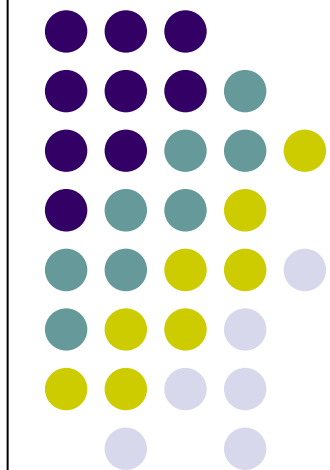
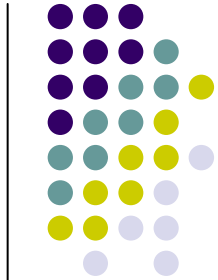
Clinical applications of molecular and flow cytometric diagnostics in myeloid leukemias



Boeckx N.
UZ Leuven

Antwerpen 01-06-2006

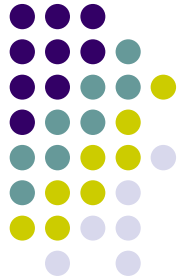


- ‘Classical’ applications
- ‘Specific’ applications
- Future applications & New developments

‘Classical’ applications of immunophenotyping in AML (CML)

- lineage assessment of leukemic cell population
- identification of leukemia-associated immunophenotypes (LAP)
- identification of antigen profiles associated with specific molecular defect
- study therapy efficacy by monitoring BM and PB samples for the occurrence of minimal residual disease (MRD)

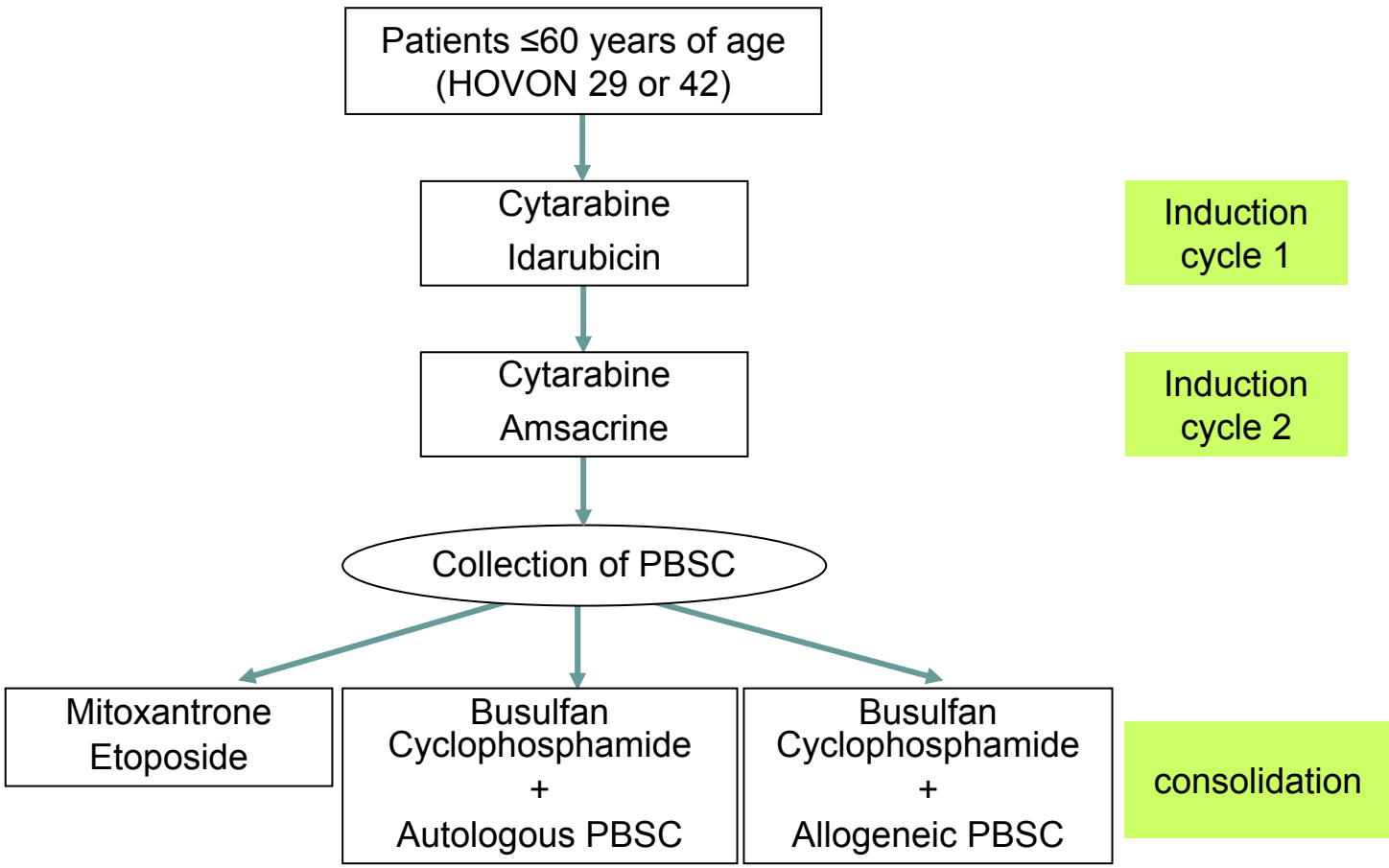
‘Classical’ applications of molecular analysis in AML/CML



- identification of chromosome aberrations with fusion genes
  - common abnormalities: t(8;21) with *AML1-ETO*, t(15;17) with *PML-RARA*, t(9;22) with *BCR-ABL*, …
  - rare abnormalities: t(6;9) with *DEK-CAN*, t(8;16) with *MOZ-CBP*, …
- identification of mutated genes
  - *FLT3* mutations (FLT3-ITD, FLT3 point-mutations), *NPM1* mutations, ….
- identification of abnormal expressed genes
  - *WT1* and *PRAME* overexpression
- study therapy efficacy by monitoring BM and PB samples for the occurrence of MRD
  - early detection of molecular relapse in APL to guide therapeutic interventions
  - evaluation of treatment effectiveness in CML after allogeneic Tx and recognition of early relapse to guide therapeutic interventions

‘Specific’ MRD applications

- Evaluation of autologous stem cell transplantation products to guide purging decisions



Evaluation of auto-PBSC products to guide purging decisions

- MRD in graft may contribute to relapse
- Auto-Tx in first remission with purged stem cells show
  - ↓ relapse rate
  - ↑ leukemia-free survival

  compared to patients receiving unpurged transplants
- Purging procedures: high toxicity for normal progenitors/SC
- MRD frequency in PBSC: varies widely, predictive for duration of relapse free survival
- Proposal of an MRD-based model to guide purging decisions

MRD in PBSC	% of patients	Relapse rate at 12 months	Desired tumorload ↓
<0.05%	31%	0%	none
0.05% - 0.5%	36%	69%	≤ 1 log
> 0.5% - 5%	33%	100%	> 1-2 log

Feller N. *et al.* Exp Hematol. 2005, 33: 120

‘Specific’ (MRD) applications

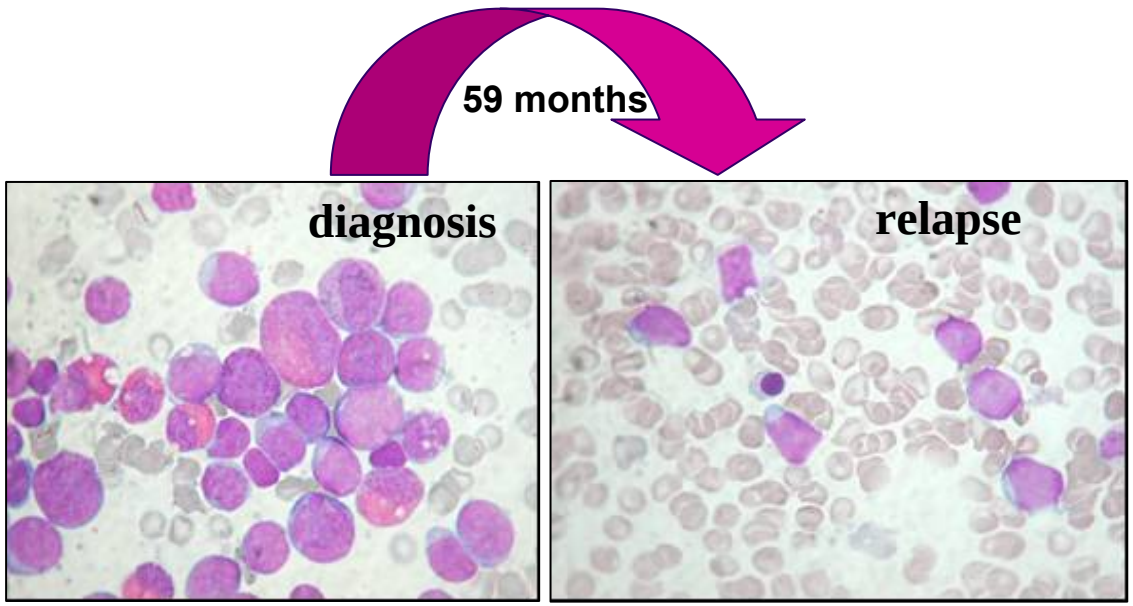
- Assessment of a common origin of two phenotypically different malignancies in the same patient

DIAGNOSIS

AML-M4Eo

68% blasts

15% eos



RELAPSE

Acute leuk

36% blasts

0.5% eos

Boeckx N. *et al.* Hematologica 2004; 89.

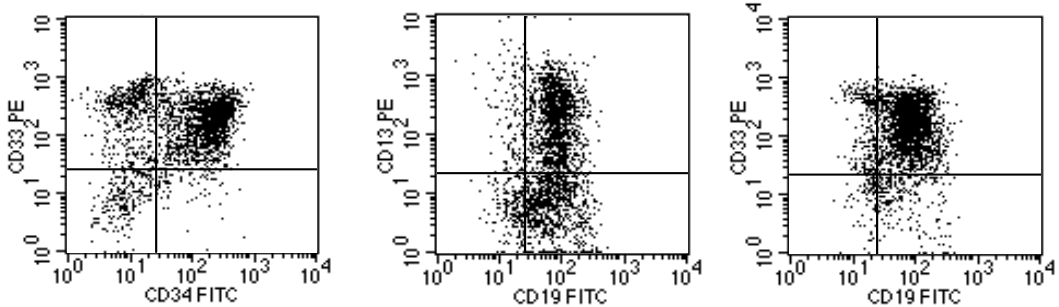
Flow cytometric analysis

DIAGNOSIS

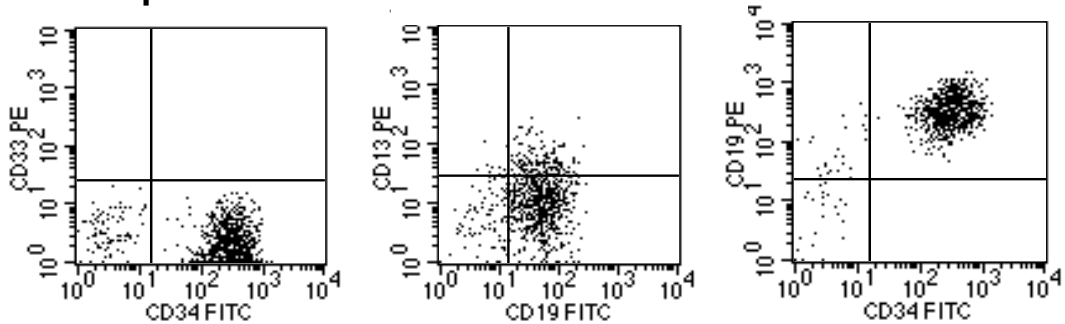
CD34
CD117
TdT
HLA-DR
CD13
CD33
MPO
CD10
CD19
CD20
CD24

AML

diagnosis



relapse



RELAPSE

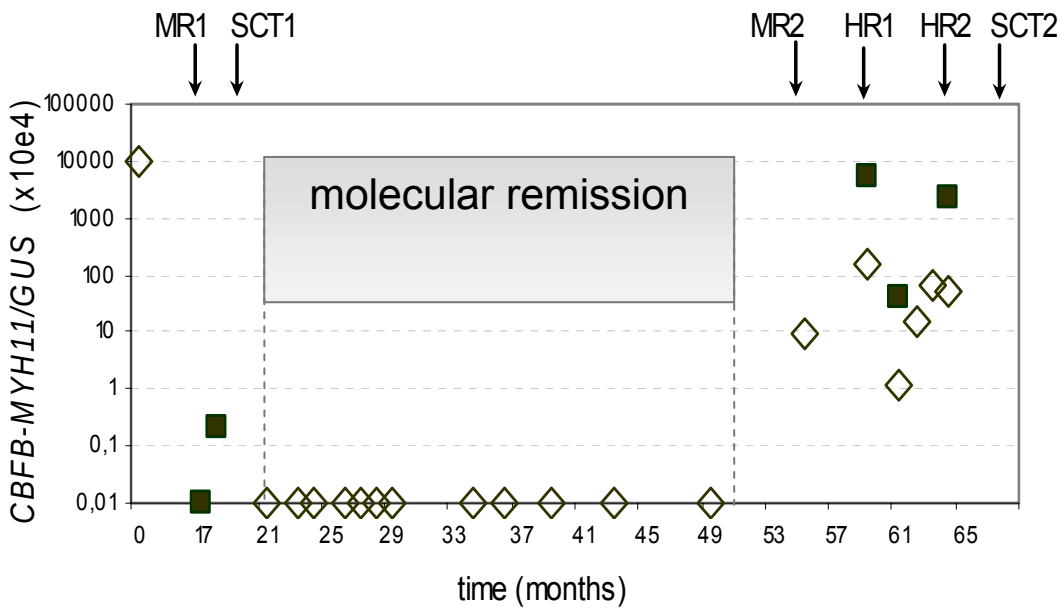
CD34
CD117
TdT
HLA-DR
CD13
CD33
MPO
CD10
CD19
CD20
CD24

Precursor B-ALL

Different phenotypes

Boeckx N. *et al.* Hematologica 2004; 89.

Karyotyping & Molecular analysis



DIAGNOSIS

46,XY,inv(16)(p13q22)

PCR: *CBFB-MYH11+*

PCR: no *IGH, IGK, TCRD, TCRG, TCRB* gene rearrangements

STR: DNA profile 100% receptor

RELAPSE

Karyotype not done

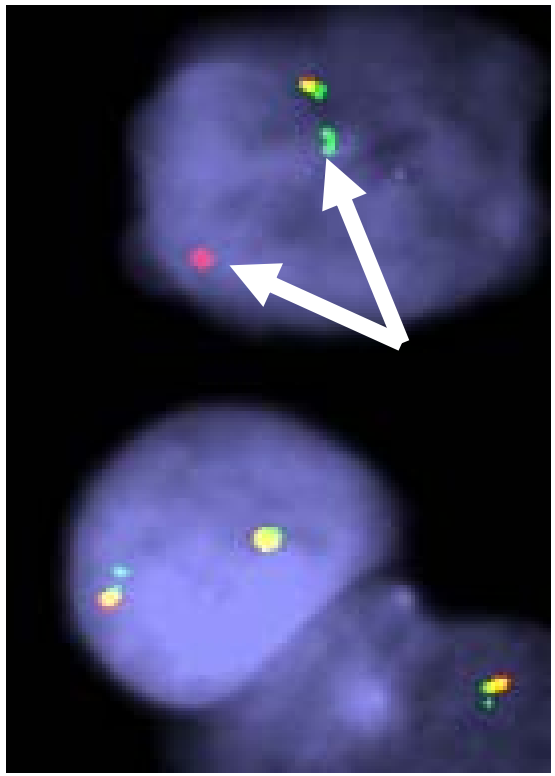
PCR: *CBFB-MYH11+*

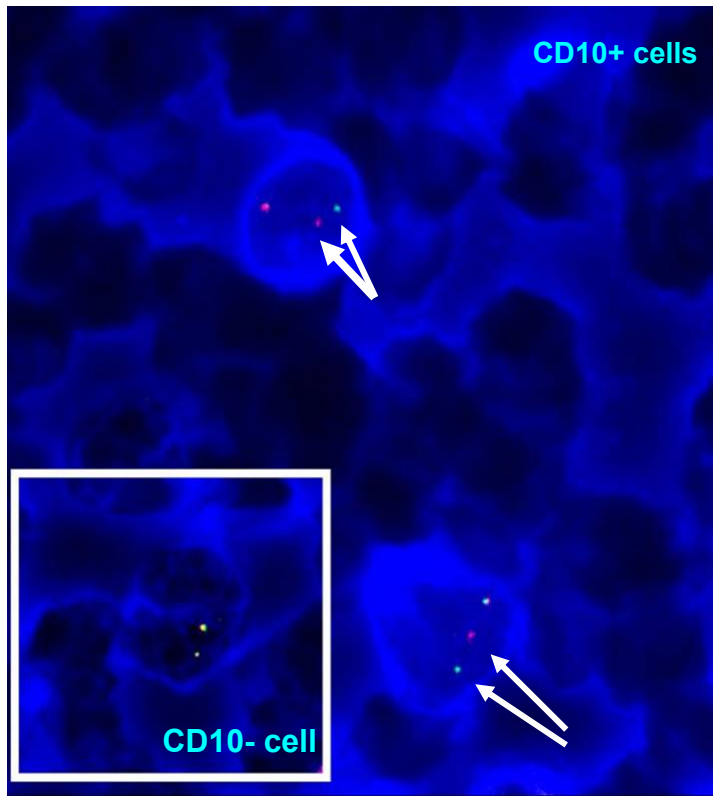
PCR: Bi-allelic incomplete *IGH* gene rearrangement

STR: DNA profile 100% receptor

Boeckx N. *et al.* Hematologica 2004; 89.

FISH & FICTION analysis





=> Clonal relationship between two phenotypically different malignancies in the same patient

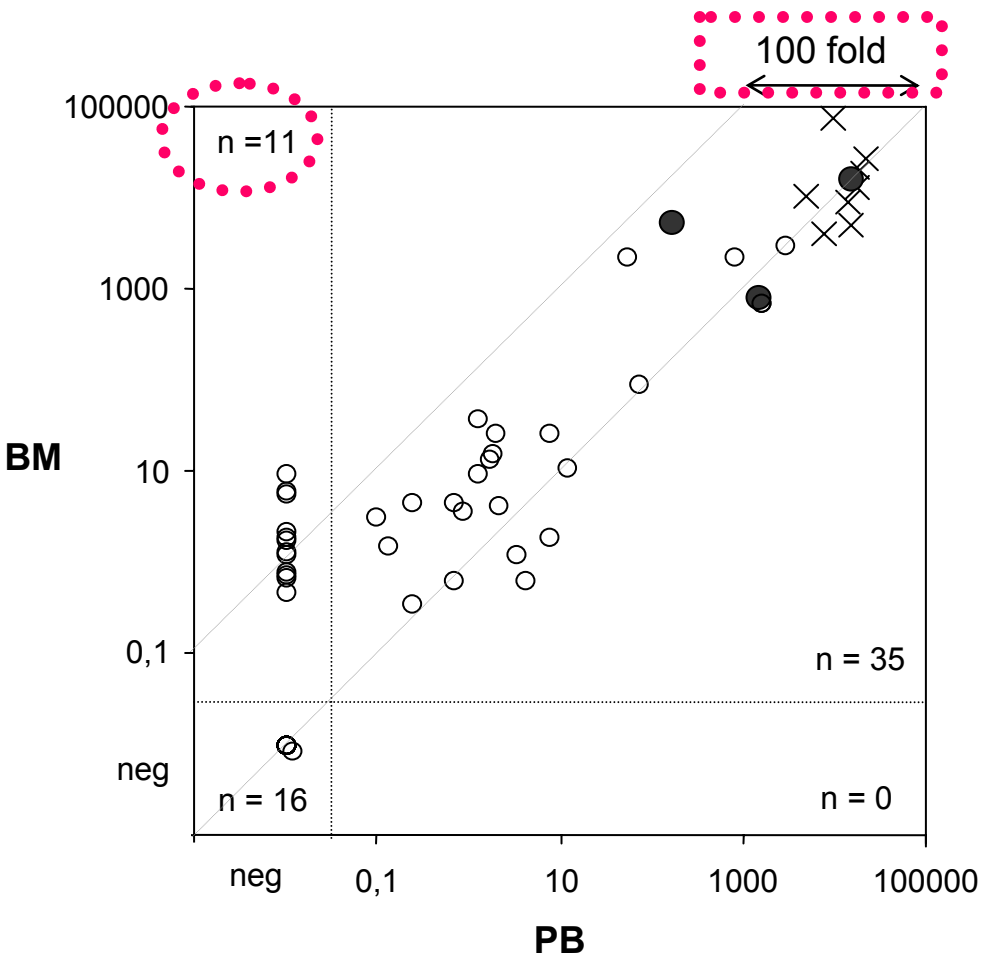
Boeckx N. *et al.* Hematologica 2004; 89.

BM or PB for MRD monitoring?

- **CML** => PB
- **AML**
  - t(8;21) with *AML1-ETO* transcripts: possibility of MRD monitoring through PB has been reported
  - t(15;17) with *PML-RARA* transcripts: paired BM-PB samples had comparable MRD levels
  - **inv(16) or t(16;16) with *CBFB-MYH11* transcripts?**

Correlation between BM and PB samples?

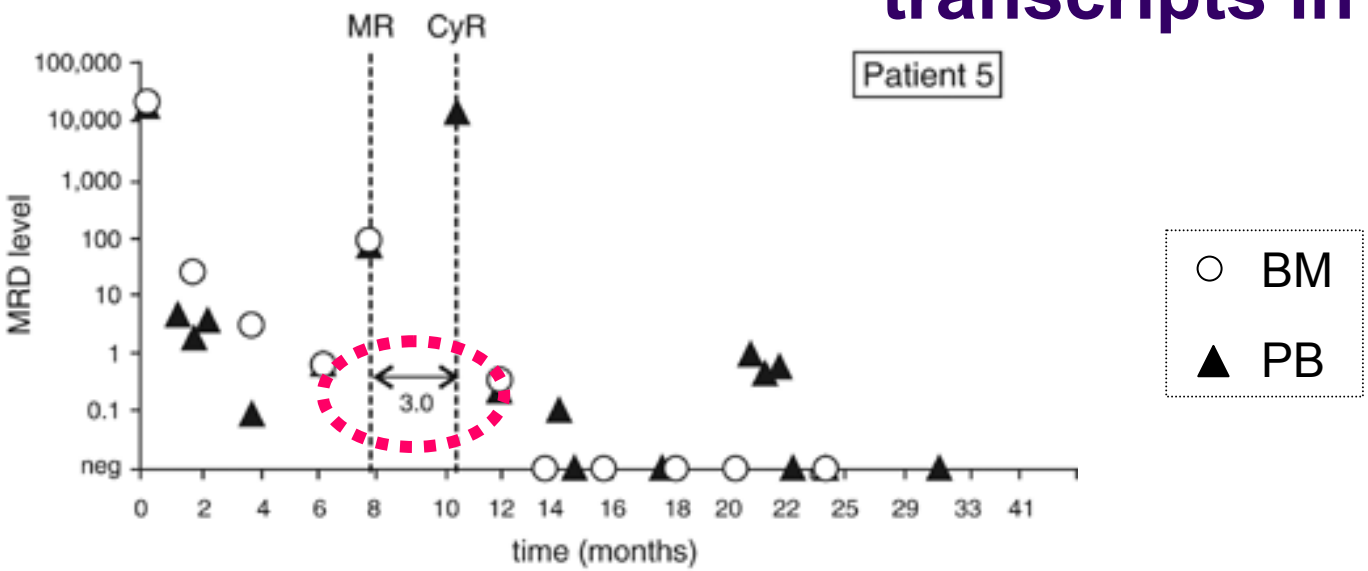
- ⇒ 10 patients (8-68 years) with AML-M4Eo
- ⇒ RQ-PCR for *CBFB-MYH11*+ (A and D)
- ⇒ 128 PB and 67 BM, 64 paired samples



No straight correlation (r_s = 0.82) between *CBFB-MYH11* MRD levels in PB and corresponding BM during follow-up.

Boeckx N. *et al.* Leukemia 2005; 19 : 1988

Clinical value of monitoring *CBFB-MYH11* transcripts in PB?



CBFB-MYH11 levels in PB predict disease re-occurrence 2.5 to 4 months prior to cytological BM relapse

Analysis of BM can predict the relapse in some patients up to 2 months earlier

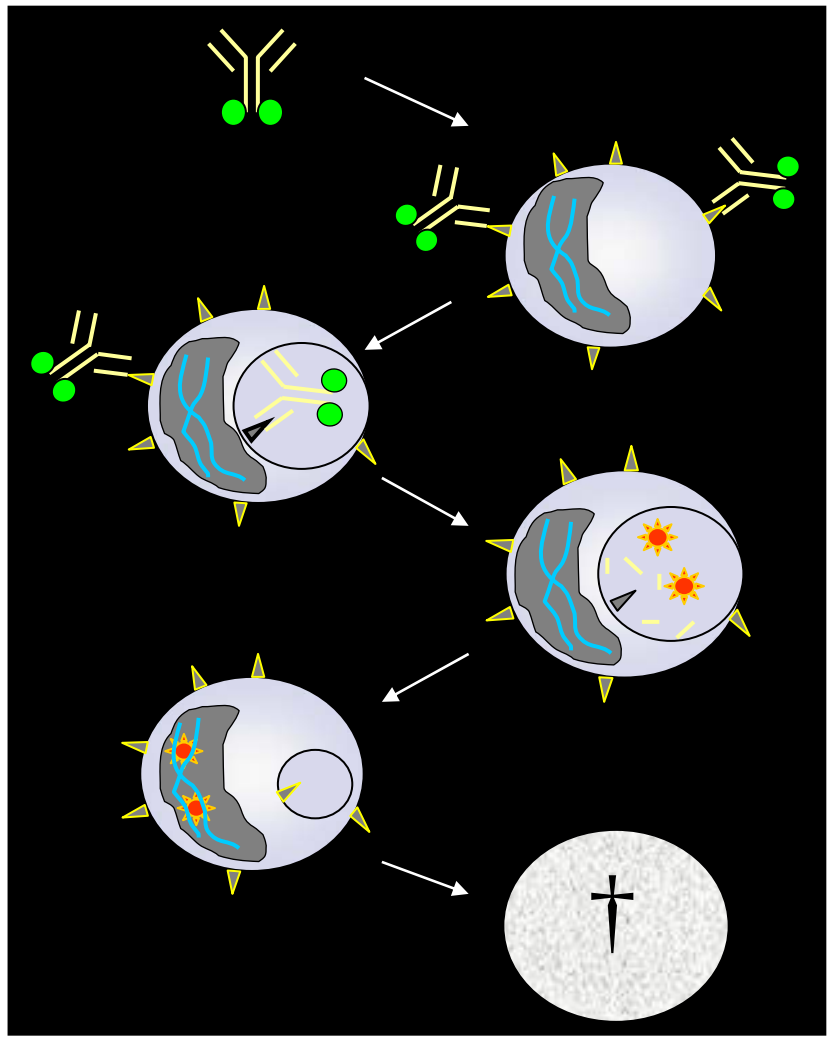
Suggested time interval between consecutive PB samples that allows an reliable early detection of molecular relapse: 2.5 to 3 months

?? Will treatment intervention at time of molecular relapse result in better clinical outcome than treatment intervention at the time of overt clinical relapse for patients with *CBFB-MYH11* transcripts

Boeckx N. *et al.* Leukemia 2005; 19 : 1988

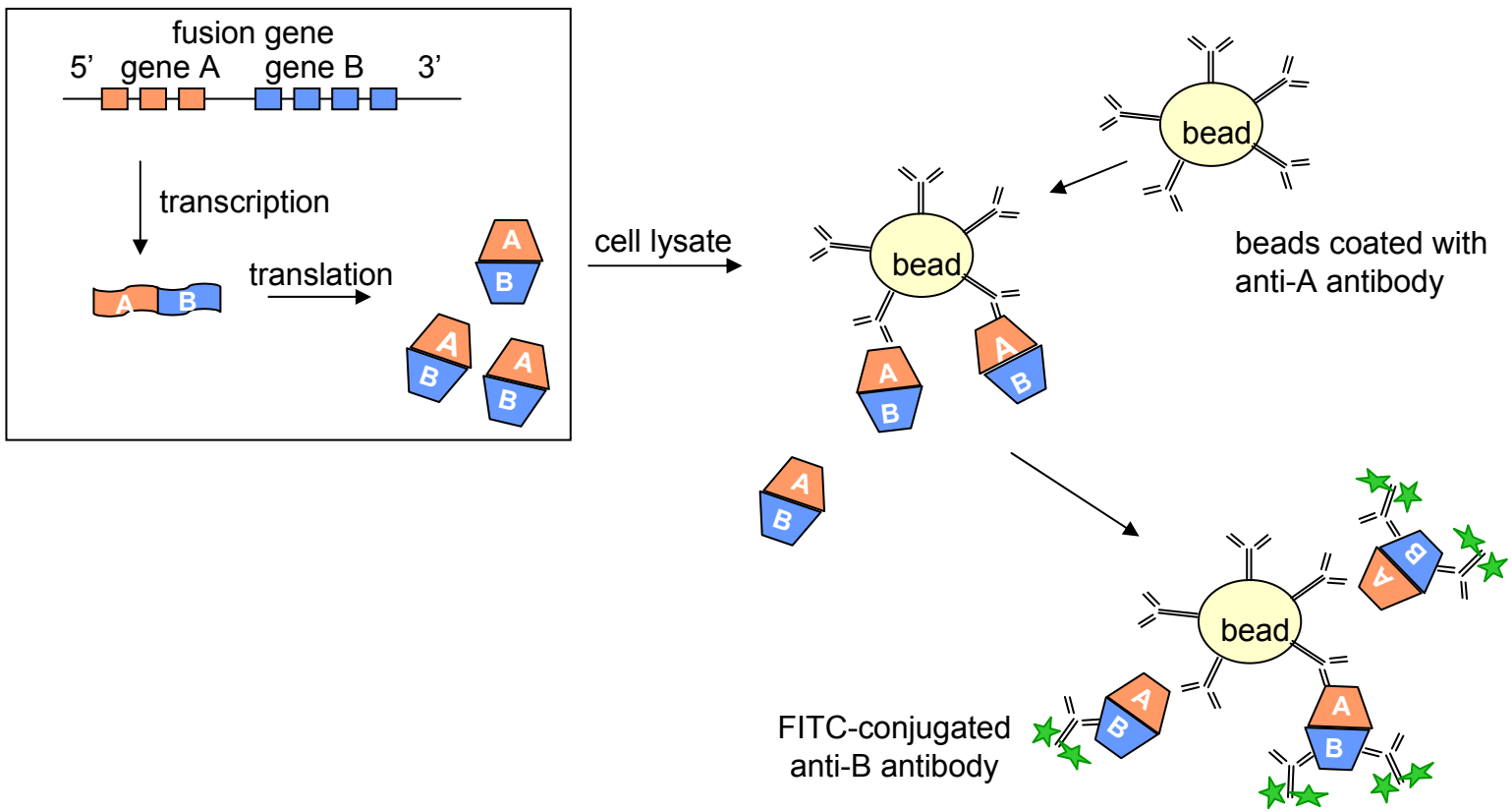
‘Specific’ (MRD) applications

- assessment of expression of certain antigens on leukemic blasts to guide therapeutic options (antibody targeted therapy)
  - CD33 (gemtuzumab ozogamicin, Mylotarg®)
  - Other potential targets: CD123 (IL-3 receptor α), CD44, CLL-1



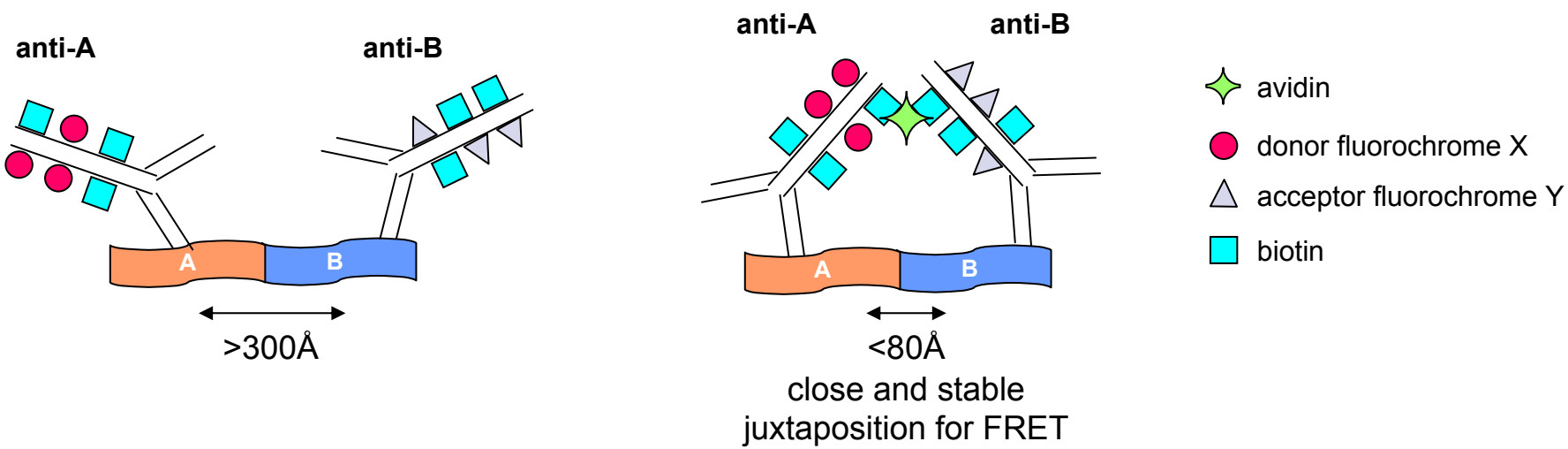
New developments

- Development of new immunobead-based assay for the detection of fusion proteins in cell lysates

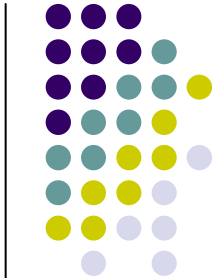


New developments

- Development of antibodies for FRET detection of intracellular fusion proteins



New developments

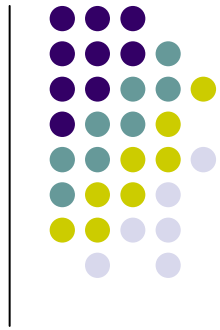


- Development of phospho-specific antibodies against particular intracellular phospho-epitopes on proteins
  - The phosphorylation state is indicative of the activation status and it correlates with its biological function
  - By measuring the phosphorylation status of the proteins by flow cytometry, one can determine which signaling cascades are used in responses to specific stimuli.

Potential applications

- analyze therapy efficacy and specificity of therapies both before and during clinical trials
- find possible leads for drug development and further research into the causes of particular diseases

HOVON 42A (amendment 5)



Patients ≤60 years of age

Cytarabine
Idarubicin
with / without G-CSF

Cytarabine
Amsacrine
with / without G-CSF

Collection of PBSC

- Mitoxantrone Etoposide
- Busulfan Cyclophosphamide + Autologous PBSC
- Busulfan Cyclophosphamide + Allogeneic PBSC

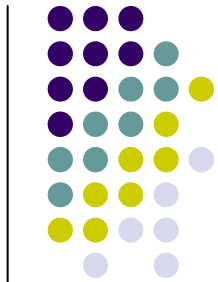
sampling for immunophenotyping

- at diagnosis → LAIP identification
- after 1st cycle (d30) → MRD?
- after 2nd cycle → MRD?
- auto-SC products → MRD?
- 3 months after 3rd cycle / Tx → MRD?
- at relapse → LAIP identification

prognostic value of flow cytometric MRD cell frequency in prospective multicenter clinical HOVON study

- Dutch/Belgian project group (5 institutions)
- Major goal
  - standardize procedures to establish leukemia-associated-phenotypes in newly diagnosed AML
  - standardize the detection and quantification of MRD in BM follow-up material

www.hematologie.nl/mrd/



Thank you for
your attention