

ERIC

**2nd Annual Symposium
of the
European LeukemiaNet
&
7th Meeting of ERIC = WP7**

Achieved deliverables for the EU funding period month 1 – 12:

- 7.2. Web-based information and communication services on CLL available
- 7.3. Creation of WP management structure with lead participant and steering committee
- 7.4. Creation of European CLL platform

Prolonged deliverables for the EU funding period month 12 – 30:

- 7.1. Establishment of WP information and communication structures
- 7.5. Regular WP meetings
- 7.6. LP report to NMC (regarding structure, trial activities and integration of national leukemia trial groups).
- 7.7. First proposal of definitions and standardization of relevant diagnostic and therapeutic procedures
- 7.8. Treatment of early high risk CLL with FCR
- 7.9. Exchange of study protocols of open clinical trials, information on structure and trial activity of national CLL trial groups

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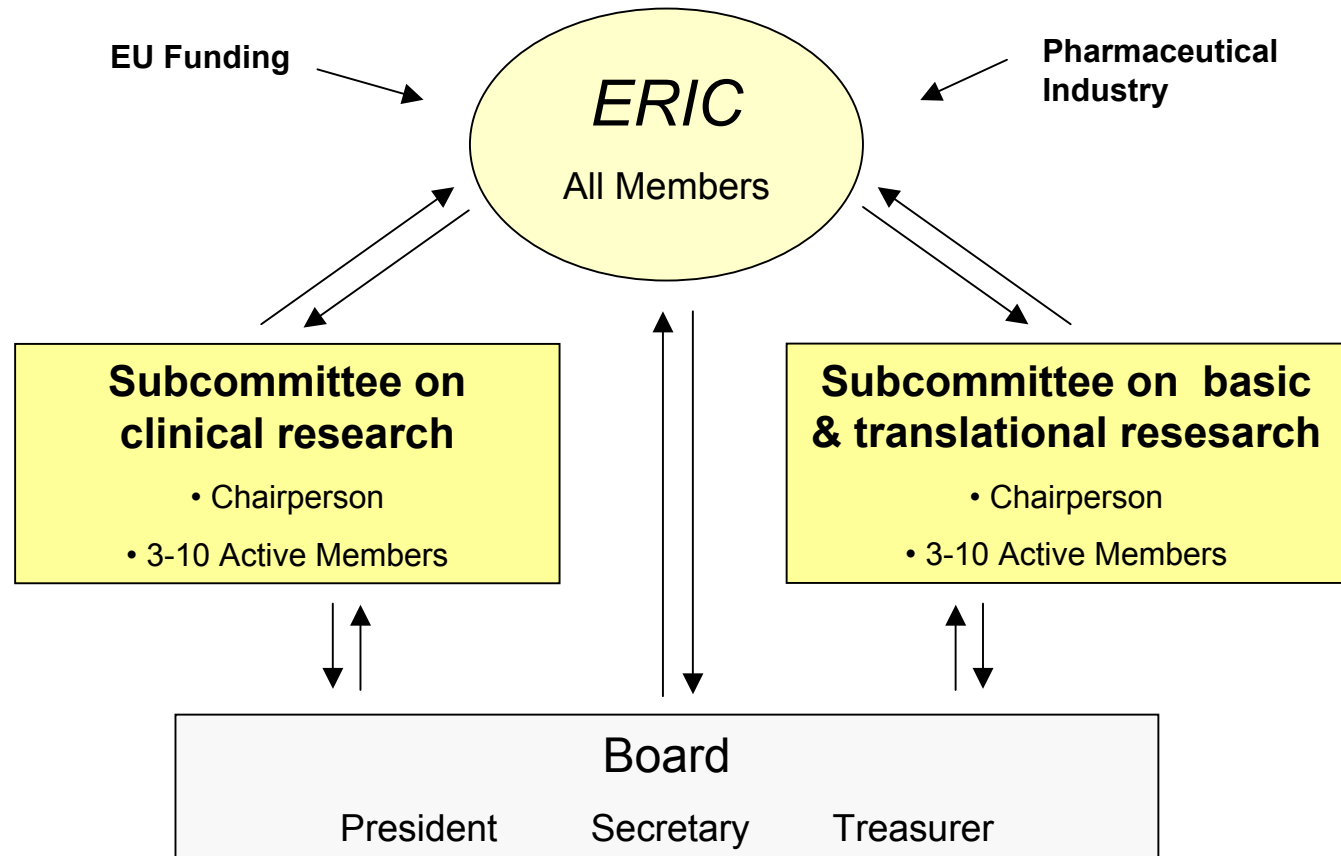
- ◆ History
- ◆ **Structure**
- ◆ Members
- ◆ Projects
- ◆ Meetings
- ◆ Contact
- ◆ Links
- ◆ Imprint

European Research Initiative on CLL

European LeukemiaNet

European CLL Study Groups

Newsletter



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EU CLL Research Consortium (ERCC)



Harmonization of diagnostic parameters



**Apply and standardize
molecular and advanced genomic technology
to analyze**

- biological and genetic prognostic factors,
- MRD,
- resistance to therapy and
- progressive disease

across Europe.

Harmonization Committee Meeting Report – ASH 2004

- **Tasks already accomplished:**
VH-mutation analysis
MRD-detection
- **Urgent future tasks:**
ZAP-70 FACS analysis
Cytogenetics & FISH
- **Tasks discussed:**
Diagnostic criteria for CLL
Collection of rare cases (familial CLL, p53-)
Target definition by expression profiling and proteomics

Harmonization Committee Overlap with Methodology Platforms

- **WP 10** Diagnostics
- **WP 11** Cytogenetics
- **WP 12** MRD
- **WP 13** Gene profiling



WP 7 CLL: Should cover specific aspects and needs.

WP7/ERCC: Visions

- **Harmonisation**
- **Translational Research**

- **Initiate basic science committee**
- **EU funding, other sources**
- **Infrastructure: e.g. cell banking**

**IgVH Gene Mutation Status and
Molecular Cytogenetic (FISH)
Analysis in CLL**

- Standardization -

Proposed Standardization of IgVH Mutation Analysis

- **Source Material:** genomic DNA / RNA (cDNA)
 - RNA mandatory if double Rearrangements etc.
- **Primer Sites:** Leader Region / Framework Region
 - LR should be attempted, FR only if LR Failure
- **Identification of clonal Rearrangement**
 - Gene Scan, SSCP, Agarose Gel,
- **Sequencing**
 - Direct Sequencing, if Failure: Cloning
- **Alignment:** Numerous online Databases available
 - V-BASE, GenBank (IgBlast), IMGT

Open Questions of IgVH Mutation Analysis

- Cut-off level for best prognostic discrimination
 - 98%, 97%, 95%,
- Prognostic Impact of specific IgVH Genes
 - V3-21, V3-23, V1-69,
- Pathogenic Role of specific Rearrangements
 - CDR3 homology, Antigenic Selection,
- Surrogate Markers
 - CD38, ZAP-70, LPL,
-

Proposed Standardization of Molecular Cytogenetic Analysis (FISH)

- **Source Material:** Direct Harvest, short-term Cultures
 - Direct Harvest more representative
- **Probe Set** (minimal required)
 - Del 13q14 (D13S272, D13S319, other); not: RB1, D13S25, ...
 - Del 11q23 (ATM, FDX1), not: CCND1, MLL, ...
 - Trisomy 12q13 (GLI), or 12 centromere
 - Del 6q21 (?), not: 6q27
 - t(11;14), if t(14q32) but not t(11;14): others (18q21, 19q13, ...)
- **Cut-off levels**
 - No Standards, must be determined on normal Controls
 - Attention: very low (i.e. 5%) cut-offs dangerous

Open Questions of Molecular Cytogenetic Analysis (FISH)

- **Source Material**
 - Representative Sample, Cell Number, (early Stage Cases)?
- **Probe Set**
 - Localization in critical Regions, Availability?
- **Cut-off levels**
 - Critical for accurate Diagnosis!
- **Cytogenetic Subclones**
 - Detection, prognostic Impact?
- **Cytogenetic Analysis (Banding) after CD40 Stimulation**
 - Evaluation in Trials
-

Standardization of IgVH Mutation Status and Molecular Cytogenetic (FISH) Analyses in CLL

- Florence Cymbalista
- Freda Stevenson
- Christian Geisler
- Richard Rosenquist
- Matthias Ritgen
- Stephan Stilgenbauer

Minimal Residual Disease (MRD)

Analysis in CLL

- Standardization -

Combinations tested for 4-Colour CLL analysis

- CD10/CD24/CD43, CD10/CD5/CD43, CD10/integrin β 7/CD43, CD10/integrin β 7/CD5, CD11a/integrin β 7/CD5, **CD20/CD38/CD5**, **CD20/CD43/CD5**, **CD20/integrin β 7/CD5**, CD21/CD48/CD43, CD21/CD48/CD5, CD21/CXCR5/CD5, CD21/integrin β 7/CD5, CD24/CCR6/CD43, CD24/CD27/CD38, CD24/CD40/CD5, CD24/CD48/CD43, CD24/CD48/CD5, **CD24/CXCR5/CD43**, CD24/CXCR5/CD5, CD24/integrin β 7/CD43, CD24/integrin β 7/CD5, CD31/CXCR5/CD5, CD37/CCR6/CD43, CD37/CD5/CD43, **CD37/CD79b/CD43**, CD37/CXCR5/CD5, CD37/integrin β 7/CD43, CD40/CD48/CD43, CD40/CD48/CD5, CD40/CXCR5/CD5, CD40/integrin β 7/CD5, **CD43/CD23/CD5**, **CD43/CD81/CD38**, CD43/CD81/CD5, CD44/integrin β 7/CD43, CD44/integrin β 7/CD5, CD48/CXCR5/CD5, CD48/integrin β 7/CD5, CD48/LAIR-1/CD5, CD48/MPC-1/CD5, CD5/CCR6/CD43, CD5/CXCR5/CD43, CD70/integrin β 7/CD5, CD79b/CD21/CD43, CD79b/CD24/CD43, CD79b/CD38/CD43, CD79b/CD39/CD43, CD79b/CD40/CD43, CD79b/CD48/CD43, **CD79b/CD5/CD43**, CD79b/CD81/CD43, CD79b/CXCR5/CD43, CD79b/CXCR5/CD5, CD79b/integrin β 7/CD5, **CD81/CD22/CD5**

Current test status

| | | | |
|-------|--------|------|-----|
| CD45 | CD56 | CD19 | CD3 |
| Kappa | Lambda | CD19 | CD5 |

Sufficient if only monoclonal B-cells are present

| | | | |
|-------|------|------|-----|
| CD20 | CD38 | CD19 | CD5 |
| CD81 | CD22 | CD19 | CD5 |
| CD79b | CD43 | CD19 | CD5 |

Required if polyclonal B-cells are present
Acquire >5,000 B-cells and up to 500,000
leucocytes

Comparison of approaches

| | MRD Flow | RQ ASO-PCR |
|---------------------------------|-------------|---------------------------------|
| Applicable patients | >95% | 85 - 95% |
| Sensitivity | 0.01% | 0.001% |
| Quantitative range | 0.1 – 0.01% | 0.01% |
| Cost & complexity | Moderate | Initially high Follow-up low |
| Pre-treatment material required | Preferable | Essential |
| Turn-round time | hours | week |

Aims

- To develop standardised methods for MRD analysis by flow cytometry and ASO-PCR that are
 - Broadly applicable
 - Defined specificity and sensitivity
 - Standardised reporting convention (“MRD negativity”, “molecular remission”)
- Provide a tool for guiding assertive therapy and a benchmark for assessing response to allow comparison of efficacy between clinical trials.

Open Questions?

- minimum events to call CLL (20-50)?
- Lyzing reagents
- Clones
- comparatison of flow and PCR
- Sensitivity level in flow samples

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**Evaluation of ZAP-70 expression
by
Flow Cytometry in B-CLL**

- Standardization -

Evaluation of ZAP-70 expression by Flow Cytometry in B-CLL

Standardization effort in a French multicentric protocol

Pitié-Salpêtrière, Paris

Hôpital l'Archet, Nice

Avicenne, Bobigny

**Magali Le Garff-Tavernier
Hélène Merle-Béral**

**Michel Ticchioni
Alain Bernard**

**Rémi Letestu
Florence Cymbalista**

National Coordinator : Prognostic factors in Binet Stage A B-CLL

Programme de Soutien aux Innovations Diagnostiques et Thérapeutiques Coûteuses 2003

Sophie Raynaud

Conclusion

- The expression of the result as **T-Ly / CLL** ratio might be helpful besides percentages, particularly in multicentric studies.
- The study of a **control cohort** is valuable in defining normal and pathological threshold of ZAP-70 expression.
- Finally multifuorescent labeling and **normal residual B cells** based expression of the result could help when T-Ly/CLL ratio fall into the inconclusive zone.

Harmonization meeting, March 15th

- **Communication on the method and results obtained in the different groups.**
- **Considering Harmonization of the technique :**
 - **Is it possible to reach a technical consensus ?**
 - **Antibodies / Fluorochromes**
 - **Expression of results : T or B cells**
 - **Definition of the positivity threshold**
 - **Exchange of samples, Quality Control**
 - **Other topics ...**
- **European Database of ZAP-70 expressing cases**
 - **Correlation with other prognostic factors**
 - **Constitution of a cohort of patients with non concordant prognostic factors : ZAP-70 Positive with mutated IgVH genes**
- **Suggestions are welcome.**

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HOVON68 CLL:

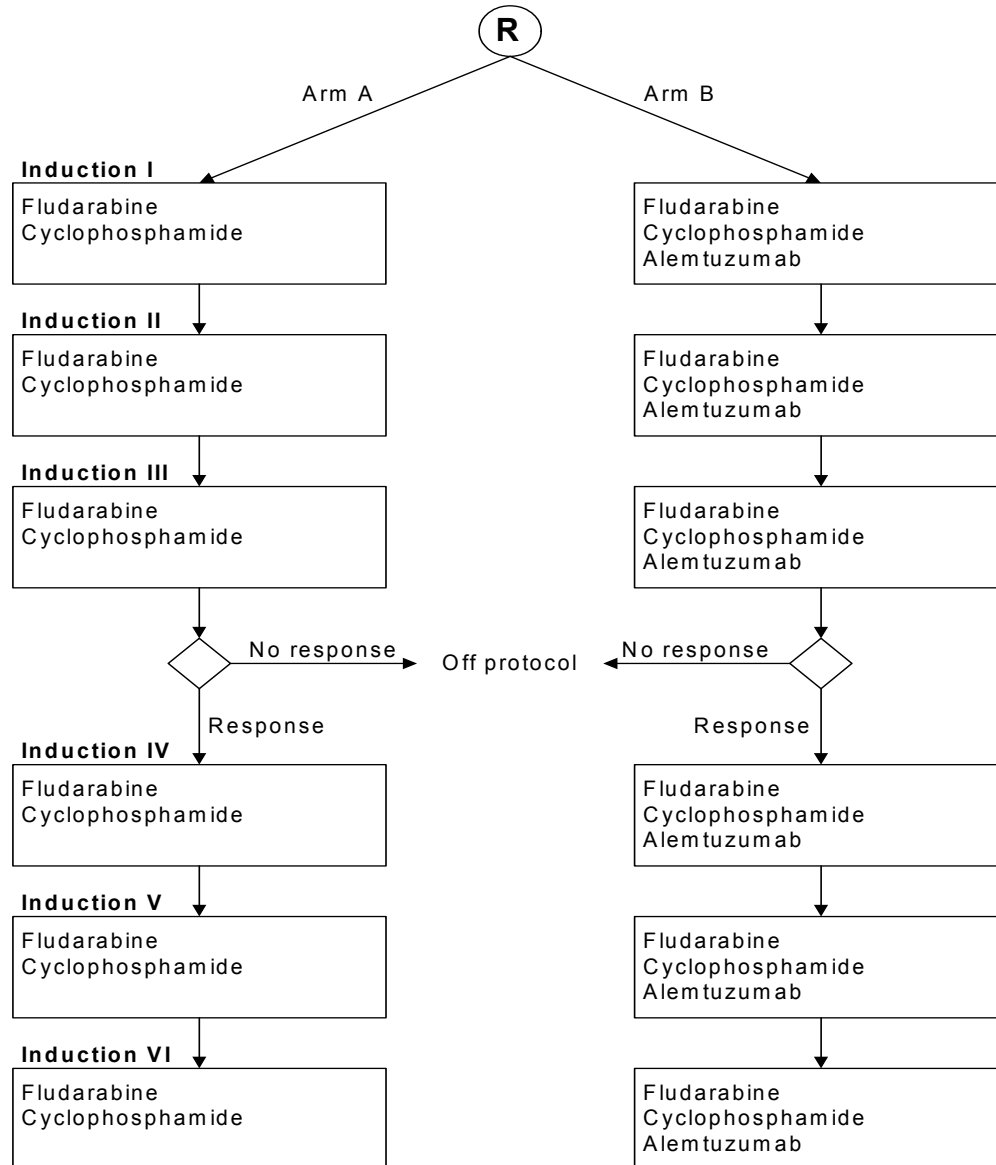
**A Nordic-Dutch-Polish
Randomised phase-III study in
Previously untreated patients
with biological high-risk CLL
who need treatment:**

**Fludarabine + Cyclophosphamide (FC)
vs FC + low-dose SC Alemtuzumab**

Supported by an unrestricted grant
from Schering Corp.

HOVON68: CLL SCHEME OF STUDY

Biological high-risk CLL
Age 18-70 years inclusive



HOVON68 CLL

Study coordinators:

- **Christian Geisler, Rigshospitalet, Copenhagen, Denmark**
- **Marinus van Oers, Academisch Medisch Centrum, Amsterdam, The Netherlands**

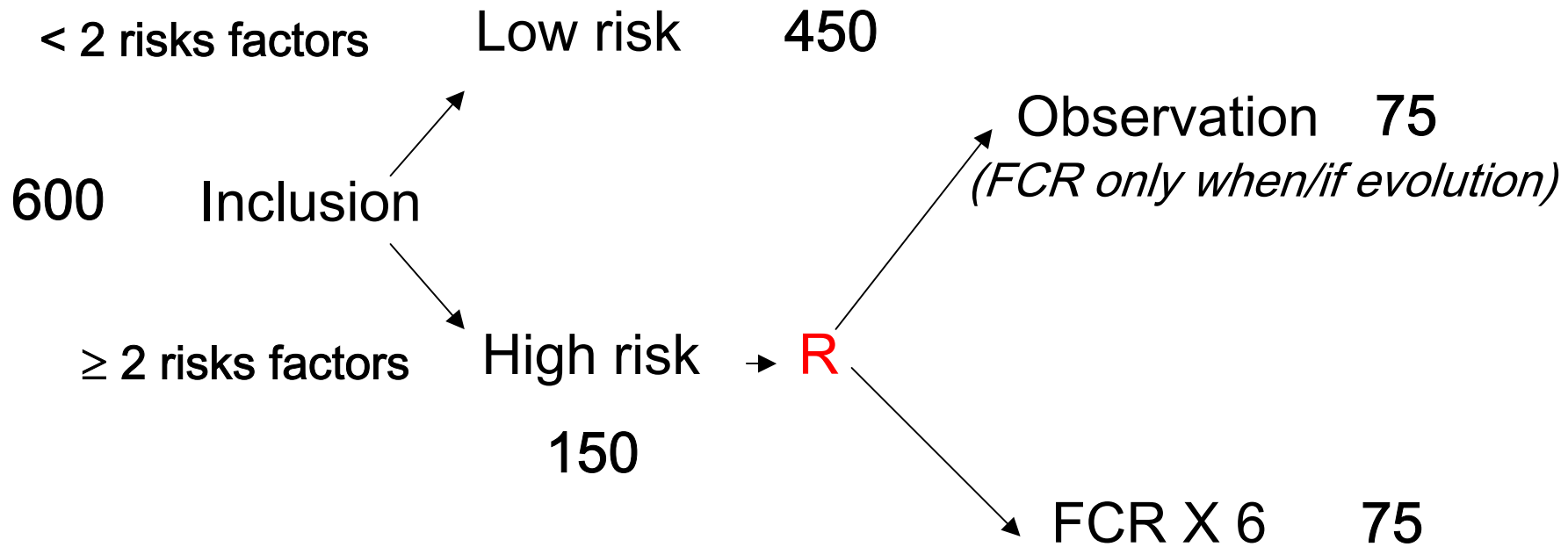
Writing Committee, additional members:

- **Maija Itälä, Turku University Hospital, Turku, Finland**
- **Jesper Jurlander, Rigshospitalet, Copenhagen, Denmark**
- **Eva Kimby, Karolinska University Hospital, Stockholm, Sweden**
- **Geir Tjønnefjord, Rikshospitalet, Oslo, Norway**
- **Mars van 't Veer, Erasmus MC, Rotterdam, The Netherlands**
- **Jan Walewski, Maria Skłodowska-Curie Memorial Cancer Centre Warsaw Poland**

Study design CLL7

- Open-labeled, prospective, multicenter risk stratified randomized phase III trial
- Evaluating intensive therapy with fludarabine/cyclophosphamide and rituximab in high risk patients with early CLL (Binet stage A) versus differed therapy
- Promoters: German and French CLL Cooperative groups (GCLLSG and FCGCLL)

Randomisation CLL7



For a common protocol, a step by step harmonization is warranted

Clinical harmonization

- Inclusion of patients
 - A, B and NCI criteria definition of progression
 - Hemolytic anemia
- Evaluation of tumoral burden
 - Clinical examination versus echography vs CTscan
- Evaluation of treatment toxicity
 - Nadir versus blood counts at D28

Biological harmonization

- Diagnostic criteria of CLL
 - Cytology, Matutes scoring system, CD20
- Cytogenetics
 - Fresh cells versus cultured cells
- VH mutational status
- Centralization versus several reference labs

New deliverables for the EU funding period month 13 – 30:

- 7.10. Common data safety monitoring board in clinical trials on CLL in Europe
- 7.11. Web-based information- and communication services on CLL refined and updated
- 7.12. Assess and create new guidelines for autologous and allogenic stemcell transplantation in CLL
- 7.13. Prepare clinical practice guidelines for autologous and allogenic stemcell transplantation in CLL
- 7.14. Final proposal of definitions and standardization of relevant diagnostic and therapeutic procedures
- 7.15. Treatment of advanced CLL with FCR/FC on the European level finished
- 7.16. Harmonisation of clinical study protocols and trial accessories between national CLL study groups

Future projects currently not included in deliverables

- Revision of the diagnostic definition of CLL (eg. atypical cases)
- Setup of a CLL registry, collection of CLL cases via webpage (eg. with unfavourable cytogenetics, familial disease)
- Definition of major diagnostic and therapeutic targets by gene expression profiling and proteomics