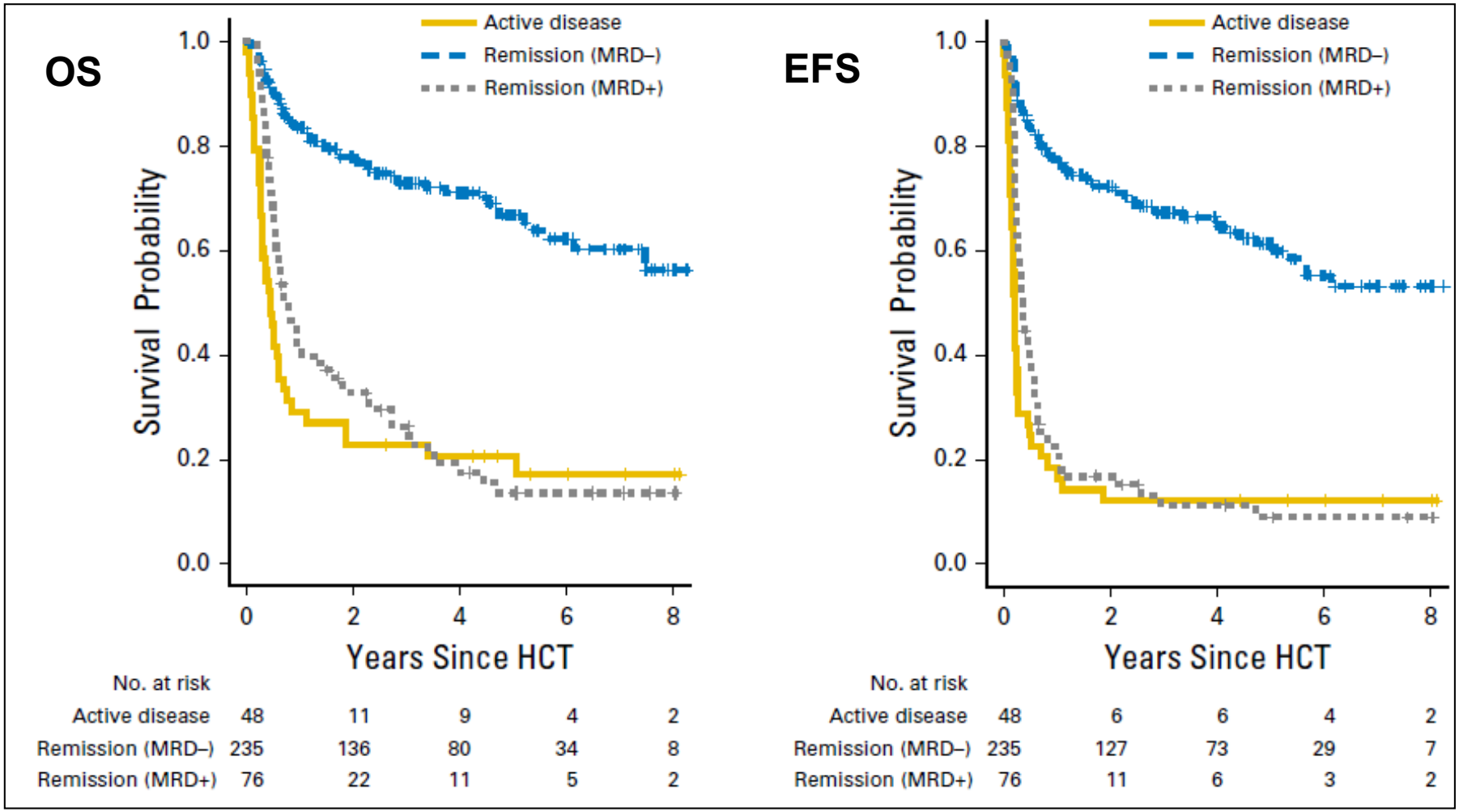


≥5% blasts by morphology



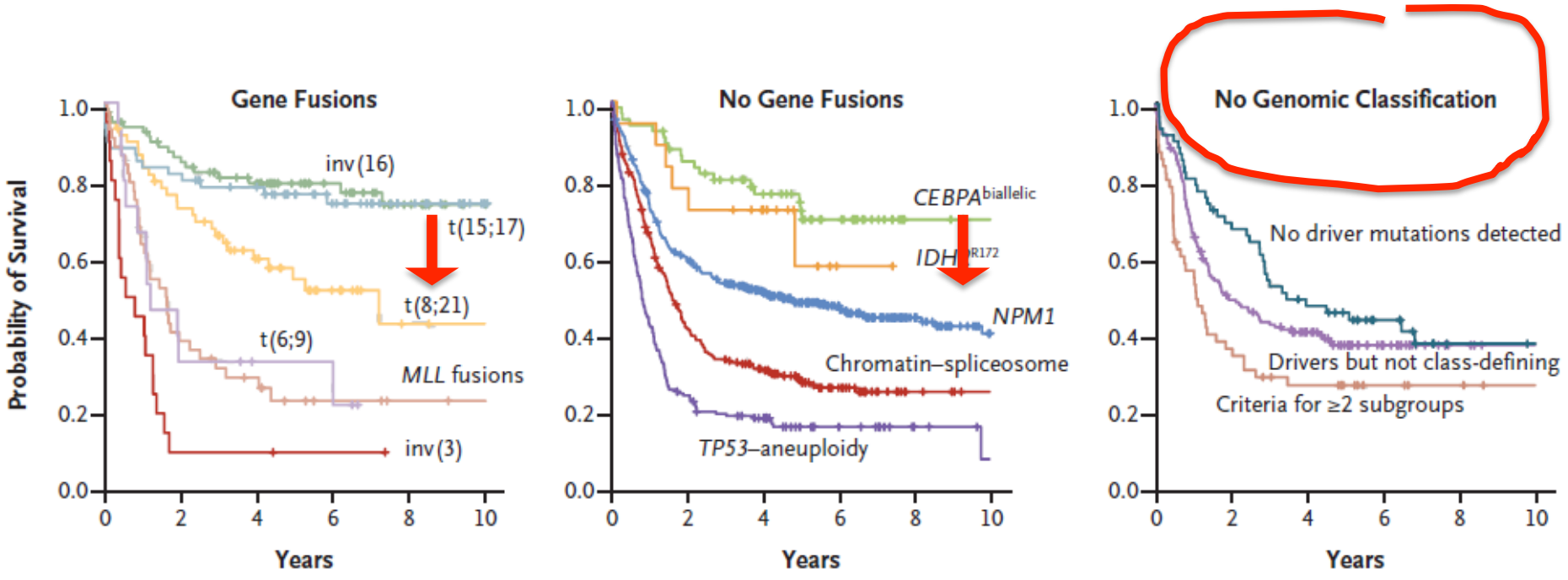
Inaba et al, J Clin Oncol 2012.

Redefining induction failure



Araki D, JCO 2015

Upfront prognostic prediction may be inadequate in some categories of patients



Patients lacking specific genetic-molecular features

Patients with intermediate prognosis

Papaemmanuil, NEJM 2016

Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel

Category	Definition	Comment
Response		
CR without minimal residual disease (CR_{MRD-})	If studied pretreatment, CR with negativity for a genetic marker by RT-qPCR, or CR with negativity by MFC	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)
Response criteria for clinical trials only		
Stable disease	Absence of CR_{MRD-}, CR, CR_i, PR, MLFS; and criteria for PD not met	Period of stable disease should last at least 3 mo
Relapse		
Hematologic relapse (after CR_{MRD-}, CR, CR_i)	Bone marrow blasts ≥5%; or reappearance of blasts in the blood; or development of extramedullary disease	
Molecular relapse (after CR_{MRD-})	If studied pretreatment, reoccurrence of MRD as assessed by RT-qPCR or by MFC	Test applied, sensitivity of the assay, and cutoff values used must be reported; analyses should be done in experienced laboratories (centralized diagnostics)

MRD can be assessed

✓ at early time points following induction and consolidation courses to assess remission status and determine kinetics of disease response,

✓ sequentially beyond consolidation to anticipate impending morphologic relapse.

blood

Dohner H, Blood 2017

Technical platforms for MRD detection

- Flow-cytometry
  - Multiparametric flow cytometry (MFC)
- PCR
  - RT-qPCR
  - Digital PCR
- NGS

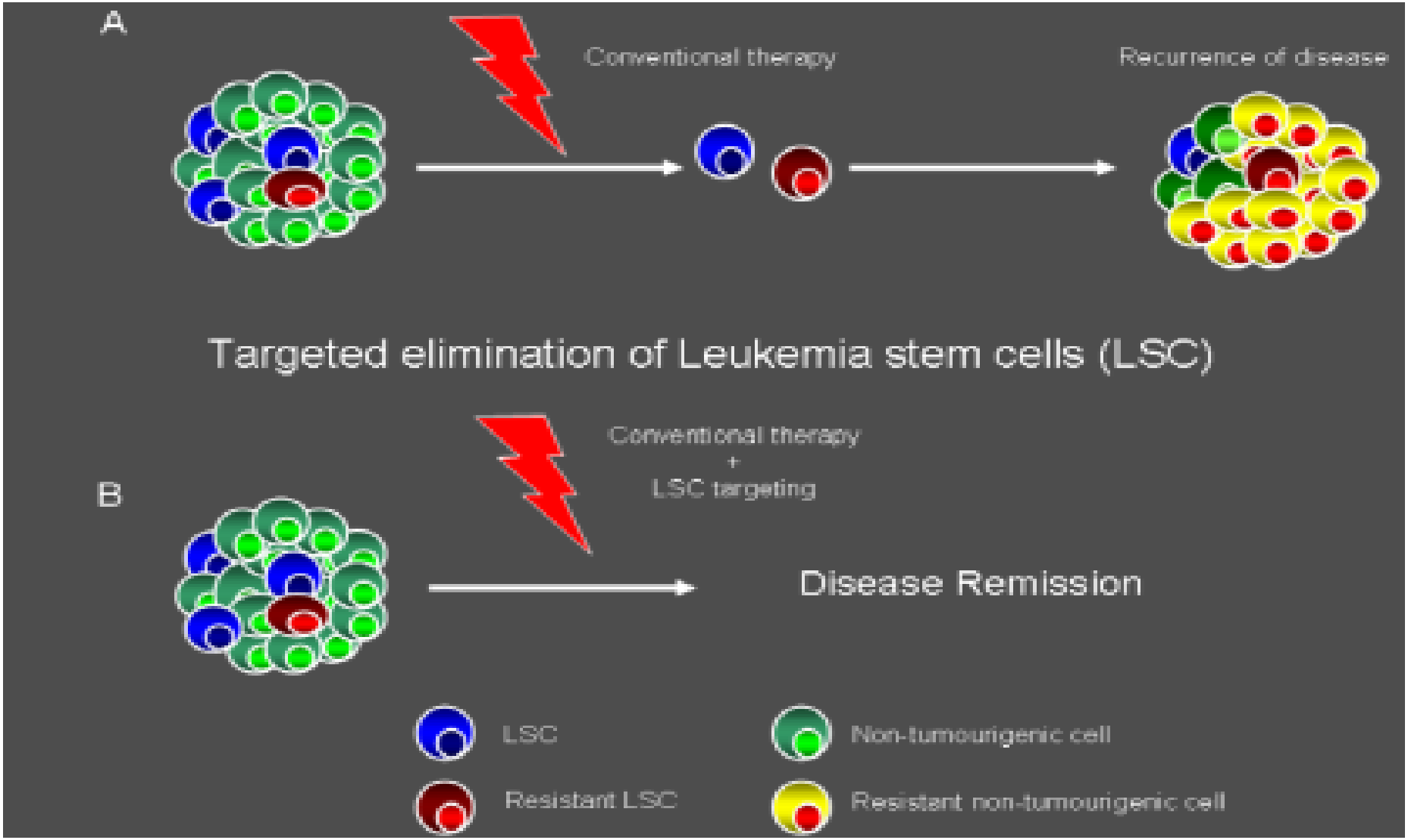
MRD detection by flow: required standards

- "Leukemia-associated immunophenotypes", that are absent or very infrequent in NBM
  - Lack of expression
  - Asynchronous expression
  - Lack/overexpression
- "Different from normal", empty spaces that are not usually occupied during normal myeloid maturation
- At least 8-color panels
  - 47 phenotypes were totally absent (<0.01% of blast cells)
  - 41 phenotypes were identified in <0.05% of blast cells

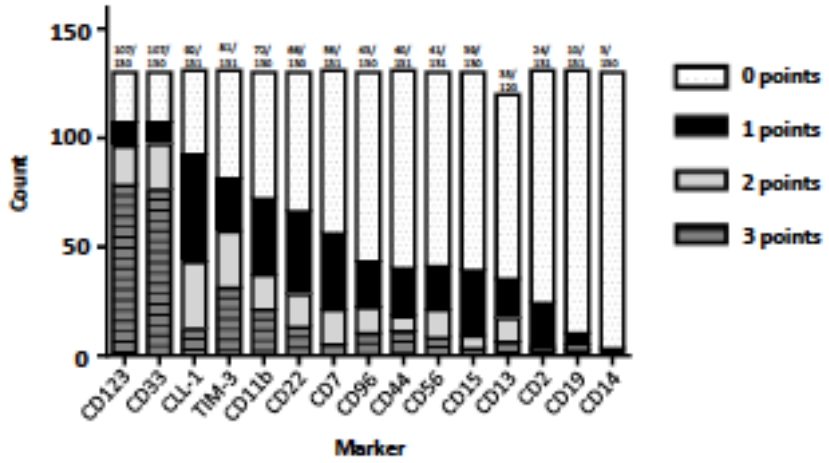
Olaru et al., Cytometry 2008

- Consider rare populations (leukemic stem cells)

Role of LSC


A
Conventional therapy
Recurrence of disease
Targeted elimination of Leukemia stem cells (LSC)
B
Conventional therapy
+
LSC targeting
Disease Remission
LSC
Non-tumourigenic cell
Resistant LSC
Resistant non-tumourigenic cell

LSC detection kit for diagnostic purposes: assessment of total stem cell load



Probability of aberrant markers expression on CD34+CD38- LSC

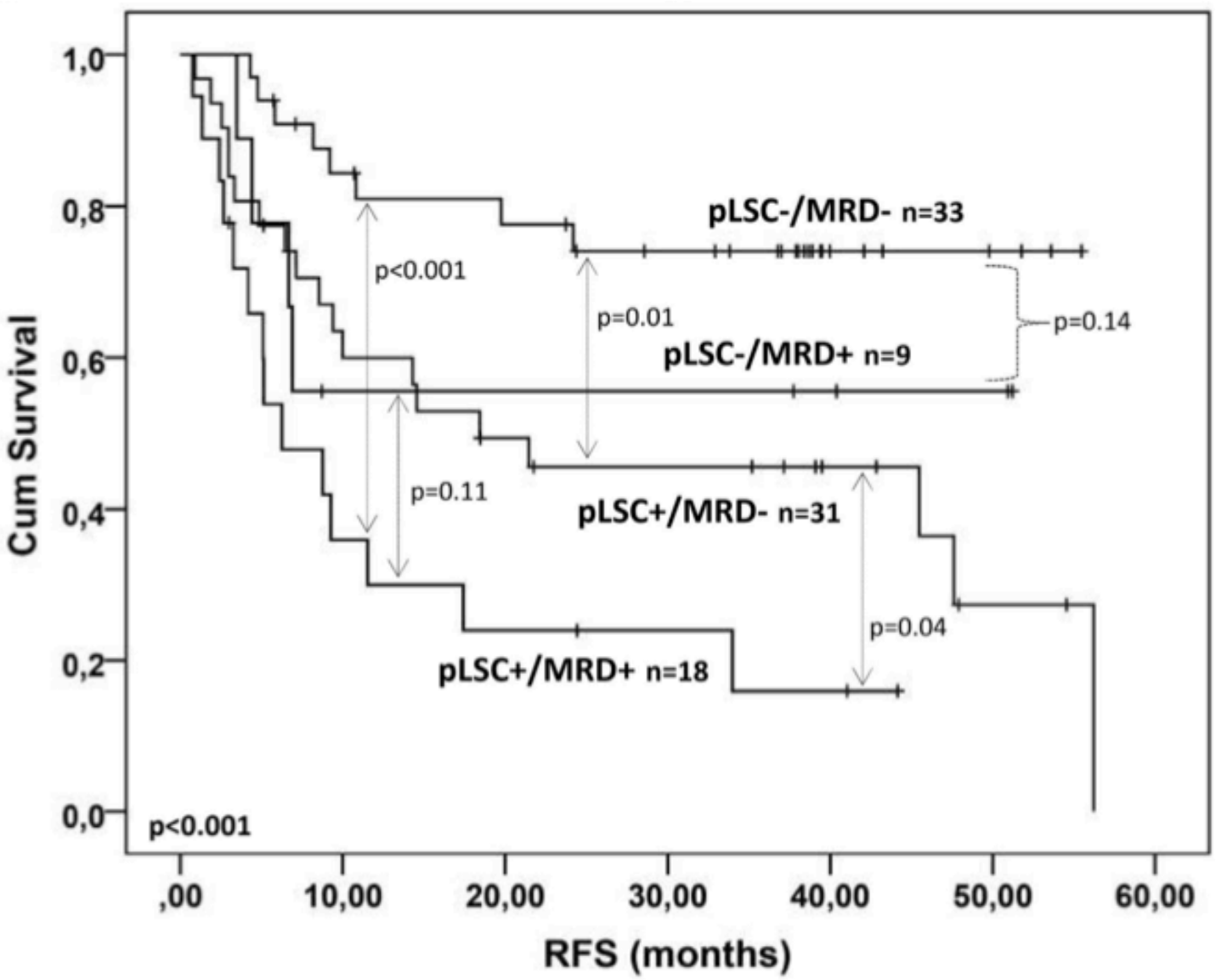
(1 tube, 8 colors, 13 markers)

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

PE channel contains antibodies negative on HSC

Zeijlemaker et al., Leukemia 2016

Combining MRD and LSC frequency improves prognostic impact of MRD



Terwijn, PLOS ONE 2014

Validation of MRD-tailored therapy

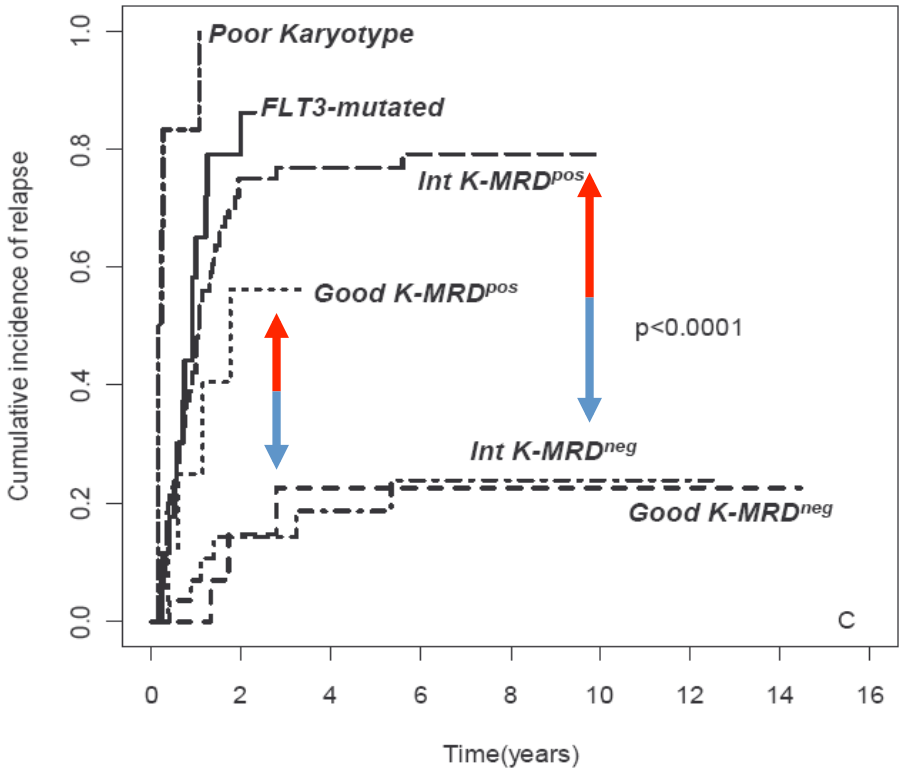
- What do we need to tailor therapy on a biomarker:
  - Measurable biological or clinical characteristics
  - Well documented risk categories
  - Robust retrospective validation
  - Prospective randomized studies showing benefits of tailoring

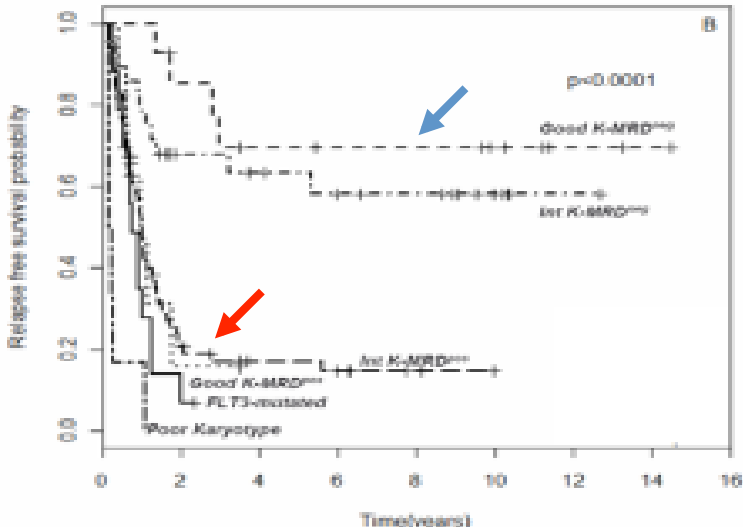
| Informed Decisions in Acute Myeloid Leukemia: Beyond Morphology and Cytogenetics |

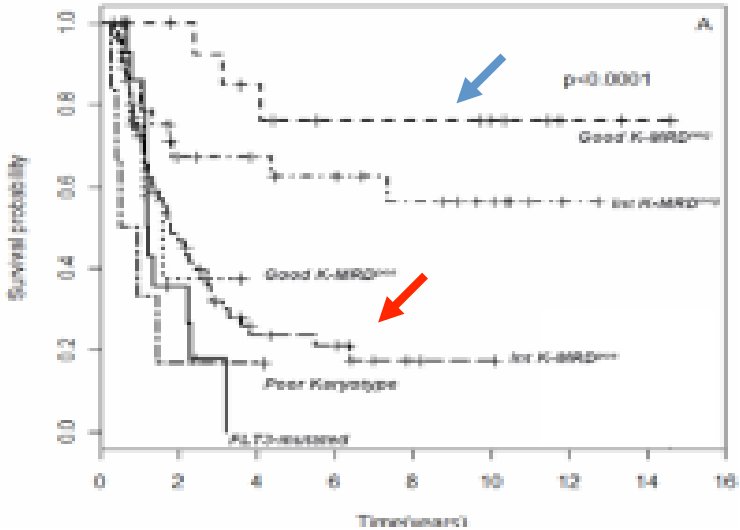
MRD in AML: does it already guide therapy decision-making?

Gert Ossenkoppele and Gerrit Jan Schuurhuis

Department of Hematology, VU University Medical Center Amsterdam, Amsterdam, The Netherlands



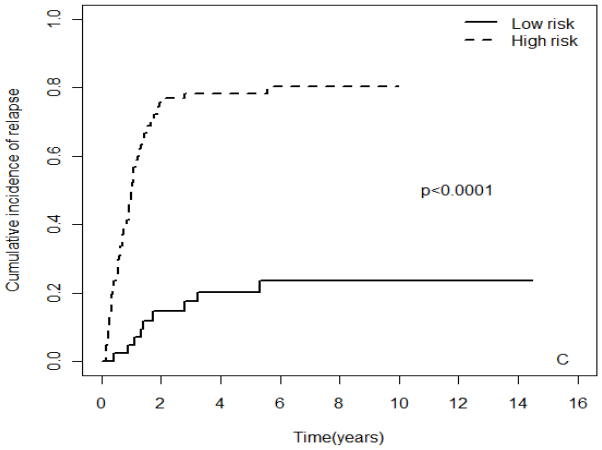


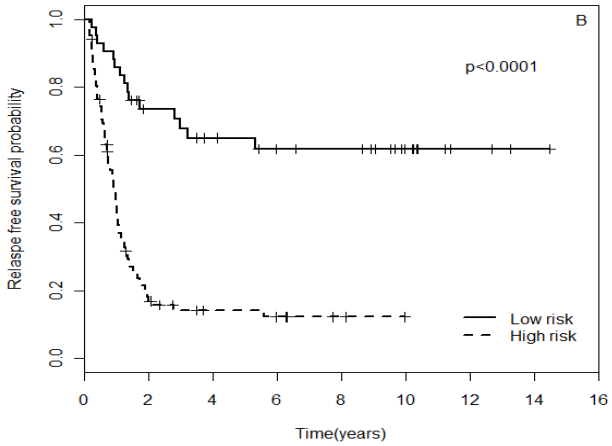


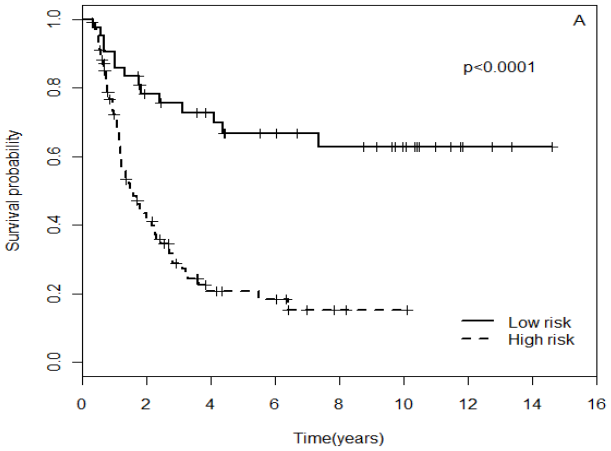
Buccisano et. al. Blood 2010

Integrated Risk-Score

Low-Risk	High-Risk
Good K / MRD- Int K / MRD-	Adverse K FLT3+ Good K / MRD+ Int K / MRD+





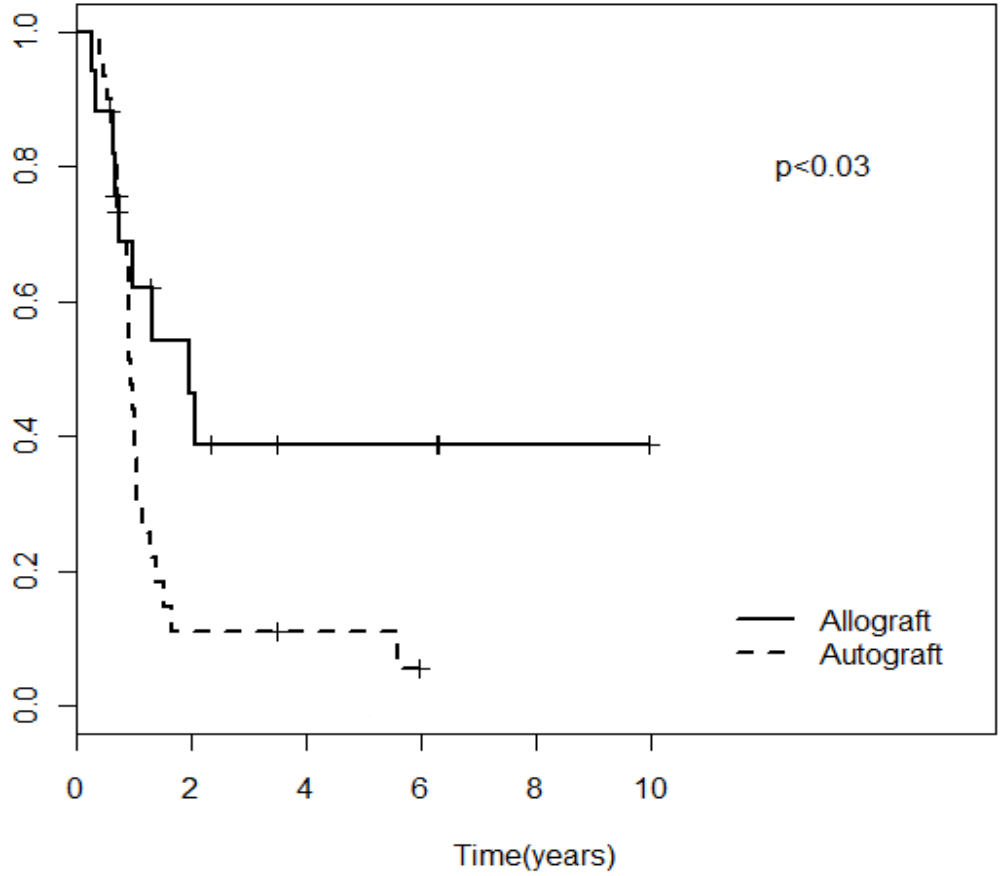


Buccisano et. al. Blood 2010

AlloSCT > AutoSCT for High-risk AML

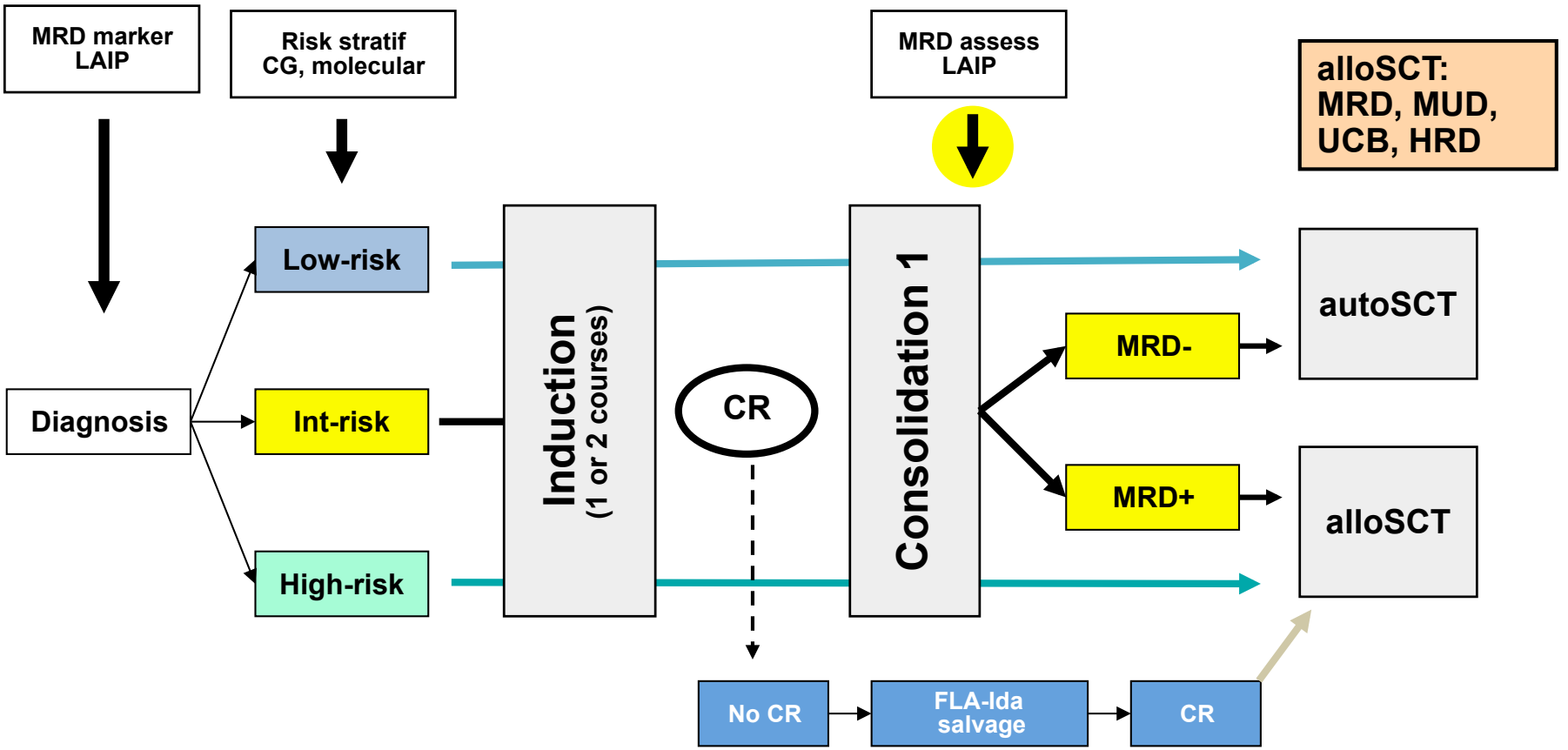
Low-Risk	High-Risk
Good K / MRD- Int K / MRD- 4 yrs. CIR = 15%	Adverse K FLT3-ITD+ Good K / MRD+ Int K / MRD+ 4 yrs. CIR = 77%

NO SCT*
Koreth, 2009
Cornelissen, 2012



Buccisano et. al. Blood 2010

AML1310 – Schedule

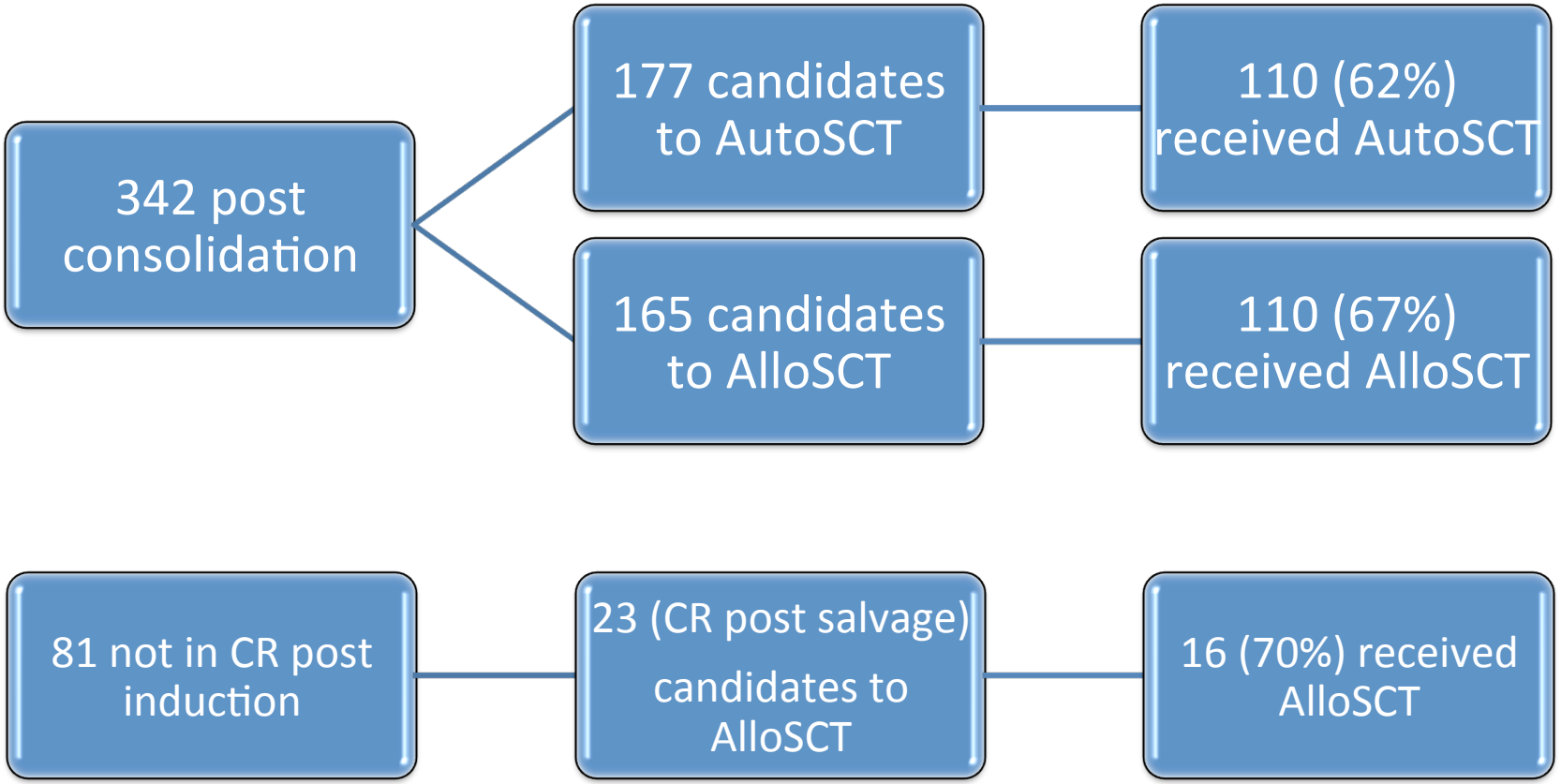


Low-risk: CBF/Kit^{wt}; NPM1+/FLT3-
Int-risk: all others
High-risk: Adverse K; FLT3-ITD

- *INDUCTION*
  - *Daunorubicin : 50 mg/m2 iv D 1,3,5*
  - *SD-Ara-C: 100 mg/m2 c.i. D 1-10,*
  - *Etoposide: 100 mg/m2 iv D 1-5*
- *CONSOLIDATION*
  - *Daunorubicin : 50 mg/m2 iv D 4-6*
  - *ID-Ara-C : 500 mg/m2/q12 hrs, over 2 hrs, D 1-6*

AML1310: results

fondazione GIMEMA onlus
per la promozione e lo sviluppo della ricerca scientifica sulle malattie ematologiche. FRANCO MANDELLI



Courtesy of A. Venditti

AML1310: results
OS and DFS by ELN category

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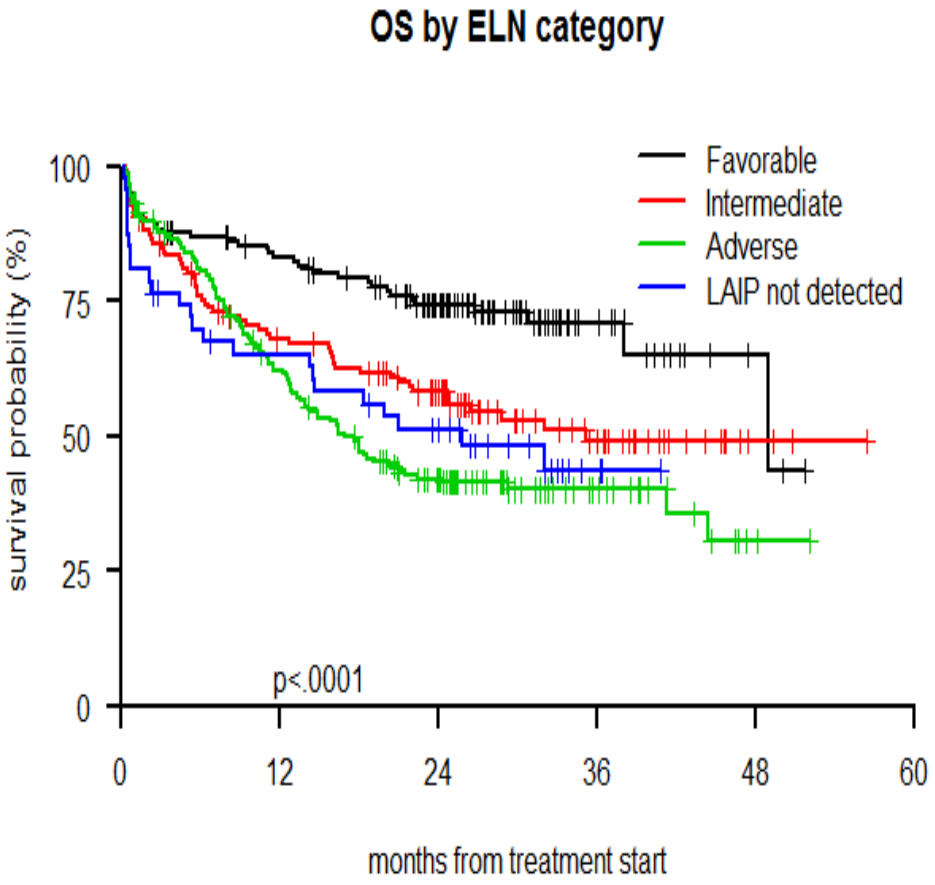

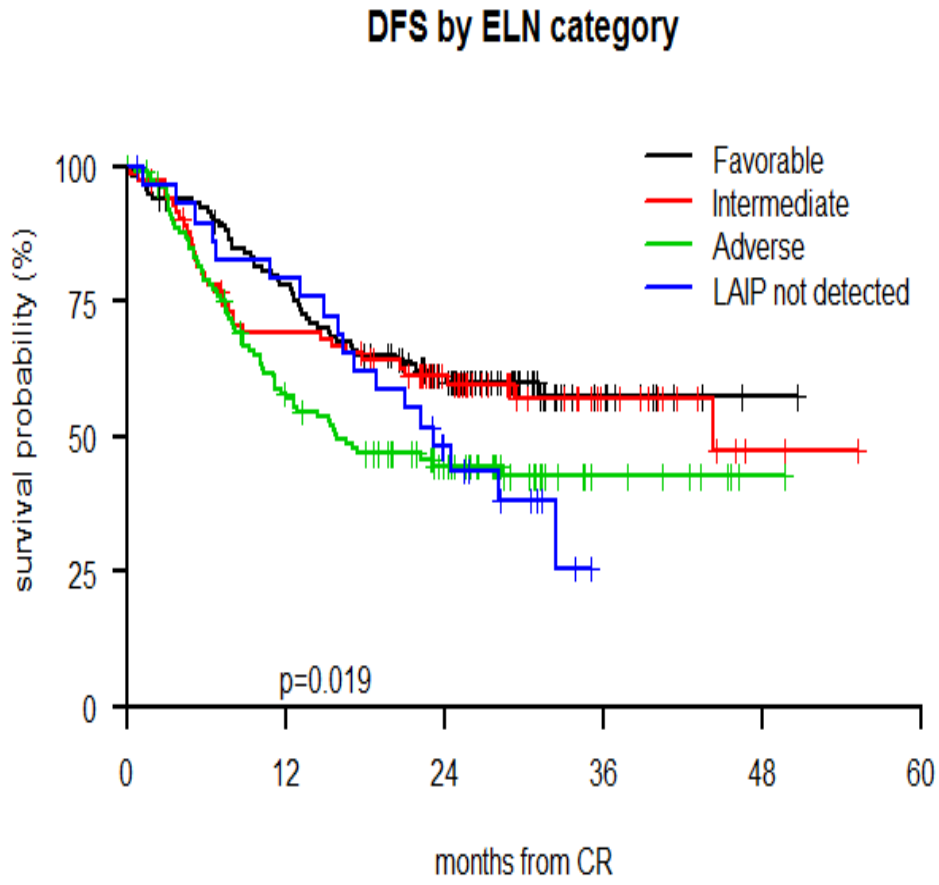

Courtesy of A. Venditti

AML1310: intermediate-risk OS and DFS by MRD status

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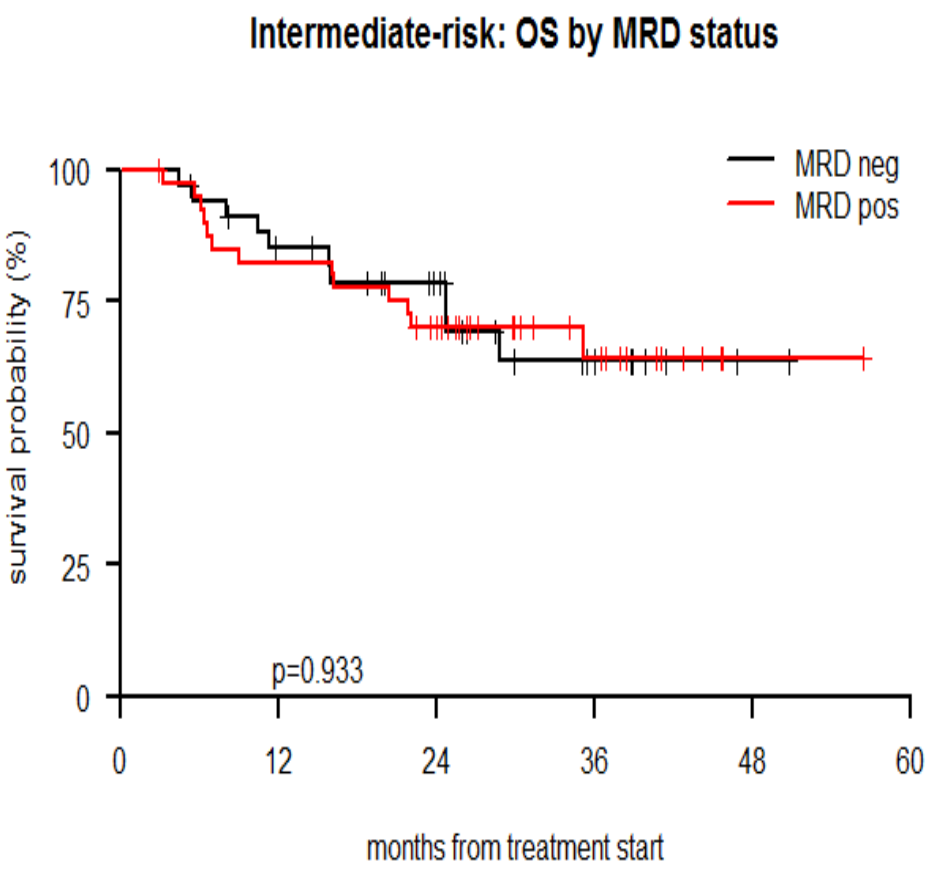
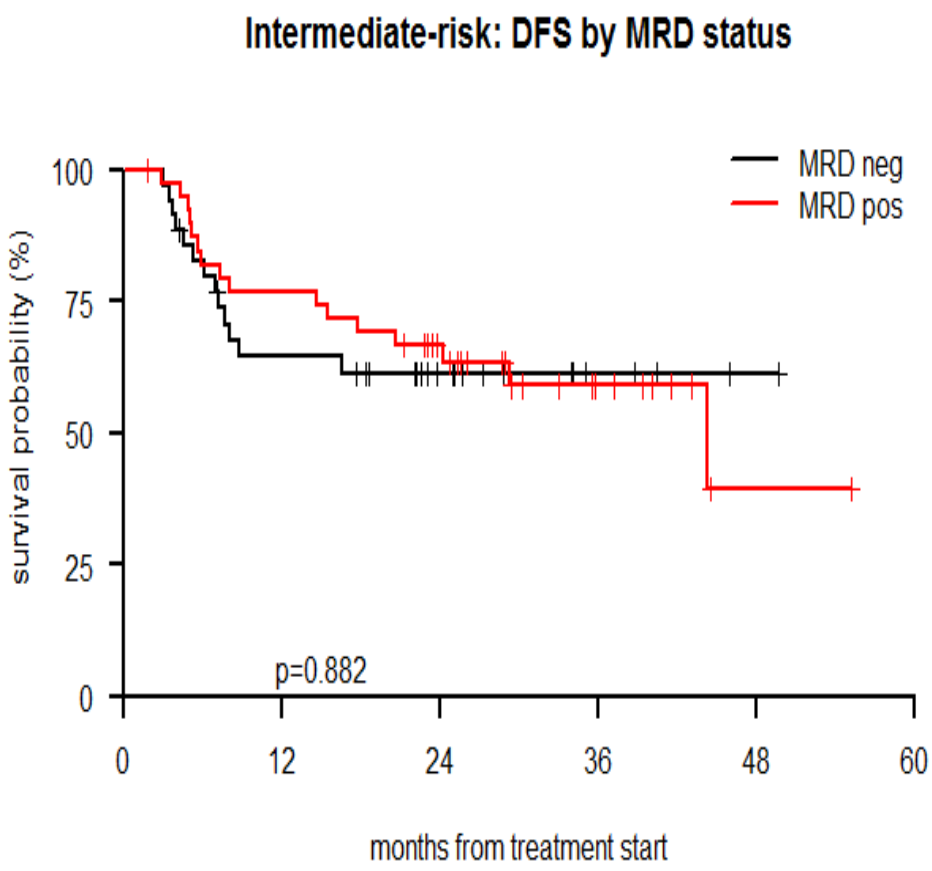
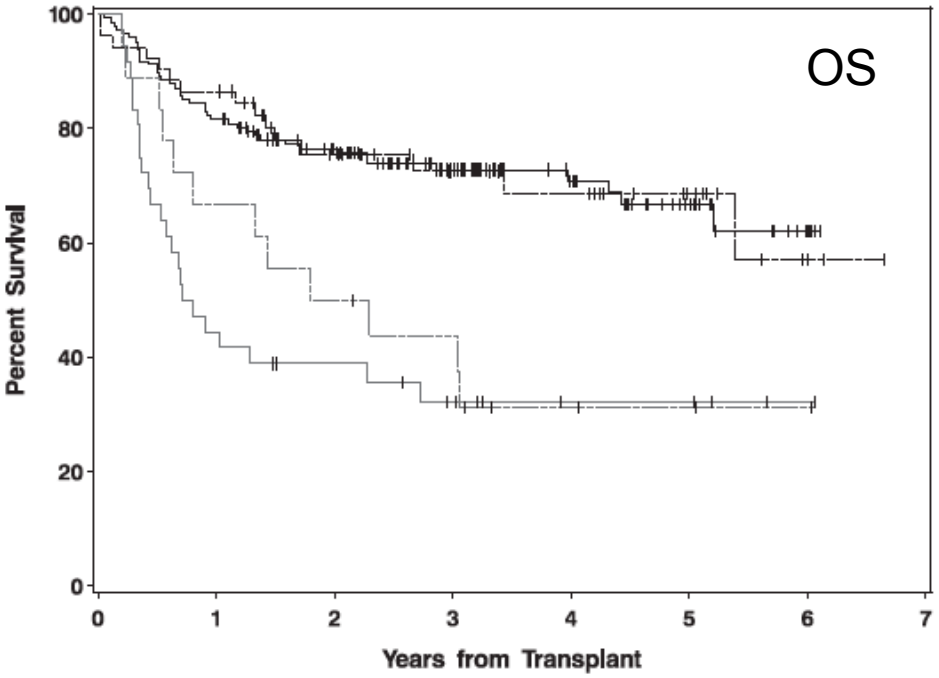


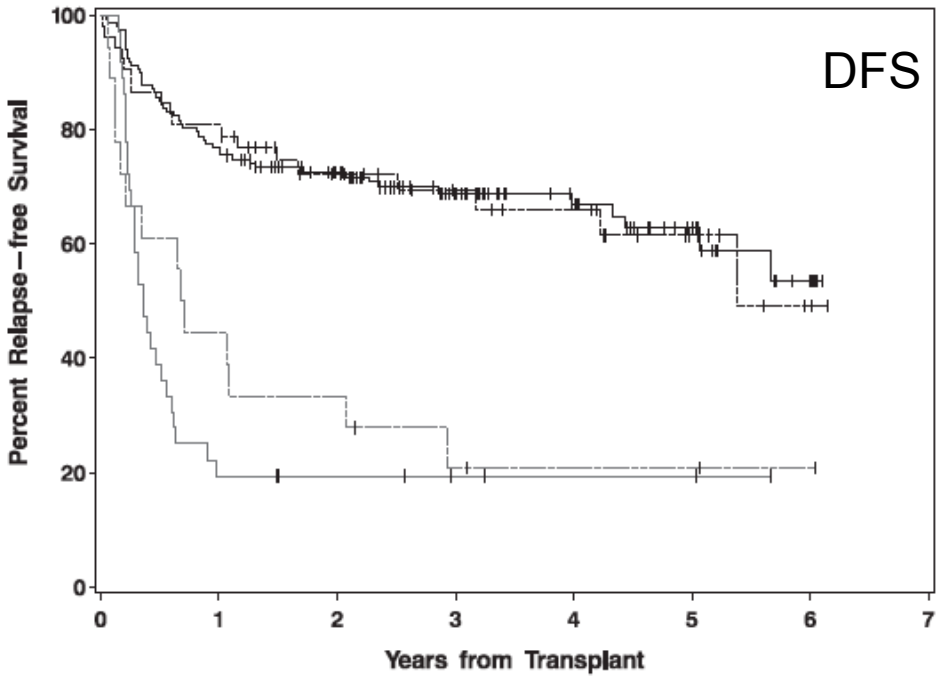


Courtesy of A. Venditti

Pre-SCT MRD positivity impacts on outcome

- ✓ 253 patients, all CR1/CR2, 33% HLA-sibling, 67% MUD
- ✓ 79% MRD negative, 21% MRD positive (any level)
- ✓ 10-color MFC pre-transplant detection of LAIP

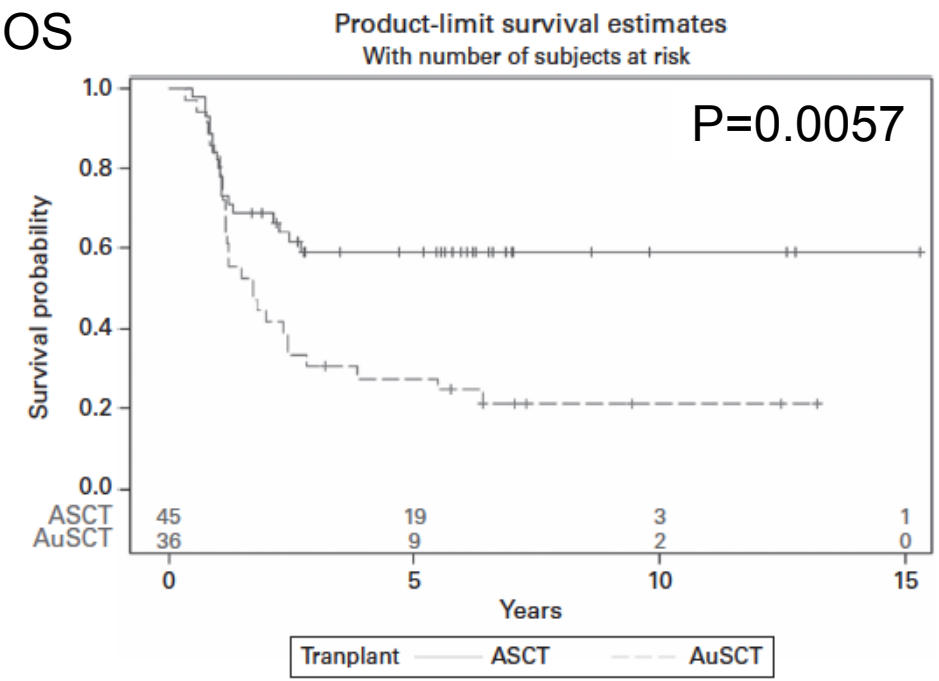




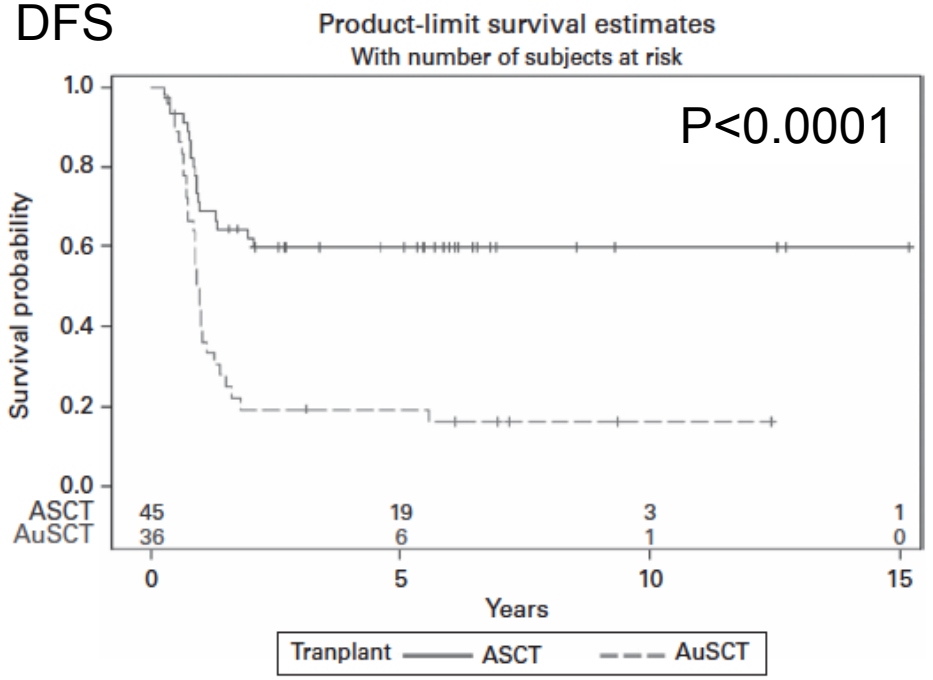
Walter RB, Blood 2013

OS and DFS of 81 AML MRD^{pos} patients stratified by type of transplant.

OS



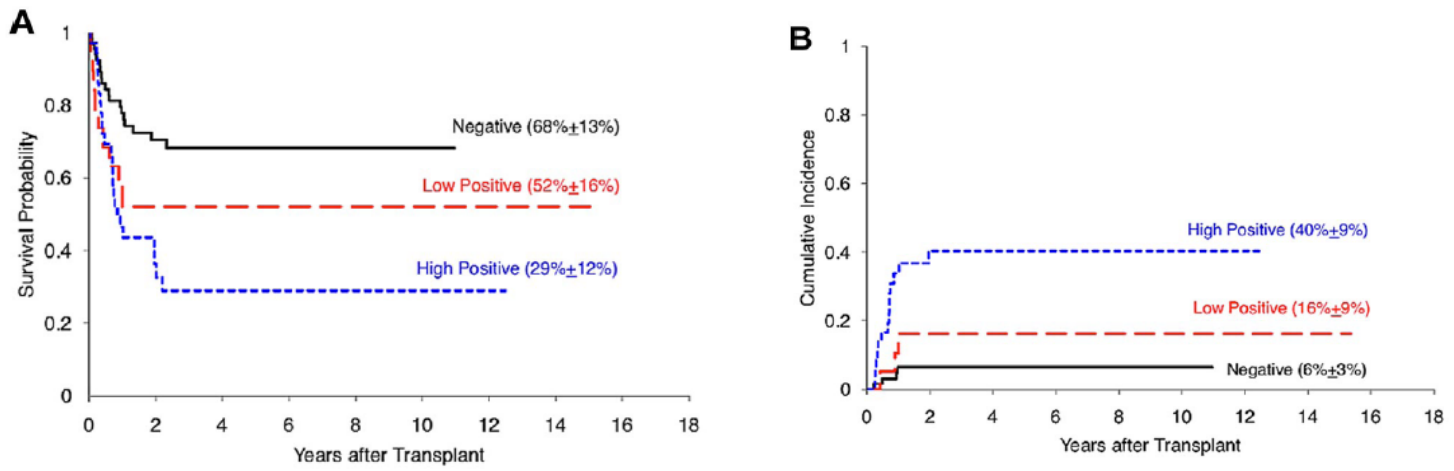
DFS



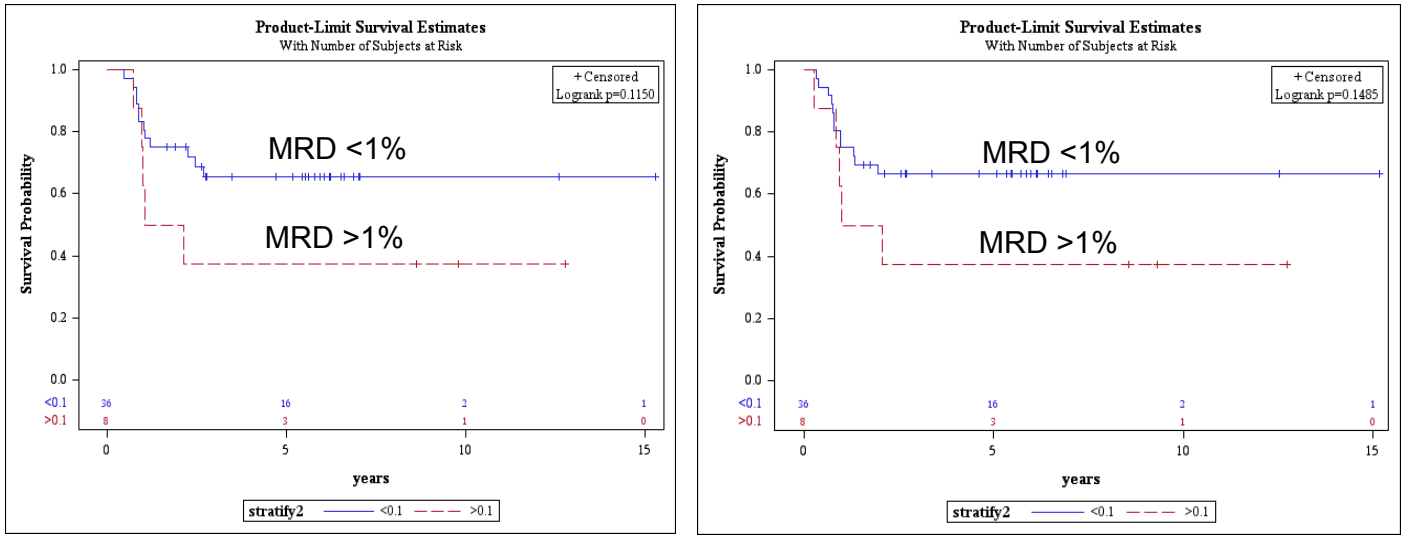
MRD positivity was defined if ≥3.5 x10^{-4} (0.035%) residual leukemic cells were detected by MFC in the BM upon full hematological recovery after consolidation cycle

Buccisano, BMT 2016

Pretransplant MRD level and clinical outcome



Leung W, Blood 2012



Buccisano et al., BMT 2016

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

AML16 (2006 – 2011)
892 AML patients
(median age 67 years)

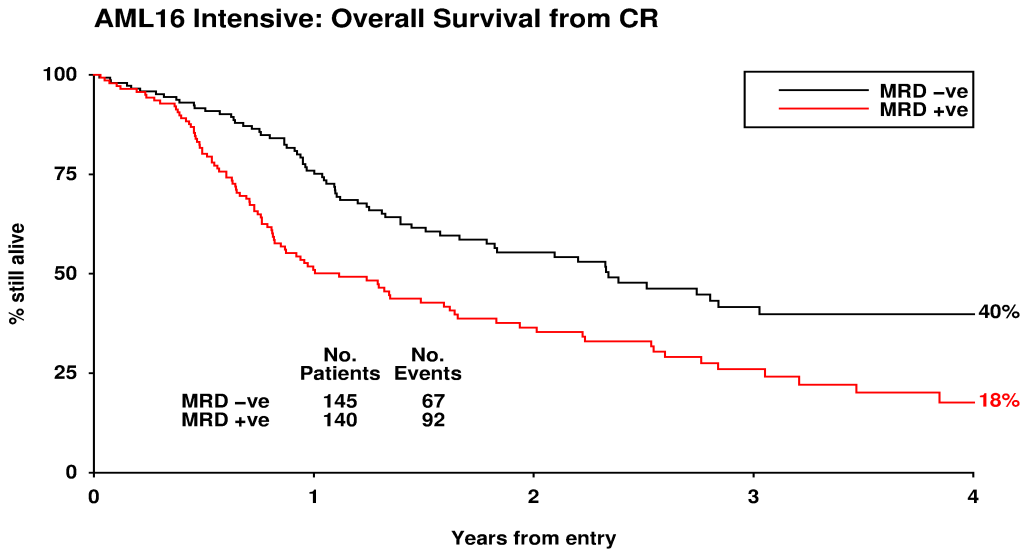
LAIP-MRD - prospectively assessed
(blind to clinical outcome)

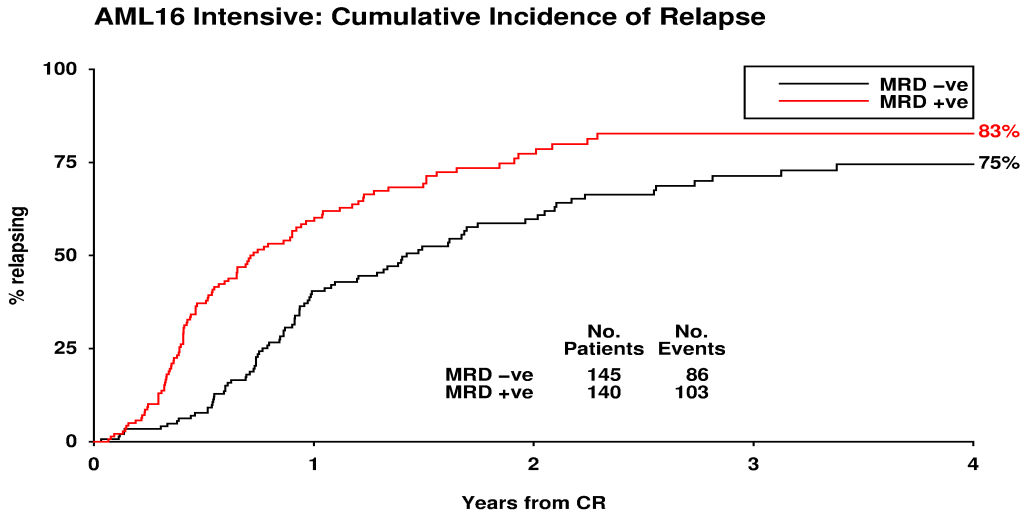
Treshold set at 0.1% residual leukemic cells

>2200 samples
>100 UK centers
2/3 labs centralised analysis

Prognostic impact of flow MRD independent of:

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

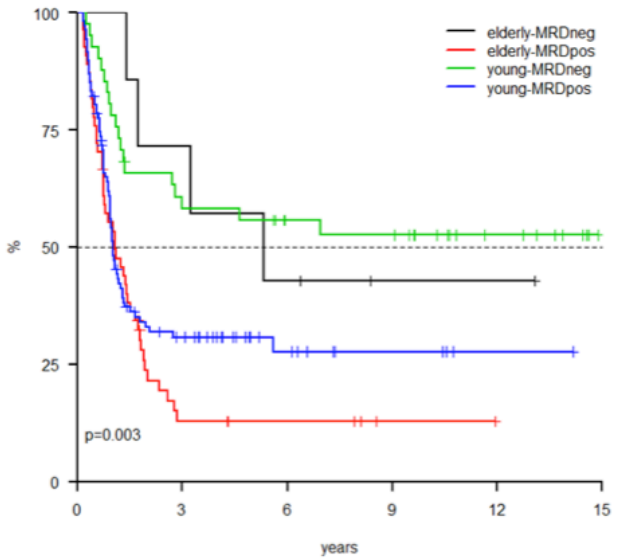




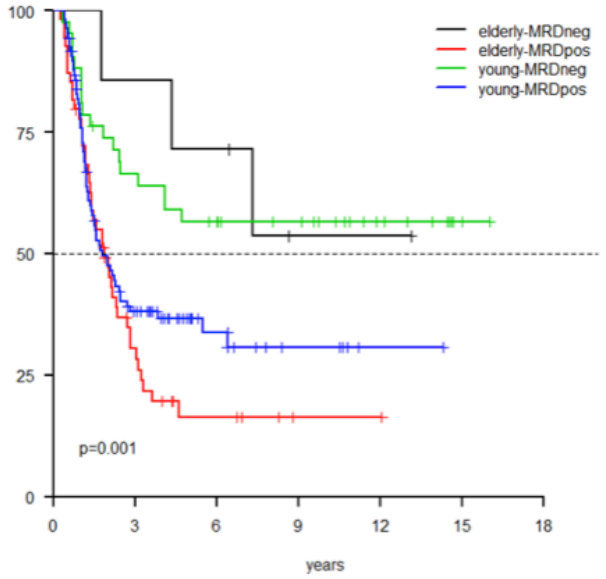
Freeman et al, J Clin Oncol 2013

MRD impact: young vs. old

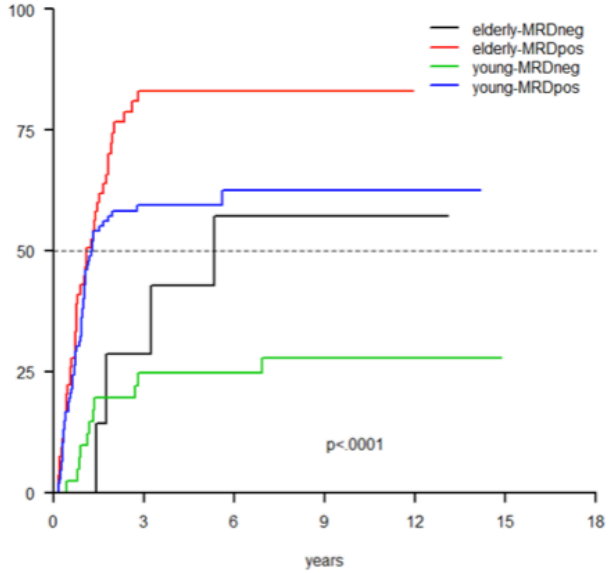
OS



RFS



CIR



- **61 older patients vs. 149 younger ones**
- **MRD negativity: < 3.5 x 10-4 (0.035%) residual leukemic cells; Time point: post-consolidation**

- **Elderly patients become MRD negative, although less frequently as compared to younger ones**
- **Relapse rate in MRD negative patients remains considerable (57% in our study, 83% in AML16)**
- **Age represents, by itself, a poor-risk features in AML.**

Buccisano, Ann H 2015

Conclusions

- MRD is a biomarker for treatment response in AML
  - Determination of MRD refines prognosis dictated by the genetic profile at diagnosis
- MFC and molecular biology are the techniques of choice
  - High technical requirement (8-color MFC)
  - Open issues: sensitivity, specificity, stability over treatment course, time-points, threshold (*ELN AML MRD WP*)
- MRD-oriented prospective clinical trials ongoing
  - Support to transplant choice
  - Elderly AML?