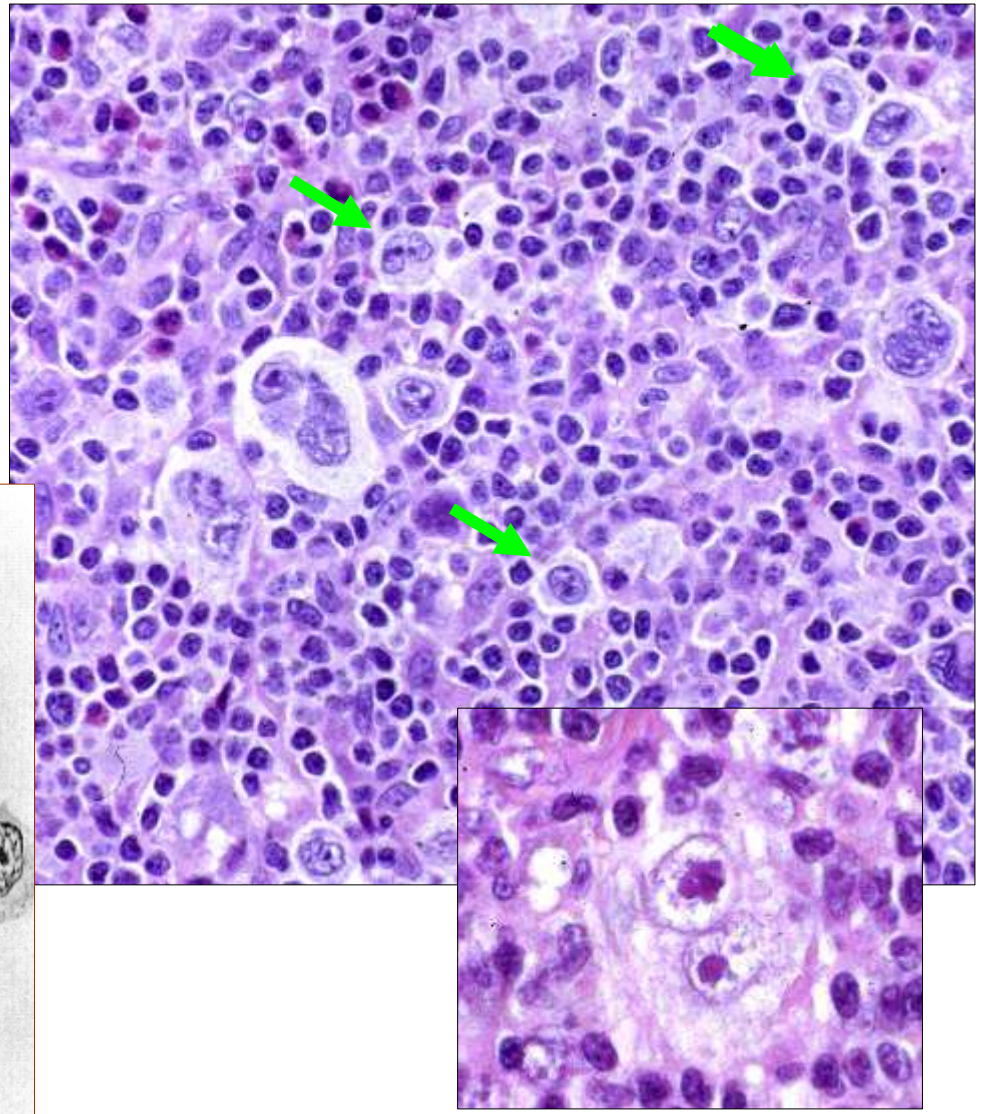
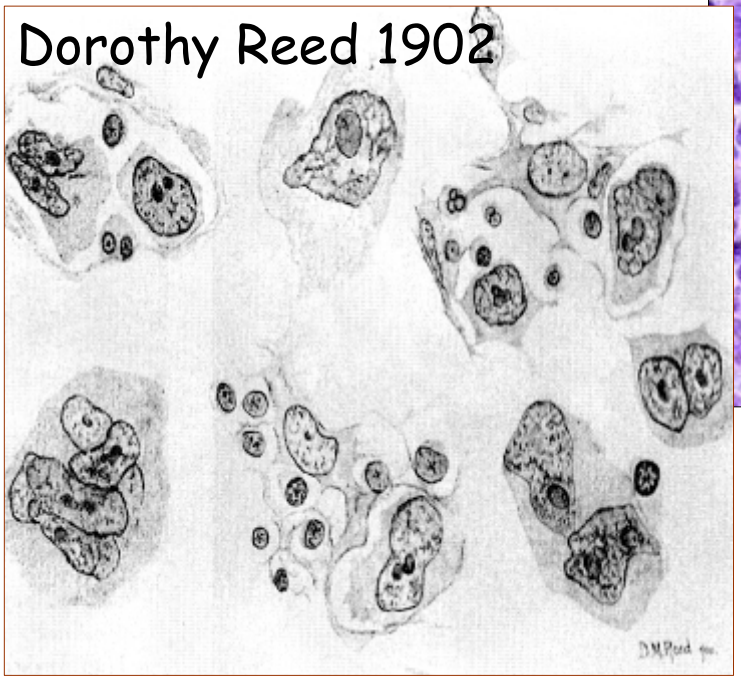
5th International Workshop on
PET in Lymphoma
Palais de l' Europe, Menton France
September 19-20, 2014

A clinical-pathological algorithm based on the combination of interim PET with biological markers in Classical Hodgkin Lymphoma

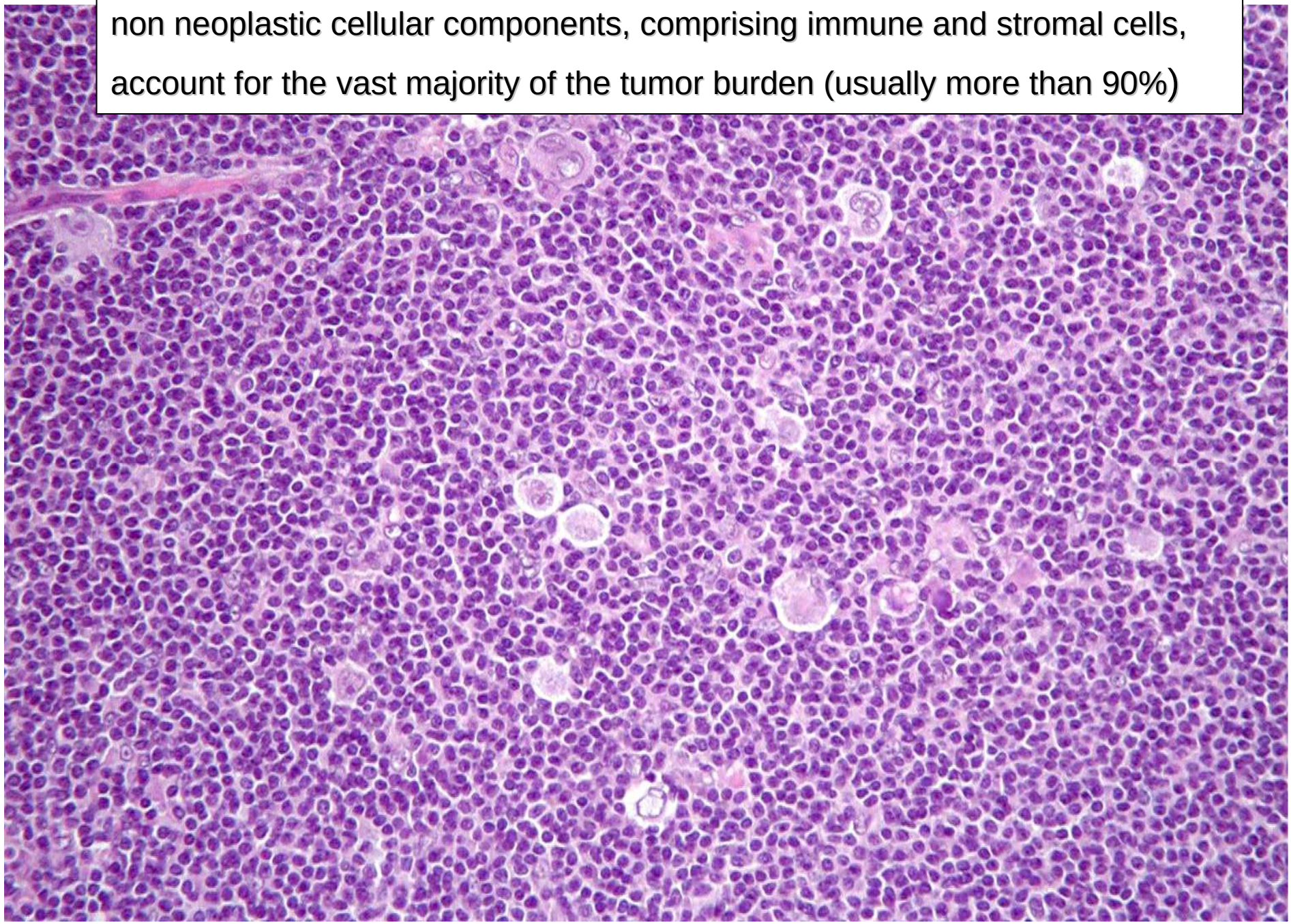
Classical Hodgkin Lymphoma

Classical Hodgkin Lymphoma is a monoclonal lymphoid neoplasm (in most instance derived from B cells) composed of mononuclear Hodgkin cells and multinucleated Reed-Sternberg cells

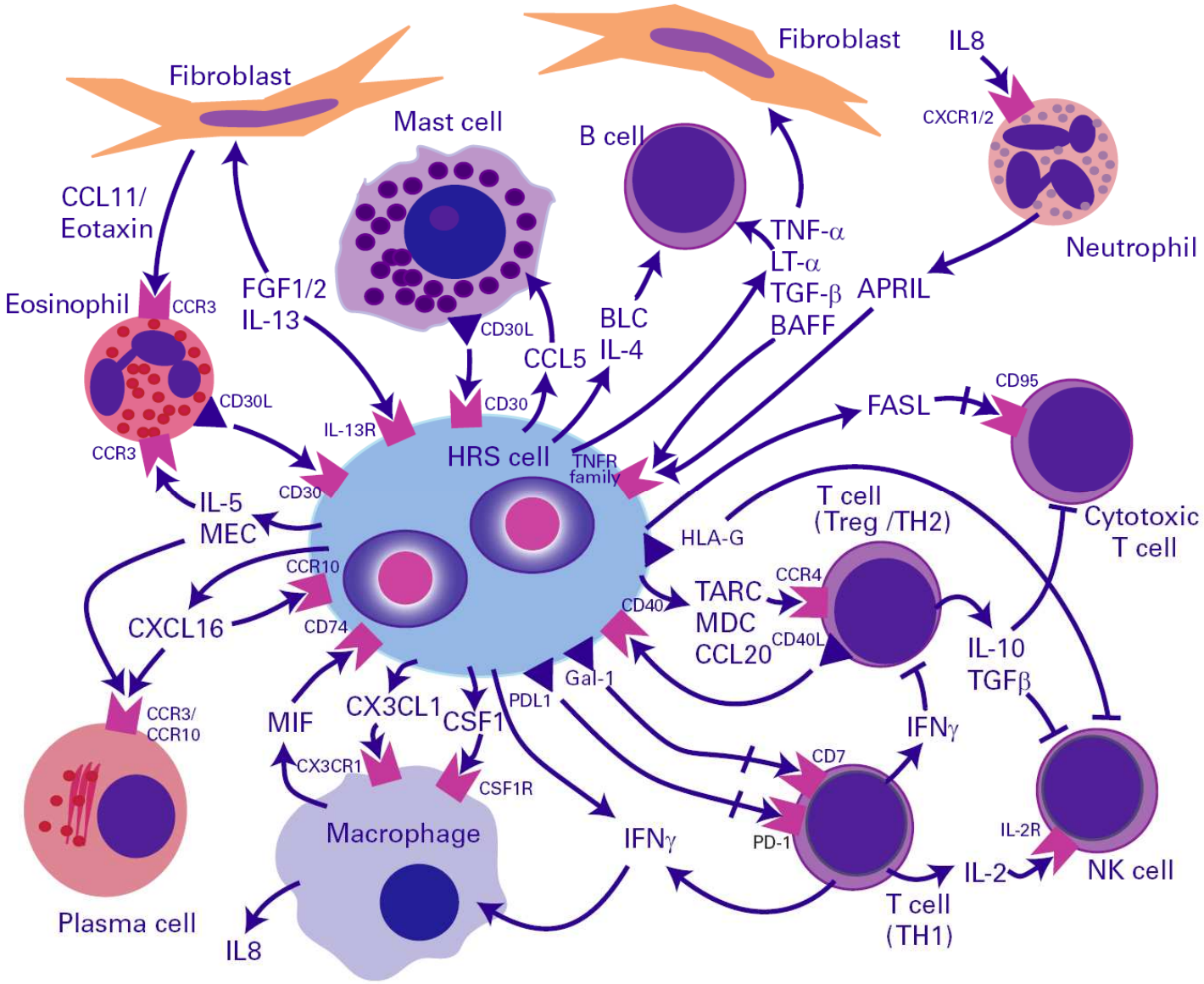




non neoplastic cellular components, comprising immune and stromal cells, account for the vast majority of the tumor burden (usually more than 90%)

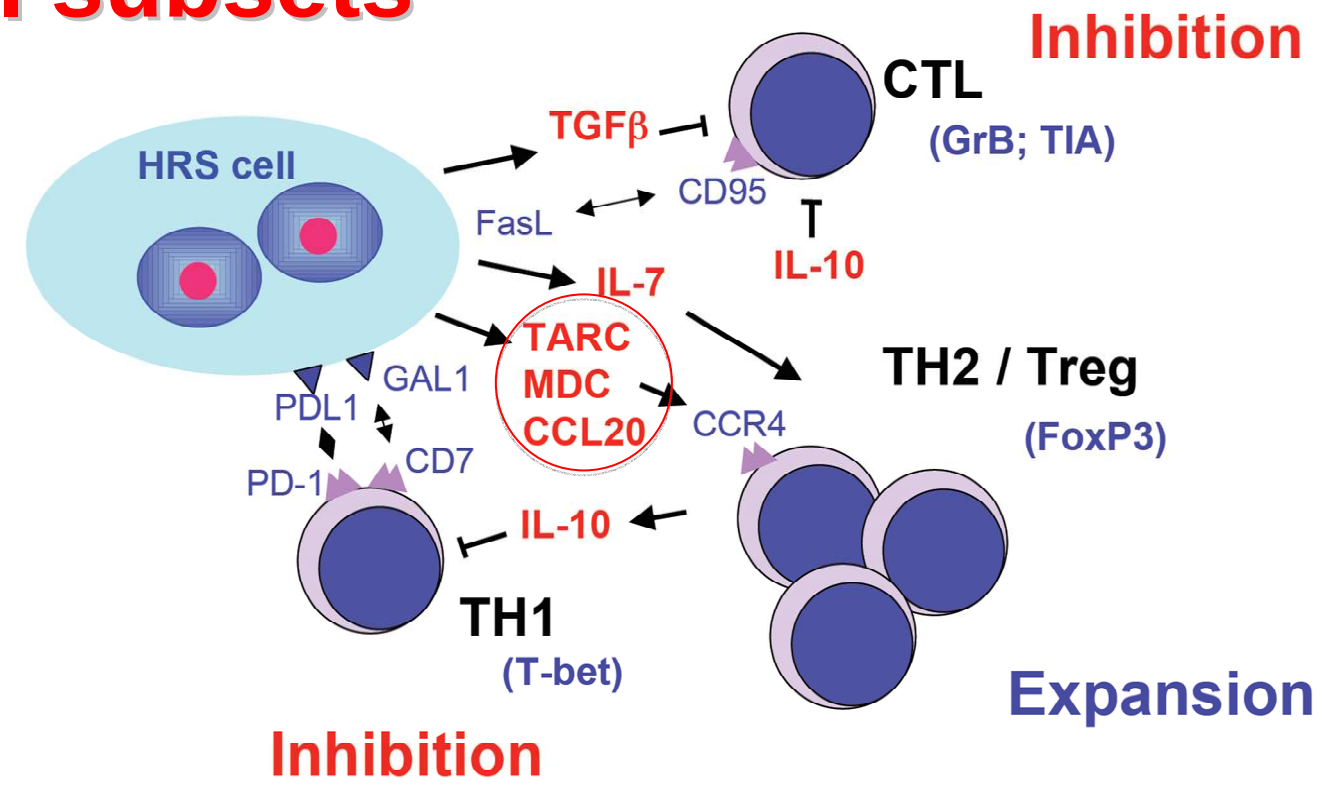


CHL: cross-talk between HRSCs and microenvironment



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

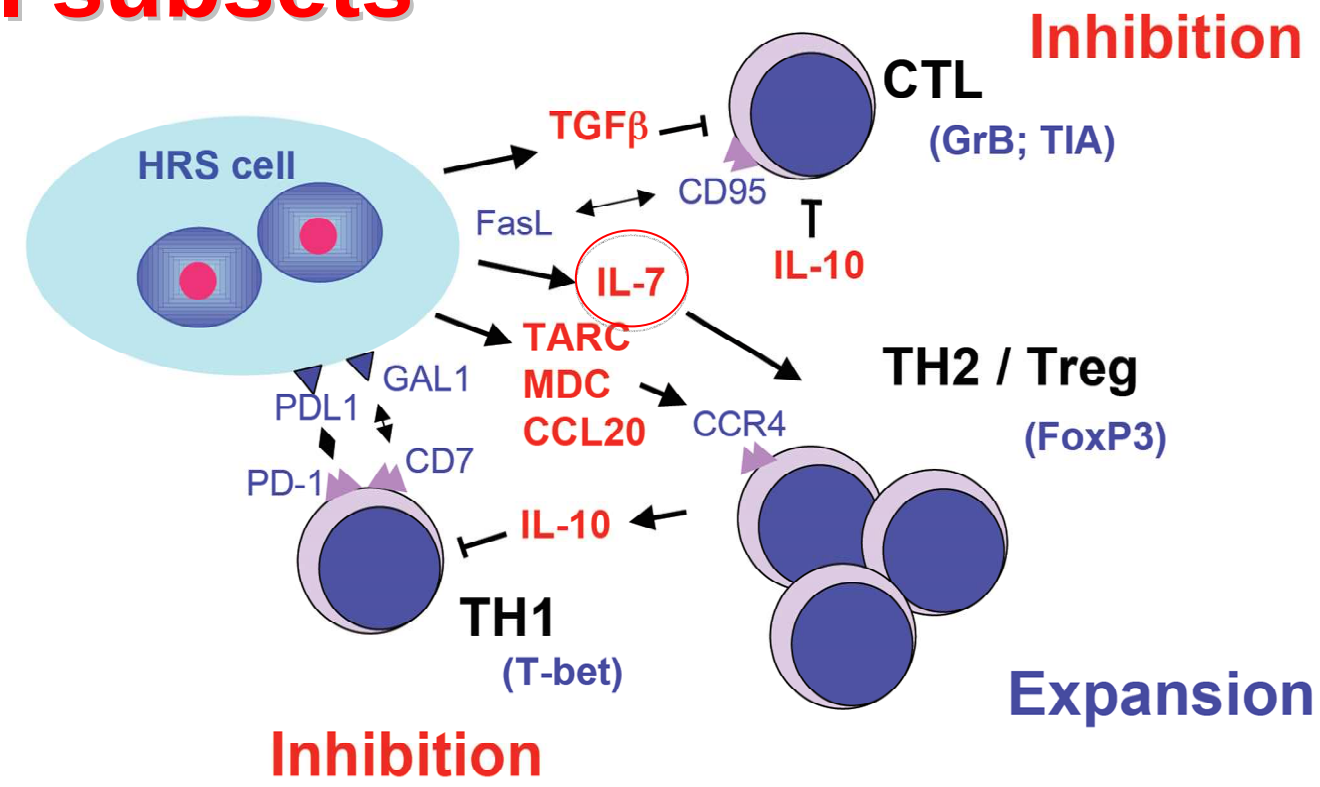
T cell subsets



HRS cells produce chemokines

- which attract TH2 and Treg cells
- capable of inducing differentiation of CD4 naiveTcells toward FOXP3^{+} T-reg cells
- which exert inhibitory effects on T-cell effector functions, especially on cytotoxic T lymphocytes

T cell subsets



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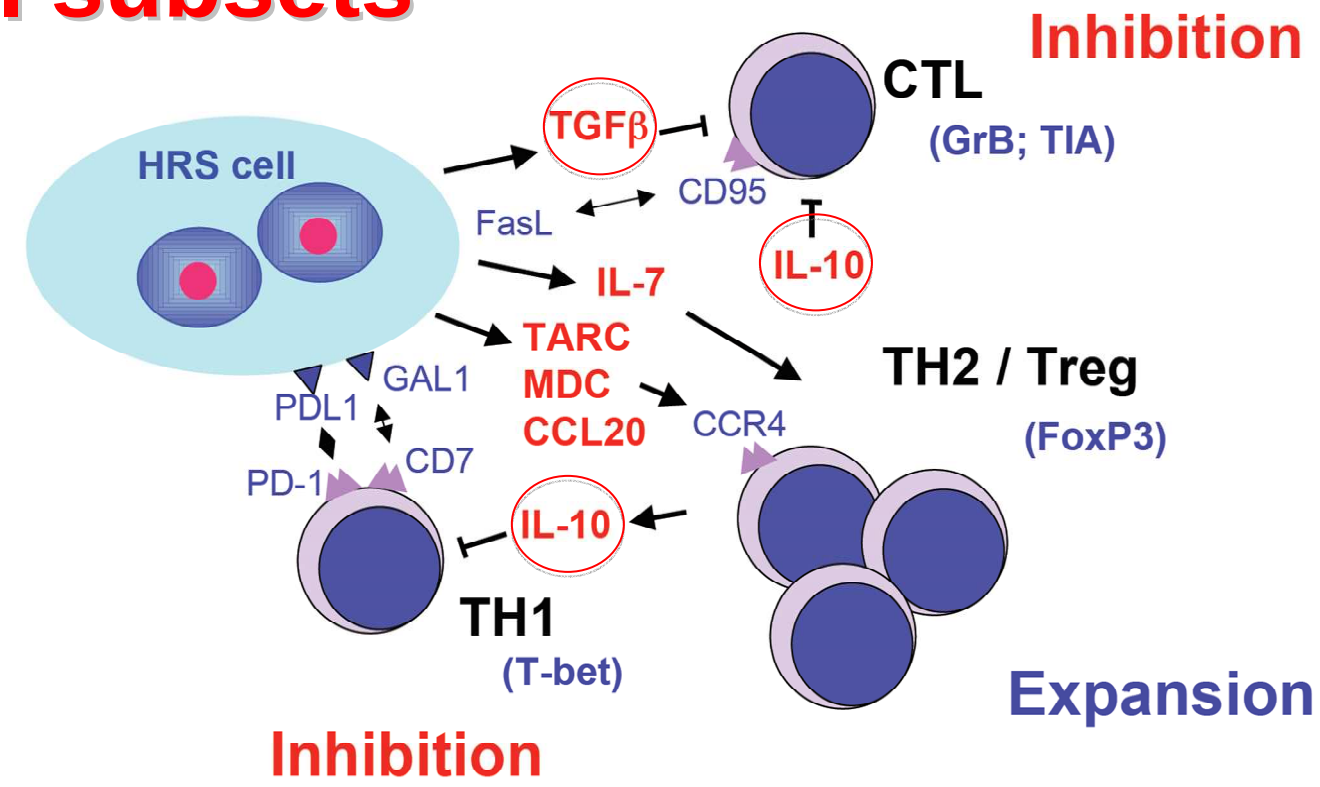

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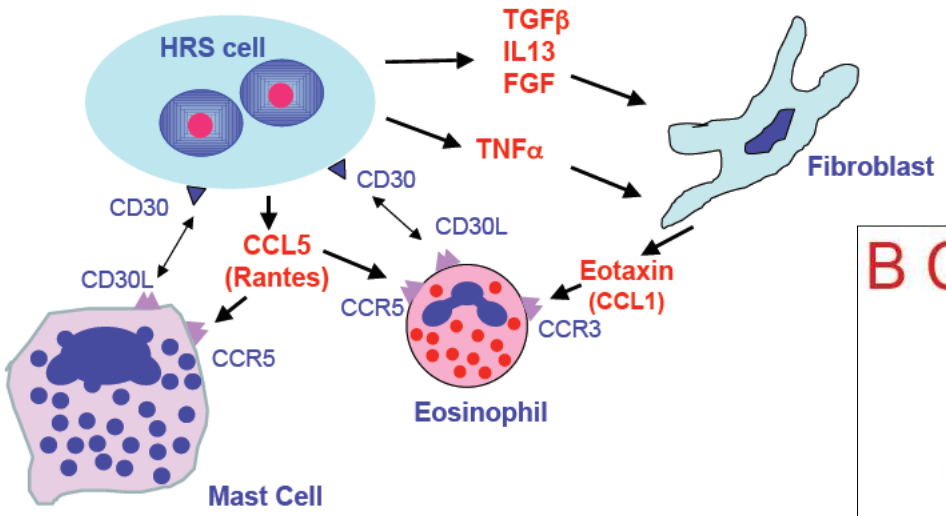
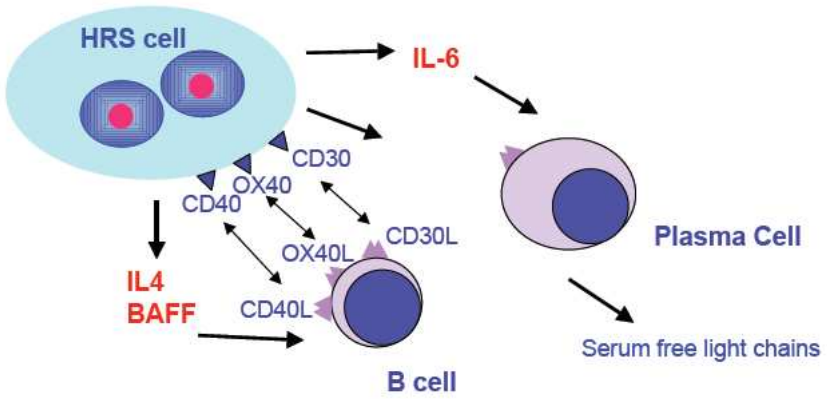
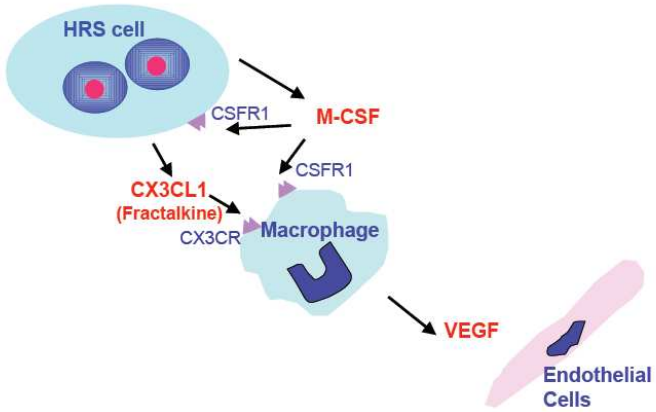
Eosinophils and Mast Cells

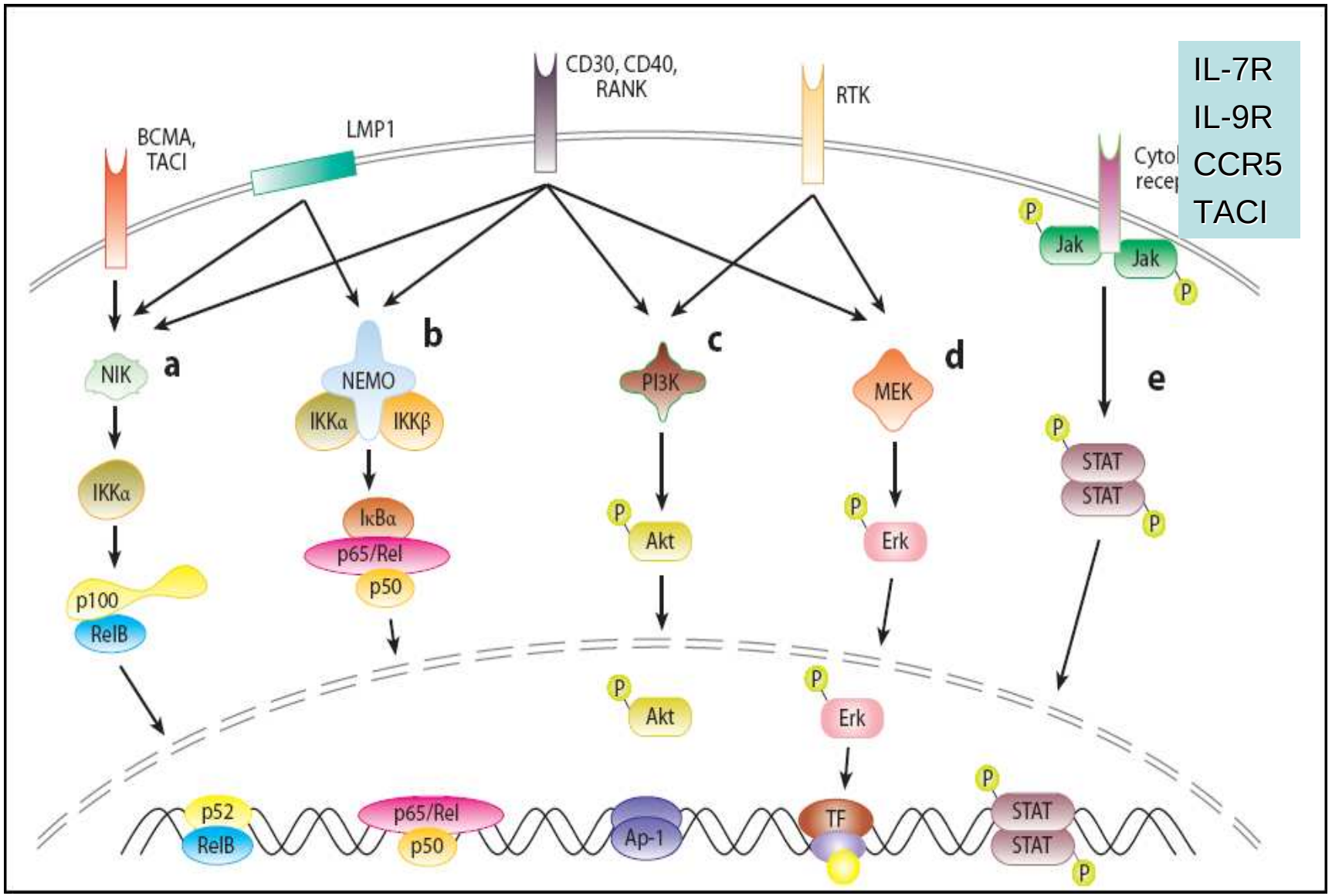


B Cells and Plasma Cells



Macrophages




BCMA,
TACI
LMP1
CD30, CD40,
RANK
RTK
Cyto
recep
IL-7R
IL-9R
CCR5
TACI
P
Jak
Jak
P
a
NIK
IKKα
p100
RelB
b
NEMO
IKKα
IKKβ
IκBα
p65/Rel
p50
c
PI3K
P
Akt
d
MEK
P
Erk
e
P
STAT
STAT
P
P
Akt
P
Erk
p52
RelB
p65/Rel
p50
Ap-1
TF
P
STAT
STAT
P

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Marker/Signature	Immunohistochemistry: Expressed on/Staining Pattern	Function	Outcome Correlation	Reference No.
Granzyme B	Cytotoxic T cells	Target cell lysis	Adverse (PFS, OS)	133-137
TIA-1	Cytotoxic T cells	Target cell lysis	Adverse (EFS, OS)	136,138
FOXP3	Regulatory T cells	Transcriptional regulation	Favorable (EFS)	137-139
CD20	Background B cells	B-cell differentiation	Favorable (OS, EFS)	132,140
BCL11A	Background B cells, plasmacytoid dendritic cells	Transcriptional regulation	Favorable (OS, EFS)	140
HLA-DR	HRS cells	Antigen presentation		
CD68, PNA	Macrophages	Scavenger receptor	Favorable (FFS)	123
ALDH1A1	Macrophages	Oxidative pathway/metabolism	Adverse (PFS, DSS)	132,141
STAT1	Macrophages	Transcriptional activation	Adverse (DSS)	142
EBV-encoded small RNAs	HRS cells	Activation of NFκB	Adverse in elderly patients, favorable in young patients (FFS, OS)	143-147
MMP11	HRS cells, macrophages, endothelial cells, extracellular	Tissue remodeling	Adverse (PFS)	132

	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

Macrophages predict treatment outcome in Hodgkin's lymphoma

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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.

Interim PET (PET-2)

5%-12% PET2 negative pts experience a treatment failure

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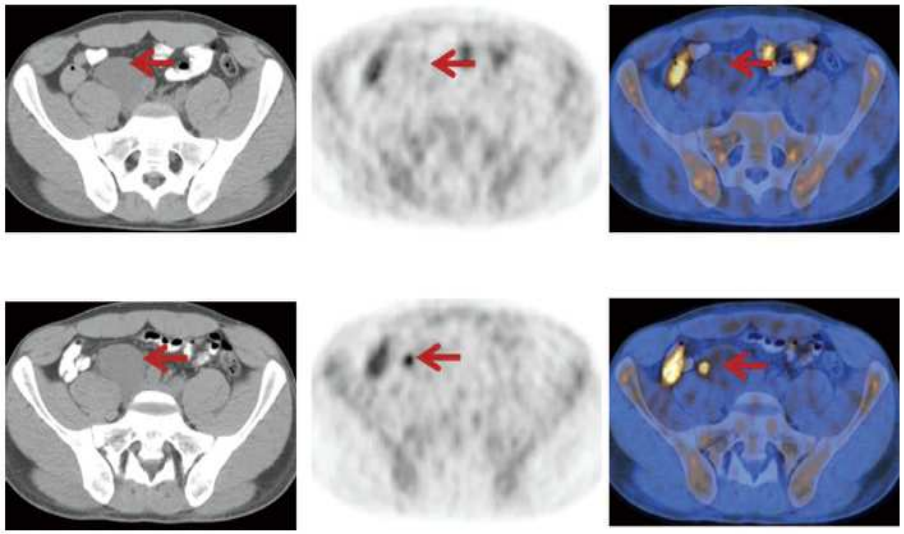
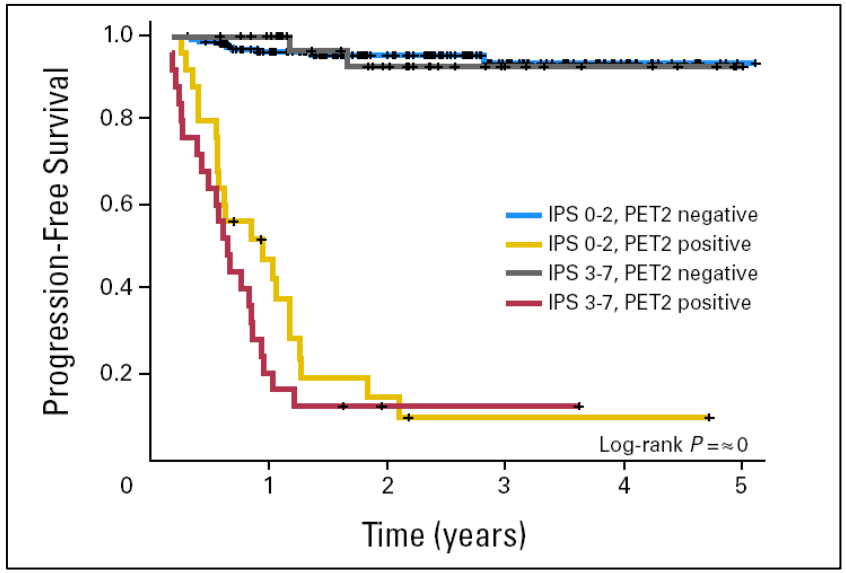
ORIGINAL REPORT

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

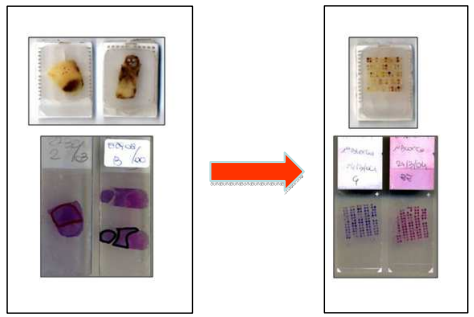
Andrea Gallamini, Martin Hutchings, Luigi Rigacci, Lena Specht, Francesco Merli, Mads Hansen, Caterina Patti, Annika Loft, Francesco Di Raimondo, Francesco D'Amore, Alberto Biggi, Umberto Vitolo, Caterina Stelitano, Rosario Sancetta, Livio Trentin, Stefano Luminari, Emilio Iannitto, Simonetta Viviani,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

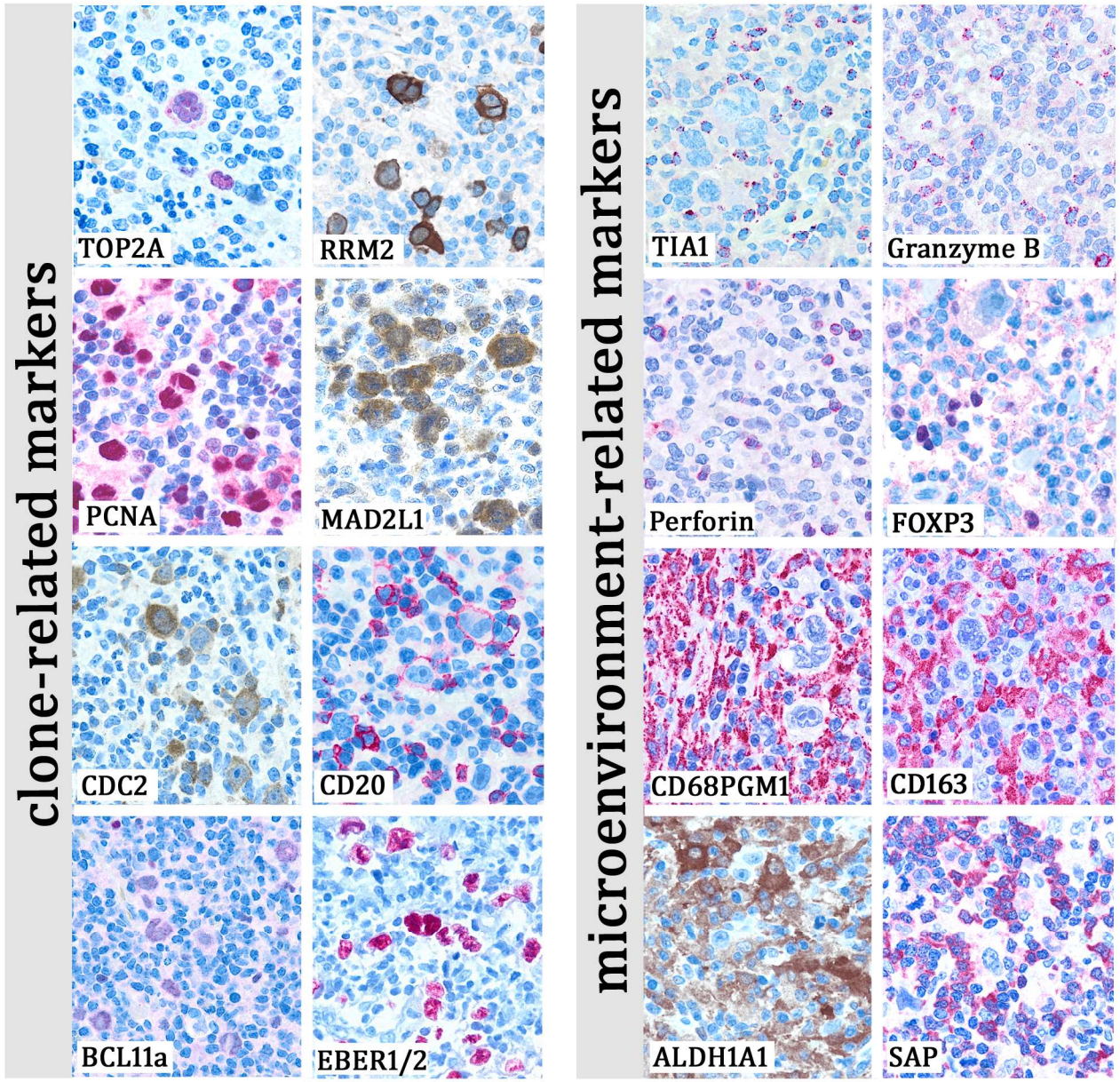




PET-2/Biologic Markers Study

- **Biopsy samples from cHL patients at diagnosis enrolled by 11 Italian and 1 Danish medical institutions and Polish Lymphoma Research Group allied centers**
- **Construction of TMAs to collect cases of interest in the same block and optimization of immunohistochemical procedures**
- We thoroughly investigated a wide set of biological markers, representative of diverse key aspects of cHL neoplastic and bystander cell biology : cell cycle regulatory proteins **(TOP2A, RRM2, MAD2L1, CDC2, PCNA)**, B-cell ontogeny-related proteins **(BCL11a, CD20)**, cell damage and apoptosis markers **(P53, BCL2)**, EBV infection status, and on macrophages related markers **(CD68, CD163, ALDH1A1, STAT1**) and T-cell cytotoxicity **(TIA1, Perforin, Granzyme B),** regulation and suppression markers **(FOXP3, PD1, SAP)**.
- **Evaluation of the prognostic impact of such markers on Hodgkin's lymphoma outcome**
- **We challenged their prognostic/predictive power versus interim PET**
- **Construction of a predictive model of lymphoma recurrence**




clone-related markers
TOP2A
RRM2
PCNA
MAD2L1
CDC2
CD20
BCL11a
EBER1/2
microenvironment-related markers
TIA1
Granzyme B
Perforin
FOXP3
CD68PGM1
CD163
ALDH1A1
SAP

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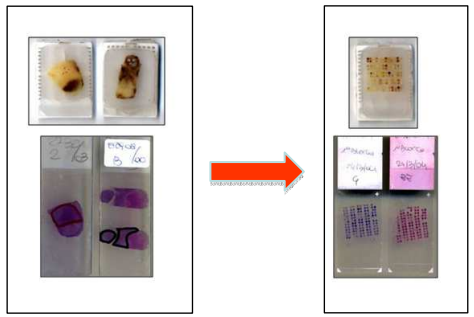
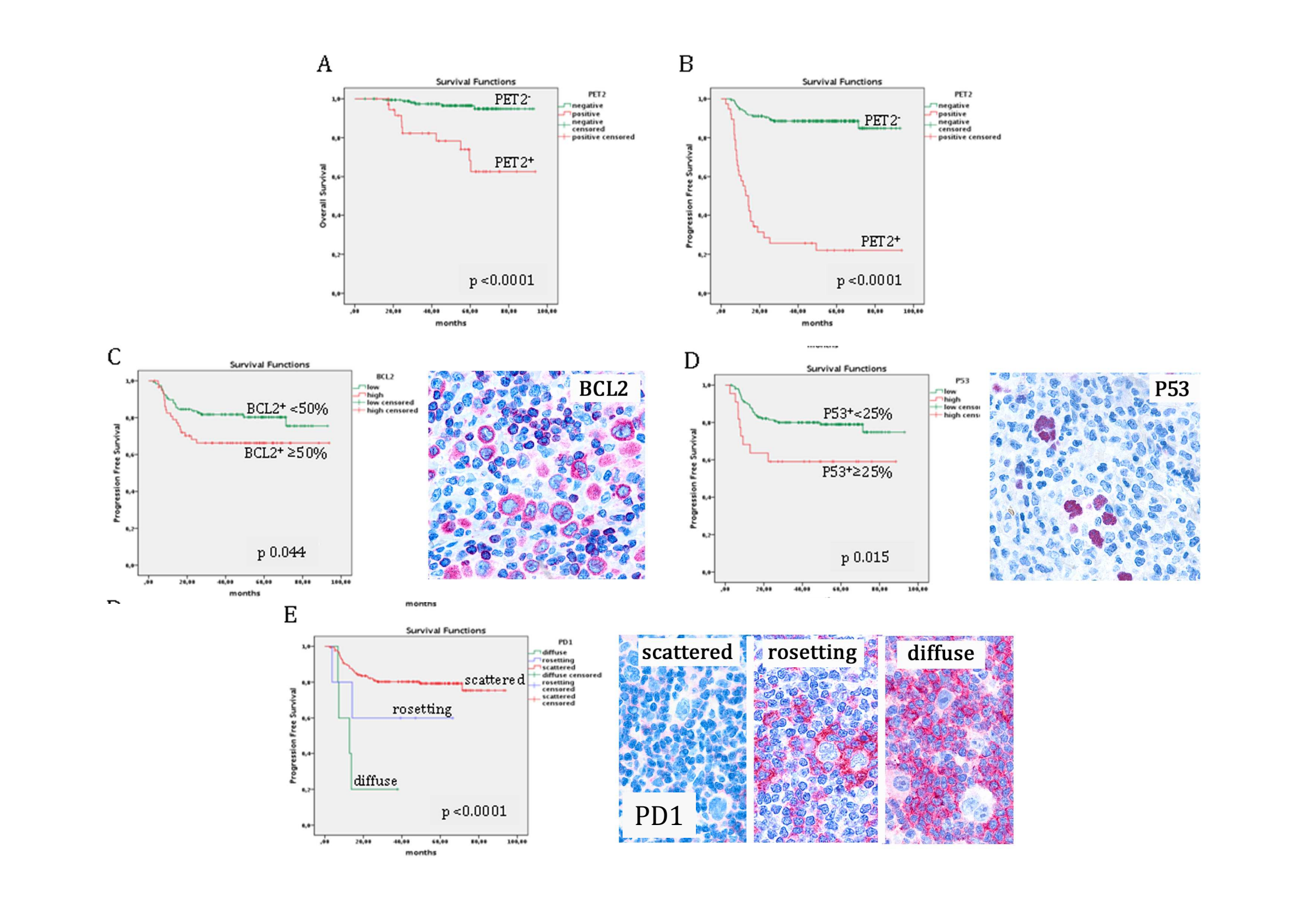


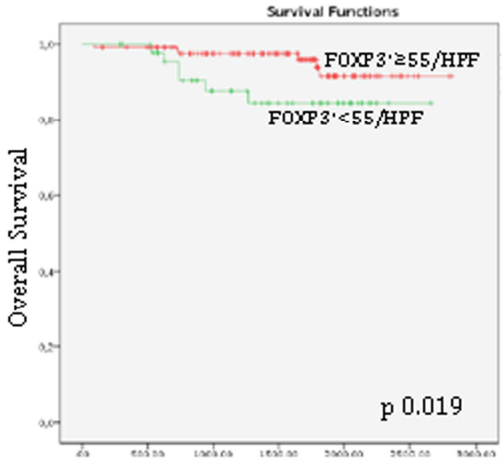
310 Patients: 208 pts + 102 pts

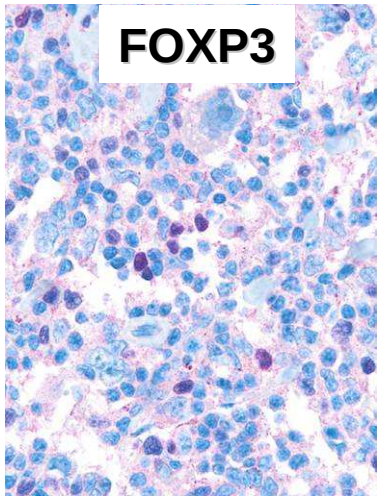
	Training set 208 pt	Test set 102 pt
Age		
mean	36	36
median	32	31
range	14-80	19-79
Sex		
male	103 (49,5%)	52 (51,5%)
female	105 (50.5%)	51(48,5%)
Follow-up months		
mean	50.26	68.28
median	52.30	68.1
range	3-93	9.9-140.7
Histologic subtype*		
cHL/nos	9 (9.1%)	3 (3%)
NS/nos	9 (4.3%)	3 (3%)
NS-cellular phase	9 (4.3%)	-
NS-1	93 (44.7%)	44 (43.1%)
NS-2	37 (17.9%)	13 (12.7%)
NS-syncytial variant	8 (3.8%)	4 (3.9%)
MC	29 (14%)	30 (29.4%)
LR	1 (0.5%)	5 (4.9%)
LD	3 (1.4%)	-

	Training set 208 pt	Test set 102 pt
Ann Arbor stage		
I	9 (4.3%)	6(5.9%)
IIA	68 (32,7)	18 (17,6)
IIB	49 (23,6)	15 (14,7)
III	49 (23.6%)	26 (25.5%)
IV	33 (15.9%)	37 (36.3%)
Symptoms		
A	110 (53%)	38 (37%)
B	98 (47%)	64 (63%)
Bulky disease		
Yes	42 (20.1%)	42 (41%)
No	166 (79.9%)	60 (59%)
First-line treatment		
ABVD	208 (100%)	102 (100%)
RT	52 (25%)	43 (42%)
PET after two cycles		
Negative	170 (81.7%)	79 (77.5%)
Positive	38 (18.3%)	23 (22.5%)
Clinical outcome		
Failure	49 (23.6%)	22 (21.6%)
Death of disease	16 (7,7%)	4 (3,9)

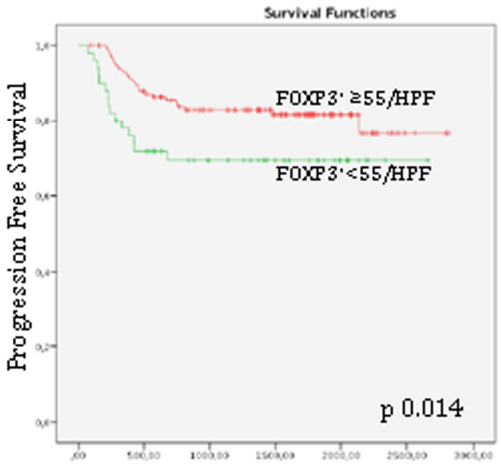

A
Survival Functions
PET2
negative
positive
negative censored
positive censored
PET2-
PET2+
p <0.0001
Overall Survival
months
B
Survival Functions
PET2
negative
positive
negative censored
positive censored
PET2-
PET2+
p <0.0001
Progression Free Survival
months
C
Survival Functions
BCL2
low
high
low censored
high censored
BCL2+ <50%
BCL2+ ≥50%
p 0.044
Progression Free Survival
months
BCL2
D
Survival Functions
P53
low
high
P53+<25%
P53+≥25%
p 0.015
Progression Free Survival
P53
E
Survival Functions
PD1
diffuse
rosetting
scattered
diffuse censored
rosetting censored
scattered censored
scattered
rosetting
diffuse
p <0.0001
Progression Free Survival
months
scattered
rosetting
diffuse
PD1

A





B



C

Cox's regression model: Overall Survival

variable	Hazard Ratio of event risk	95% C.I for HR	p
PET-2+	0.032	(0.04-0.274)	0.002

D

Cox's regression model: Progression Free Survival

variable	Hazard Ratio of event risk	95% C.I for HR	p
Stage II			0.001
Stage III	2.138	(1.756-2.522)	0.004
Stage IV	3.562	(3.235-3.888)	0.003
FOXP3^{high}	0,324	(0.147-0.715)	0.005
P53+	2,624	(1.043-6.579)	0.040
PET-2+	33.333	(13.513-83.333)	0.000

E

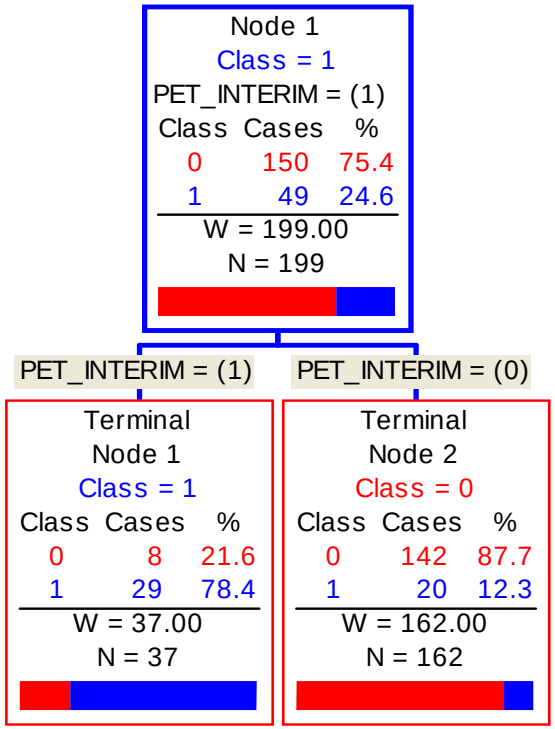
Progression Free Survival after 5 years for different categories

Variable	Category	PFS
Stage	Stage=2	74.12%
Stage	Stage=3	59.46%
Stage	Stage=4	52.17%
FOXP3	Low:<55/HPF	58.83%
FOXP3	High:>55/HPF	76.34%
P53	Low: <25%	71.43%
P53	High: ≥25%	50.00%
PET2	Negative	83.33%
PET2	Positive	14.71%

Classification and Regression Tree (CART) analysis

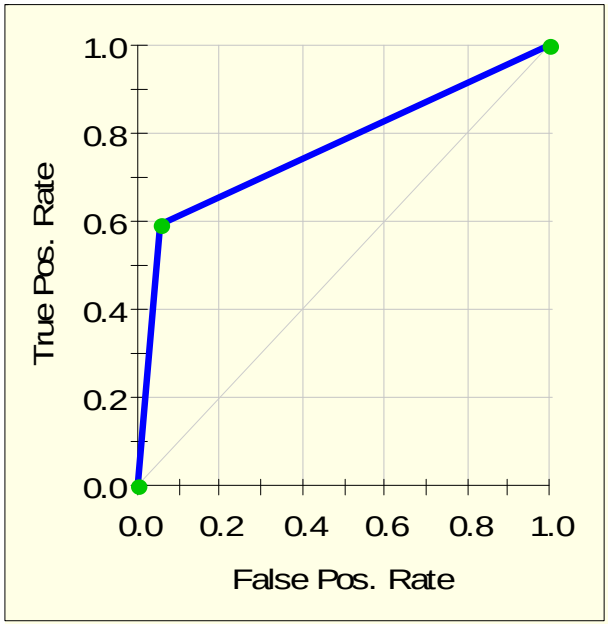
Misclassification for Learn and Test Data

PET-2 alone based model

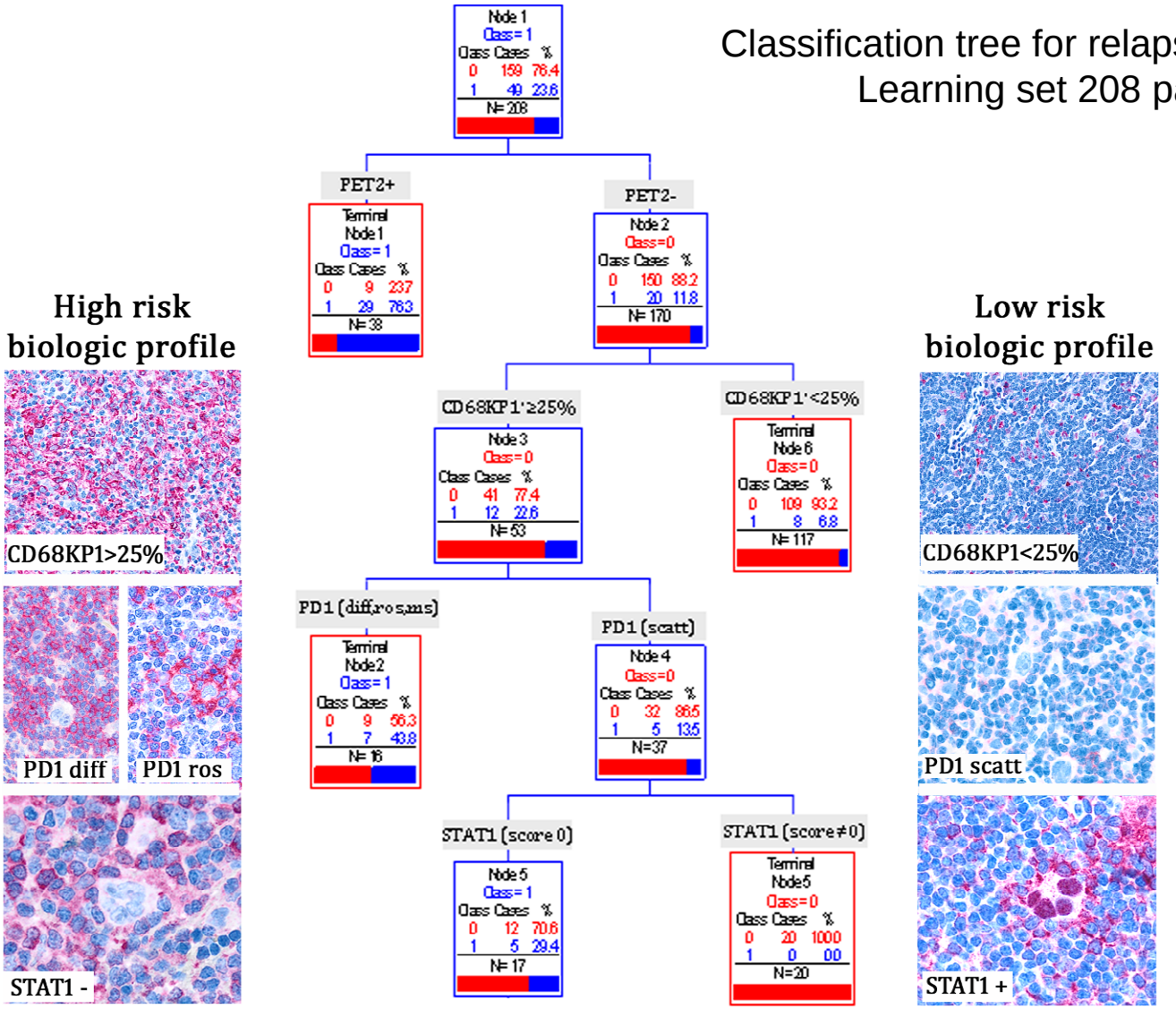


Class	N Cases	N Mis-Classed	Pct Error
0	150	8	5.33
1	49	20	40.82
Tot	199	**28**	**14.07**

ROC Integral: 0.770



Classification tree for relapse/progression
Learning set 208 patients



low-risk class:

a) patients that, in addition to a negative PET-interim, had low values of CD68KP1 percentage < 25% ;

b) patients that, in addition to a negative PET-interim, and despite CD68KP1 ≥ 25%, had jointly scattered PD1 pattern and positive STAT1 in RCS;

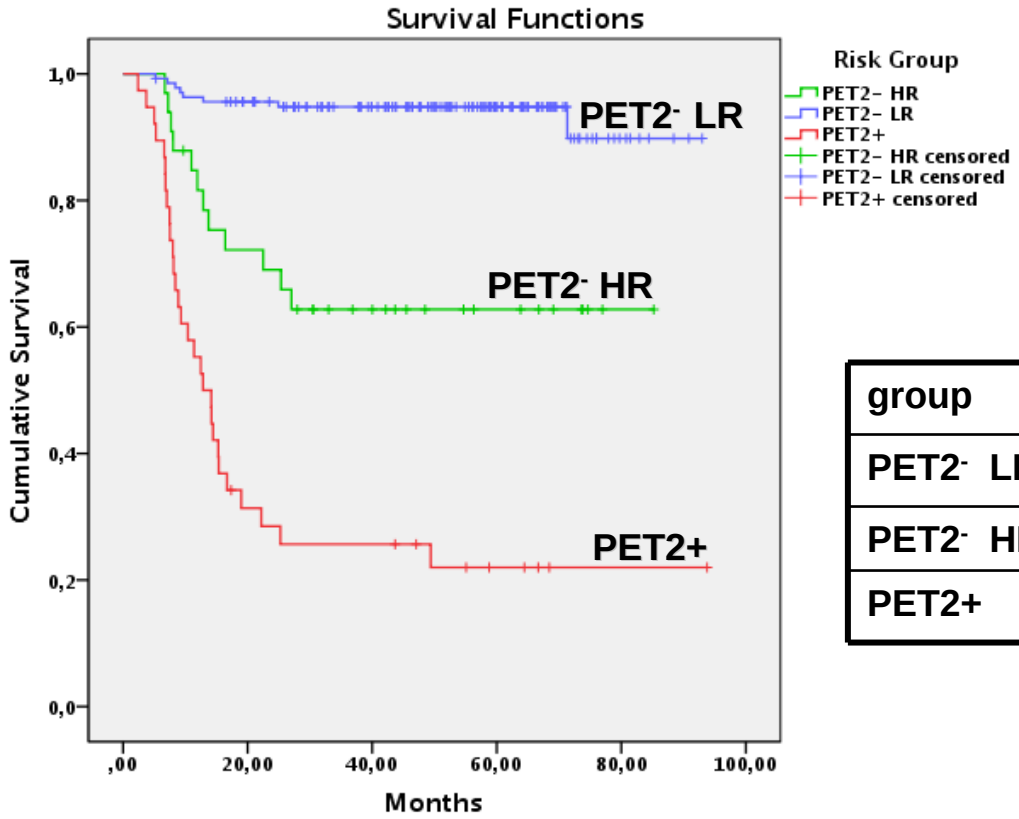
high-risk class:

a)patients that, despite a negative PET-interim, were characterised by CD68KP1 ≥ 25% and diffuse or rosetting PD1 pattern;

b) subjects that, despite a negative PET-interim, had jointly CD68KP1 ≥ 25%, scattered PD1 pattern and were negative for STAT1.

misclassification rates

Class	Pet-2 based classification scheme in learning set: 208 pts			Pet-2 + biologic markers classification scheme					
				Learning set: 208 patients 10-fold cross validation			Test set: 102 patients		
	number of cases	n of misclass	misclass rate (%)	number of cases	n of misclass	misclass rate (%)	number of cases	n of misclass	misclass rate (%)
0	159	9	5.66%	159	26	16.35%	80	10	12.50%
1	49	20	40.82%	49	11	22.45%	22	3	13.63%
total	208	29	13.94%	208	37	17.79%	102	13	12.74%



group	5 yrs PFS
PET2- LR	94,7%
PET2- HR	63,6%
PET2+	23,7%

Conclusions

- none of the biologic factors evaluated could perform better than PET-2 in predicting outcome.

- a clinical-pathological algorithm based on the combination of PET-2 with some of the investigated biological markers, allowed the identification of poor prognosis patients that were misclassified by PET-2 alone.

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