Third international workshop on
interim-PET in lymphoma

# PET AND BIOMARKERS


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# FDG-PET studies

## Evaluate the prognostic role of an early interim fluorodeoxyglucose-PET scan in advanced Hodgkin's Lymphoma

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JOURNAL OF CLINICAL ONCOLOGY

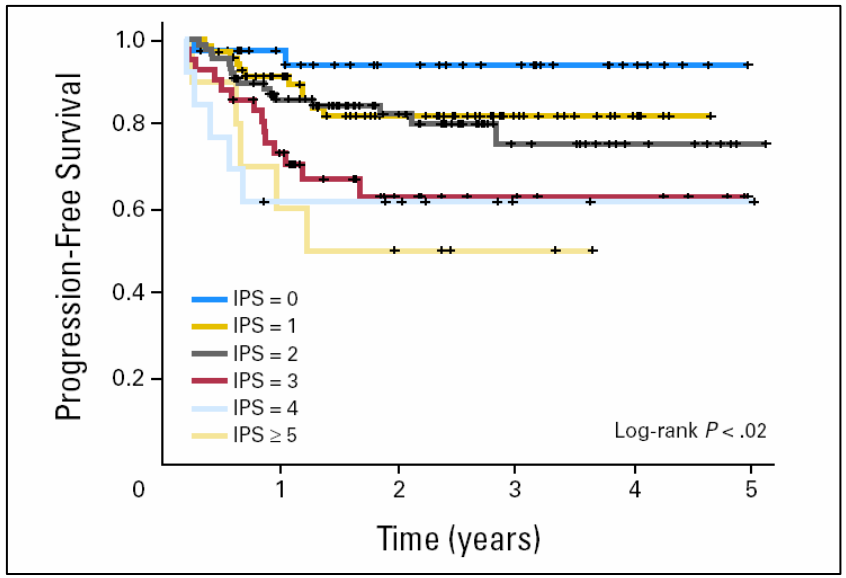
ORIGINAL REPORT

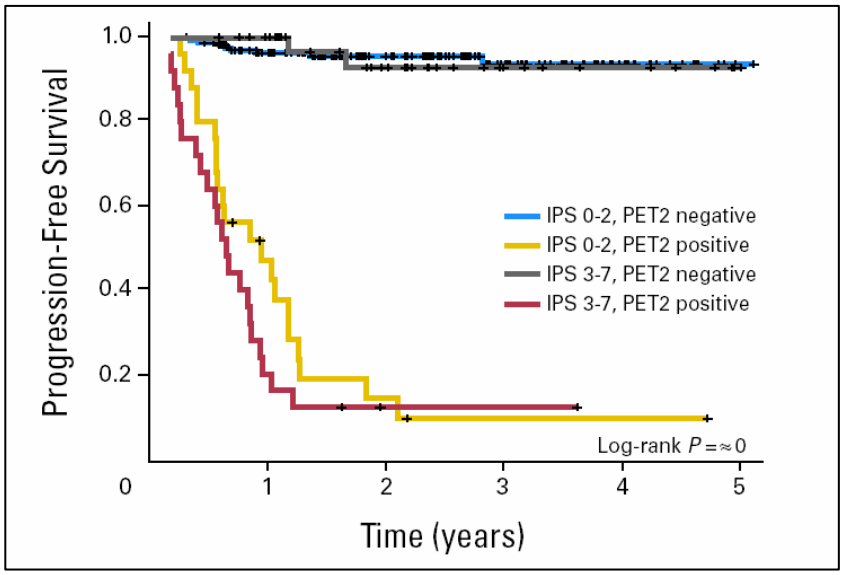
Early Interim 2-[$^{18}$F]Fluoro-2-Deoxy-D-Glucose Positron Emission Tomography Is Prognostically Superior to International Prognostic Score in Advanced-Stage Hodgkin's Lymphoma: A Report From a Joint Italian-Danish Study

*Andrea Gallamini, Martin Hutchings, Luigi Rigacci, Lena Specht, Francesco Merli, Mads Hansen, Caterina Patti, Annika Loft, Francesco Di Raimondo, Francesco D'Amore, Alberto Biggi, Umberto Vitolo, Caterina Stelitano, Rosario Sancetta, Livio Trentin, Stefano Luminari, Emilio Iannitto, Simonetta Viviani, Ivana Pierri, and Alessandro Levis*

Prognostic biologic factors in Hodgkin's lymphoma

# Progression-Free Survival




According to International Prognostic Score

According to PET-2 results for patients with a low or a high IPS

Prognostic biologic factors in Hodgkin's lymphoma

PubMed interim PET and Hodgkin lymphoma

Display Settings: Summary, 20 per page, Sorted by Recently Added

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**Results: 1 to 20 of 45**

1. The role of FDG-**PET** in early and late therapy assessment of patients with advanced **Hodgkin Lymphoma** treated with BEACOPP.
   Markova J, Kahraman D, Kobe C, Skopalova M, Mocikova H, Klaskova K, Dedeckova K, Eich H, B LI B, Dietlein M, Kozak T.
   Leuk **Lymphoma**. 2011 Jul 7. [Epub ahead of print]
   PMID: 21740300 [PubMed - as supplied by publisher]

2. Epratuzumab with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy (ER-CHOP) in patients with previously untreated diffuse large B-cell **lymphoma**.
   Micallef IN, Maurer MJ, Wiseman GA, Nikcevich DA, Kurtin PJ, Cannon MW, Perez DG, Soori GS, Link BK, Habermann TM, Witzig TE.
   Blood. 2011 Jun 14. [Epub ahead of print]
   PMID: 21673350 [PubMed - as supplied by publisher]

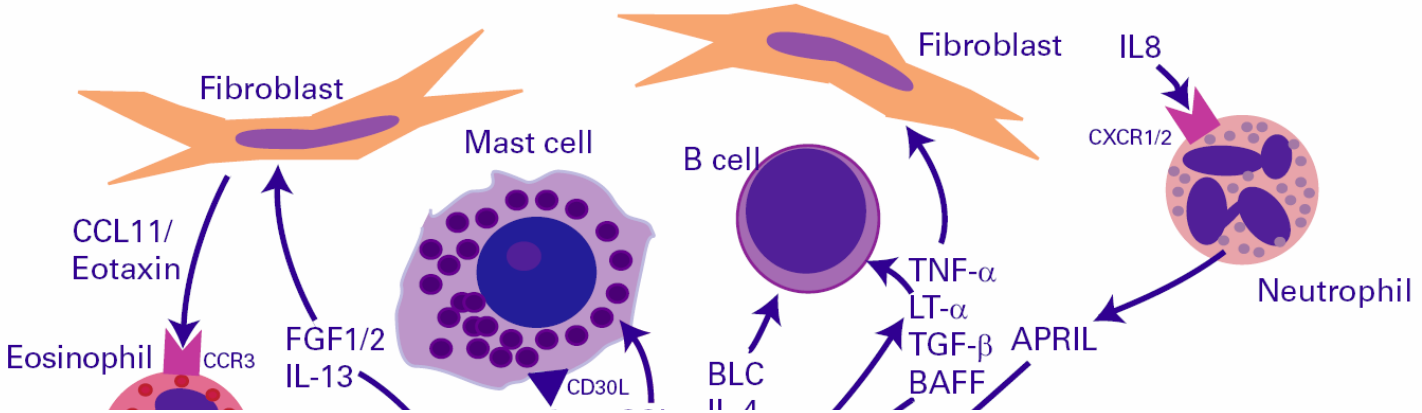
3. Report of satellite workshop on **interim**-**PET** in **Hodgkin lymphoma**: 8th International Symposium on **Hodgkin Lymphoma**, Cologne, 23 October 2010.
   Gallamini A, O'Doherty M.
   Leuk **Lymphoma**. 2011 Apr;52(4):583-6. Review. No abstract available.
   PMID: 21438829 [PubMed - indexed for MEDLINE]

4. Radioguided lymph node biopsy of a chemoresistant lymph node detected on **interim** FDG **PET**-CT in **Hodgkin lymphoma**.
   Györke T, Kollár A, Bottlik G, Szepesi A, Bodó I, Masszi T, Bérczi V, Garai I.
   Int J Hematol. 2011 Apr;93(4):545-50. Epub 2011 Mar 1.
   PMID: 21360009 [PubMed - indexed for MEDLINE]

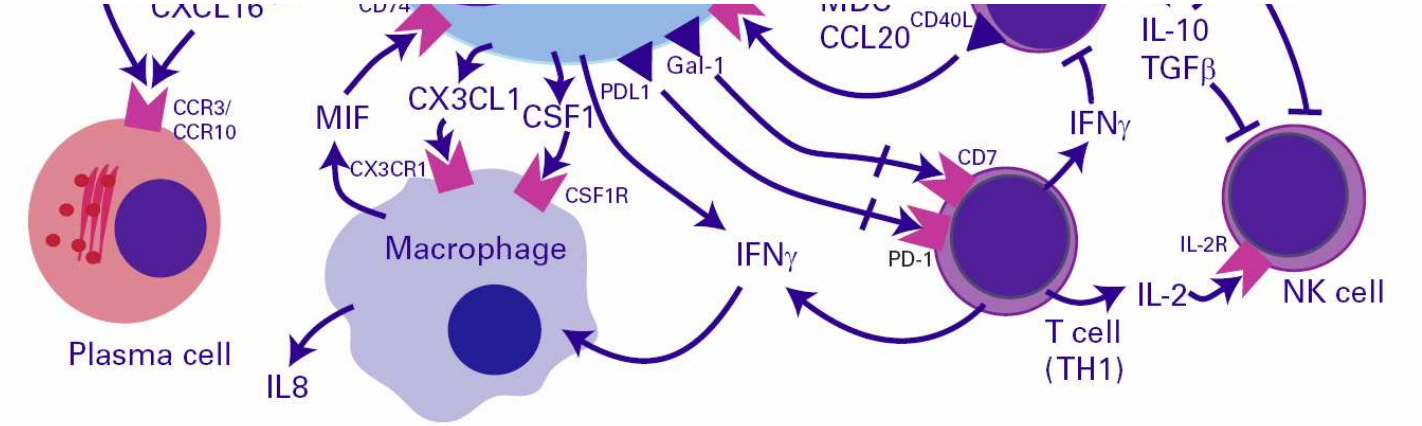
**Problems**: costs and availability as well as *ad interim* test

"If at time of diagnosis we could identify patients who are destined to have a poor response to treatment, most patients could be spared a combination of therapies or radiotherapy with its attendant long-term toxic effects". De Vita NEJM 2010

# CHL: cross-talk between HRSCs and microenvironment




## Biomarkers referred to neoplastic cells and microenvironmental components




Steidl C, Connors JM, Gascoyne RD. JCO 2011, 29:1812-26

# Immunoistochemical studies

- BCL2
- CD20
- p53
- EBV
- TOP2A
- HGAL
- IRF4
- HLA class II
- FOXP3
- Tia1/GyB

BLOOD, 15 JANUARY 2003 • VOLUME 101, NUMBER 2

Hodgkin and Reed-Sternberg cells harbor alterations in the major tumor suppressor pathways and cell-cycle checkpoints: analyses using tissue microarrays

Juan F. García, Francisca I. Camacho, Manuel Morente, Máximo Fraga, Carlos Montalbán, Tomás Álvaro, Carmen Bellas, Ángel Castaño, Ana Díez, Teresa Flores, Carmen Martín, Miguel A. Martínez, Fr
Ana I. Sáez, Lydia Sánchez, and Miguel A. Piris, for the Spanish

Vol. 11, 1467–1473, February 15, 2005 Clinical Cancer Research

**Outcome in Hodgkin's Lymphoma Can Be Predicted from the Presence of Accompanying Cytotoxic and Regulatory T Cells**

Tomás Álvaro,[1] Marylène Lejeune,[1] Mª Teresa Salvadó,[1] Ramón Bosch,[1] Juan F. García,[2] Joaquín Jaén,[1] Alison H. Banham,[5] Giovanna Roncador,[3] Carlos Montalbán,[4] and Miguel A. Piris[2]

VOLUME 22 · NUMBER 9 · MAY 1 2004

JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Influence of Biologic Markers on the Outcome of Hodgkin's Lymphoma: A Study by the Spanish Hodgkin's Lymphoma Study Group

*Carlos Montalbán, Juan F. García, Víctor Abraira, Leocricia González-Camacho, Manuel M. Morente, Jose L. Bello, Eulogio Conde, Miguel A. Cruz, Ramón García-Sanz, José García-Laraña, Carlos Grande,*

BLOOD, 1 OCTOBER 2005 • VOLUME 106, NUMBER 7

Impact of tumor Epstein-Barr virus status on presenting features and outcome in age-defined subgroups of patients with classic Hodgkin lymphoma: a population-based study

Ruth F. Jarrett, Gail L. Stark, Jo White, Brian Angus, Freda E. Alexander, Andrew S. Krajewski, June Freeland, G. Malcolm Taylor, and Penelope R. A. Taylor, for the Sc

Prognostic Significance of Cell Proliferation and Apoptosis-Regulating Proteins in Epstein–Barr Virus Positive and Negative Pediatric Hodgkin Lymphoma

SAFİYE AKTAŞ, M.D., Ph.D.,[1] AYDANUR KARGI, M.D.,[2] NUR OLGUN, M.D.,[2] GULDEN DİNİZ, M.D.,[1] AYŞE ERBAY, M.D.,[1] and CANAN VERGIN, M.D.[1]

haematologica | 2008; 93(2) | 193 |

**Correlation of high numbers of intratumoral FOXP3+ regulatory T cells with improved survival in germinal center-like diffuse large B-cell lymphoma, follicular lymphoma and classical Hodgkin's lymphoma**

Alexandar Tzankov,[1] Cecile Meier,[1] Petra Hirschmann,[1] Philip Went,[2] Stefano A. Pileri,[3] and Stephan Dirnhofer[1]

CLINICAL OBSERVATIONS, INTERVENTIONS, AND THERAPEUTIC TRIALS

BCL-2 expression in Hodgkin and Reed-Sternberg cells of classical Hodgkin disease predicts a poorer prognosis in patients treated with ABVD or equivalent regimens

George Z. Rassidakis, L. Jeffrey Medeiros, Theodoros P. Vassilakopoulos, Simonetta Viviani, Valeria Bonfante, Gianpaolo Nadali, Marco Herling, Maria K. Angelopoulou, Roberto Giardini, Marco Chilosi, Christos Kittas, Timothy J. McDonnell, Gianni Bonadonna, Alessandro M. Gianni, Giovanni Pizzolo, Gerassimos A. Pangalis, Fernando Cabanillas, and Andreas H. Sarris

VOLUME 23 · NUMBER 16 · JUNE 1 2005

JOURNAL OF CLINICAL ONCOLOGY ORIGINAL REPORT

Expression of bcl-2 in Classical Hodgkin's Lymphoma: An Independent Predictor of Poor Outcome

*Stephen J. Sup, Carlos A. Alemany, Brad Pohlman, Paul Elson, Serena Malhi, Snehal Thakkar, Roxanne Steinle, and Eric D. Hsi*

Prognostic biologic factors in Hodgkin's lymphoma

# Steidl C, Connors JM, Gascoyne RD. JCO 2011, 29:1812-26

**Table 2.** Biomarkers With Outcome Correlations Described in the Literature

| | Gene Expression Profiling | | |
|---|---|---|---|
| | Main Gene Components | Outcome Correlation | Reference No. |
| Angiogenic signature | *ADH1B, CD93, SRPX, PLA2G2A, GPR126* | Adverse (primary treatment failure) | 132 |
| Adipocyte signature | *GLUL, MGST1, COL1A2, FABP4* | Adverse (primary treatment failure) | 132 |
| Fibroblast function/extracellular matrix remodeling | Adverse: *MMP2, MMP3, TIMP1, COL1A1, COL4A1, COL4A2, COL5A1, COL18A1, COL16A1, MFAP2, THBS1/2, FN1, EDNRA, ITGB5, LAMA4*; favorable: *TIMP4, SPON1, LAMB1, TACR1, CCL26* | Discordant: adverse/favorable (primary treatment outcome) | 142,148 |
| B-cell signature | *BCL11A, BANK1, STAP1, BLNK, FCER2, CD24, CCL21* | Favorable (primary treatment outcome) | 132,140 |
| Cytotoxic T-cell signature | *CD3D, CD8B1, CTSL, CD26, SH2D1A, IFI16, RGS13, CR2, ELL3, CCDC23, PPM1L, TRA@, PIK3CA* | Adverse (primary treatment outcome) | 131,132,142 |
| Plasmacytoid dendritic cells | *ITM2A, SRPX, CTSB, APP* | Adverse (primary treatment outcome) | 132 |
| Macrophage signature | *ALDH1A1, LYZ, STAT1, ITGA4, CCL13, MS4A4A, CCL23, VCAN, HSP90AB3P, HSP90AB1, CTSB, CFL1, JMJD6, MAPK7, IKBKG, RAB7A, RXRA, MAPK13* | Adverse (primary treatment outcome) | 131,132,142 |


**131.** Sánchez-Espiridión B, Sánchez-Aguilera A, Montalbán C, et al: A TaqMan low-density array to predict outcome in advanced Hodgkin's lymphoma using paraffin-embedded samples. Clin Cancer Res 15:1367-1375, 2009

**132.** Steidl C, Lee T, Shah SP, et al: Tumor-associated macrophages and survival in classic Hodgkin's lymphoma. N Engl J Med 362:875-885, 2010

**140.** Chetaille B, Bertucci F, Finetti P, et al: Molecular profiling of classical Hodgkin lymphoma tissues uncovers variations in the tumor microenvironment and correlations with EBV infection and outcome. Blood 113:2765-3775, 2009

**142.** Sánchez-Aguilera A, Montalbán C, de la Cueva P, et al: Tumor microenvironment and mitotic checkpoint are key factors in the outcome of classic Hodgkin lymphoma. Blood 108:662-668, 2006

**148.** Devilard E, Bertucci F, Trempat P, et al: Gene expression profiling defines molecular subtypes of classical Hodgkin's disease. Oncogene 21:3095-3102, 2002

# Macrophages predict treatment outcome in Hodgkin's lymphoma

**Christian Steidl, Pedro Farinha, and Randy D. Gascoyne**

*Departments of Pathology and Experimental Therapeutics, British Columbia Cancer Agency and the BC Cancer Research Centre, BC Cancer Agency, University of British Columbia, Vancouver, BC, Canada*

*E-mail: rgascoyn@bccancer.bc.ca* *doi:10.3324/haematol.2010.033316*

**Haematologica 2010, 96:186-9**

| Markers used | Method | # | Outcome correlation | Reference |
|---|---|---|---|---|
| PNA | Histochemistry | 43 | Adverse (refractory disease, early relapse) | Ree *et al.*, Cancer 1985[10] |
| STAT1, ALDH1A1 | GE, IHC | 235 | Adverse (disease-specific survival) | Sanchez-Aguilera *et al.*, Blood 2006[11] |
| LYZ, STAT1, ALDH1A1 | GE, IHC | 194 | Adverse (refractory disease, early relapse) | Sanchez-Espiridion *et al.*, Clincial Cancer Research 2009[13] |
| CD68 | IHC | 166 | Adverse (progression-free survival, disease-specific survival) | Steidl *et al.*, NEJM 2010[9] |
| LYZ, STAT1 | GE | 262 | Favorable (failure-free survival) | Sanchez-Espiridion *et al.*, Blood 2010[12] |
| CD68, CD163 | IHC | 288 | Adverse (event-free survival, overall survival) | Kamper *et al.*, Haematologica 2011[8] |
| CD68 | IHC | 59 | Adverse (refractory disease) | Benedicte *et al.*, Blood 2010 [abstr.][34] |
| CD68 (also in combination with FOXP3) | IHC | 122 | Adverse (freedom from treatment failure, overall survival) | Greaves *et al.*, Blood 2010 [abstr.][25] |
| CD68 | IHC | 144 | Adverse (event-free survival, disease-specific survival) | Yoon *et al.*, Blood 2010 [abstr.][35] |
| CD68 | IHC | 105 | Adverse (overall survival) | Tzankov *et al.* [personal communication] |
| CD68 | IHC | 45 | Adverse (progression-free survival) | Hohaus & Larocca [personal communication] |
| CD68 | IHC | 153 | Adverse (overall survival, progression-free survival) | Farinha *et al.* [abstr.][36] |

*PNA: peanut agglutinin, GE: gene expression (mRNA), IHC: immunohistochemistry.*

# Macrophages predict treatment outcome in Hodgkin's lymphoma

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*Departments of Pathology and Experimental Therapeutics, British Columbia Cancer Agency and the BC Cancer Research Centre, BC Cancer Agency, University of British Columbia, Vancouver, BC, Canada*

*E-mail: rgascoyn@bccancer.bc.ca* *doi:10.3324/haematol.2010.033316*

**Haematologica 2010, 96:186-9**

## Antibody and scoring system

Further study is needed to determine the optimal antigen (e.g. CD68 *versus* CD163), anti-CD68 antibody clone (e.g. KP1 *versus* PGM1) and scoring thresholds (e.g. manual *versus* computer-assisted) for detecting HL-associated macrophages.

## Biomarker combination

The preliminary results of an immunohistochemistry study combining two markers, CD68 and FOXP3 (a marker for regulatory T cells), were presented at the ASH 2010 meeting. The authors showed that a combined FOXP3/CD68 immunohistochemistry score was an improvement over the predictive value of the individual markers alone and that this score was applicable to both limited and advanced-stage disease. The value of this com-

## EBV infection

In a re-analysis of our data we were able to confirm a relationship between increased tumor-associated macrophages and EBV positivity; however, virtually all of our cases were of the nodular sclerosis subtype and EBV alone was not associated with treatment outcome (*unpublished observations, 2010*). EBV infection of HRS cells has been reported in up to 60% of patients and is more frequent in mixed cellularity subtype, although varying with geographical location, age, gender, clinical stage and histological subtype.[14] The impact of EBV infection on outcome remains controversial, but appears to be dependent on age.

# Bologna study

- Biopsy samples from cHL patients at diagnosis enrolled by 13 Italian and 3 Danish haematological centres
- Construction of TMAs to collect cases of interest in the same block and optimization of immunohistochemical procedures
- Ab tested:

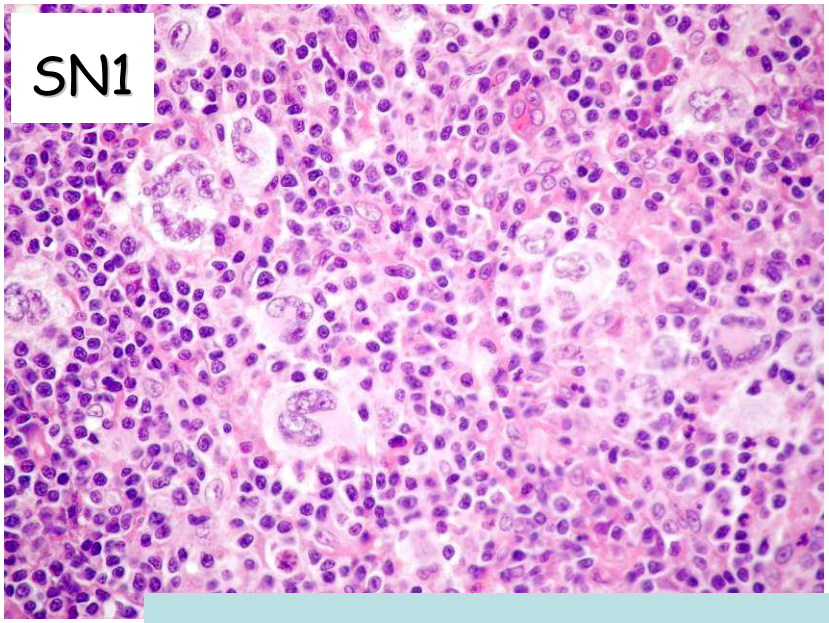
  - 11 proteins encoded by genes shown as prognostically relevant by DNA-microarray studies (STAT1, PCNA, SAP, TOP2A, RRM2, CDC2, MAD2L1, ALDH1A1, CD68, CD163, and BCL11a)

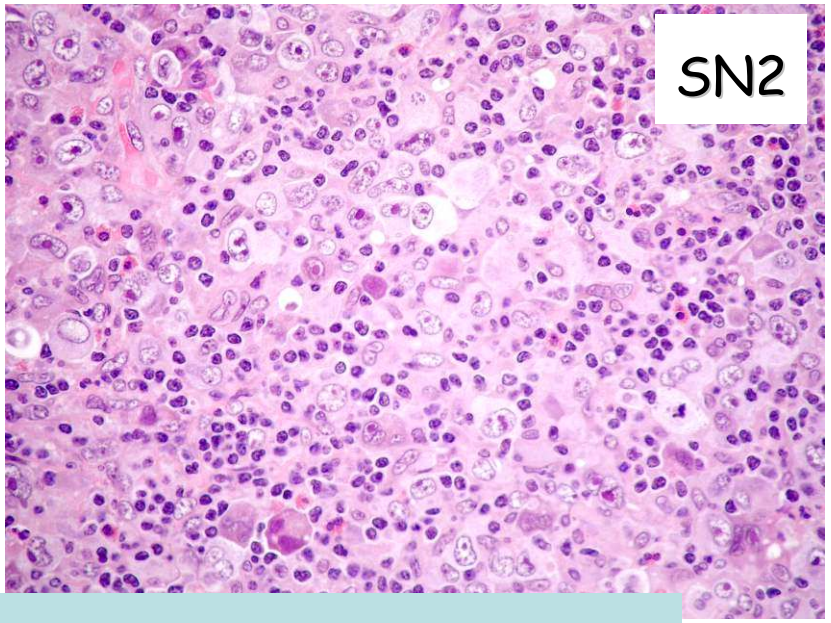
  - 9 markers previously reported to have prognostic value in conventional studies (CD20, EBER, Bcl-2, p53, PD1, FOXP3, TIA1, Granzyme B, and Perforin)
- The molecules were assessed in both neoplastic (HRSC) and micro-environmental cell (MEC) components
- Evaluation of the prognostic impact of such markers on Hodgkin's lymphoma outcome
- Comparison with the predictive value of ad interim PET
- Construction of a predictive model

# Inclusion criteria

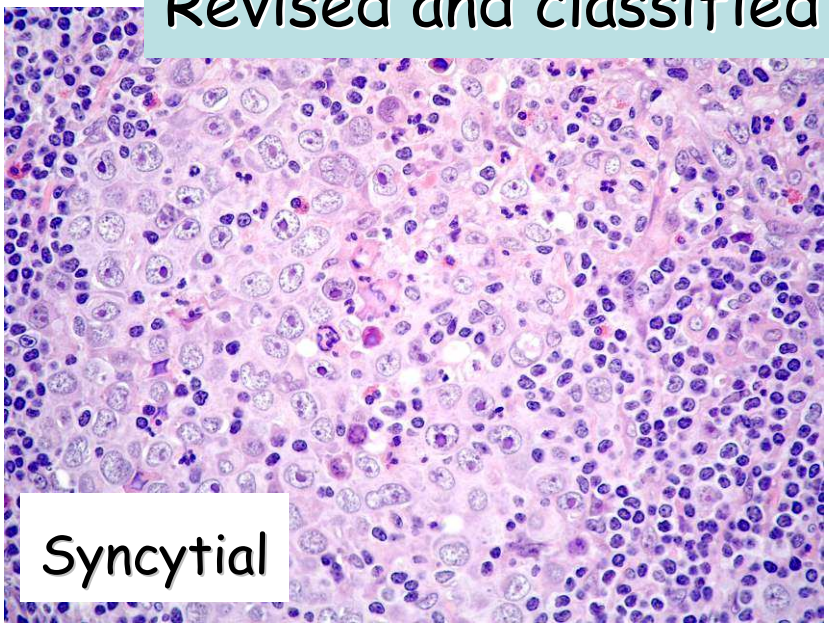
- Diagnosis of cHL
- HIV negative status
- Biopsy sample at diagnosis available
- Clinical and follow-up (FU) data were always available
- Treatment with courses of ABVD with or without radiotherapy
- FDG-PET appraisal of the treatment response performed after two courses of chemotherapy (PET-2) available

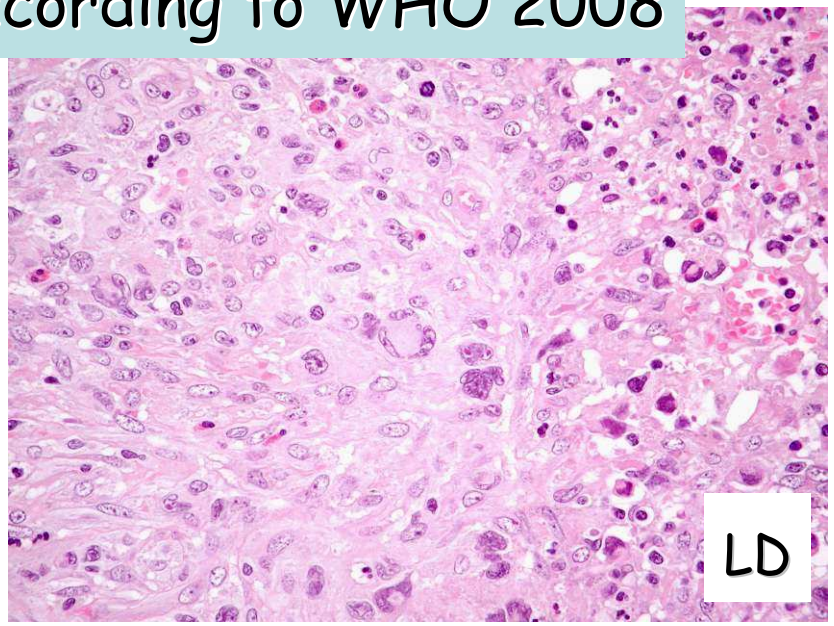
209 cases enrolled




Revised and classified according to WHO 2008



# Patients' characteristics

| Age, years | |
|---|---|
| mean | 36 |
| median | 32 |
| range | 14-80 |
| Sex | |
| male | 103 (49,3%) |
| female | 106 (50,7%) |
| Follow-up, months | months |
| mean | 50,26 |
| median | 52,30 |
| range | 3-93 |
| Histologic subtype | |
| CHL, nos | 19 (9,1%) |
| NS-nos | 10 (4,8%) |
| NS-cellular phase | 9 (4,3%) |
| NS-1 | 93 (44,5%) |
| NS-2 | 37 (17,7%) |
| NS-syncytial variant | 8 (3,8%) |
| MC | 29 (13,9%) |
| LR | 1 (0,5%) |
| LD | 3 (1,4%) |

| Ann Arbor Stage | |
|---|---|
| I | 9 (4,3%) |
| II | 118 (56,5%) |
| III | 50 (23,9%) |
| IV | 32 (15,3) |
| Constitutional symptoms | |
| A | 110 (52,6%) |
| B | 99 (47,4) |
| Bulky disease | |
| Yes | 42 (20.1%) |
| No | 167 (79,9%) |
| First-line treatment | |
| ABVD | 209 (100%) |
| RT | 52 (24,9) |
| PET after 2 cycles | |
| Negative | 171 (81,9%) |
| Positive | 38 (18,1%) |
| Clinical outcome | |
| Failure | 49 (23.4%) |
| *progression* | 31 |
| *relapse* | 18 |

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- Comparison with the predictive value of ad interim PET
- Construction of a predictive model

## Scoring system for HRSC markers

score 0 : 0%(+)

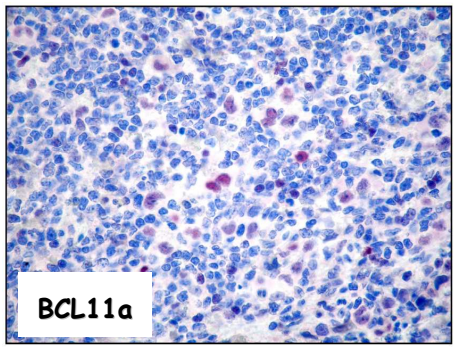
score 1 : 1-9%(+)

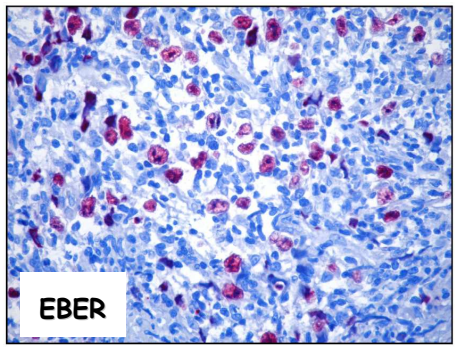
score 2: 10-24%(+)

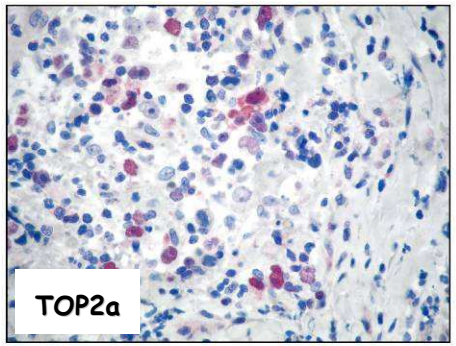
score 3: 25-49%(+)

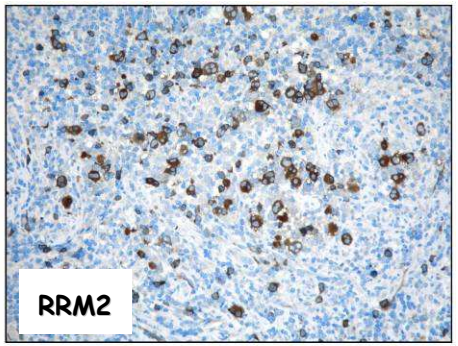
score 4: 50-74%(+)

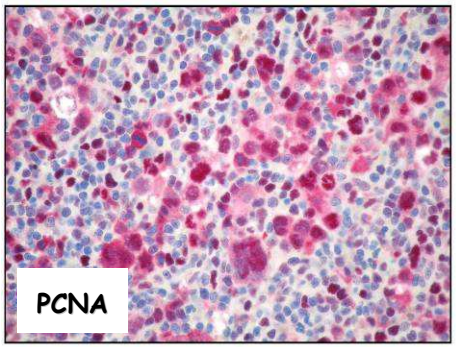
score 5: >75%(+)

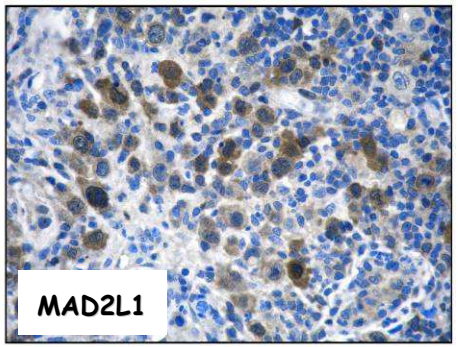


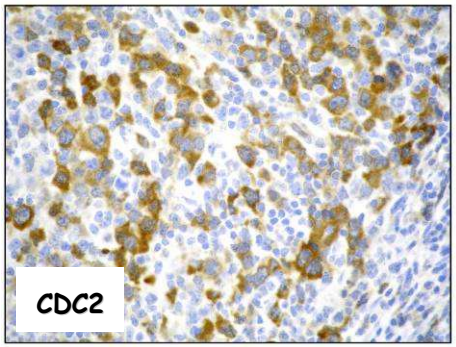



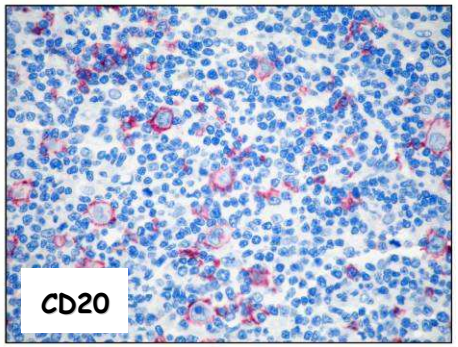



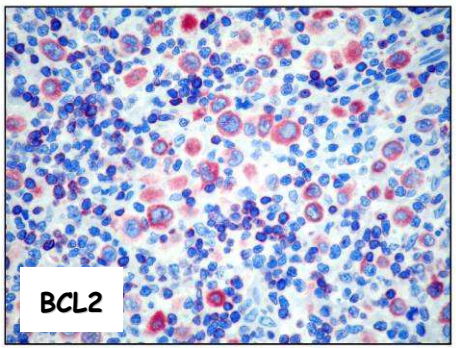



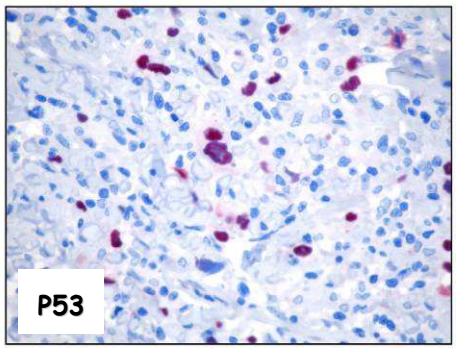



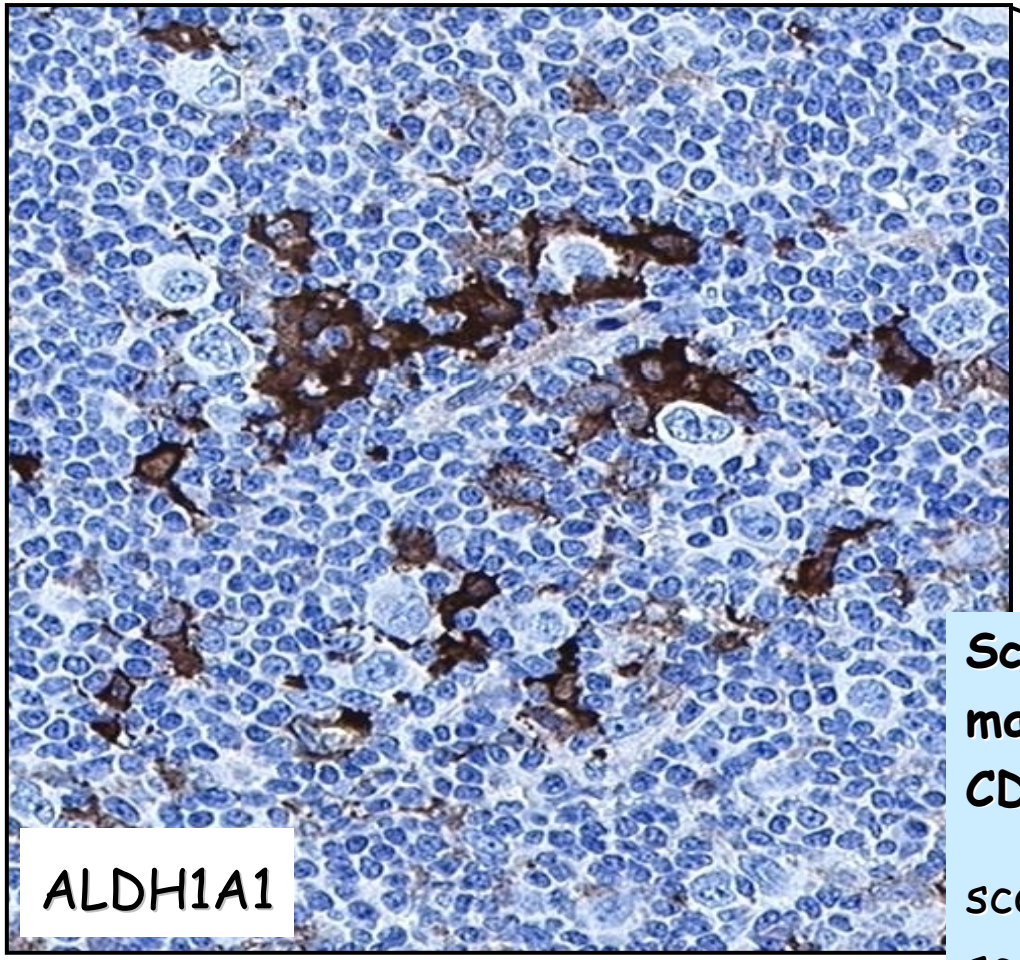



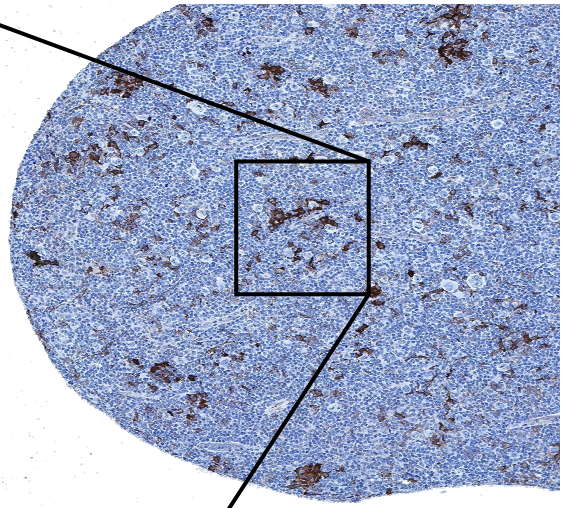





**Scoring system for macrophage markers (ALDH1A1, CD68/PGM1, CD68/KP1, CD163)**

score 0 : 0%(+)

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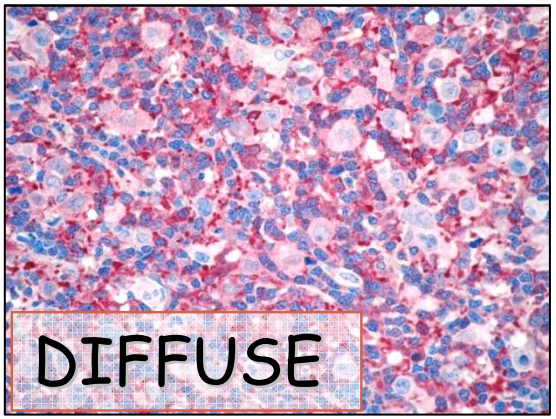
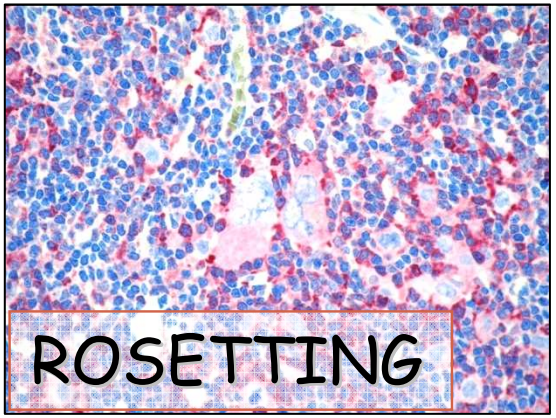
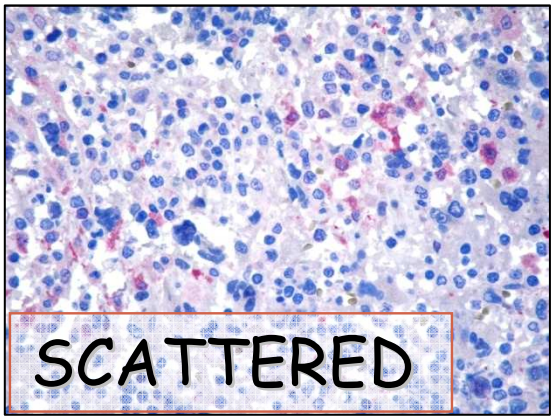
score 5: >75%(+)



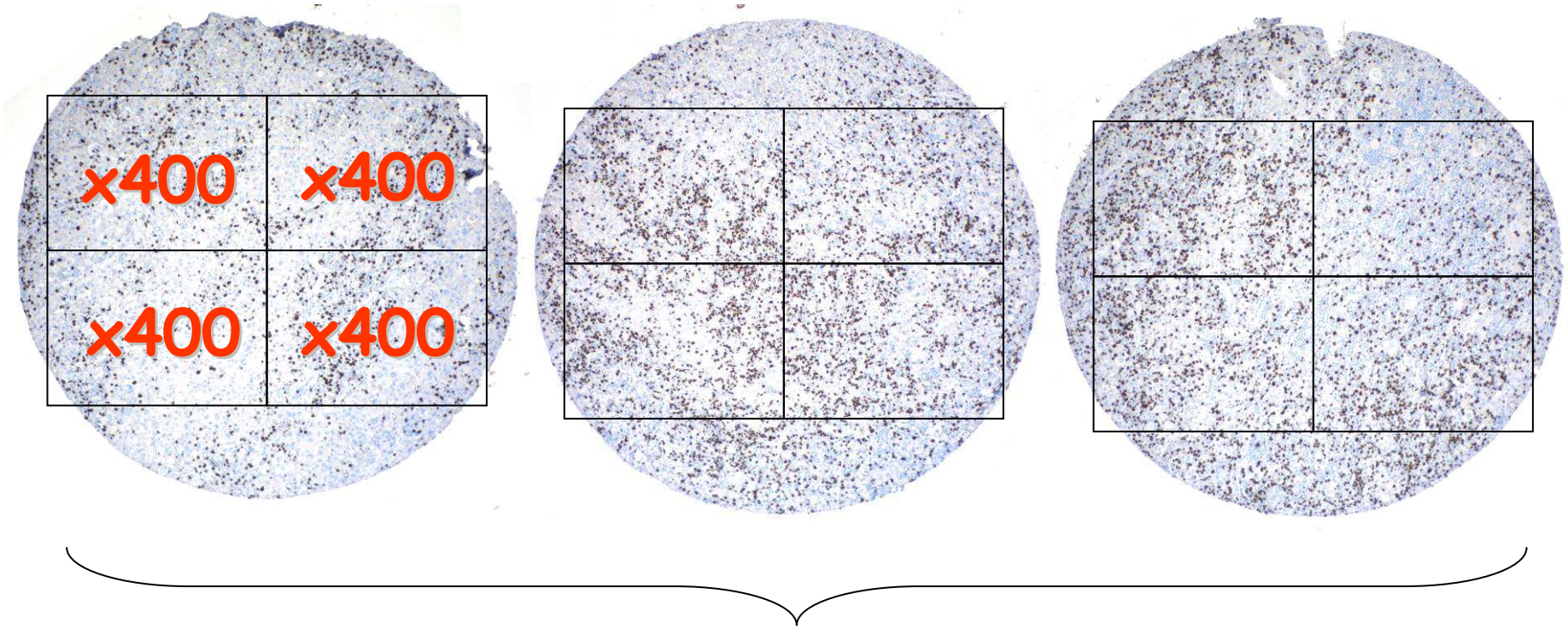




## STAT1, SAP, PD1 microenvironment expression patterns

- **Diffuse** : diffuse pattern of staining in MC cells between and surrounding neoplastic cells
- **Rosetting** : expressed only in cells forming rosettes around neoplastic cells
- **Scattered** : few cells positive in the microenvironment

# FOXP3 and Cytotoxic markers (Tia1, GyB, Perforin) evaluation

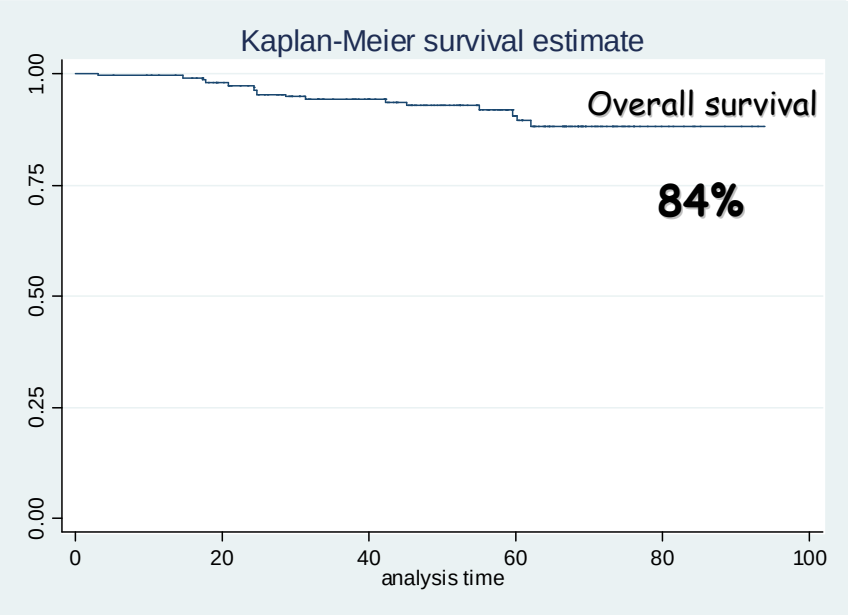


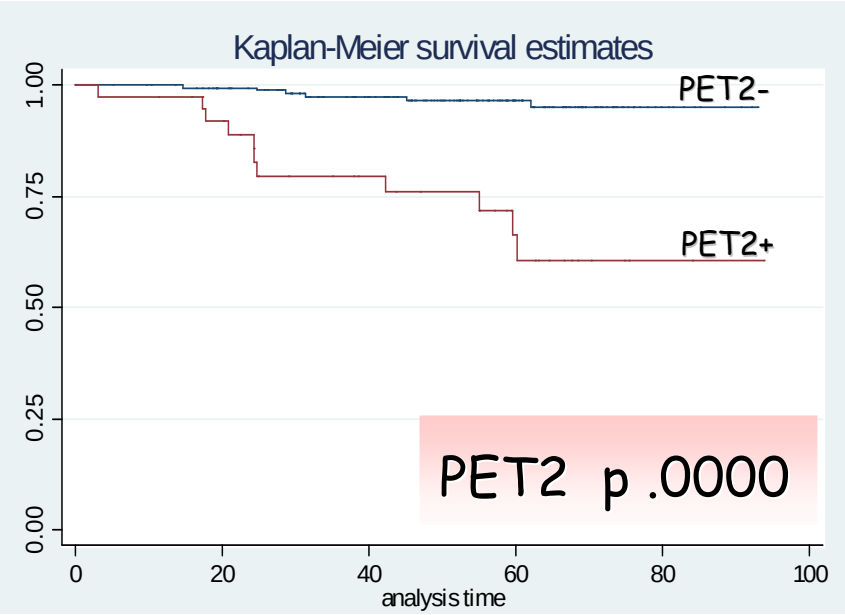

mean value
calculated on evaluable cores

# Data processing

- Every result evaluated to find correlation with patients' outcome: percentage and intensity of expression, nuclear or cytoplasmic localization, both in tumour cells and microenvironment
- Every cut-off assessed
- Every pattern tested

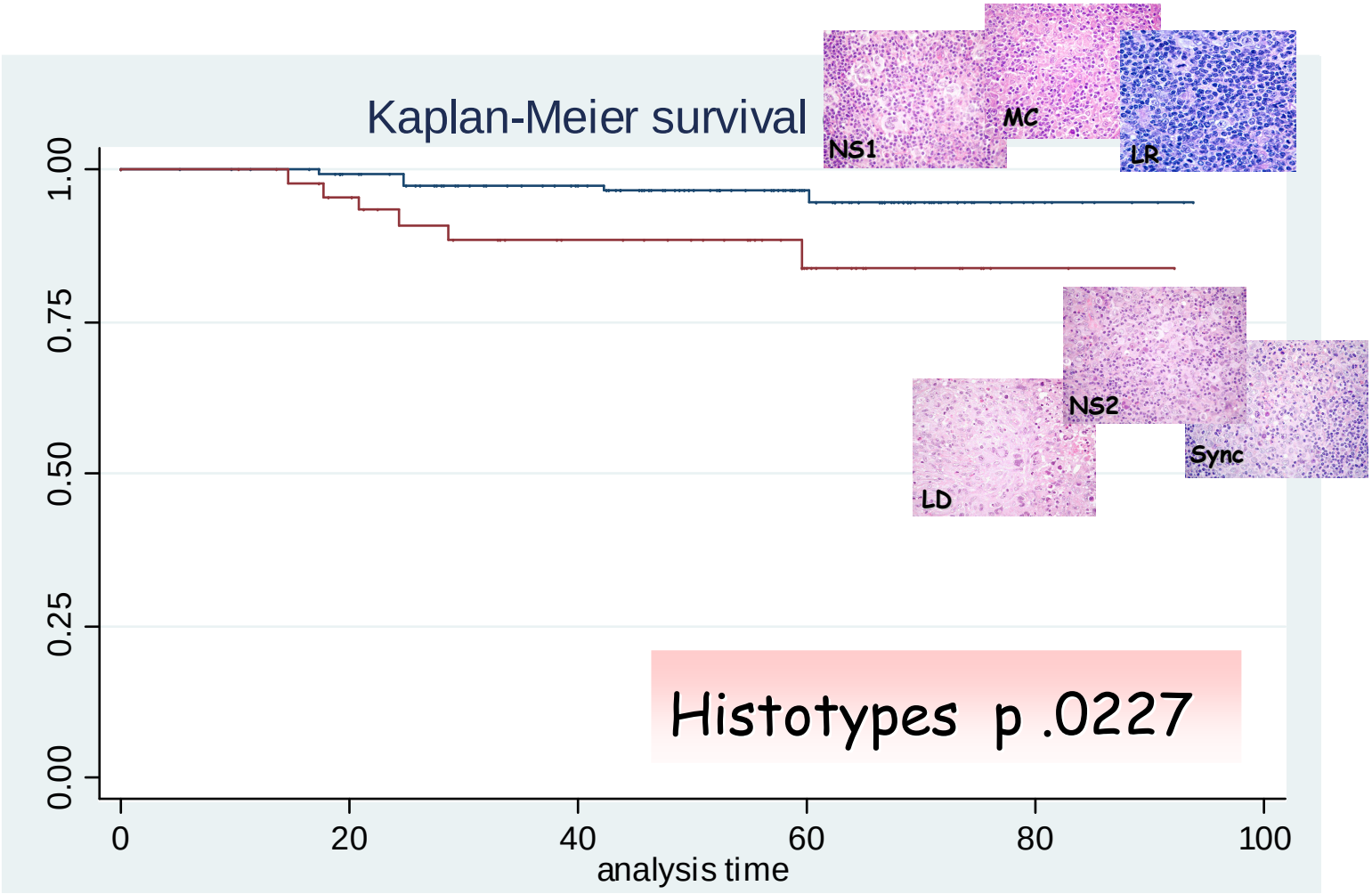
# Overall survival



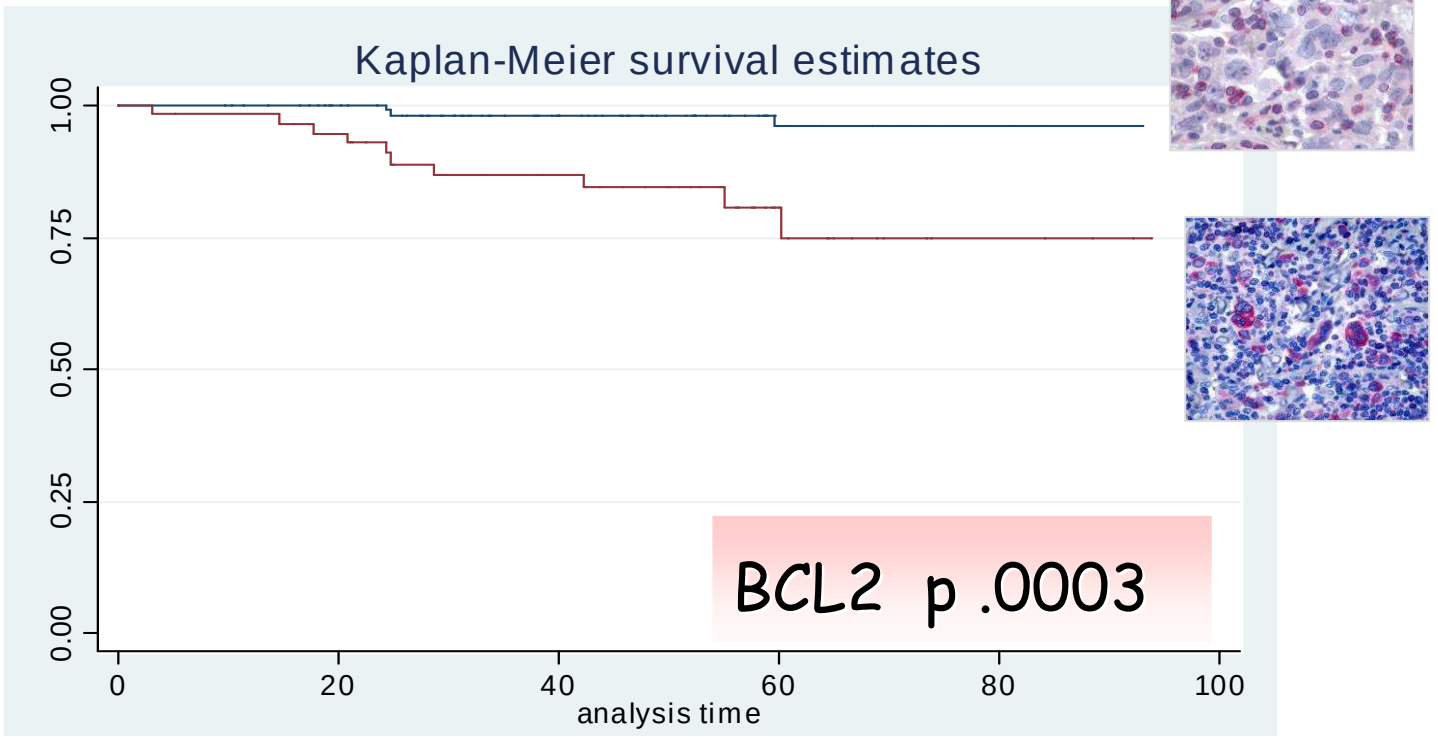

**Median follow-up: 62.3 months**

# Overall survival



Uni-variate analysis

# Overall survival



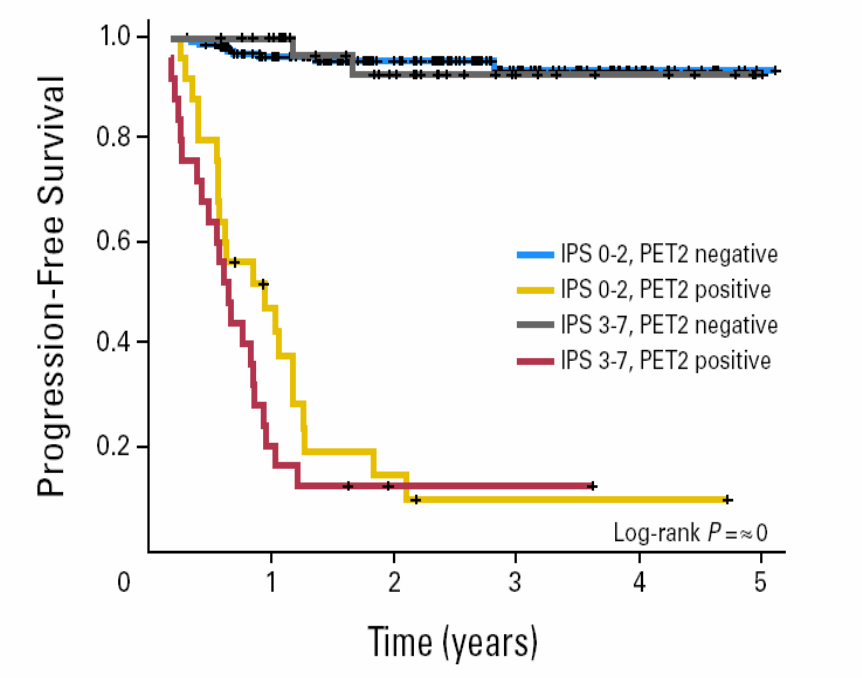


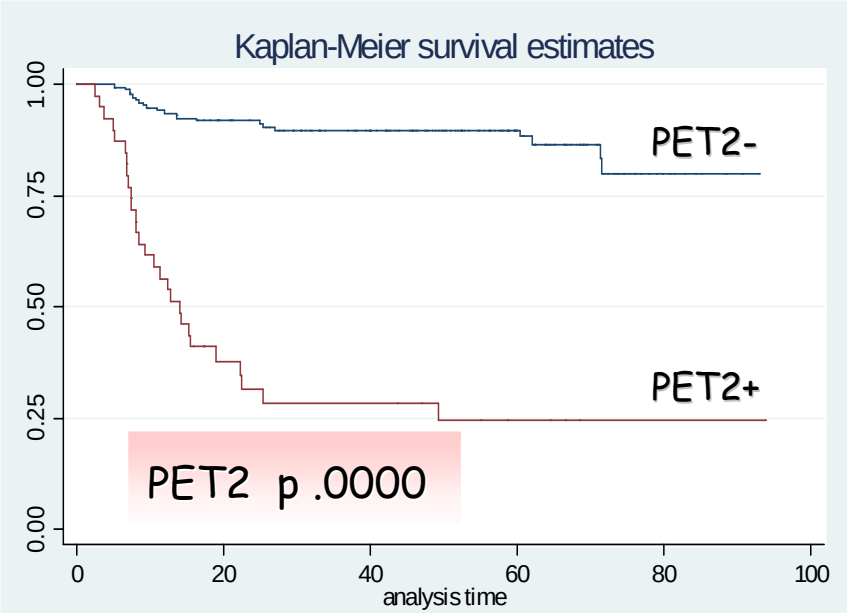



| variable | cut-off | n | Hazard ratio of event risk | 95% C.I. | P |
|---|---|---|---|---|---|
| BCL2 | ≥ 50% | 33,5% | 7.63 | (2.09-27.80) | .0003 |

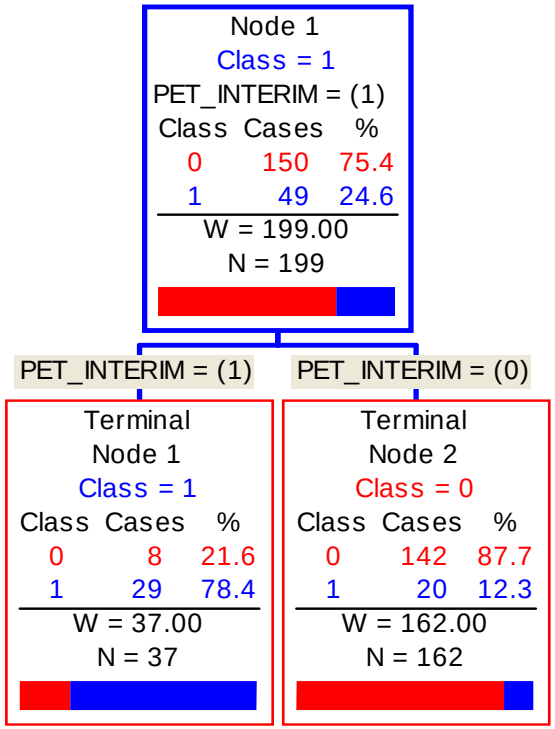
| variable | cut-off | OS- 93 months | 95% C.I. |
|---|---|---|---|
| BCL2 | < 50% | 96% | (87.2-98.8) |
| BCL2 | ≥ 50% | 75% | (55.4-86.9) |

# Progression Free Survival



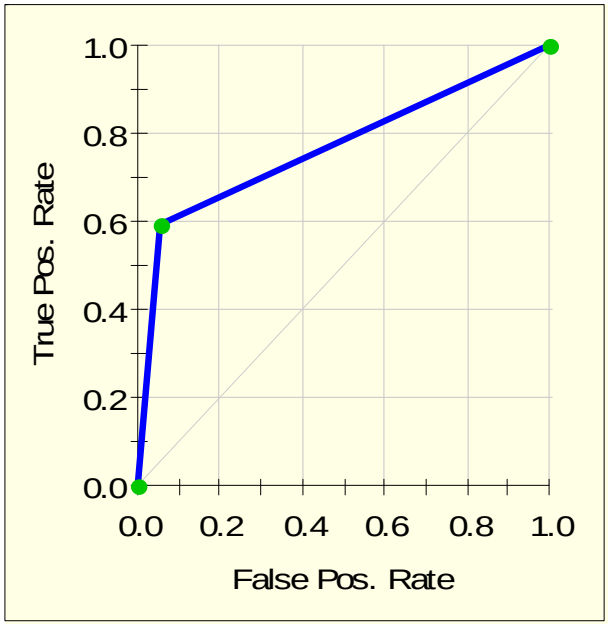



# Misclassification for Learn and Test Data



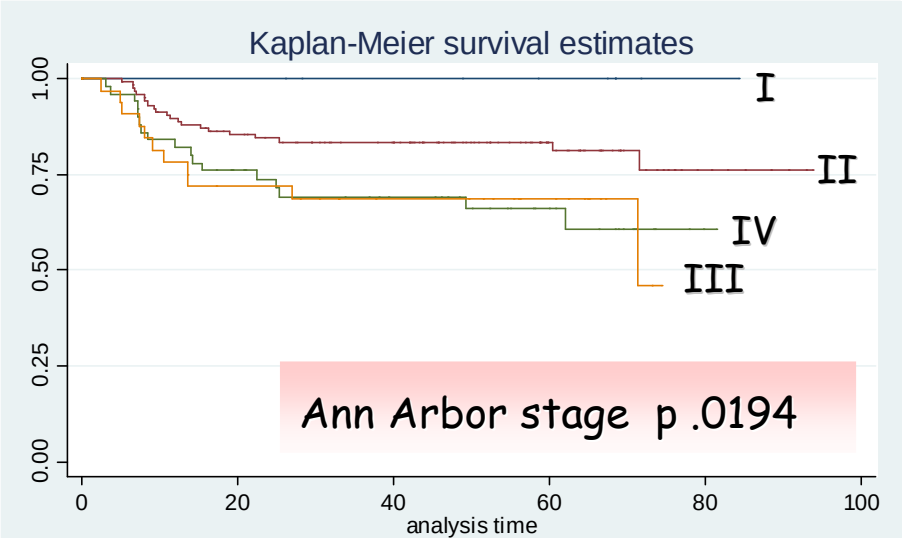

| Class | N Cases | N Mis-Classed | Pct Error |
|---|---|---|---|
| 0 | 150 | 8 | 5.33 |
| 1 | 49 | 20 | 40.82 |
| Tot | 199 | **28** | **14.07** |

## ROC Integral: 0.770

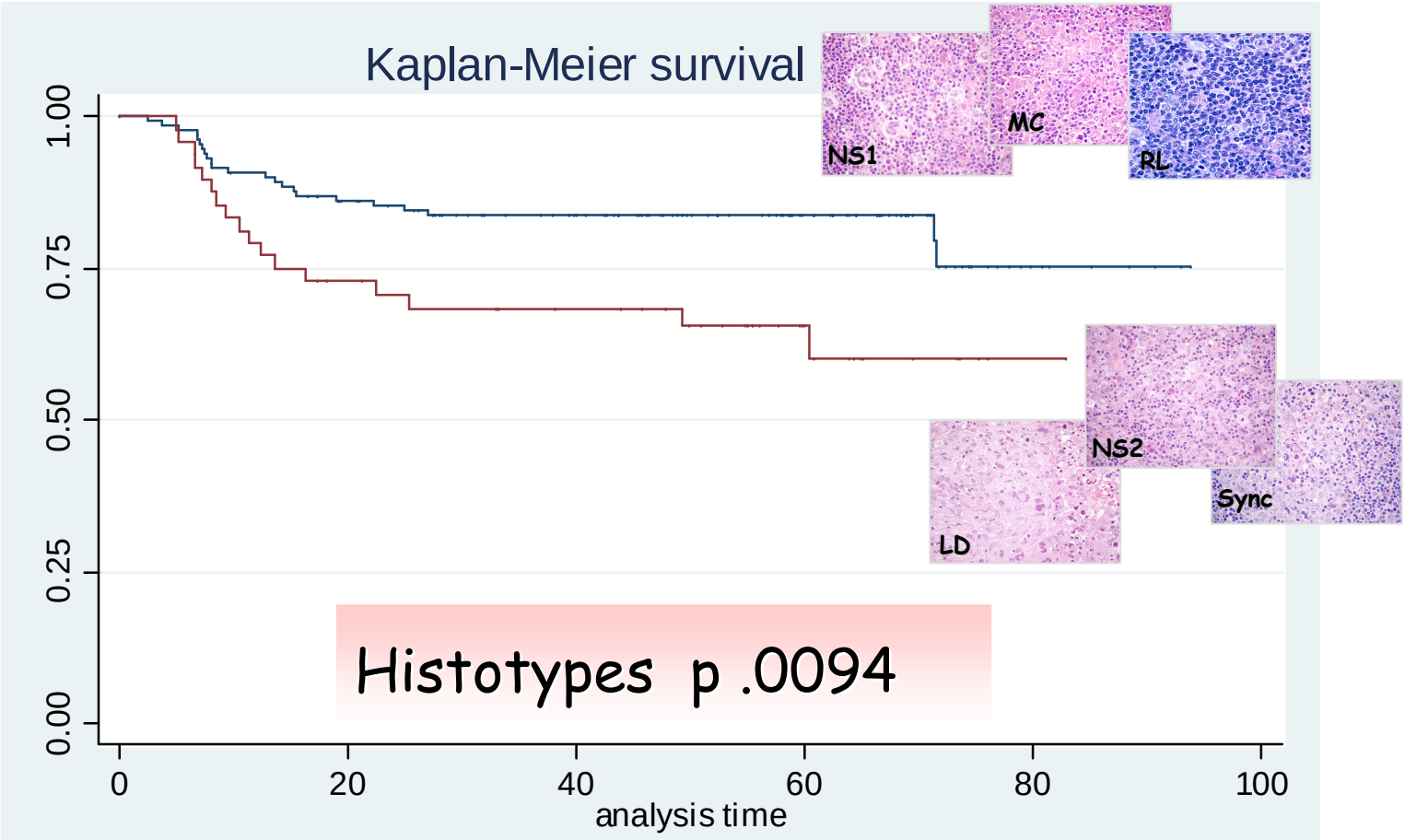


# Progression Free Survival

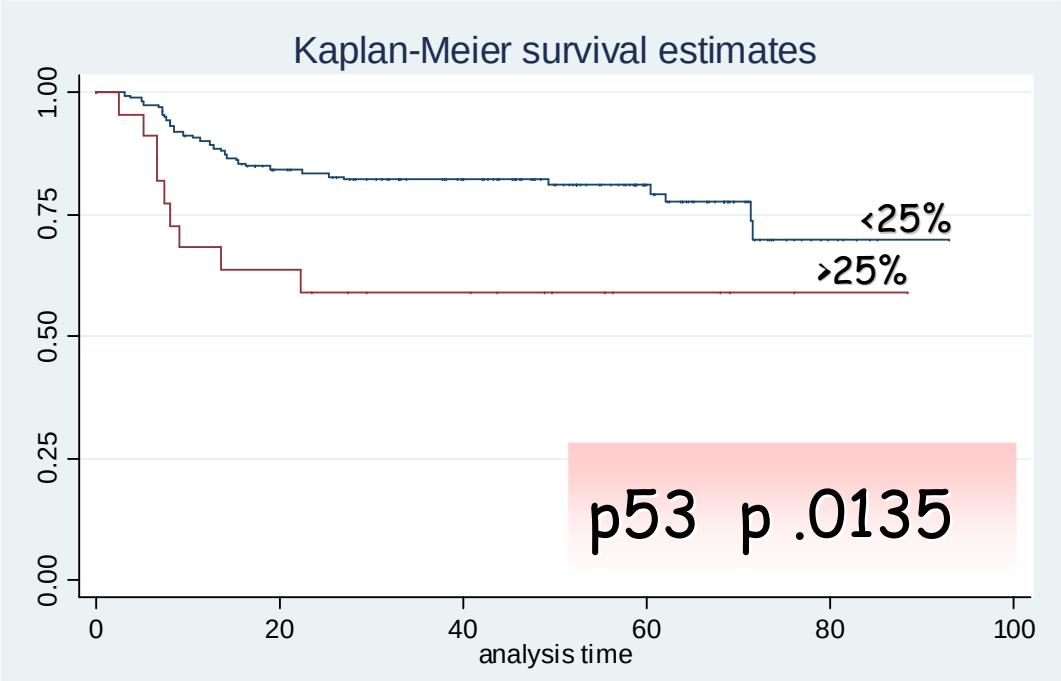
| variable | Hazard ratio of event risk | 95% C.I. | P |
|---|---|---|---|
| Stage I | 0 | | |
| Stage II | 4.03 | (1.94-8.36) | .000 |
| Stage III | 8.31 | (3.89-17.80) | .000 |
| Stage IV | 8.59 | (4.93-11.17) | .000 |



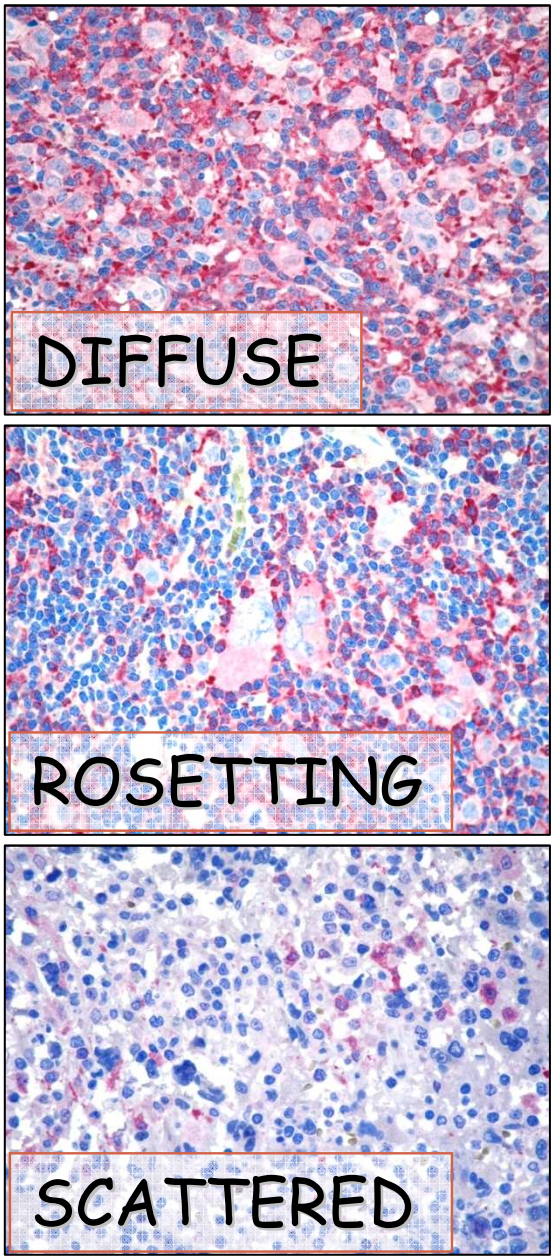
# Progression Free Survival



# Progression Free Survival



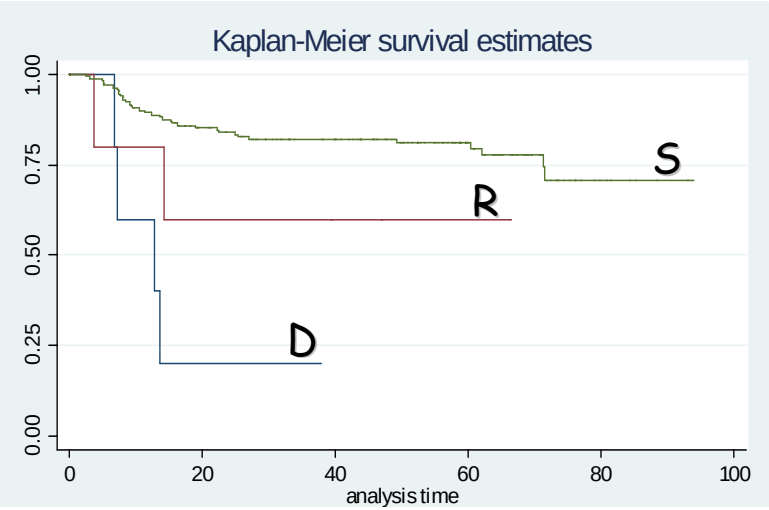

| variable | cut-off | PFS- 93 months | 95% C.I. |
|---|---|---|---|
| p53 | < 25% | 69.3% | (55.3-79.6) |
| p53 | ≥ 25% | 61.1% | (35.3-79.1) |



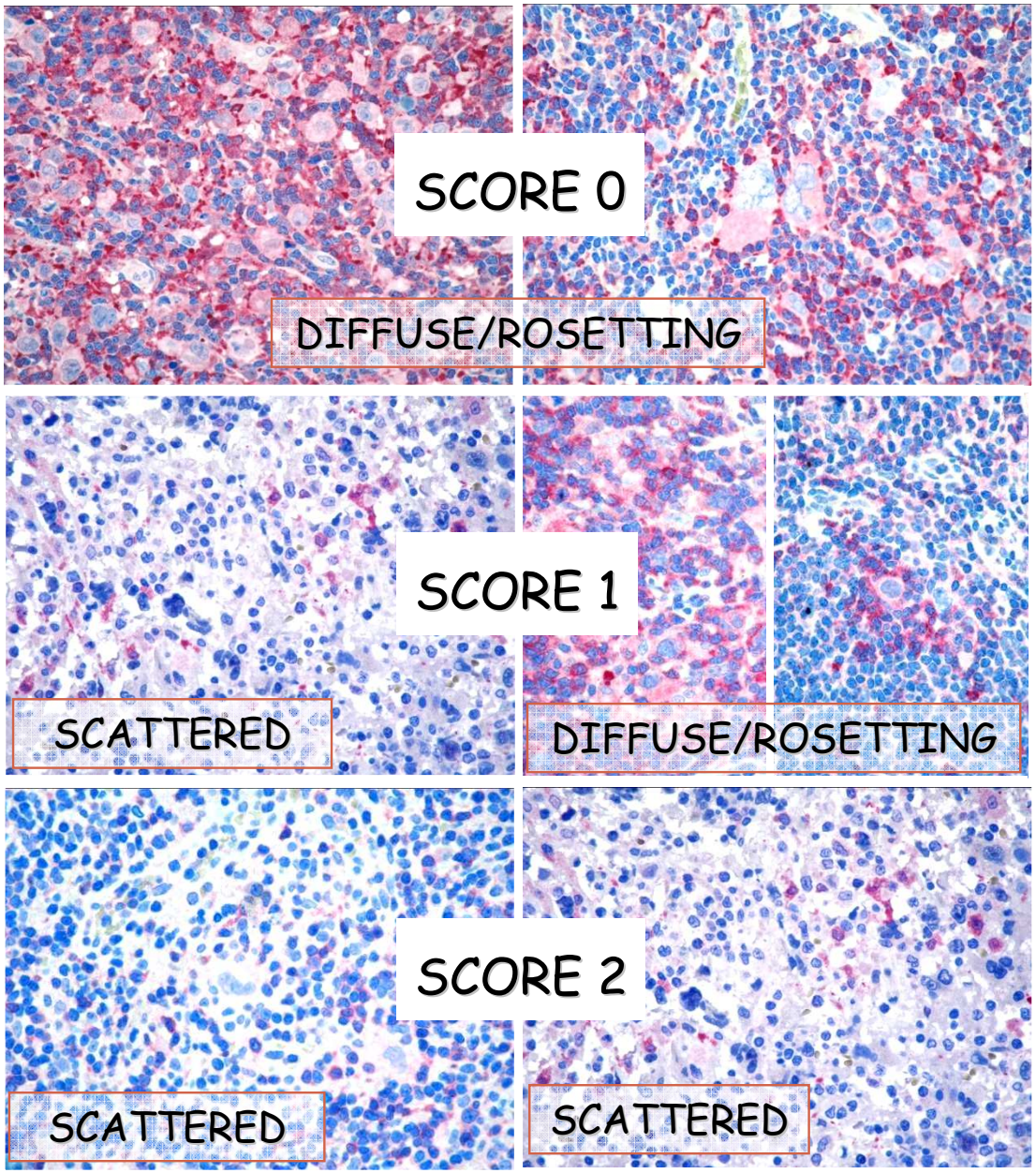

# PD1 and PFS

Membranous staining

- PD1 is involved in regulation of TCR-signaling
- Expressed by follicular helper T-cells
- In our series, the expression of PD1 by lymphocytes of microenvironment is related to adverse outcome (p.0000)




Prognostic biologic factors in Hodgkin's lymphoma

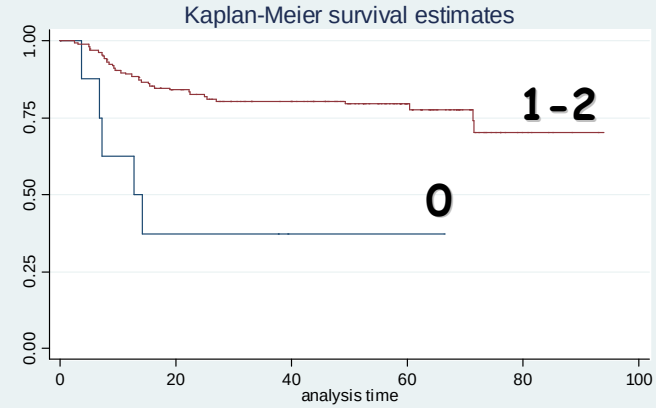



# PD1/SAP

## uni-variate analysis PFS

Combined expression of FTH markers in microenvironment is associated with worse prognosis ( p .0018 ):

Score 0 = worse prognosis




Prognostic biologic factors in Hodgkin's lymphoma

# Multivariate analysis

## Cox's regression model:

### Overall Survival

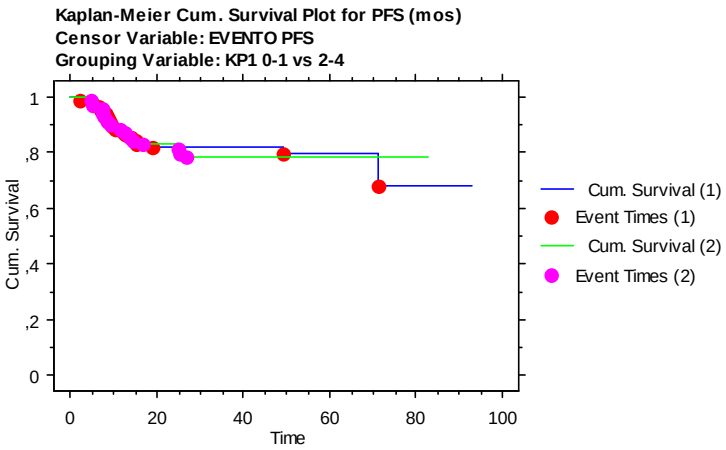
| variable | Hazard ratio of event risk | 95% C.I. | P |
|---|---|---|---|
| BCL2 | 1.51 | (1.06-2.15) | .021 |
| PET2 | 11.5 | (3.0-43.5) | .000 |

### Progression Free Survival

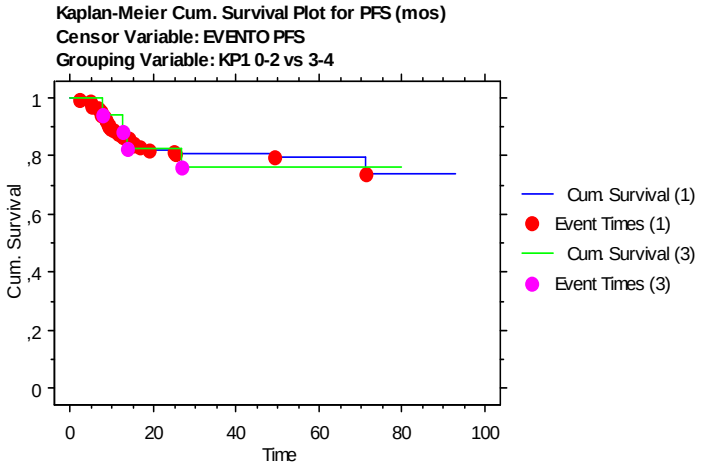
| variable | Hazard ratio of event risk | 95% C.I. | P |
|---|---|---|---|
| Stage | 2.16 | (1.46-3.09) | .000 |
| P53 | 3.64 | (1.55-8.51) | .003 |
| PET2 | 14.97 | (7.53-29.78 ) | .000 |

Prognostic biologic factors in Hodgkin's lymphoma

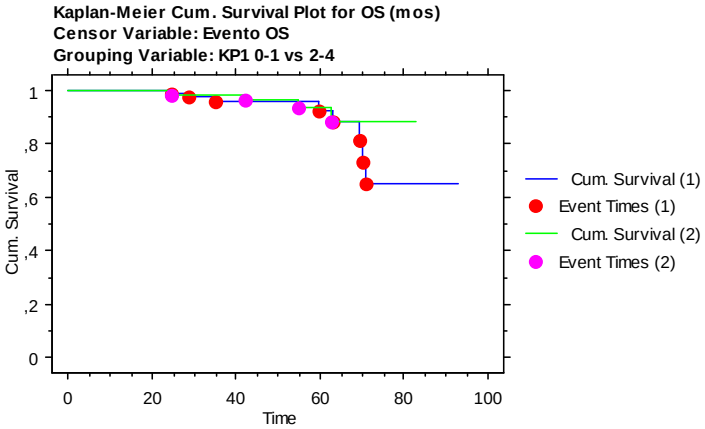
# CD68/KP1



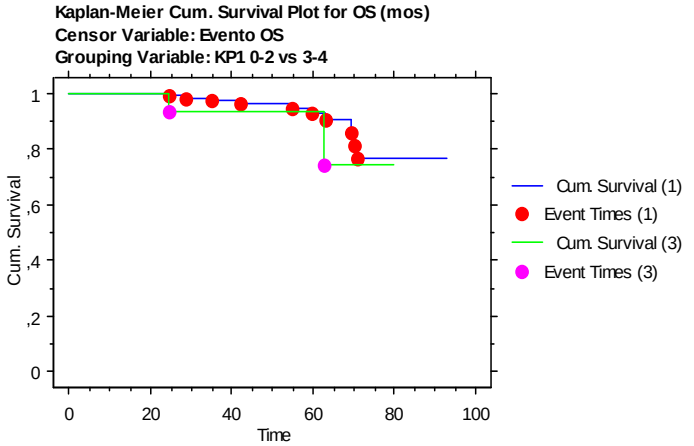

PFS, Cut off 5%, p=0.95




PFS, Cut off 25%, p=0.81



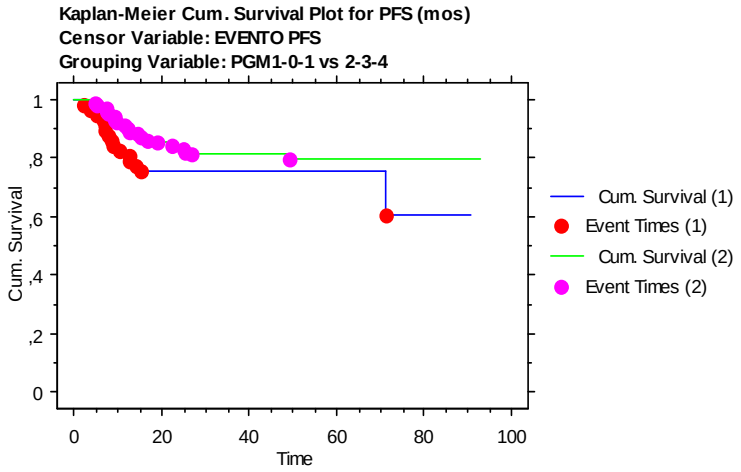

OS, Cut off 5%, p=0.36



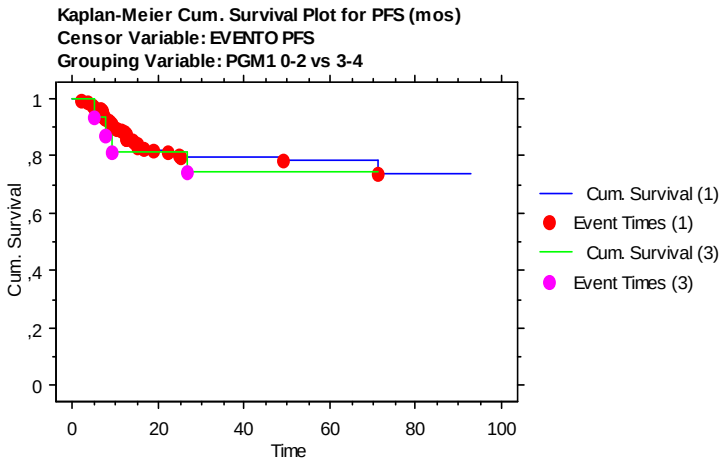

OS, Cut off 25%, p=0.5

Prognostic biologic factors in Hodgkin's lymphoma

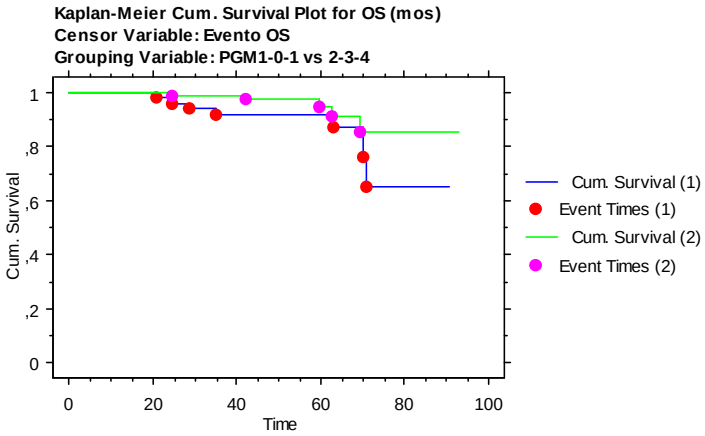
# CD68/PGM1



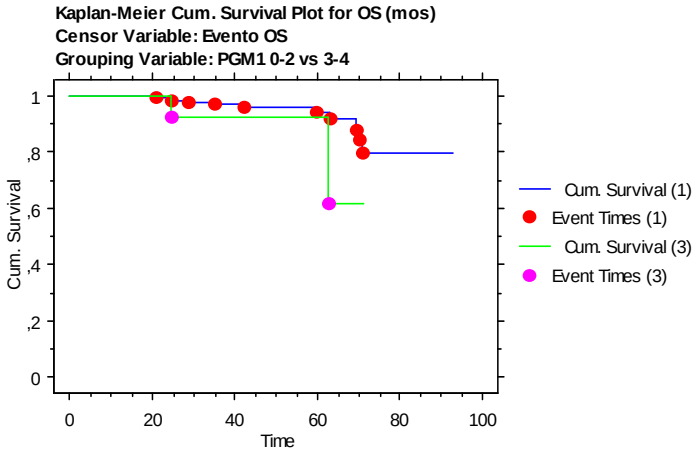

**PFS, Cut off 5%, p=0.26**




**PFS, Cut off 25%, p=0.67**



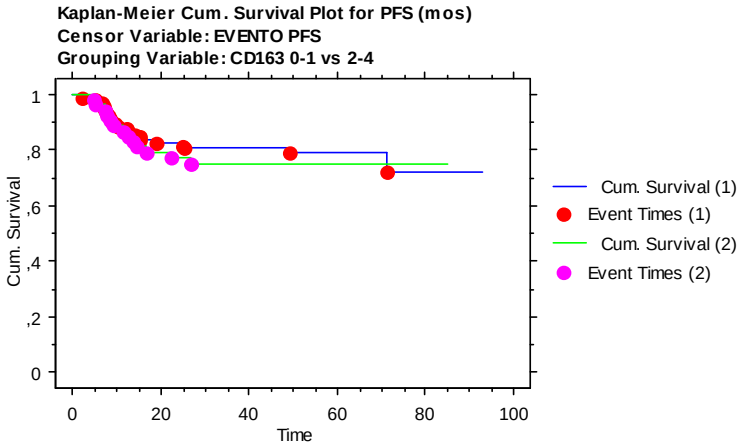

**OS, Cut off 5%, p=0.1**



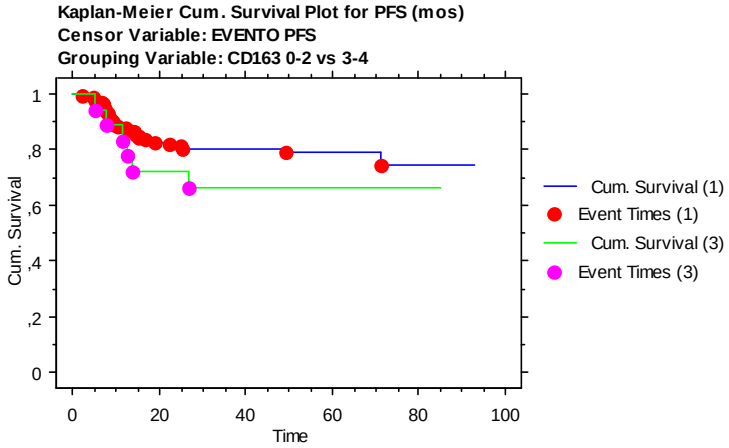

**OS, Cut off 25%, p=0.2**

Prognostic biologic factors in Hodgkin's lymphoma

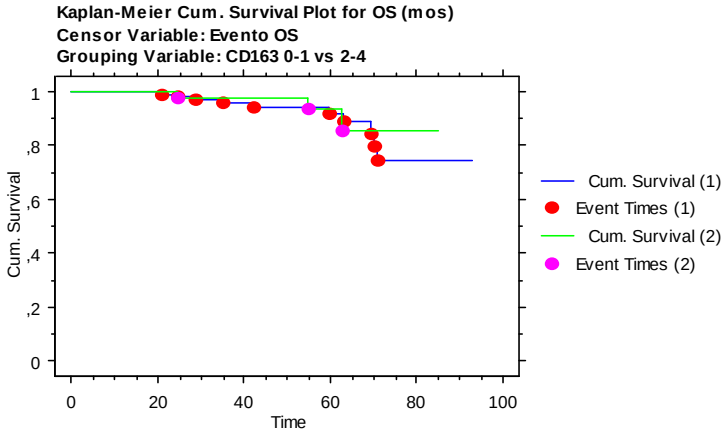
# CD163



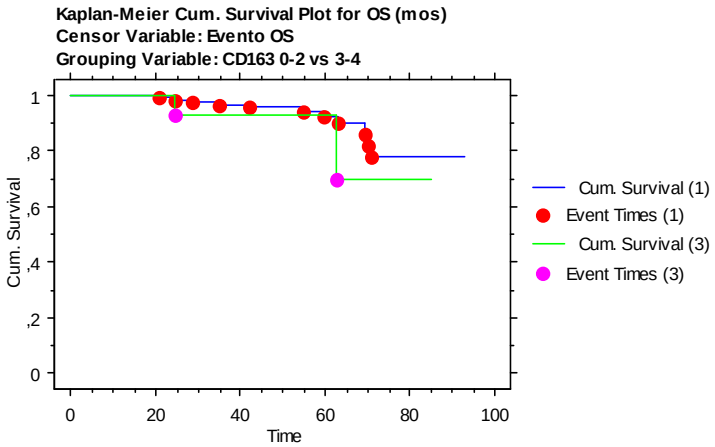

PFS, Cut off 5%, p=0.64




PFS, Cut off 25%, p=0.2



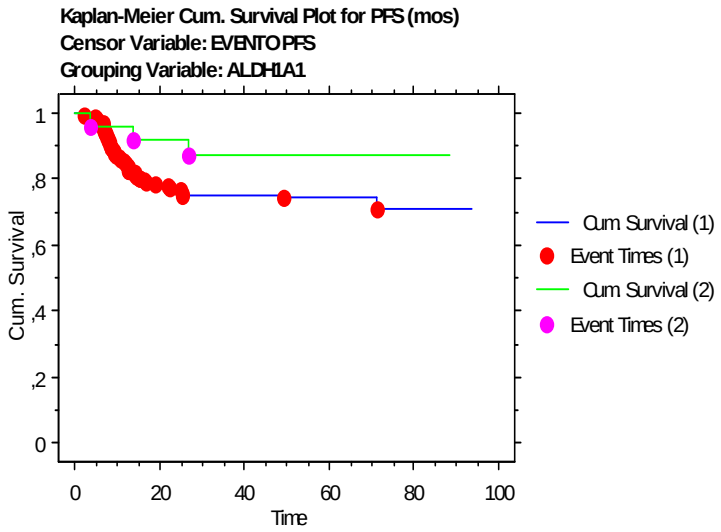

OS, Cut off 5%, p=0.36



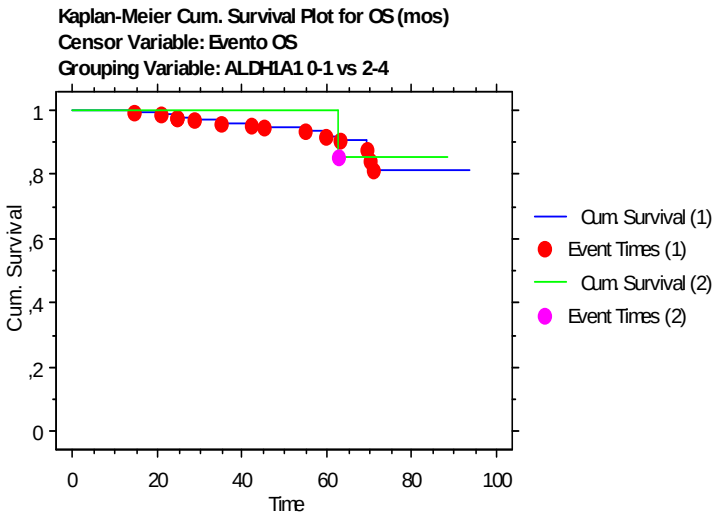

OS, Cut off 25%, p=0.34

Prognostic biologic factors in Hodgkin's lymphoma

# ALDH1A1




**PFS, Cut off 5%, p=0.1**




**OS, Cut off 5%, p=0.5**

Ann Oncol (2011) doi: 10.1093/annonc/mdr157
First published online: May 20, 2011

# Lack of association of tumor-associated macrophages with clinical outcome in patients with classical Hodgkin's lymphoma

D. Azambuja[1], Y. Natkunam[2], I. Biasoli[3], I. S. Lossos[4], M. W. Anderson[2], J. C. Morais[3] and N. Spector[3,*]

## Abstract

**Background**: A recent study demonstrated that an increased number of CD68+ macrophages were correlated with primary treatment failure, shortened progression-free survival (PFS) and disease-specific survival (DSS) in patients with classical Hodgkin's lymphoma (cHL).

**Patients and methods**: The aim of the present study was to verify the relationship between the number of CD68+ and CD163+ macrophages with clinical outcomes in a cohort of 265 well-characterized patients with cHL treated uniformly with the standard doxorubicin, bleomycin, vinblastine and dacarbazine chemotherapy regimen. Two pairs of hematopathologists carried out independent pathological evaluations of tissue microarray slides.

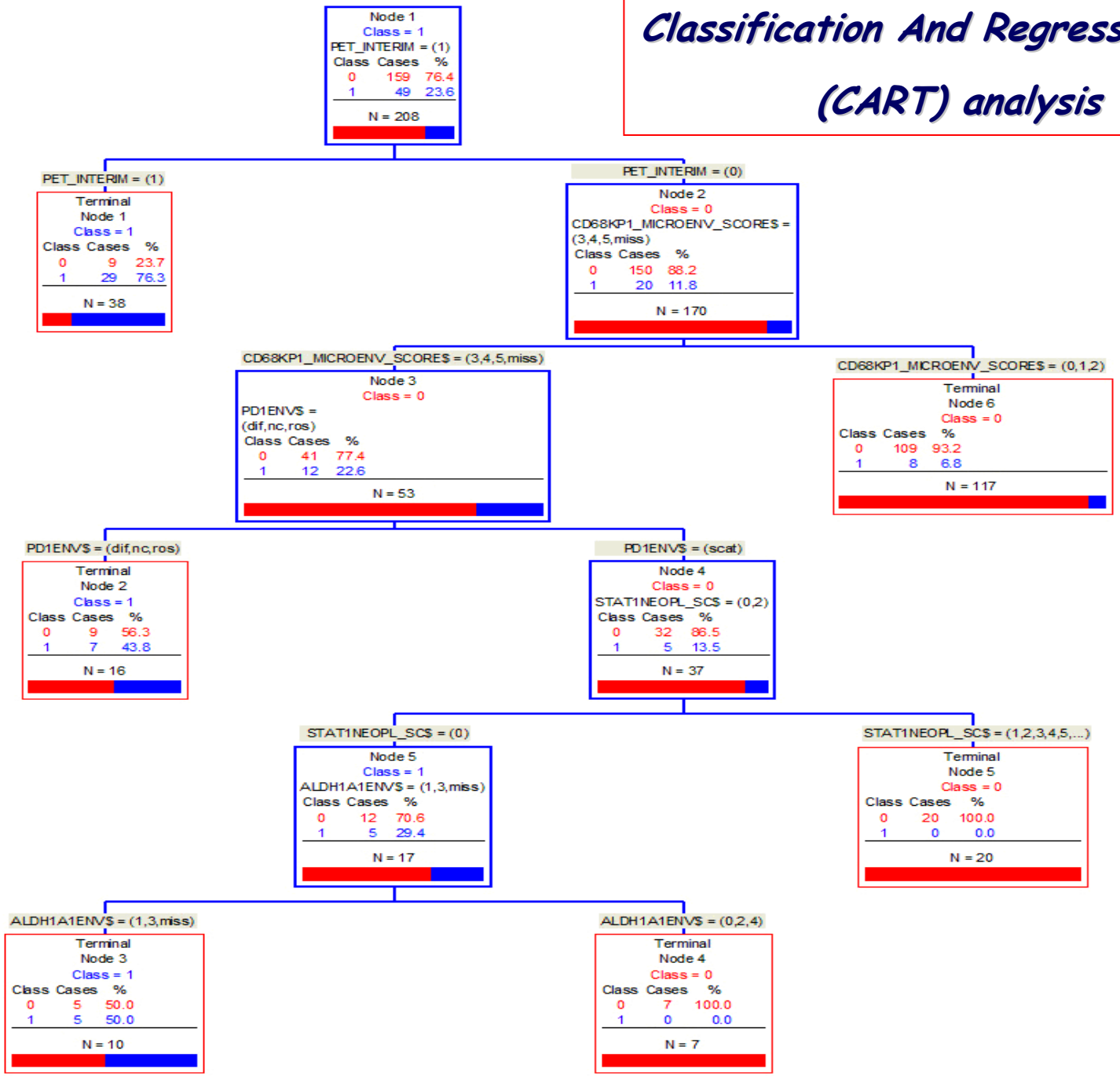
**Results**: There were no associations between clinical characteristics and the expression of CD68 or CD163. However, higher levels of CD68 and CD163 expression were correlated with the presence of Epstein-Barr virus-positive Hodgkin tumor cells ($P = 0.01$ and 0.037, respectively). The expression of CD68 or CD163 was not associated with either the PFS or the DSS.

**Conclusion**: CD68 and CD163 expression require further evaluation before their use can be recommended for prognostic stratification of patients with cHL.

# Comments on macrophages

- Steidl et al. (NEJM) found that the macrophage content correlates with DFS in a cohort of patients with a median follow-up of 16.4 years.
- In two other papers, Kamper et al (Haematologica) and Tzankov et al (Pathobiology) also observed that the amount of macrophages correlates with OS by using however different counting systems; the two series spanned over 17 and 26 years, respectively.
- It is likely that the relatively short follow-up (median 62.3 months) due to the selection criteria used (i.e. cases with PET-2 available), has limited the statistical power of the analysis.

# Classification And Regression Tree (CART) analysis



# Macrophages predict treatment outcome in Hodgkin's lymphoma

**Christian Steidl, Pedro Farinha, and Randy D. Gascoyne**

*Departments of Pathology and Experimental Therapeutics, British Columbia Cancer Agency and the BC Cancer Research Centre, BC Cancer Agency, University of British Columbia, Vancouver, BC, Canada*

*E-mail: rgascoyn@bccancer.bc.ca* *doi:10.3324/haematol.2010.033316*

**Haematologica 2010, 96:186-9**

## Antibody and scoring system

Further study is needed to determine the optimal antigen (e.g. CD68 *versus* CD163), anti-CD68 antibody clone (e.g. KP1 *versus* PGM1) and scoring thresholds (e.g. manual *versus* computer-assisted) for detecting HL-associated macrophages.

## ⇨ Biomarker combination

The preliminary results of an immunohistochemistry study combining two markers, CD68 and FOXP3 (a marker for regulatory T cells), were presented at the ASH 2010 meeting. The authors showed that a combined FOXP3/CD68 immunohistochemistry score was an improvement over the predictive value of the individual markers alone and that this score was applicable to both limited and advanced-stage disease. The value of this com-

## EBV infection

In a re-analysis of our data we were able to confirm a relationship between increased tumor-associated macrophages and EBV positivity; however, virtually all of our cases were of the nodular sclerosis subtype and EBV alone was not associated with treatment outcome (*unpublished observations, 2010*). EBV infection of HRS cells has been reported in up to 60% of patients and is more frequent in mixed cellularity subtype, although varying with geographical location, age, gender, clinical stage and histological subtype.[14] The impact of EBV infection on outcome remains controversial, but appears to be dependent on age.

# Conclusions

- PET2 still maintains the highest predictive value but remains an *ad interim* parameter that doesn't avoid the risk of induced chemo-resistance produced by two cycles of ABVD.
- Several promising up-front prognostic markers are proposed by the present study, including p53 and Bcl2 that have a bit been neglected during the last few years.
- The impact of microenvironment including macrophages, is certainly relevant; however, some further work (e.g. standardization of cut-off values and markers) seems needed.
- CART analysis allowed the retrieval of most patients misclassified by interim PET as negative and may therefore represent an interesting operational tool .


C Agostinelli, PP Piccaluga, E Sabattini, F Bacci, C Sagramoso, M Rossi, S Righi, A Gazzola, T Sista, M Piccioli, MR Sapienza, C Mannu, F Sandri, P Artioli, G De Biase, G Da Pozzo, C Tigrini and I Barese

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