Proteomics & PET and lymphoma prognosis

Where are we in the field of biomarkers ? where do we go?

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Issues/questions (DLBCL)

- **Do we need prognostic or predictive markers in lymphomas (DLBCL, but also other entities)?**

- **How does interim-PET compare with biomarkers ?**

- **Current status and perspectives in the field of candidate prognostic or predictive biomarkers ?**

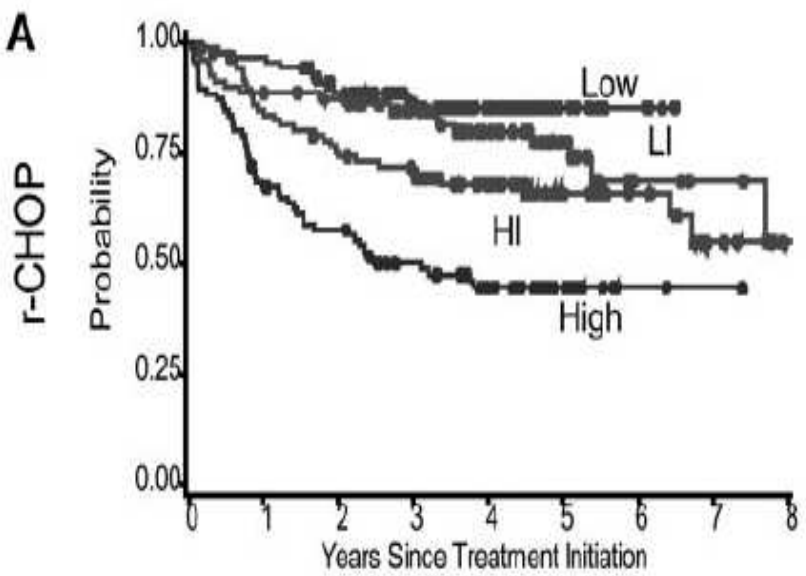
- **What are the prerequisites for a candidate biomarker .... in daily practice?**

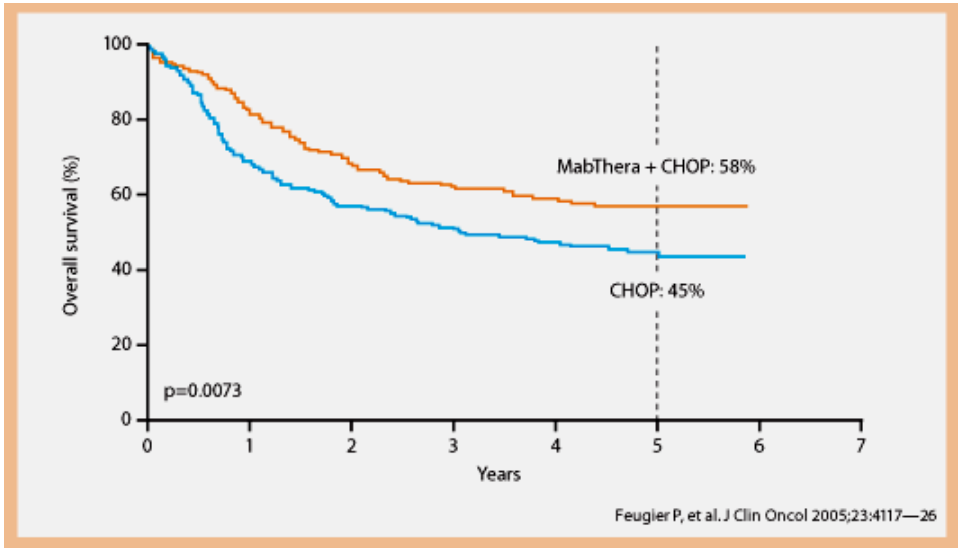
Requirements for a prognostic « marker » (DLBCL)

- Identify as soon as possible patients at risk for failure after a « conventional » therapy [R-CHOP(-like)] --→ Candidates to an experimental approach

- Identify patients, candidates for less aggressive therapies (« dose-desescalade »)

Even in the Rituximab era, around 30% of DLBCL patients are not cured…



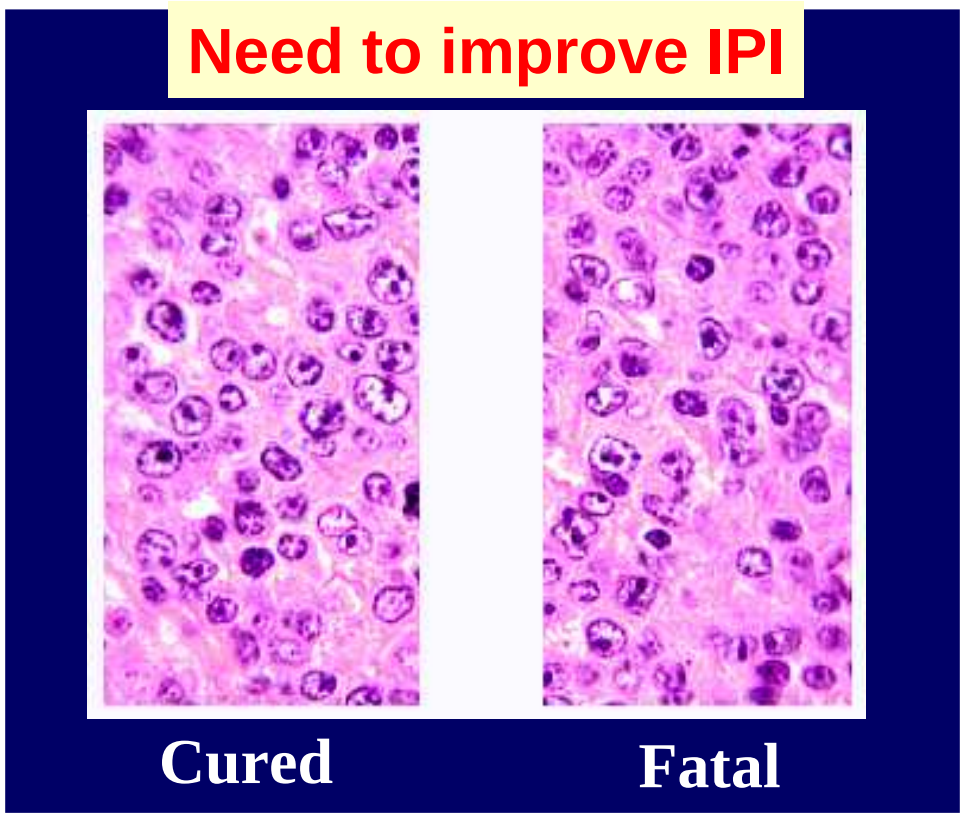


IPI still works….., but…

G Salles et al. Blood 2011

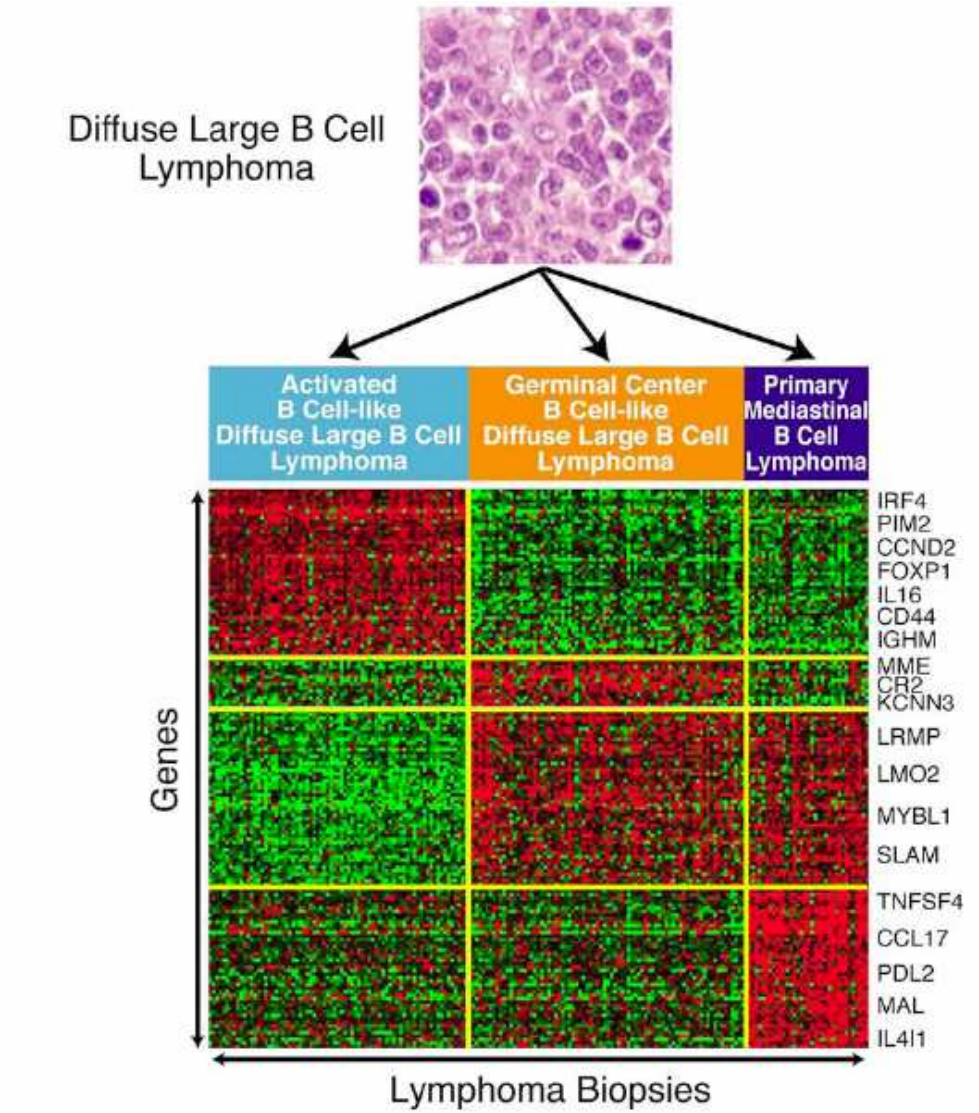
Coiffier B et al. NEJM. 2003; Feugier P et al. J Clin. Oncol 2005
Sehn L, Blood 2007 ; Pfreundschuh et al, Lancet Oncol 2006
Pfreundschuh et al, Lancet Oncol 2008; Lunenburg Consortium, unpublished data

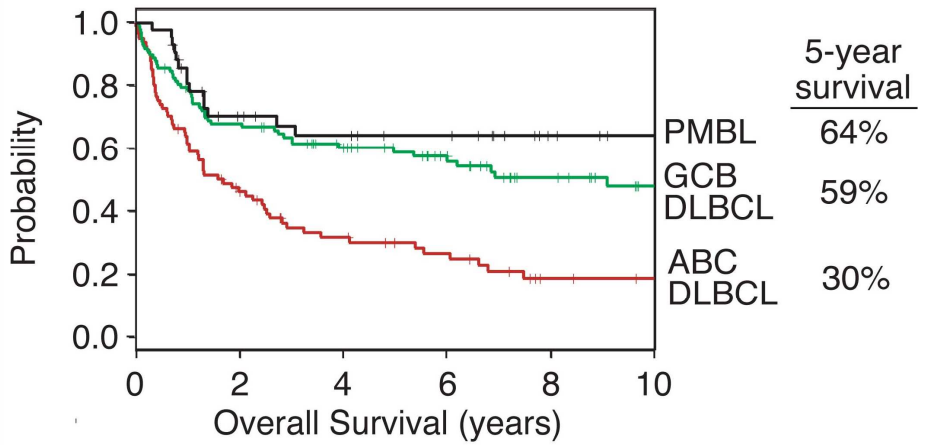
However, need for additional markers to identify curable from refractory/fatal patients?



- Biomarkers with prognostic relevance
- Biomarkers predictive for response to therapy

DLBCL is far from being a single entity, but a heterogeneous disease with – at least – 3 molecular subtypes





- **Cell of origin**
- **Clinical relevance independently of the IPI**
- **Distinct genetic features & oncogenic pathways**

Rosenwald et al. NEJM 2002
Rosenwald et al. J Exp Med 2003

Heterogeneity in DLBCL

(updated WHO classification, 2008)

- **Diffuse large B-cell lymphoma, not otherwise specified (NOS)**
- T-cell/histiocyte rich large B-cell lymphoma
- DLBCL associated with chronic inflammation
- ALK-positive DLBCL
- **Mediastinal (thymic) large B-cell lymphoma**
- Plasmablastic lymphoma
- Intravascular large B-cell lymphoma
- Primary DLBCL of the CNS
- Primary cutaneous DLBCL, leg type
- EBV+ DLBCL of the elderly
- Primary effusion lymphoma
- B-cell lymphoma, with features intermediate between DLBCL and BL
- B-cell lymphoma, with features intermediate between DLBCL and cHL

Interim PET vs Biomarker ?

- Many studies have emphasized the prognostic value of interim-PET
- Efforts have been made to establish guidelines for response criteria (SUV, visual assessment)

Respective prognostic values of germinal center phenotype and early PET scanning in previously untreated patients with DLBCL

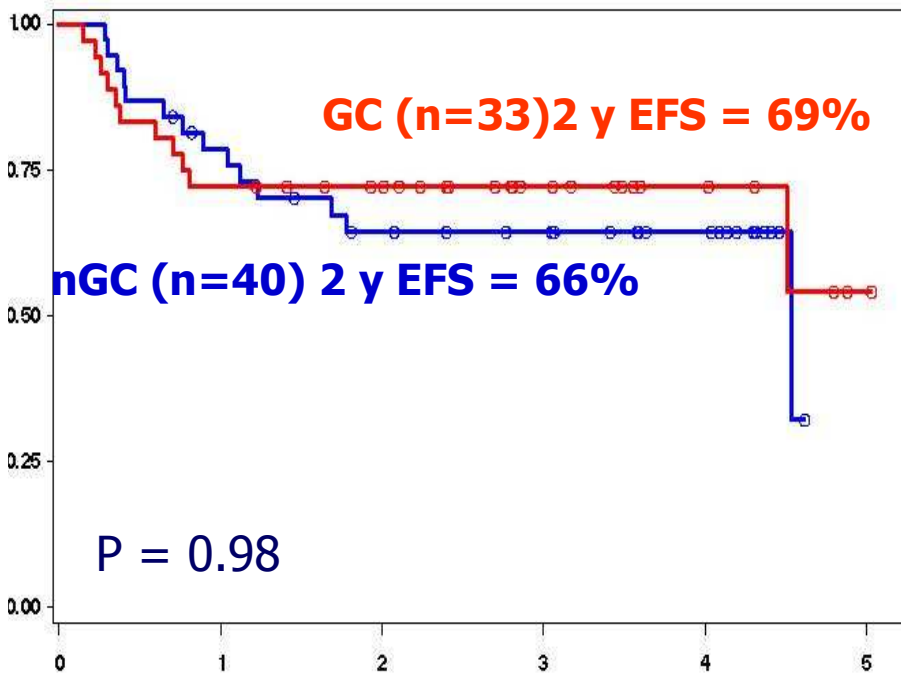
- **81 DLBCL pts (*Haioun, Blood 2005*)**
- **Median age 52 y,**
- **aaIPI >1: 72%.**
- **nodal (56%), extranodal (36%) mediastinal (14%)**
- **CHOP ou CHOP-like (100%),**
- **Rituximab (46%), Frontline HDT (41%)**

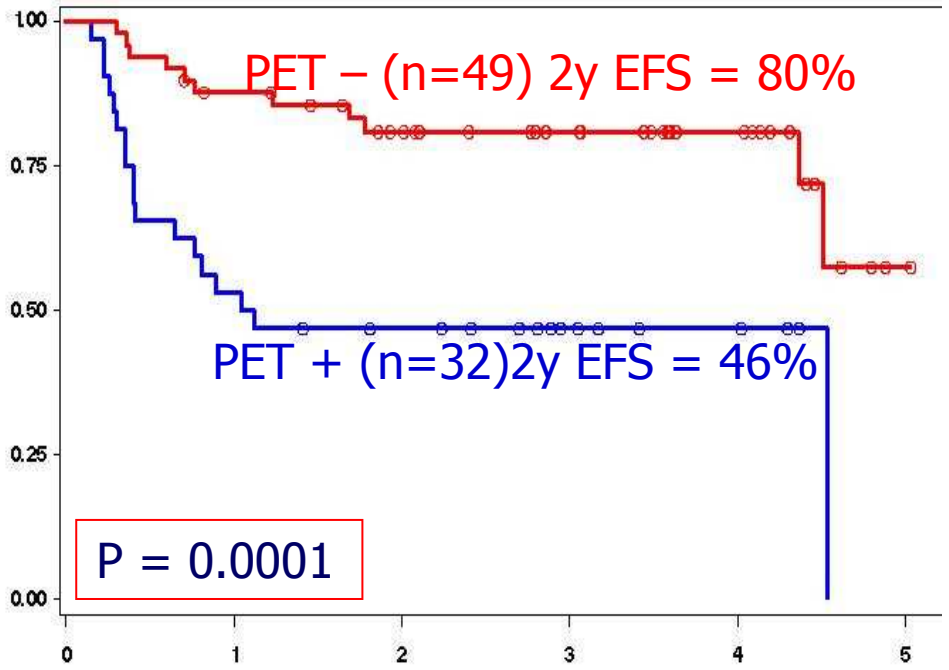
- **Bcl2+ : 53%**
- **CD10+ : 36%**
- **Bcl6+ : 58%**
- **MUM1+ : 45%**

GC : 52%
nGC : 48%

J Dupuis et al. Haematologica 2007

RESULTS





	p	RR
Bcl2 +	0.31	1.60
PET +	0.014	5.26
IPI 3-5	0.61	1.28

J Dupuis et al. Haematologica 2007

No way for the pathologist ….?

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- How does interim-PET compare for biomarkers ?

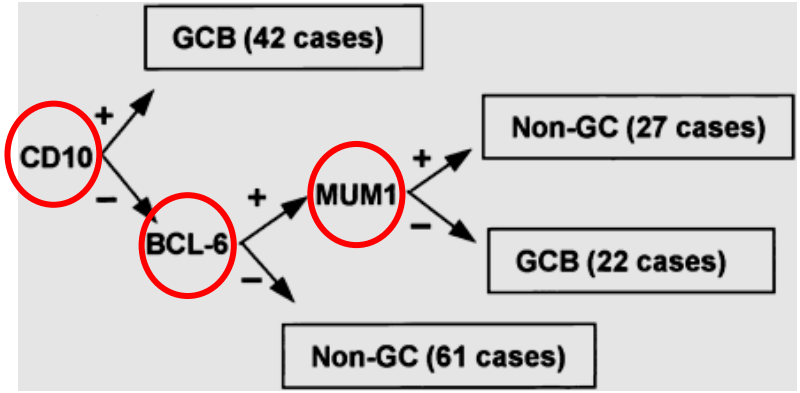
- **Current status and perspectives in the field of candidate prognostic or predictive biomarkers ?**

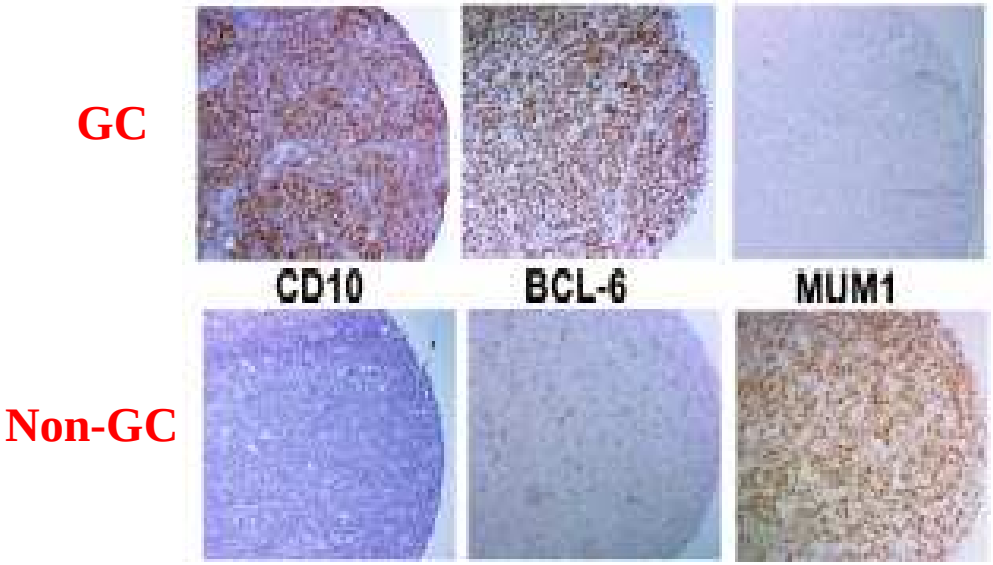
- What are the prerequisites for a candidate biomarker .... in daily practice?

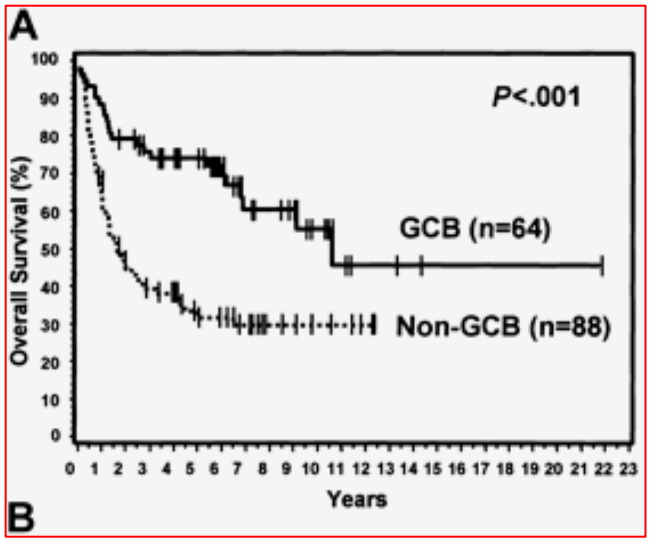
COO and other biomarkers to predict outcome of DLBCL (R-CHOP) patients ?

- Cell of origin (GCB / non GCB) represents a separation of 2 different disease entities, with distinct biology (supported by CGH / NGS data) more than a prognostic tool
- Most likely, this parameter will be included in DLBCL future trials
- Assessment by immunohistochemistry is therotically “easy”, but likely to be less reproducible than GEE or RT-PCR:
  - optimal biomarker(s)?
  - optimal algorithm (Hans classifier & others)?
  - optimal technique & quantification measurement?
- There are other prognostic models based on additional biomarkers unrelated to COO
- Other feasible techniques on FFPE are being developed (FISH, RT-PCR, mutations analysis, …)

An example: Can immunostochemistry be a surrogate for the GC/ABC classification ?







C Hans et al, Blood 2004, 103:275

The Hans algorythm: controversies in the CHOP and R-CHOP era

Immunohistochemical Prognostic Markers in Diffuse Large B-Cell Lymphoma: Validation of Tissue Microarray As a Prerequisite for Broad Clinical Applications—A Study From the Lunenburg Lymphoma Biomarker Consortium

Daphne de Jong, Andreas Rosenwald, Mukesh Chhanabhai, Philippe Gaulard, Wolfram Klapper, Abigail Lee, Birgitta Sander, Christoph Thorns, Elias Campo, Thierry Molina, Andrew Norton, Anton Hagenbeek, Sandra Horning, Andrew Lister, John Raemaekers, Randy D. Gascoyne, Gilles Salles, and Edie Weller

Conclusion

This study shows that semiquantitative immunohistochemistry for subclassification of DLBCL is feasible and reproducible, but exhibits varying rates of concordance for different markers. These findings may explain the wide variation of biomarker prognostic impact reported in the literature. Harmonization of techniques and centralized consensus review appears mandatory when using immunohistochemical biomarkers for treatment stratification.

J Clin Oncol 25:805-812. © 2007 by American Society of Clinical Oncology

A challenge for the pathologist

Why these discrepancies ?

- **Patients:**
  - Patients heterogeneity : nodal/EN/xPMBL, therapy, single/multicentric study,…

- **Methods :**
  - Fixation : fixative, over/underfixation,..
  - IHC optimization/standardization: pretreatment, Ab,..
  - TMA or not

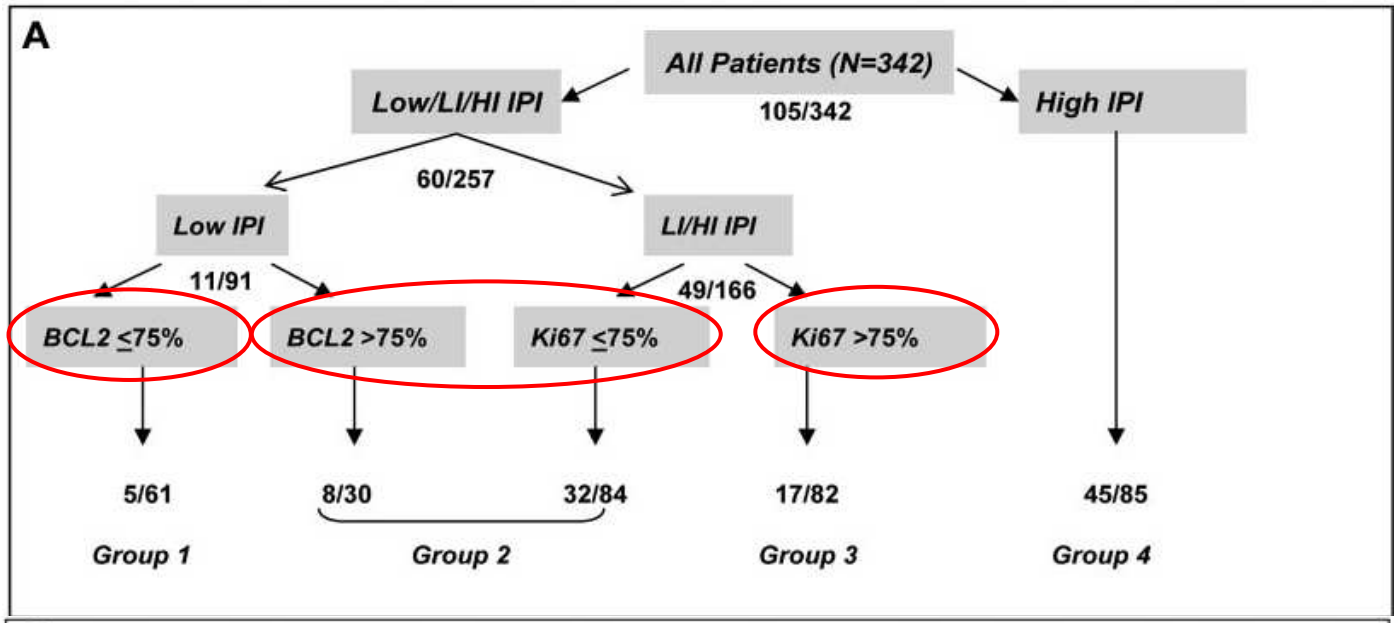
- **Interpretation of the results :**
  - lack of standard system: scoring, cut-off, algorythm
  - intra/inter-observer reproducibility ++

A challenge for the pathologist

GCB - nonGCB IHC algorithms ?

- Hans algorithm (CD10 / BCL6 / MUM1)
- Choi algorithm (**GCET** ; then MUM1 if + / CD10 if neg ; then BCl6 / Foxp1 if GECT/CD10 neg)
- Tally count
  - CD10 and GCET positive (0,1,2) points towards GCB
  - MUM1 and **Foxp1** positive (0,1,2) points towards ABC
  - **LMO2** as a tie breaker
- Visco/Young algorythm (CD10,**FoxP1**, BCL6) *(Abstract #078, Lugano, 2011)*
- LMO2 prognostic marker; correlates with GCB but does not discriminate GCB / non GCB

Other prognostic biomarkers…



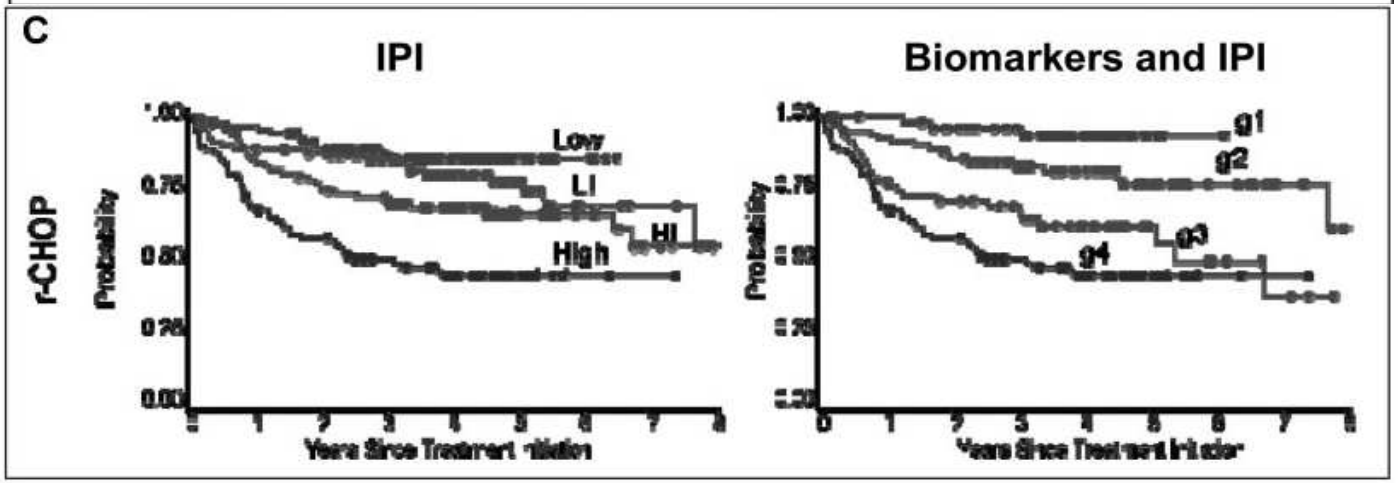


Figure 5. Prognostic model using biomarkers and IPI. (A) Hierarchical tree model. Numbers indicate the number of deaths observed at each level in the population at risk. (B) Number of patients, 4-year OS, and HR for the risk of death (without and with imputation for samples with missing scores) in the r-CHOP cohort. (C) OS for r-CHOP patients according to the IPI and the biomarker and IPI model, respectively. Log-rank *P* values for both models were < .0001.

G Salles et al. Blood 2011

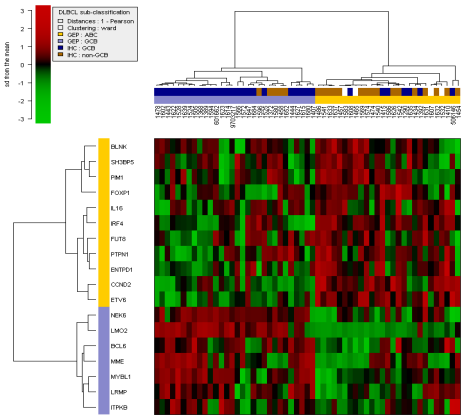
The Germinal Center B-cell signature is associated to a higher [^{18}F]-FDG uptake and improves the prognosis value of TEP scan in DLBCL treated by rituximab and anthracyclines-based chemotherapy.

Lugano, ICML, June 2011. H Lanic et al. abstract #044

3rd International workshop on interim-PET in Lymphoma, Menton, 2011

ABC (n=27)

FOXP1, SH3BP5, IRF4, IL16, BLNK, ENTPD1, ETV6, CCND2, PIM1, FUT8 PTPN1



GCB (n=30)

MME, LRMP, NEK6, BCL6, LMO2, ITPKB MYBL1

IHC GC-non GC (*Hans algorithm*)

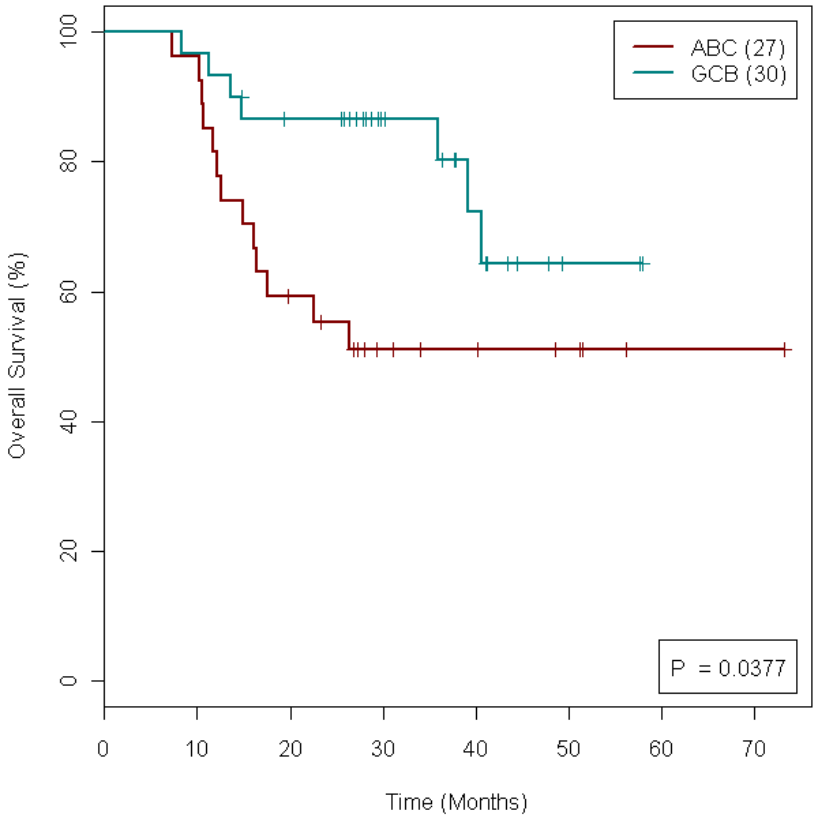
Glucose transporters

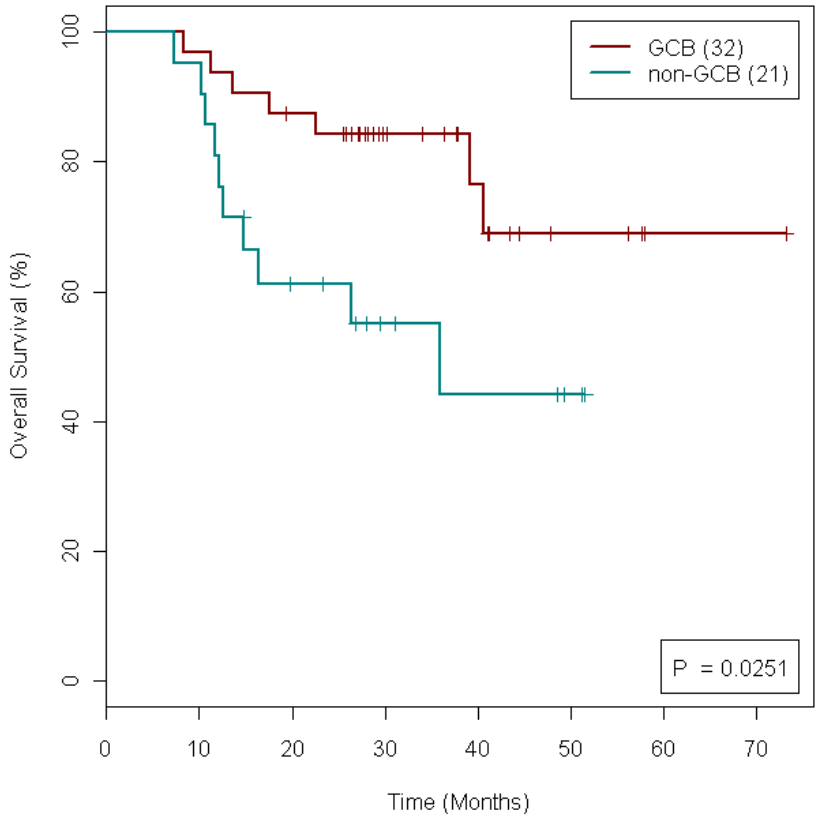
SLC2A1, SLC2A2, SLC2A3, SLC2A4, SLC2A5

PET at diagnosis, interim PET (3/4 cycles) and final PET

$$\text{SUV reduction}\,(\%) = 100 \times \frac{\text{SUVmax}\,(\text{PET0}) - \text{SUVmax}\,(\text{PET4})}{\text{SUVmax}\,(\text{PET0})}$$

Prognostic relevance of the GC/ABC signature and of the immunohistochemical GC/nonGC profile (*Hans classifier*)





Lugano, ICML, June 2011. H Lanic et al. abstract #044
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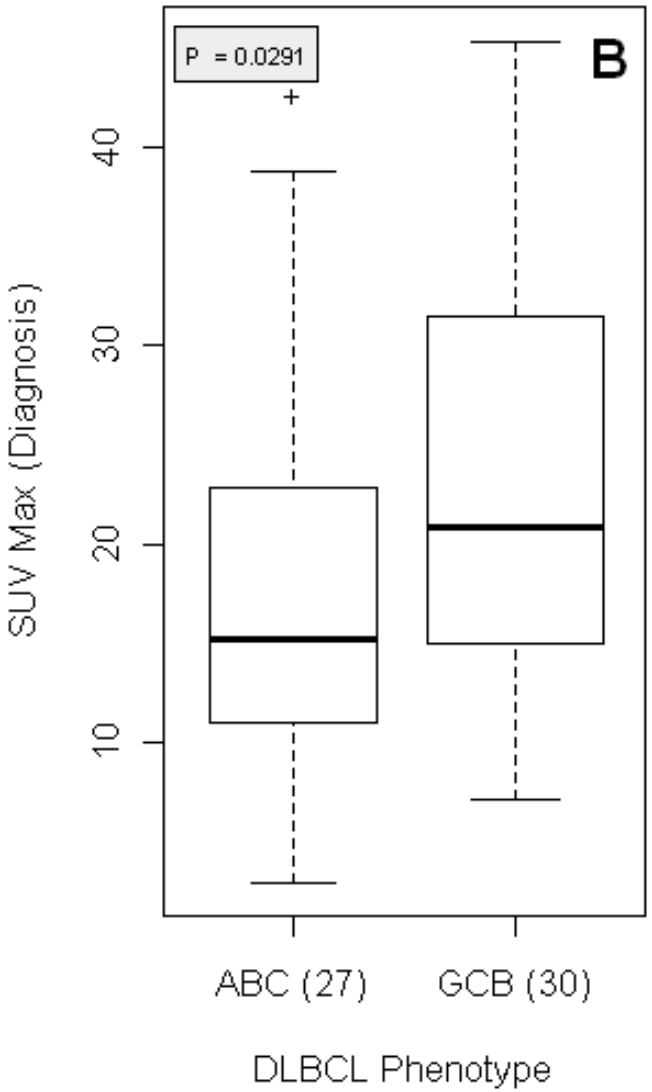
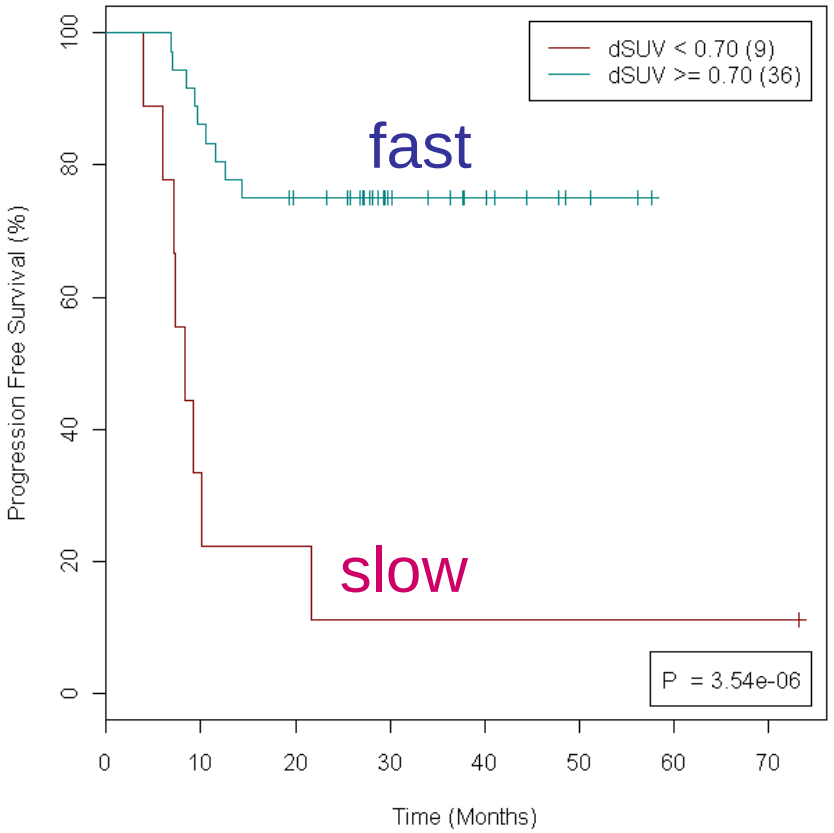
GCB/ABC subtype and SUV max at baseline

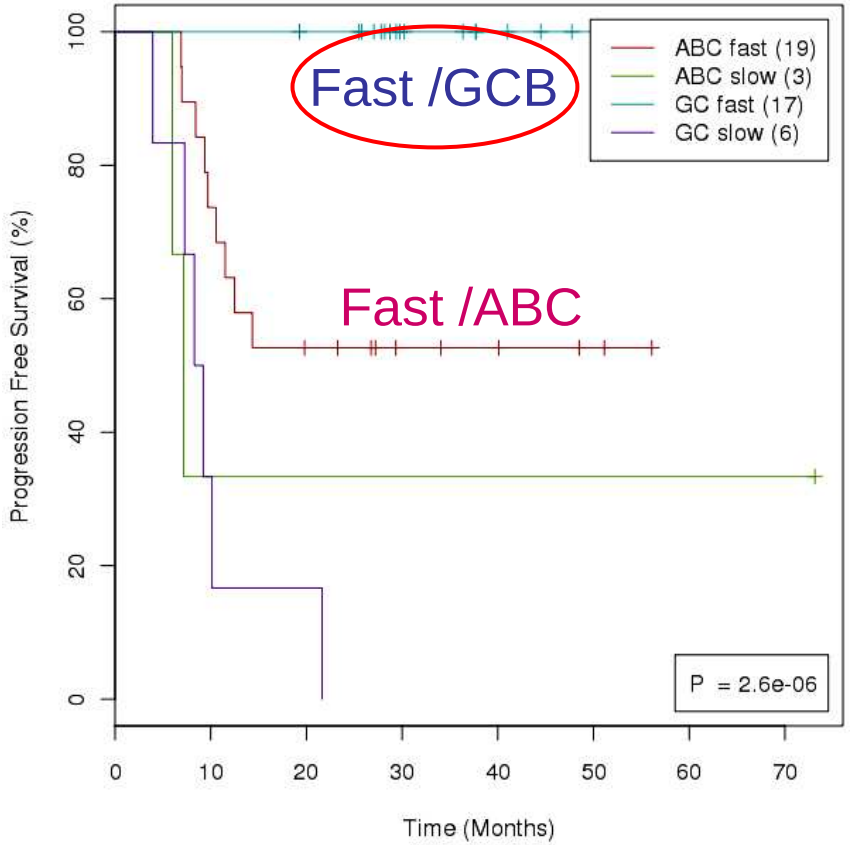


1. High SUV max at diagnosis correlates with GC profile
2. SUV max base line = correlation with GLU2 expression
3. GLU expression = no correlation with GCB/ABC classification

Lugano, ICML, June 2011. H Lanic et al. abstract #044

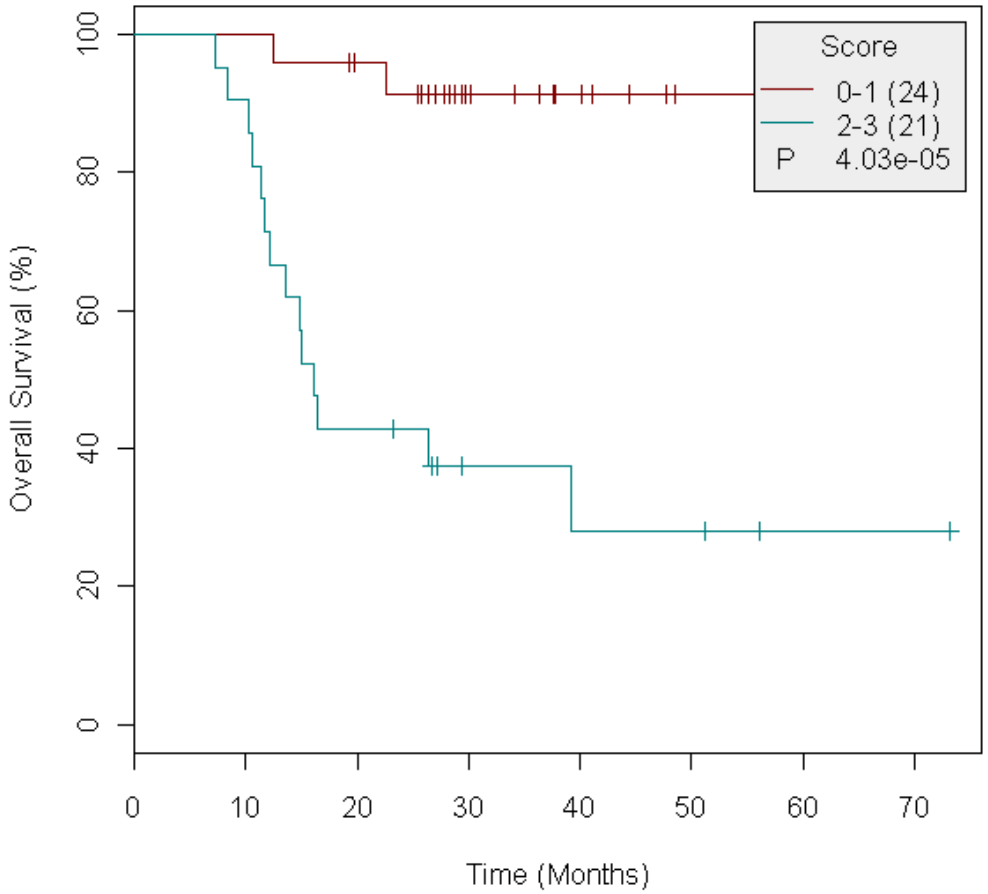
Survival according Interim-Pet and GCB/ABC subtypes





- **Good risk group**
  Fast / GCB
- **Poor risk group**
  Slow GCB and ABC

Toward a novel index based on IPI, GC/ABC profile and PETscan…?



Prognostic Score

1. IPIaa (0-1/2-3)
2. GCB/ABC
3. Slow/fast

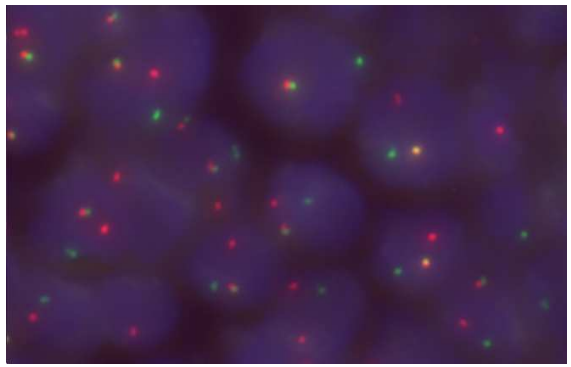
H Lanic et al. Rouen, abstract #044

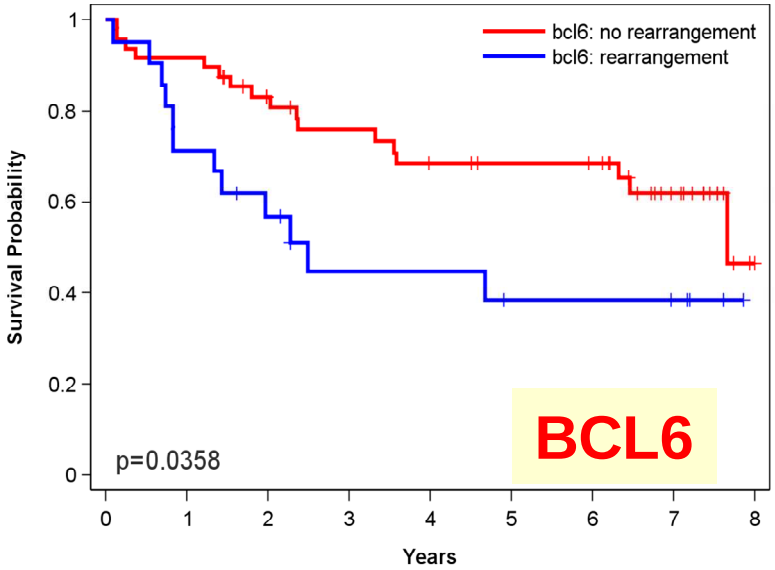
Are there other biomarkers using tools more robust than immunohistochemistry to predict outcome of DLBCL (R-CHOP) patients ?

GELA: LNH98-5 et 01-5B (R-CHOP patients)

- FISH is a robust technique
- DLBCL with BCL2 or BCL6 rearrangements are biologically distinct

BCL2	14/71 (19%)
BCL6	21/69 (30%)
C-MYC	2/68 (3%)





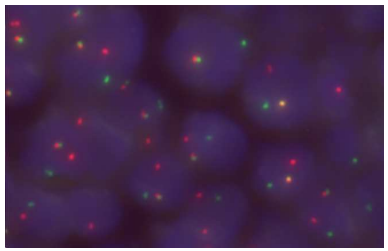
GELA

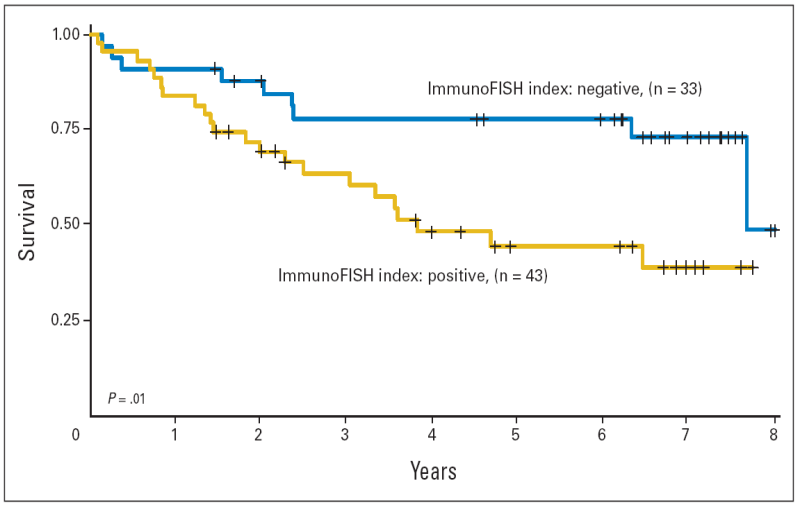
C Copie-Bergman et al. J Clin Oncol 2009

Are there other biomarkers using tools more robust than immunohistochemistry to predict outcome of DLBCL (R-CHOP) patients ?

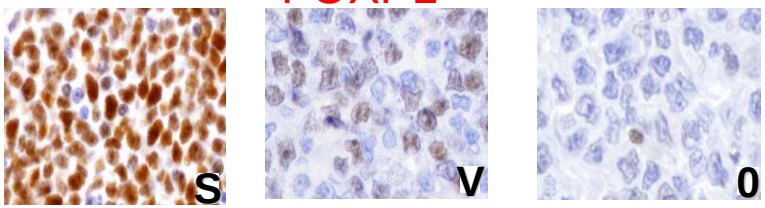
GELA: LNH98-5 et 01-5B (R-CHOP patients)

Prognostic Immuno-FISH index :
at least 2 / 3 biomarkers positive
[FOXP1, MUM1/IRF4 by IHC and *BCL6* gene rearrangement by FISH]





FOXP1

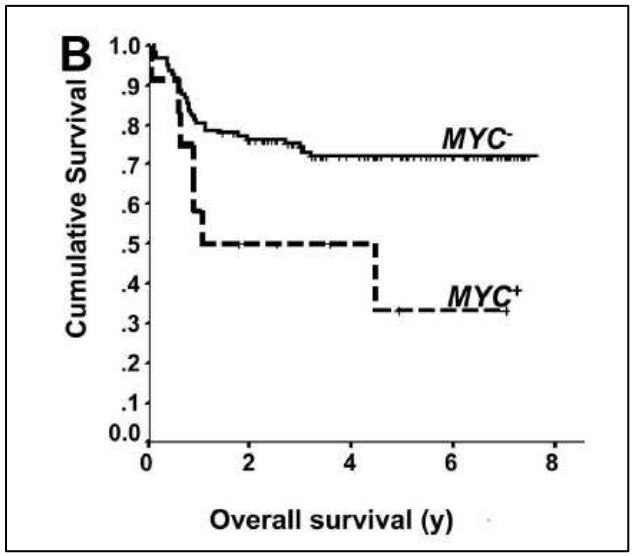


GELA

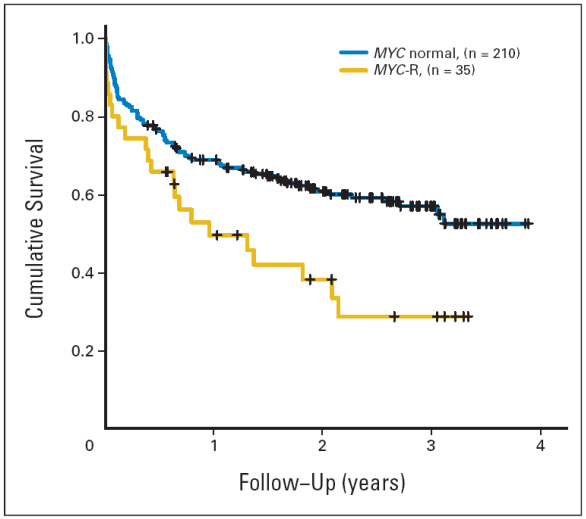
C Copie-Bergman et al. J Clin Oncol 2009

MYC break & clinical significance in DLBCL

- 8-20% of de novo DLBCL, R-CHOP
- include « double hits » *BCL2/MYC*
- inferior survival
- higher risk of CNS relapse
- cMYC expression (IHC) as an alternative prognostic marker?



Savage et al, Blood 2009



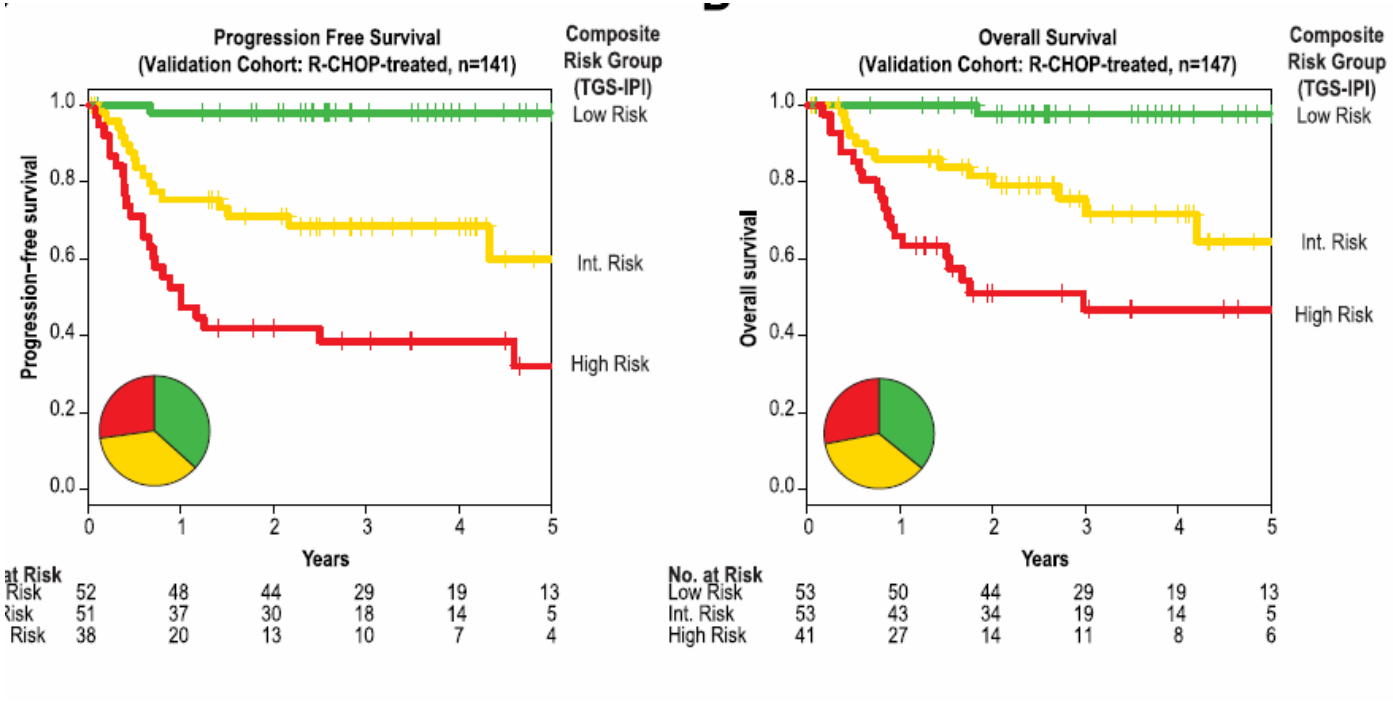
Barrans et al, J Clin Oncol 2010

Toward improvements in optimal tools for measuring a biomarker from FFPE

Paraffin-based 2-gene model based on the expression of 2 genes:

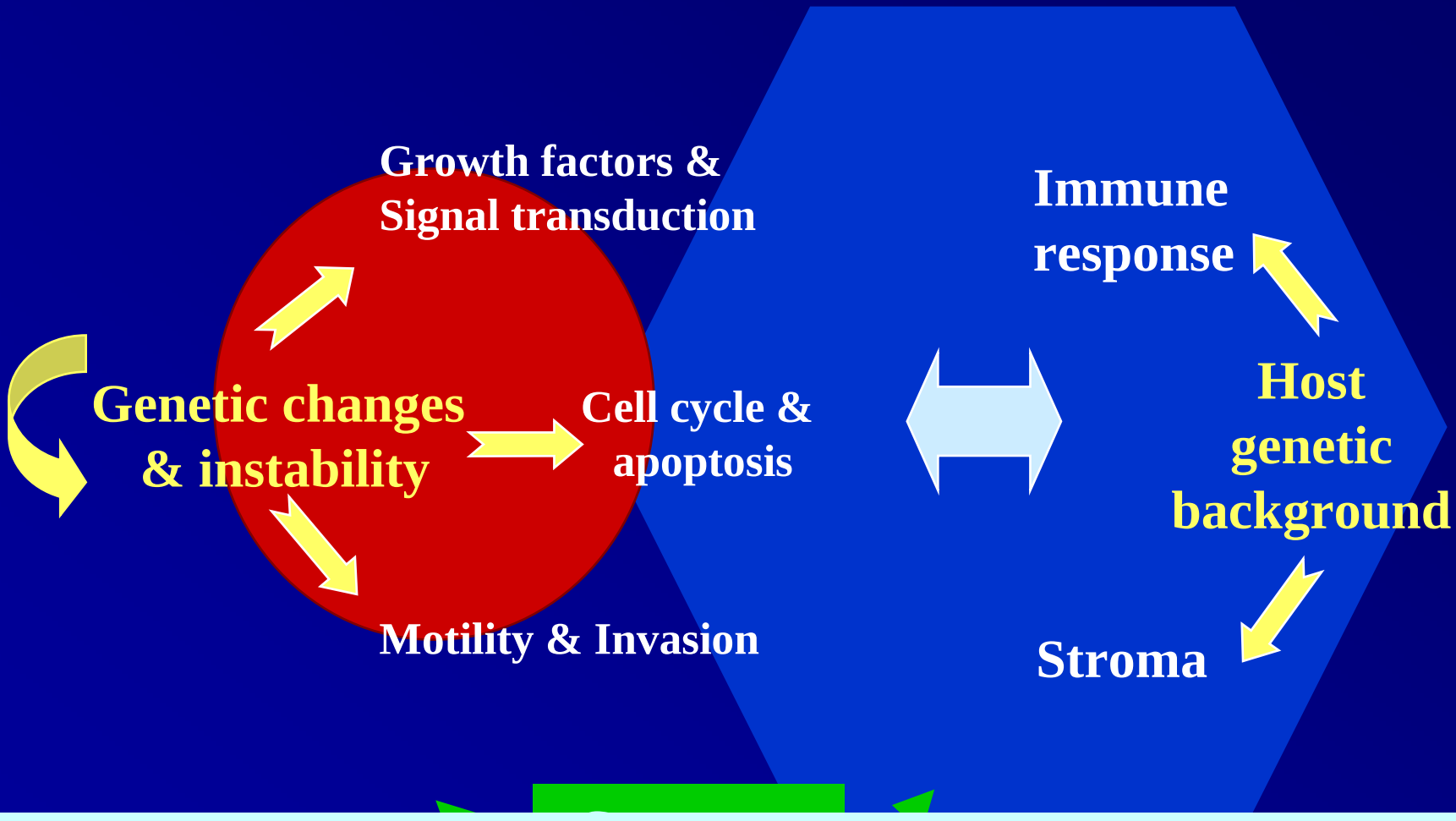
- LMO2 targeting neoplastic B cells (LMO2, GC),
- CD137 (TNFRSF9) targeting CD45RO T cells of the microenvironement)

→ **Pc score based on IPI and these 2 genes**



A Alizadeh et al. Blood 2011

Microenvironement : a possible target for DLBCL ?



Expression of lymph node signature predicts outcome in R-CHOP DLBCL patients
(Lenz et al. NEJM, 2008)

Biomarkers: why do we need them ?

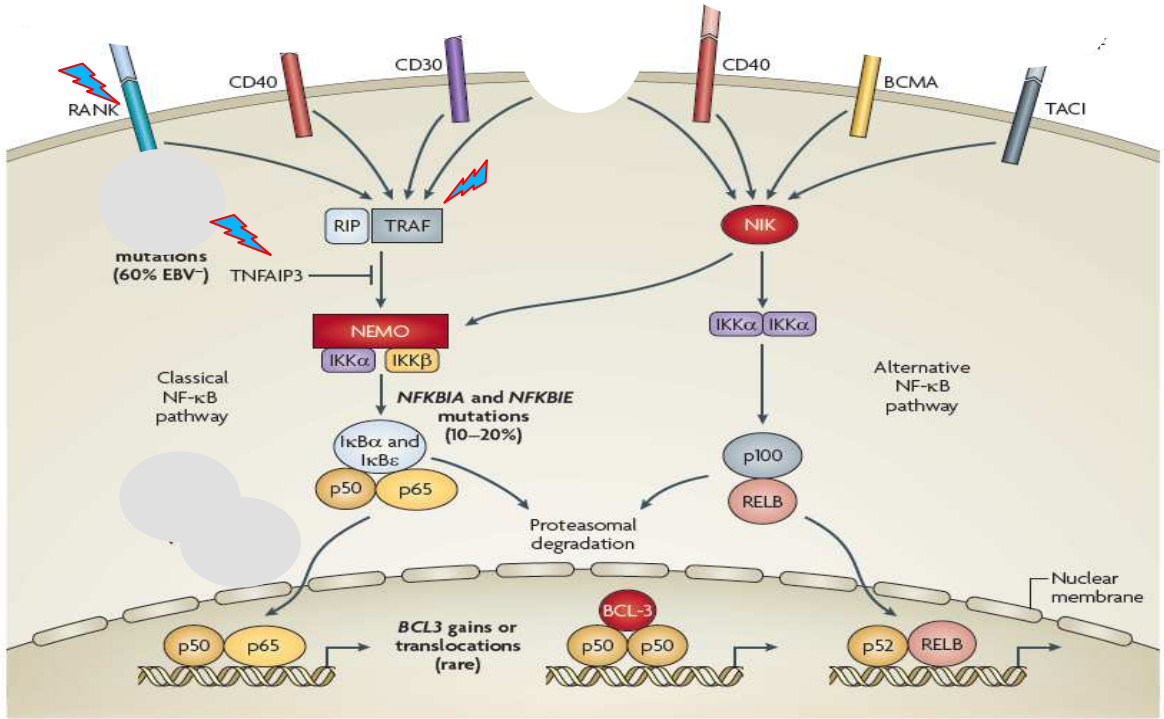
1. Diagnostic / staging
   - To know disease natural history, choose treatment
2. Prognostic biomarkers
   - who will do well or not ?
3. **Predictive biomarkers**
   - **who will respond to a given drug / regimen ?**

From empiric therapies to more rational targeted therapies

NFkB signalling pathway is constitutively activated in ABC DLBCL

- ABC DLBCL are **less curable**
- **More than 50% ABC DLBCL carry mutations in positive or negative regulators of NFkB** (*Compagno et al. Nature 2009*)
- anti-apoptotic effect and can inhibit chemotherapy
- pharmaceutical agents targeting components of the NFkB pathway are being developed

- **A20 (TNFAIP3): 24%**
- **CARD11: 11%**
- **RANK: 8%**
- **TRAF5: 5%**
- **TRAF2: 3%**
- **MAP3K7 (TAK1): 5%**
- **MYD88: 29%**

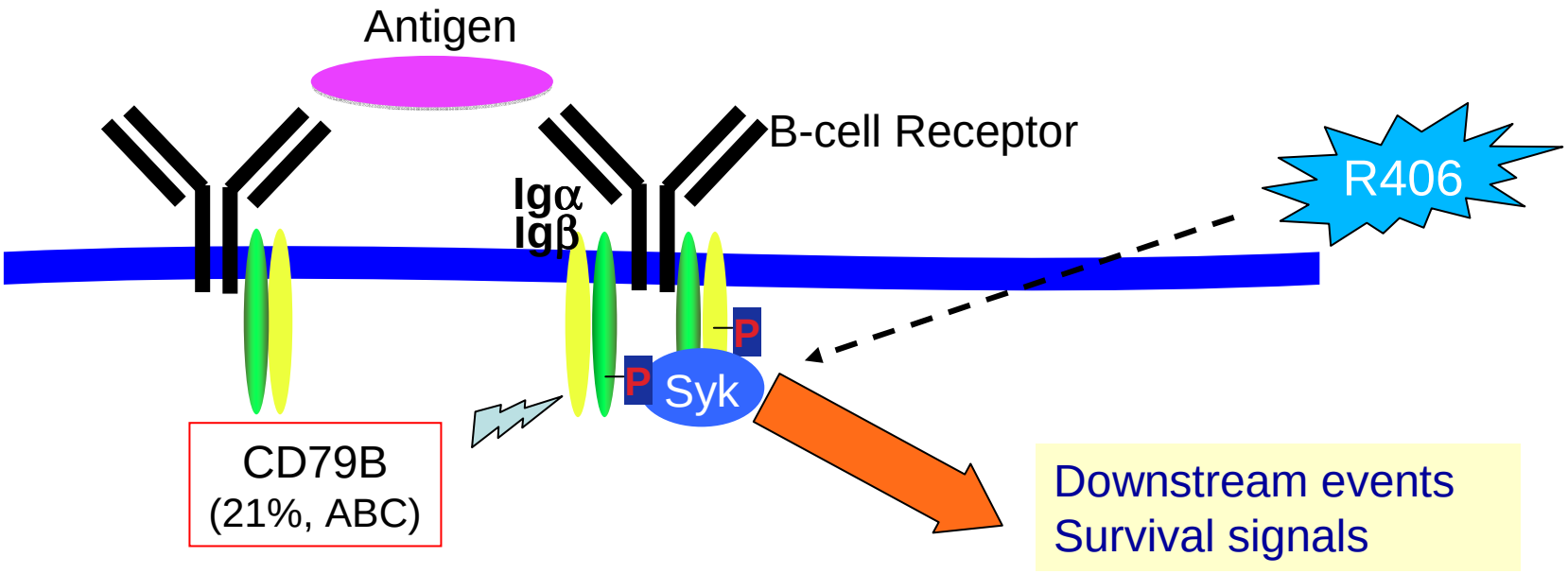


Compano, Nature 2009; Ngo, Nature 2011

Adapted from Küppers et al. 2009

« Tonic » or « chronic active » BCR signalling and DLBCL

- “BCR” BLBCL can be identified by gene expression profile
- “chronic active” BCR signaling is required for cell survival in ABC DLBCL
- Tonic BCR signaling requires Syk expression & phosphorylation



Tonic BCR signaling can be targeted with a SYK inhibitor (R406)

Clinical trials of SYK/BCR, BTK,… inhibitions are promising

“BCR” DLBCL are good candidate for BCL6 inhibitor *(Cerchietti. Blood 2009)*

(Chen et al. Blood, 2008; Davis RE et al. Nature 2010)

Other perspectives at the era of genome-wide sequencing?

- Recurrent somatic mutations, specially in genes with roles in histone modification (methylation *MML2, EZH2*; acetylation *MEF2B, CREBBP, EP300*,…)
- Found both in DLBCL (GC) and FL
- May have direct implications for
  - the use of drugs targeting acetylation/deacetylation mechanisms
  - the development of molecular tests on FFPE to identify patients to receive targeted therapy

Morin et al. Nature Genetics 2010; Pasqualucci et al. Nature 2011; Morin et al. Nature 2011

Conclusions

1) **Interim PET** is a strong prognostic parameter that compares favorably to biological parameters yet tested.
2) **DLBCL is a heterogeneous category** with distinct subtypes or entities; the determination of the **cell of origin (GCB / non GCB)** is important and most likely should be included in future DLBCL trials
3) Novel therapeutic strategies will include **more extensive molecular characterization**, to determine whether the therapy has a preferential activity in a peculiar subset of patients **(“Predictive biomarker”, before therapy)**
4) The choice of the **biomarker(s)** and the choice of the optimal technique(s) for its assessment still remain a challenging issue and will largely depend on the therapeutic situations/implications

Prerequisites for a **biomarker** in the context of targeted therapies

1) The **biomarker** should be easy to assess:
   . peripheral blood; tumor samples (**FFPE** > frozen > fresh)
   . Techniques available in every center, rapidly available
   . Interpretation easy; the cost should be reasonable

2) The **biomarker** assessment should be robust:
   . It should provide clear cut results (positive/negative)
   . Reproducible

3) The **biomarker** needs a strong clinical validation:
   . in multiple centers and in multiple studies
   . within the context of different treatments

4) The **biomarker** needs to help clinician to improve patient care

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Hervé Tilly, F Jardin,
Christian Gisselbrecht
Bertrand Coiffier

and many others…

?