Universitair Ziekenhuis Gent

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MRD as prognostic marker in CLL

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Therapeutic strategies in CLL

Table 3 | Treatment principles in CLL patients based on physical fitness, comorbidity and life expectancy

Physical fitness	Comorbidity	Life expectancy	Principle of action	Therapeutic goal and treatment options
Functionally independent	None or mild	Normal (reduced by malignant disease only)	'Go go'	Therapy aimed at prolonging survival, such as FCR
Reduced physical fitness	≥1 comorbidity	Intermediate (impaired by malignancy, comorbidity and/or unfit state of health)	'Slow go'	Therapy aimed at achieving optimal symptom control, such as monotherapies (chlorambucil or bendamustine) or dose-reduced combinations
Severely handicapped	Multiple or severe comorbidities	Short (severely reduced by comorbidities and/or frail state of health)	'No go'	Best supportive palliative care

Abbreviation: FCR, fludarabine, cyclophosphamide and rituximab.

Cramer & Hallek
Nat Rev Clin Oncol 2010

When to treat?

If CLL disease is <u>progressive</u>

CLL: therapeutic opportunities

No curative therapy

Alkylating agents, McAbies, Corticoids

However,

- *Except allogeneic BM transplantation (6 in UZ Gent) (long term remission)*
- *new therapeutics with the potential to reach complete molecular remission*
  - *Anti-CD20 mAb (Rituximab,Ofatumumab, …)*
  - *Anti-CD23 mAb (Lumiliximab)*
  - *Anti-CD40 (Dacetuzumab)*
  - *Anti-CD52 (Alemtuzumab)*
  - *Bcl-2 family of inhibitors (Oblimersen, Obatoclax)*
  - *Immunomodulating agents (Lenalidomide (anti-TNF, anti-angiogenic, anti-T_{act}, …))*
  - *Protein kinase inh (flavopiridol)*

Garcia-Escobar et al.
Crit Rev in Oncol/Hematol 2010

How to monitor CLL?

- Without progression:
  - 'Watch and wait' → cell count is sufficient
- In 'slow go' → symptom control
- If CR/molRem is attempted → MRD analyses
  - Quantitative monitoring with RQ-PCR
  - Quantitative monitoring with flow cytometry

What is the ideal MRD assay?

-CLL-specific

-High sensitivity, also in the presence of normal B cells

-Simplicity

-Possibility for quantification

Molecular methods used for follow-up in CLL

Consensus PCR

Consensus primers against the framework regions and the J_H region

drawbacks: limited sensitivity (1%)
no quantification

Clone-specific PCR (nested)

1e step consensus IgH PCR

2e step with 1 or 2 allele-specific primers (CDR2 and CDR3)

advantage: high sensitivity (10^{-6})

drawback: need for individual sequencing
no quantification

Clone-specific real-time quantitative PCR (RQ-PCR)

Combination of patient-specific primers (CDR2 en CDR3)
and a consensus probe (FR3)

advantage quantification

drawback labor intensive (sequencing, primer-probe test)
decreased sensitivity (10^{-4})

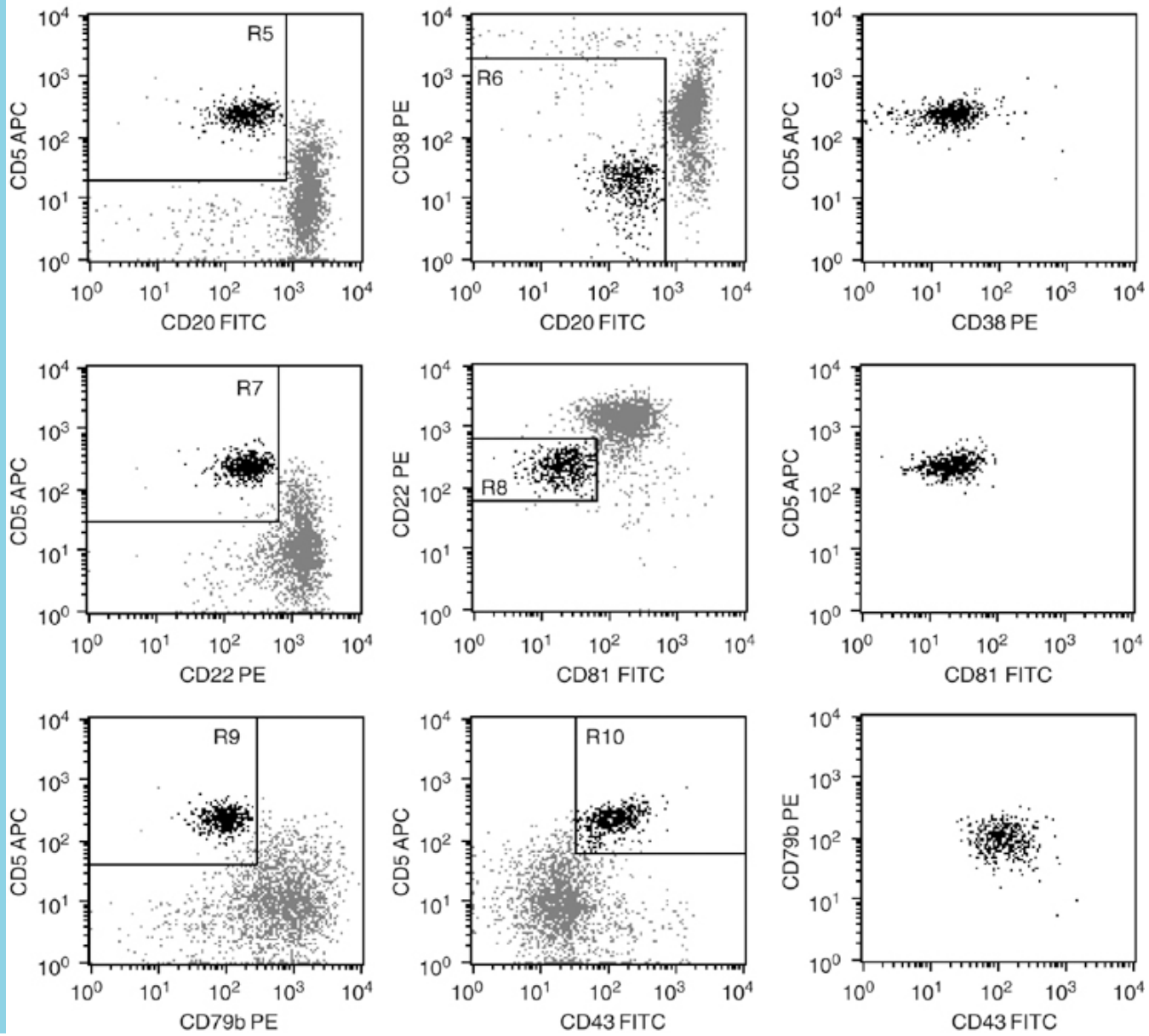
International standardized approach for FC residual disease monitoring in CLL *(A. Rawstron et al. Leukemia 2007)*

- **50 CLL specific antibody combinations were tested for 4-color FC**

- **Protocol:**
  - Start with 10^6 cells, use whole blood or BM
  - NH_4Cl lysis (10') followed by 2 washings
  - Staining
  - FACSLyse (according to the manufacturer's protocol)
  - Analysis performed on CD19+ gated B cells

International standardized approach for FC Residual disease monitoring in CLL *(A. Rawstron et al. Leukemia 2007)*

- **3 combinations turned out to have low inter-laboratory variation and false detection rates**
  - FITC / PE / PerCP / APC
  - CD20 / CD38 / CD19 / CD5
  - CD81 / CD22 / CD19 / CD5
  - CD43 / CD79b/ CD19 / CD5



Rawstron et al.
Leukemia 2007

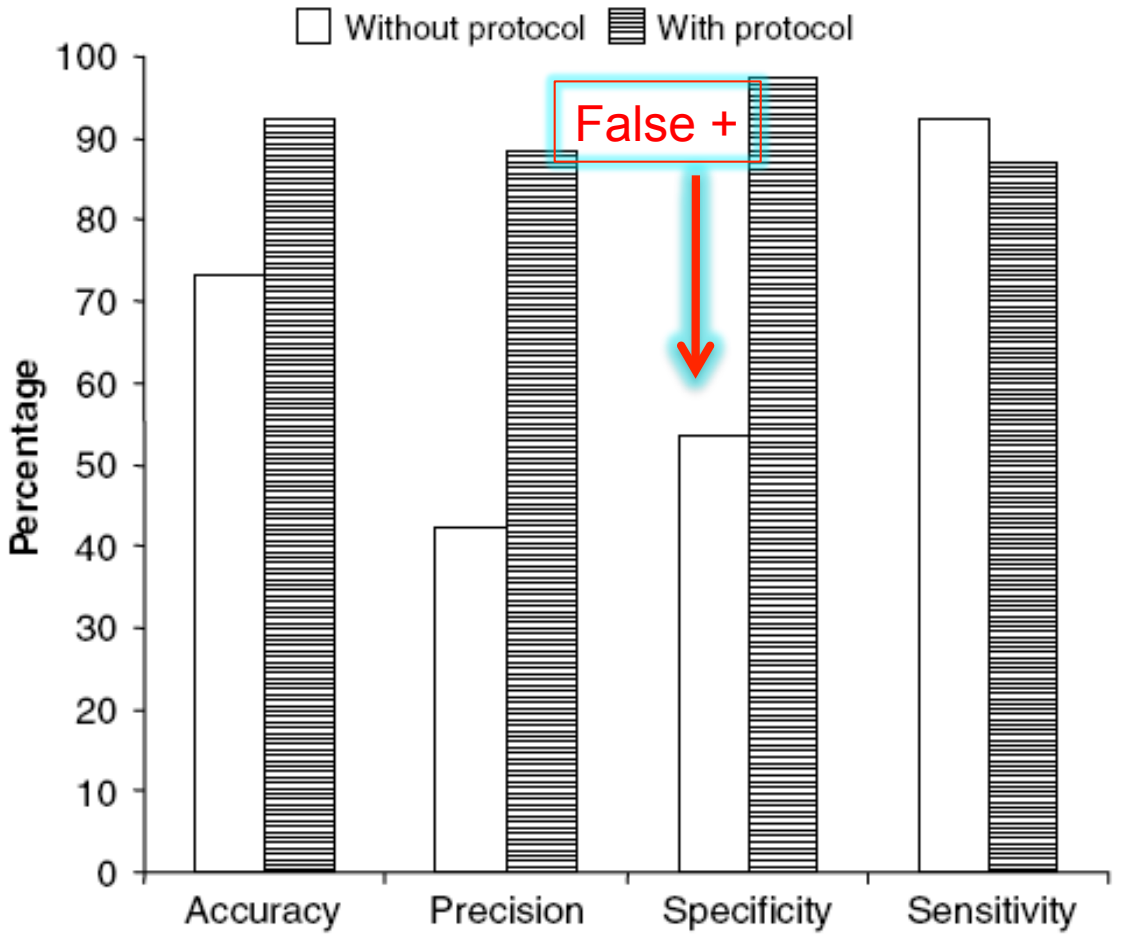


Figure 3 Use of a consensus protocol improves precision, consistency and specificity for detection of MRD by operators who are experienced in flow cytometric analysis of CLL.

Rawstron et al. Leukemia 2007

Standardized MRD flow and ASO IgH RQ-PCR for MRD quantification in CLL (*S Böttcher et al, Leukemia 2009*)

- **Protocol based upon previous papers**
  - 200 µL of whole blood ($2x10^6$ cells) are washed
  - Incubation with rabbit serum
  - Stained with 4 colors
  - + FACSLyse & 2 washings
  - Use of isotype controls

- FITC / PE / PerCP / APC
- CD20 / CD5 / CD19 / CD43
- CD81 / CD22 / CD19 / CD5
- CD79b / CD20 / CD19 / CD5
- K / λ / CD19 / CD5

Standardized MRD flow and ASO IgH RQ-PCR for MRD quantification in CLL (*S Böttcher et al, Leukemia 2009*)

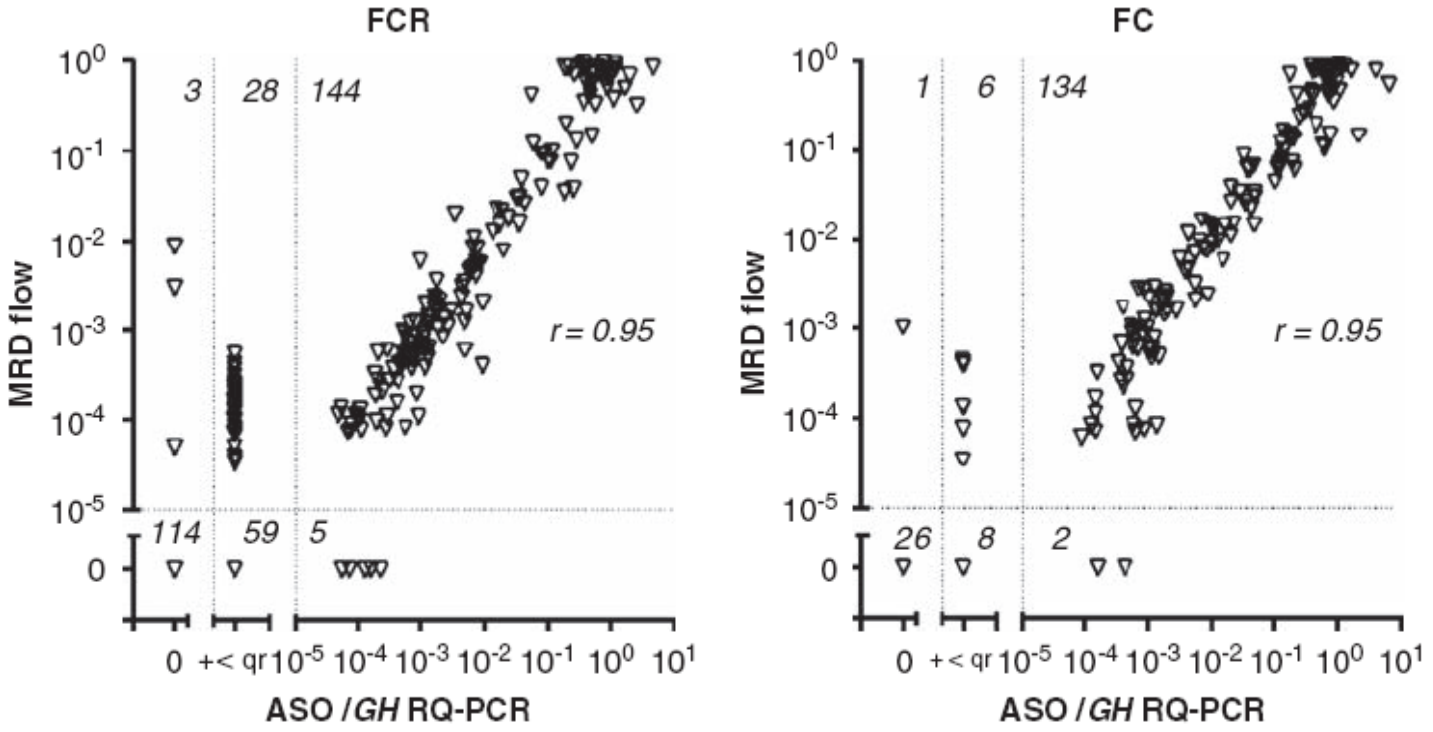


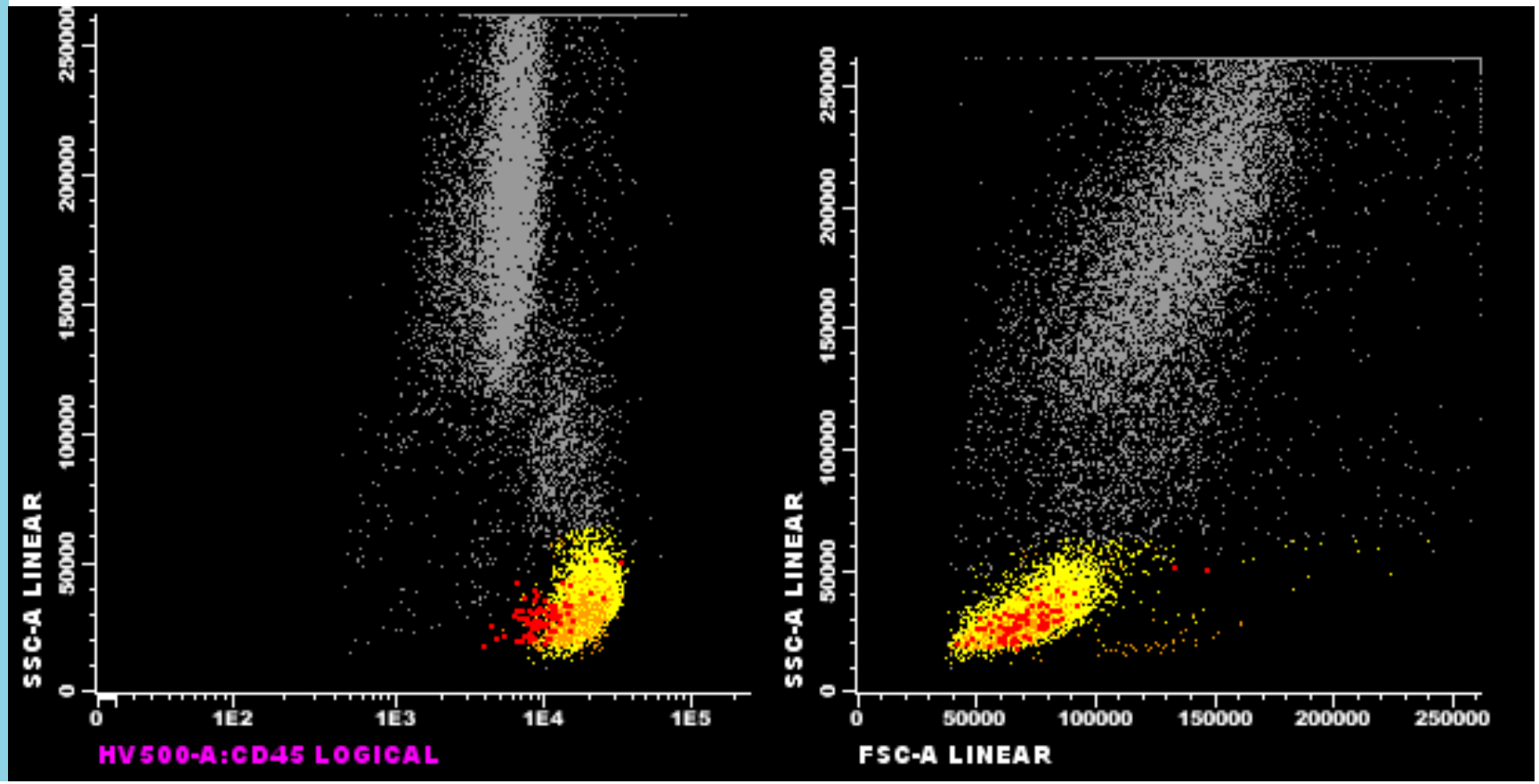
Figure 1 Correlation of minimal residual disease (MRD) results obtained by MRD flow and by allele-specific oligonucleotide primer *IGH* real-time quantitative (RQ)-PCR (ASO *IGH* RQ-PCR) according to treatment arm ($^{+}$ < qr: positive, outside quantitative range). The numbers in the graphs give sample numbers according to MRD flow and RQ-PCR results. Concordantly, positive samples show a significant quantitative correlation in both treatment arms: $r=0.95$, $P<0.0001$ for each treatment arm.

ESCCA 2010: S. Böttcher (Kiel)

- Euroflow based approach

FITC	PE	PerCP Cy5.5	PE-Cy7	APC	APCH7	V450	V500
IgM	CD5	CD79b	CD19	CD200	---------	CD20	CD45

Follow-up sample from a patient, 1 year post allotransplant



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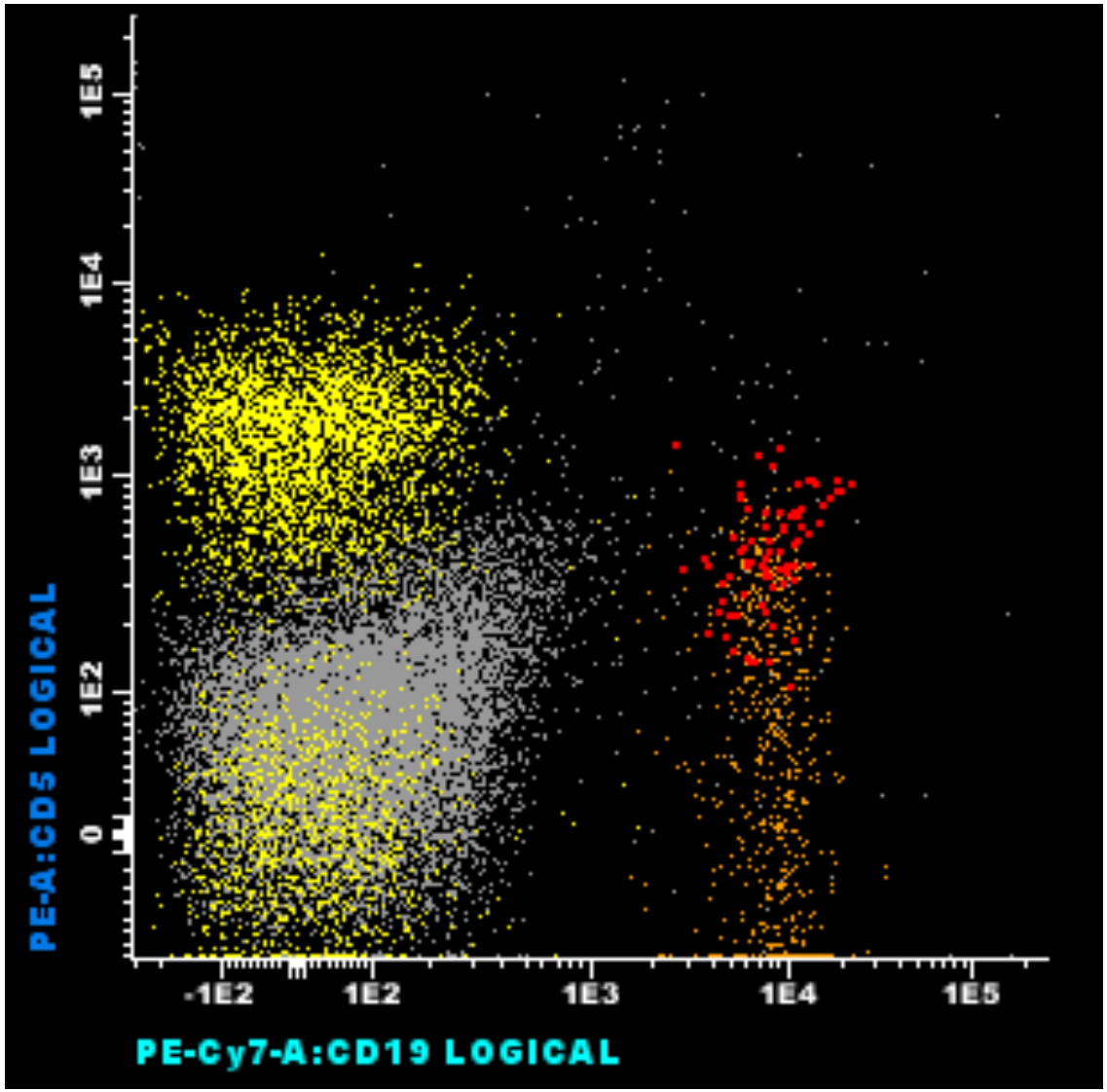

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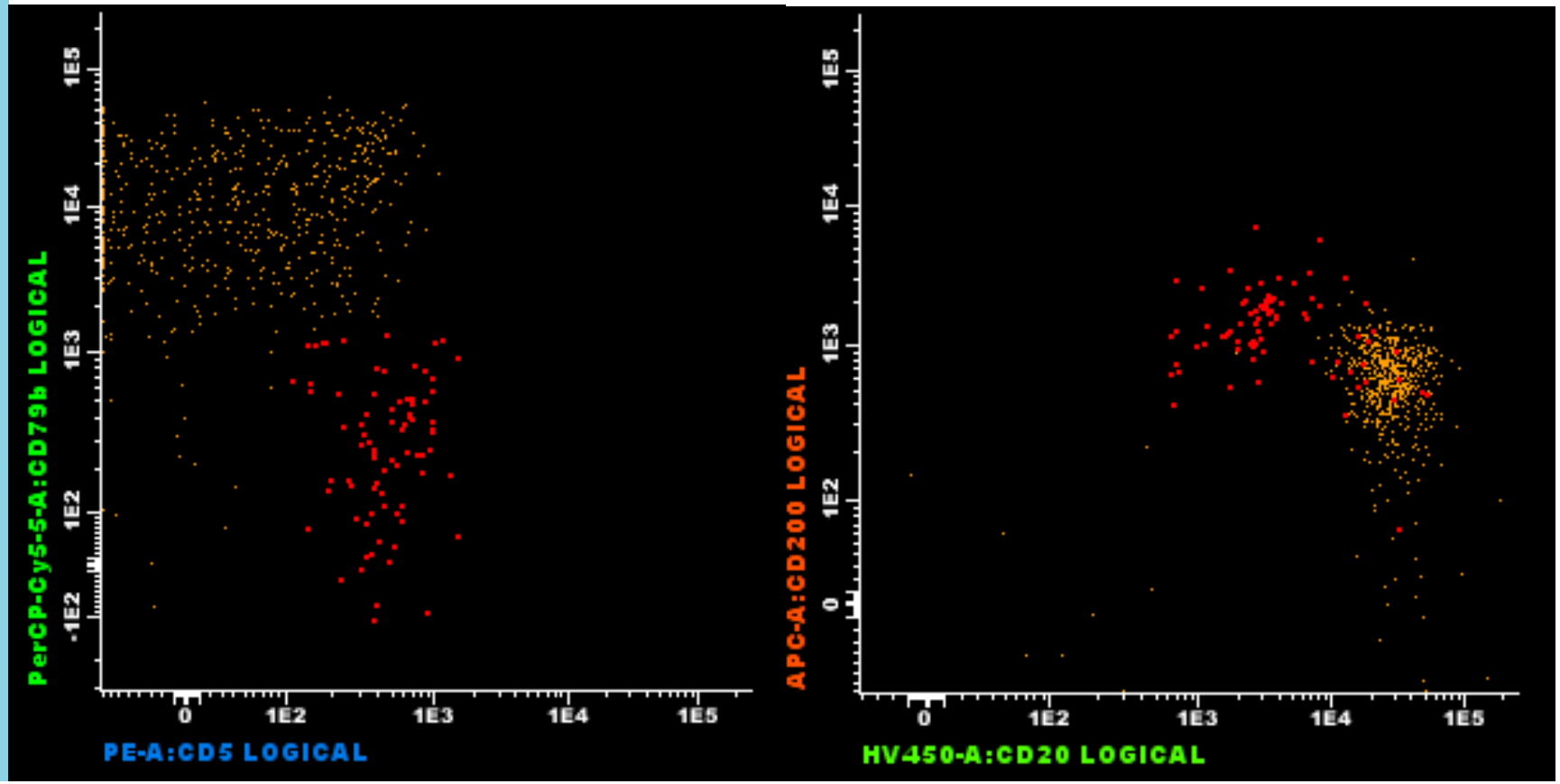
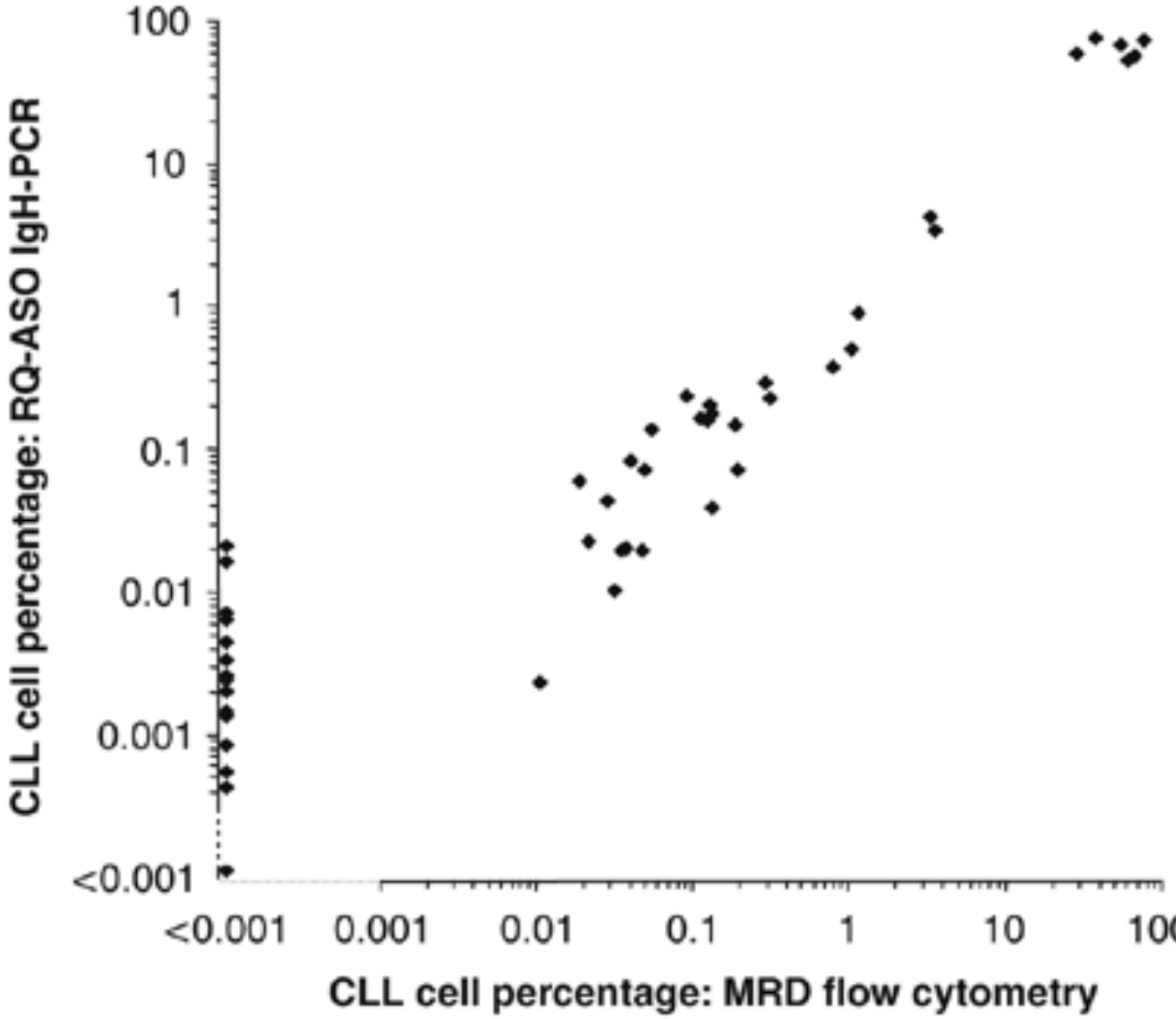
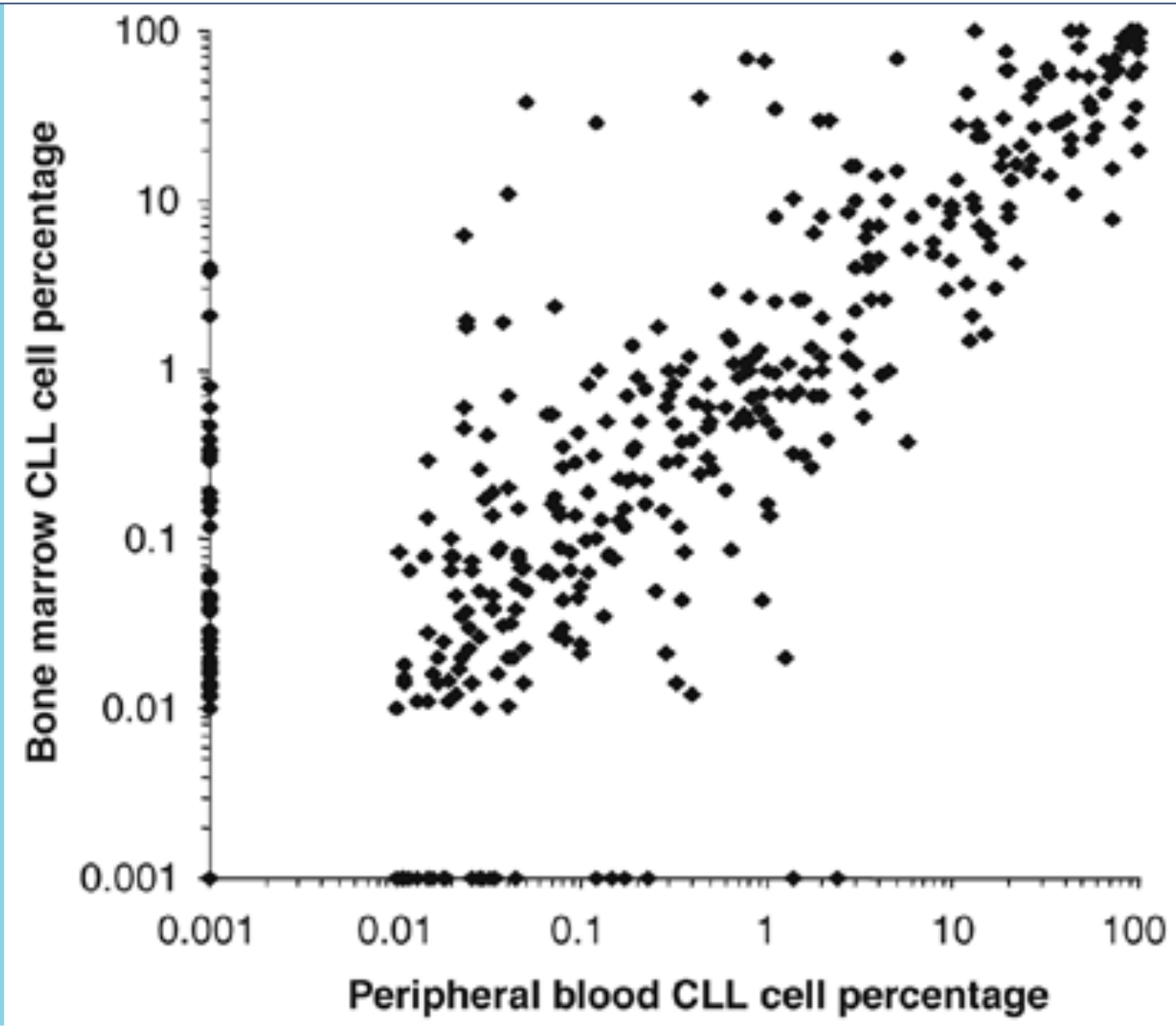


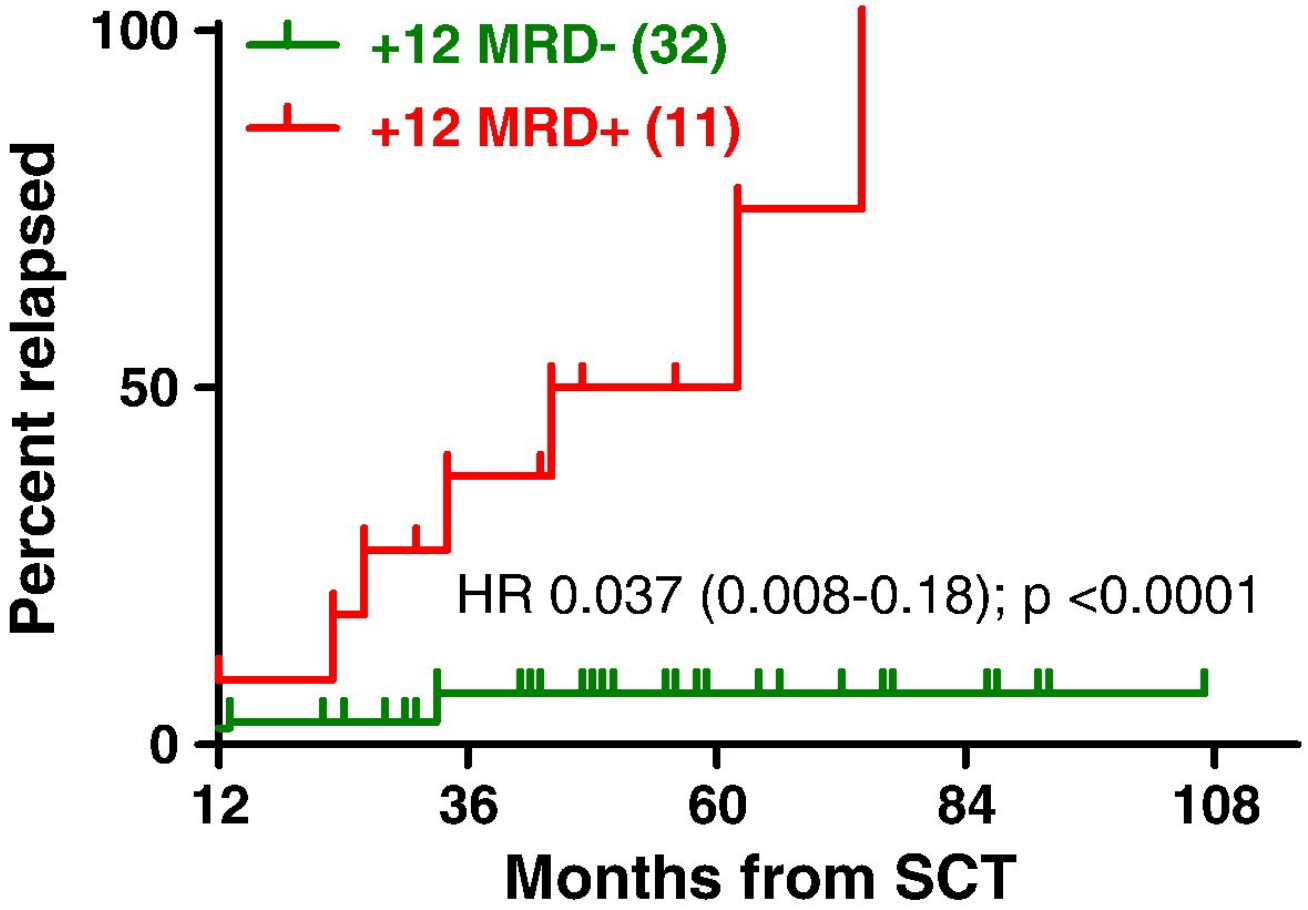


Boettcher et al
Blood Rev 2011



Boettcher et al
Blood Rev 2011

Impact of MRD status 12 months after allotransplant
N=43, event-free after12 m



Boettcher et al
Blood Rev 2011

Thank you

Questions?