Utilidad del inmunofenotipo en las gammapatías monoclonales (MGUS y mieloma múltiple)

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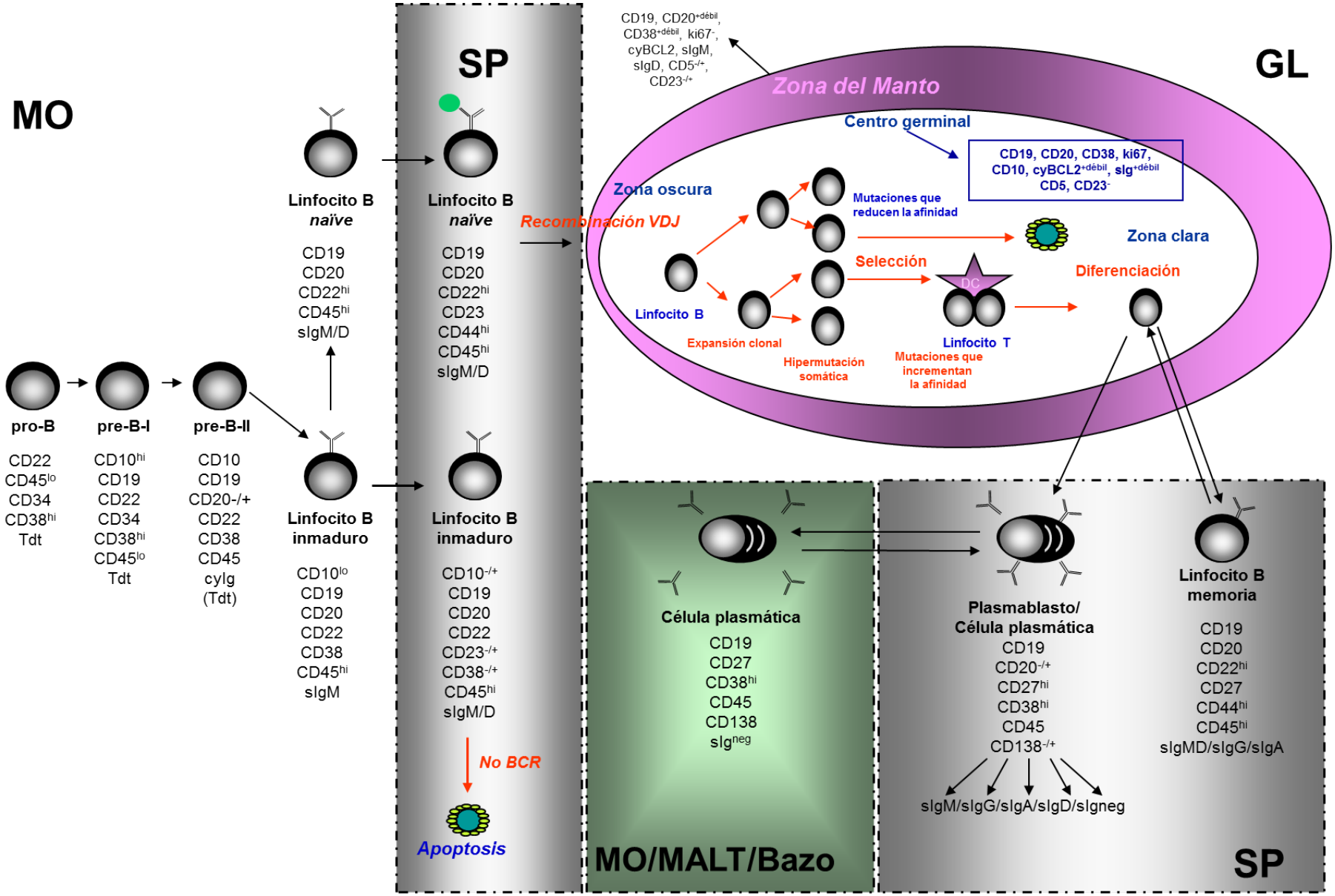
EuroFlow™ Consortium

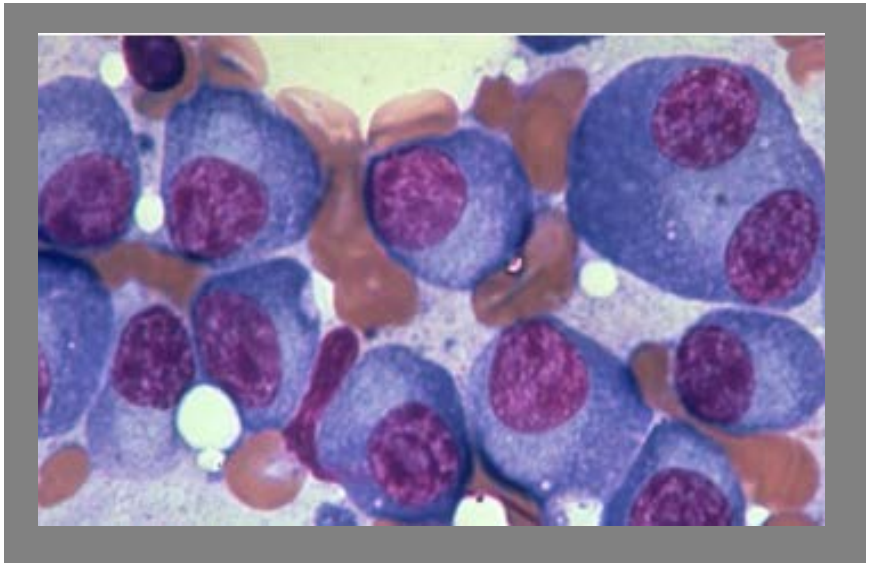
Curso avanzado de Actualización en Onco Hematología. Buenos Aires, Mayo, 30 a Junio, 1, 2011

Normal B-cell diferentiation

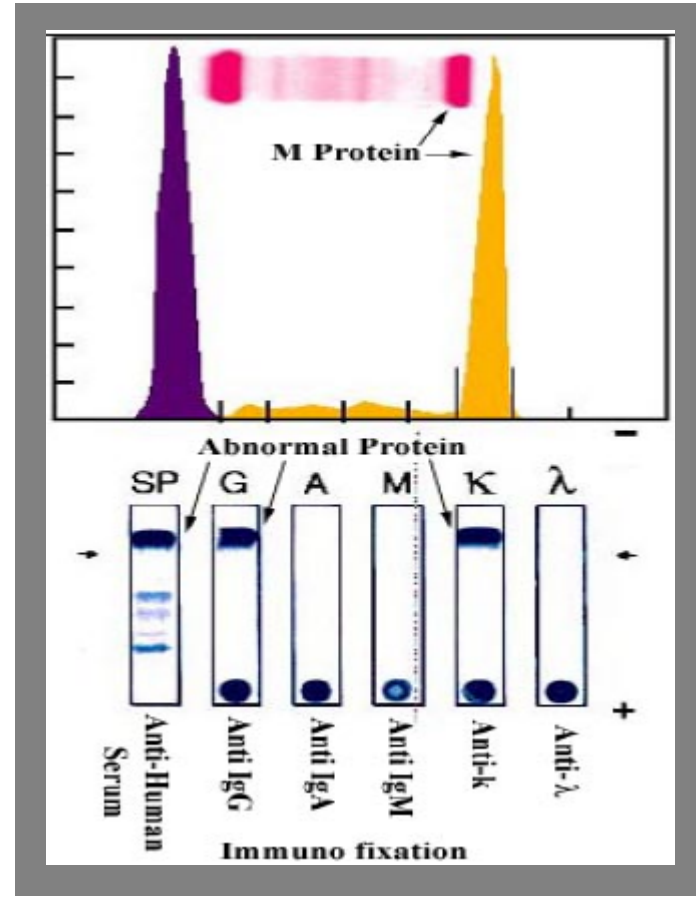


Monoclonal gammopathies

Bone Marrow infiltration by PC



Detection of monoclonal Ig (paraprotein) in serum and/or urine



Monoclonal gammopathies

Most relevant disease categories and their diagnostic criteria

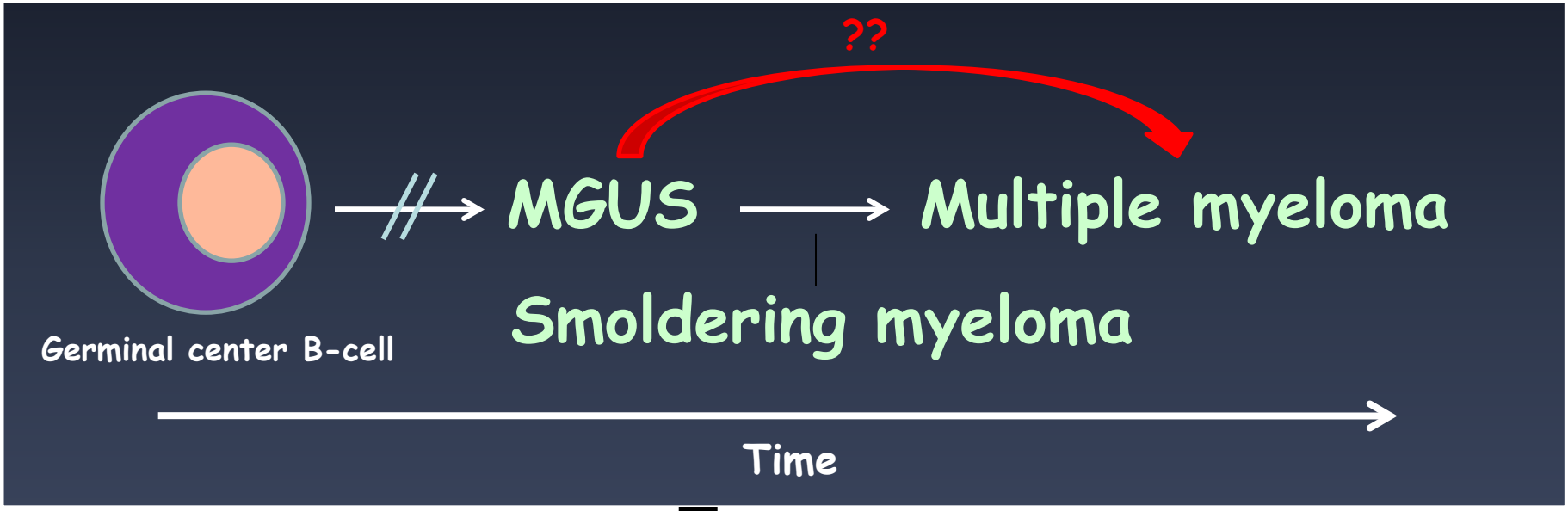
Parameter	Disease category		
	MGUS	SMM	MM
M Component	< 30 g/L in serum	> 30 g/L in serum	Present in serum/urine
	and	*or*	
% of plasma cells	< 10%	≥ 10%	> 10%
	and	*and*	*and*
End organ damage	Absence	Absence	Present

MGUS: monoclonal gammopathies of undetermine significance; SMM: smouldering myeloma; MM: multiple myeloma

International Working Group (BJH 2003; 121:749)

Monoclonal gammopathies

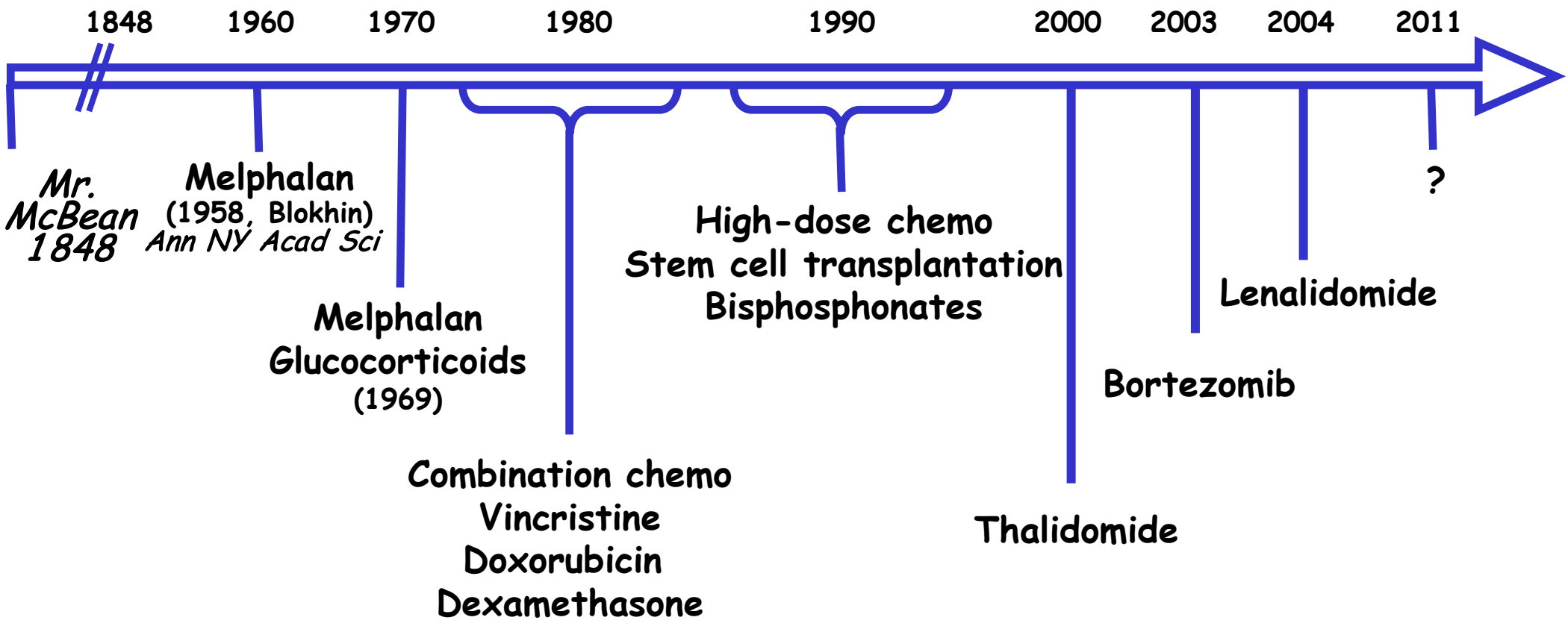
Natural history ?



prevalence of 3.2%; ≥50y → risk of transformation:
1% per year MGUS to MM
10% per year SMM to MM

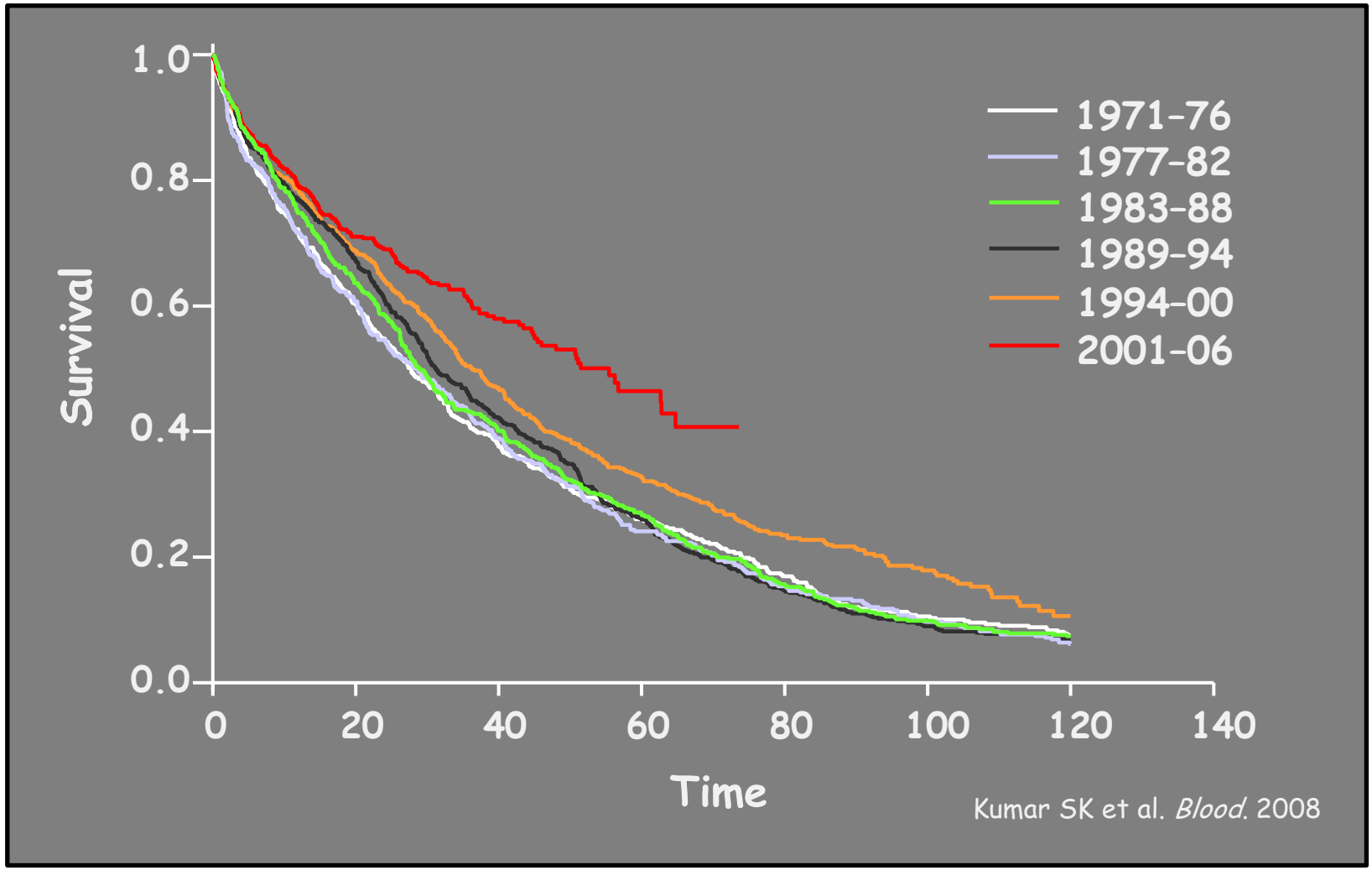
Kyle RA, et al. N Engl J Med. 2007;356:2582-90.
Weiss BM, et al. Blood. 2009;113(22):5418-22

MM. Treatment



MM. Treatment

OS from time of diagnosis in 6-year intervals based on date of diagnosis



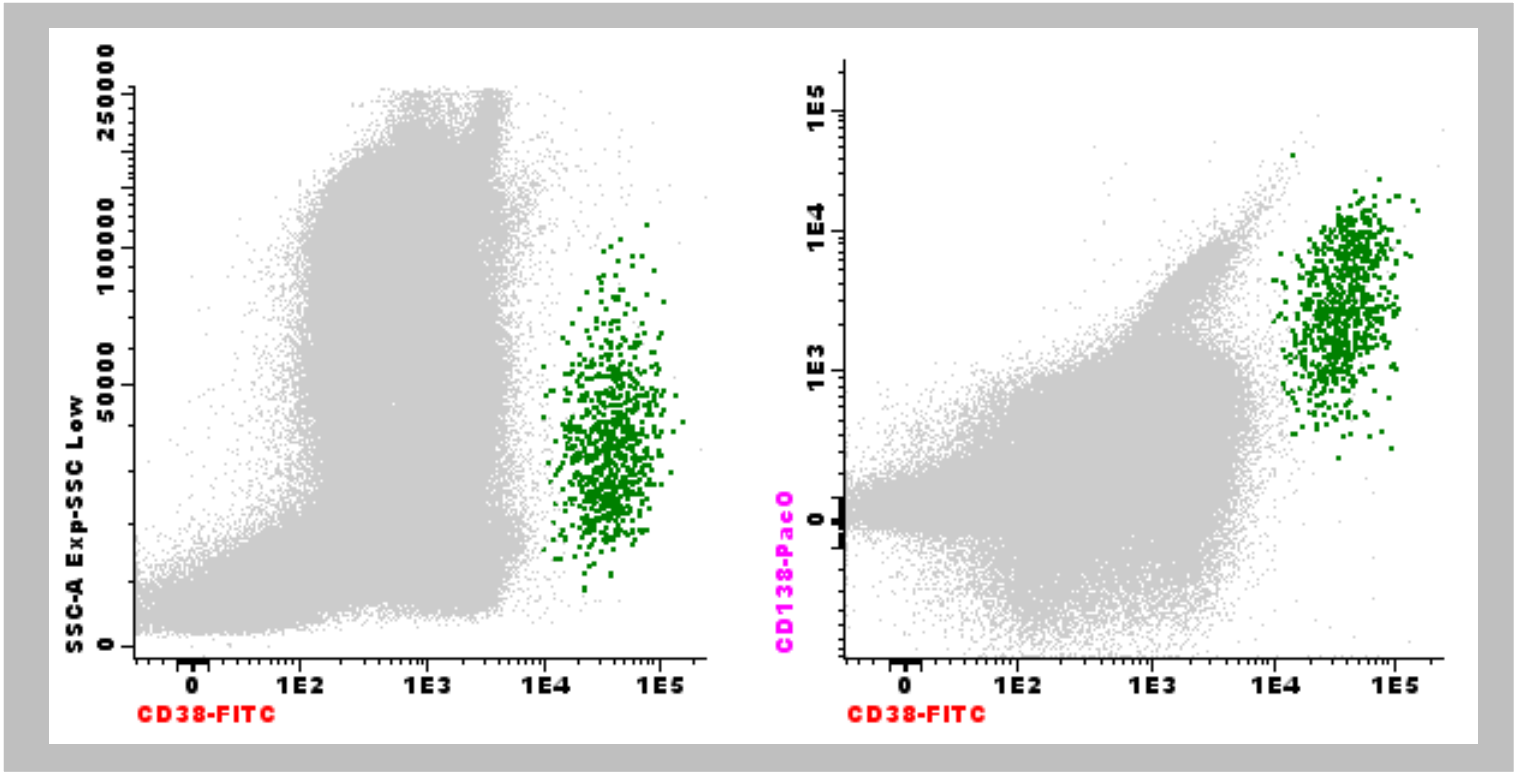
FCM Immunophenotyping of PC

- **Identification and characterization of PC**
  - *Prognostic influence of number of PC* [1]
  - *Prognostic influence of specific antigens* [2]
  - *Analysis of DNA ploidy and cell cycle* [3]
- **Discrimination between normal vs. clonal PC:**
  - *Differential diagnosis of MGUS & MM* [4,5]
  - *Risk of transformation of MGUS & smoldering MM* [6,7]
  - *Favourable prognosis subgroup of symptomatic MM* [8]
  - *MRD investigations in MM* [9-12]
  - *Differential diagnosis of AL vs. other forms of amyloidosis* [13]
  - *Differential diagnosis of IgM monoclonal gammopathies*

1. Paiva et al. Haematologica 2009;94(11):1599-1602.
2. Mateo et al. J.Clin.Oncol. 2008;26(16):2737-2744.
3. San Miguel et al. Blood 1995;85(2):448-455.
4. Ocqueteau et al. Am.J.Pathol. 1998;152(6):1655-1665.
5. Olteanu et al. BMC.Clin.Pathol. 2008;8:13.
6. Perez-Persona et al. Blood 2007;110(7):2586-2592.
7. Perez-Persona et al. Br J Haematol. 2010 Jan;148(1):110-4.
8. Paiva et al. Blood 2009;114(20):4369-72.
9. San Miguel et al. Blood 2002;99(5):1853-1856.
10. Rawstron et al. Blood 2002;100(9):3095-3100.
11. Paiva et al. Blood 2008;112(10):4017-4023.
12. Paiva et al. J Clin Oncol. 2011 Apr 20;29(12):1627-33.
13. Paiva et al. Blood 2011 Epub ahed of print
14. Ocio et al. Clin Lymphoma Myeloma Leuk. 2011

MONOCLONAL GAMMOPATHIES: IDENTIFICATION OF PLASMA CELLS

Specific positive identification. CD38/CD138 gating strategy

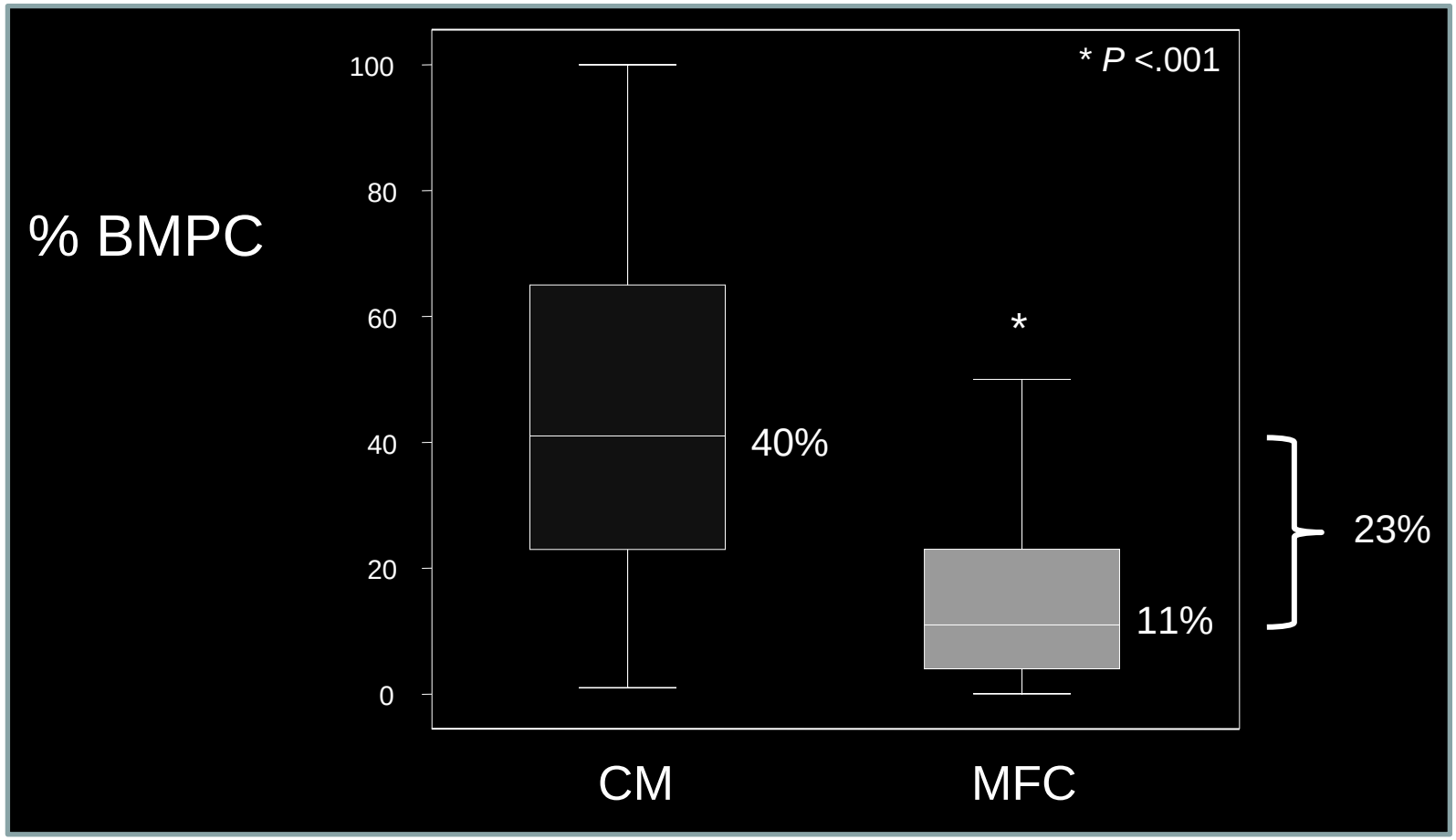


EuroFlow

FCM Immunophenotyping of PC

Identification and characterization of PC. Quantitation

Differences in BMPC counts between CM and MFC in 765 MM patients at diagnosis

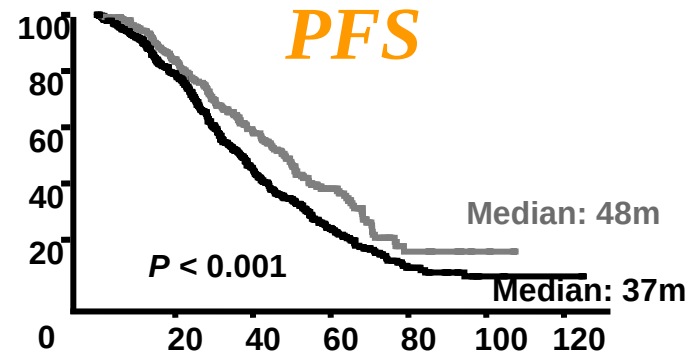


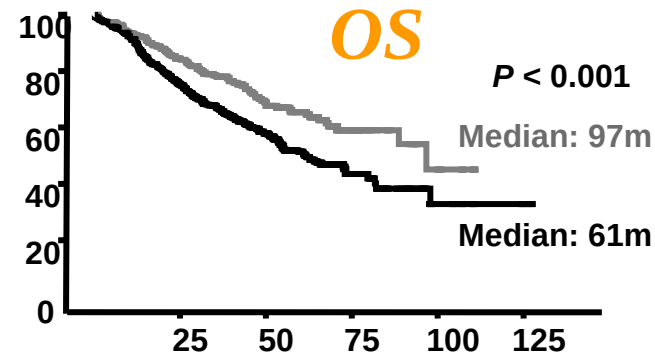
Paiva et al. Haematologica 2009;94(11):1599-1602.

FCM Immunophenotyping of PC

Identification and characterization of PC. Quantitation. Clinical impact

≥ 30% BMPC by CM

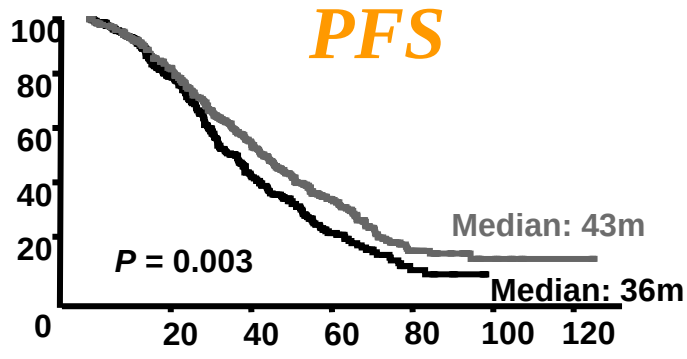


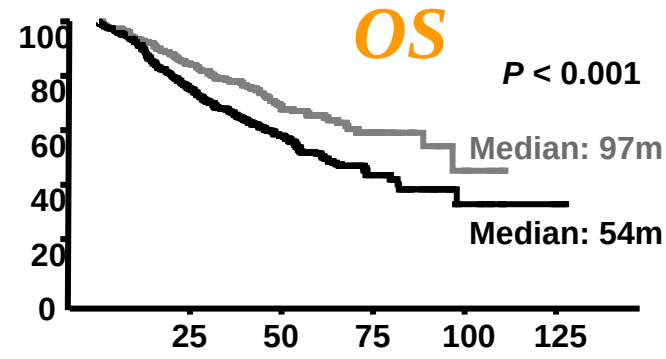


< 30% BMPC (n=253)

≥ 30% BMPC (n=512)

≥ 15% BMPC by MFC





< 15% BMPC (n=438)

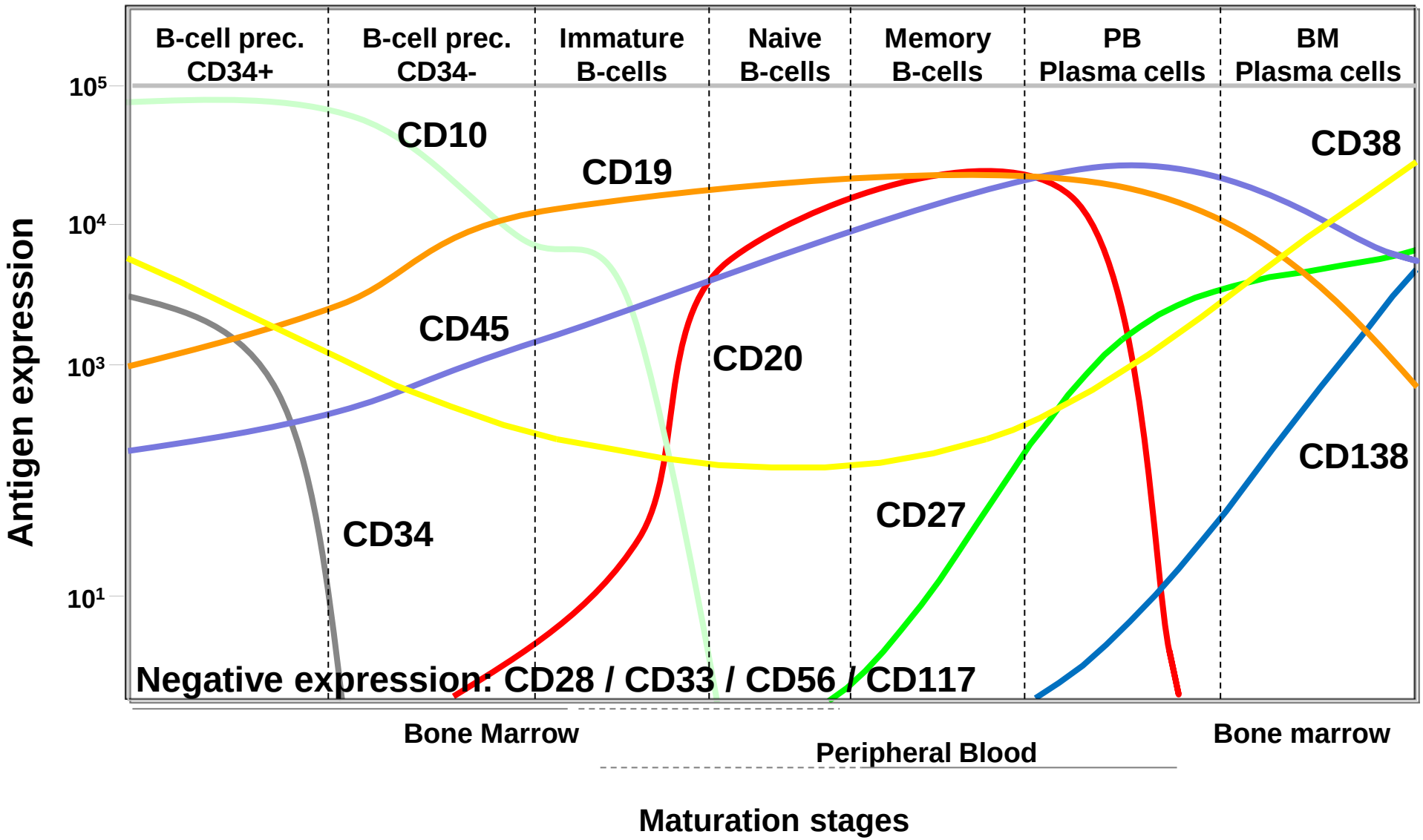
≥ 15% BMPC (n=327)

ONLY BMPC QUANTIFICATION BY MFC IS OF INDEPENDENT PROGNOSTIC VALUE

Paiva et al. Haematologica 2009;94(11):1599-1602.

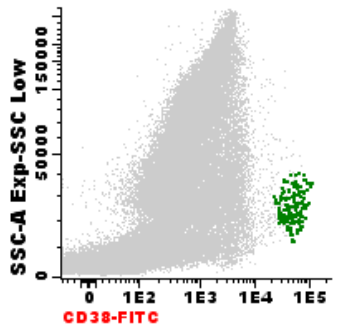
FCM Immunophenotyping of PC

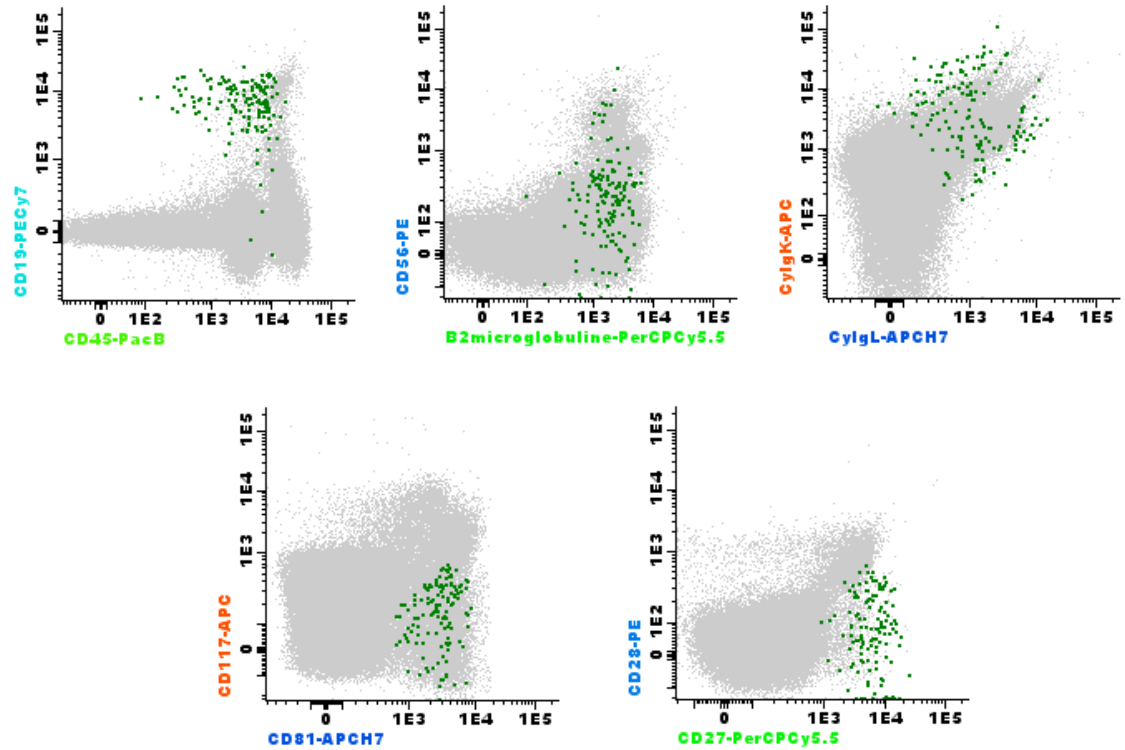
PC Immunophenotypic profile



FCM Immunophenotyping of PC

PC Immunophenotypic profile. Panel EuroFlow

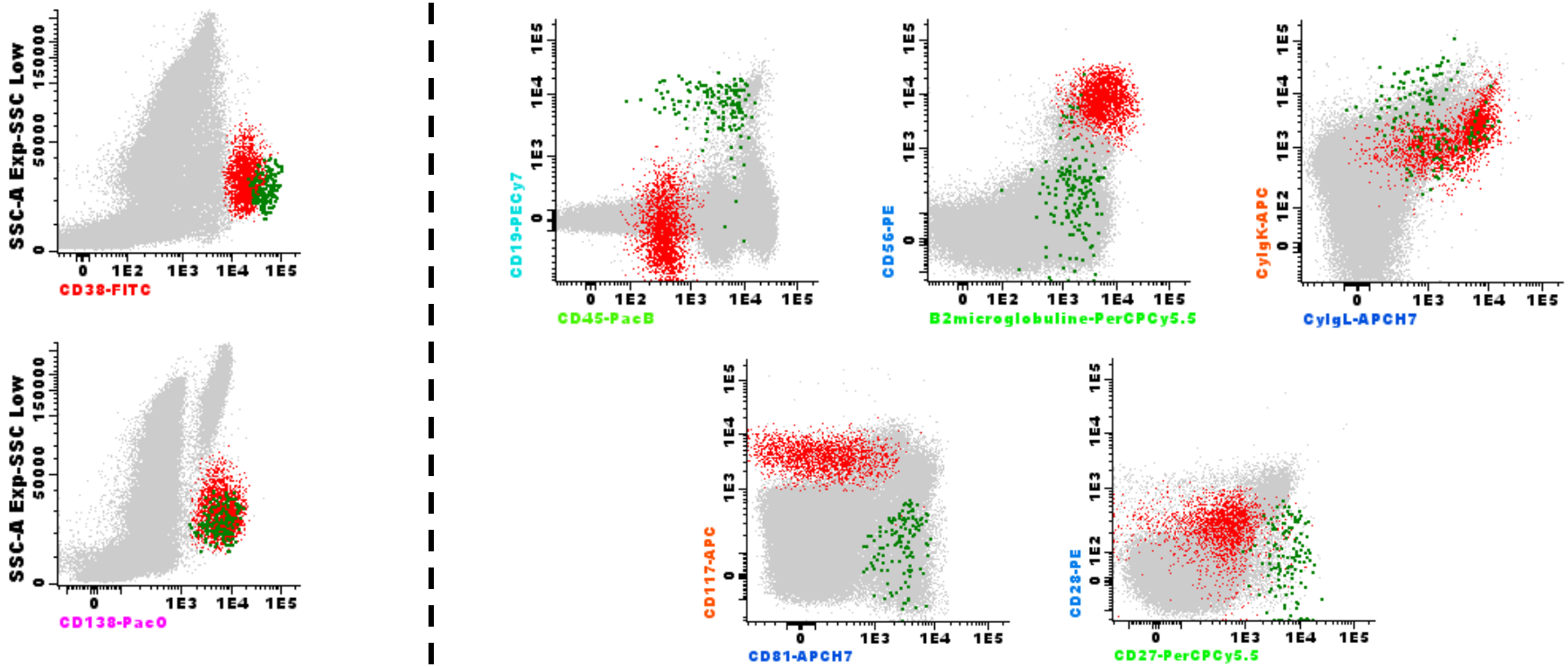




EuroFlow

FCM Immunophenotyping of PC

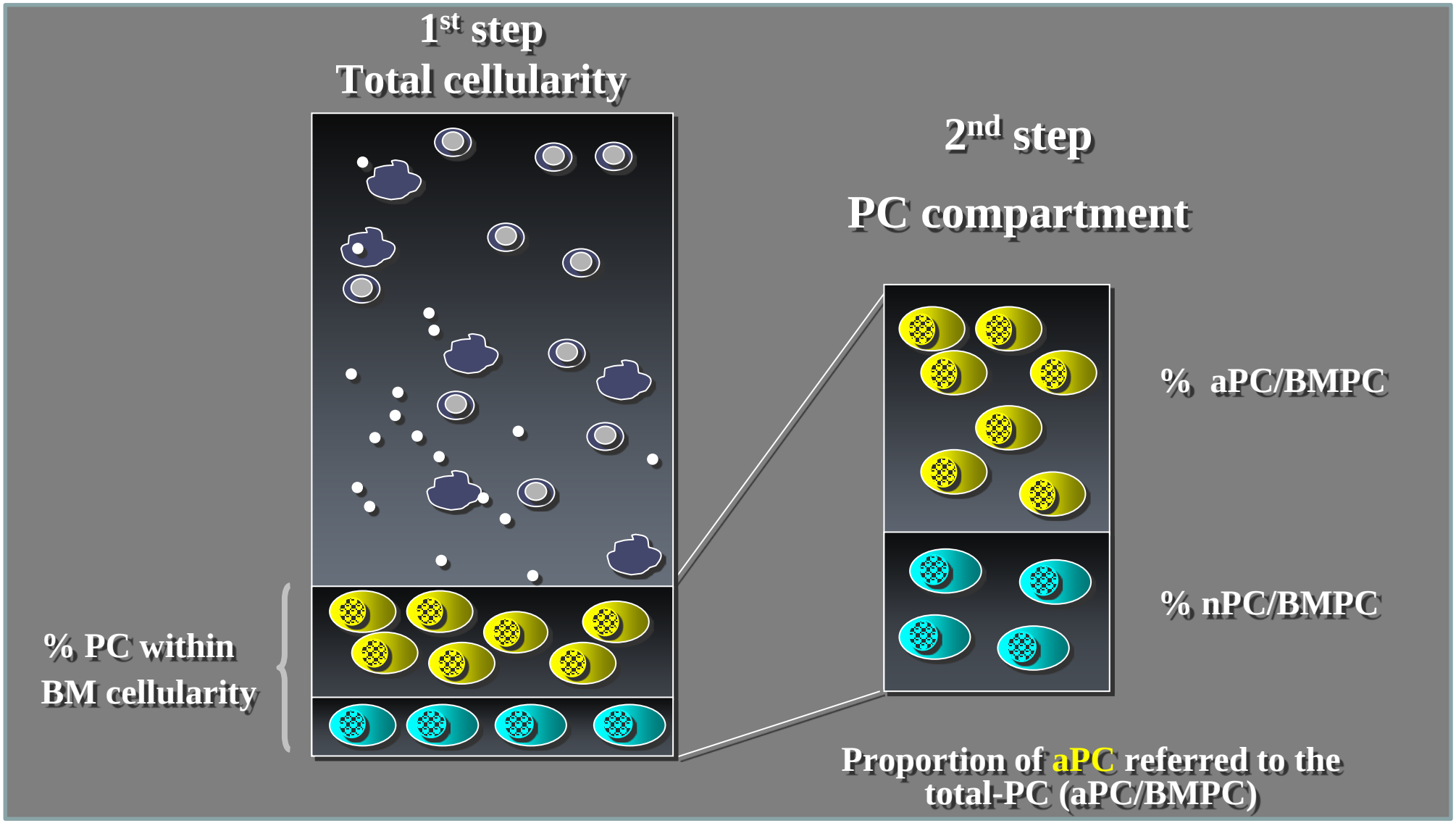
PC Immunophenotypic profile: Normal versus clonal PC



EuroFlow

FCM Immunophenotyping of PC

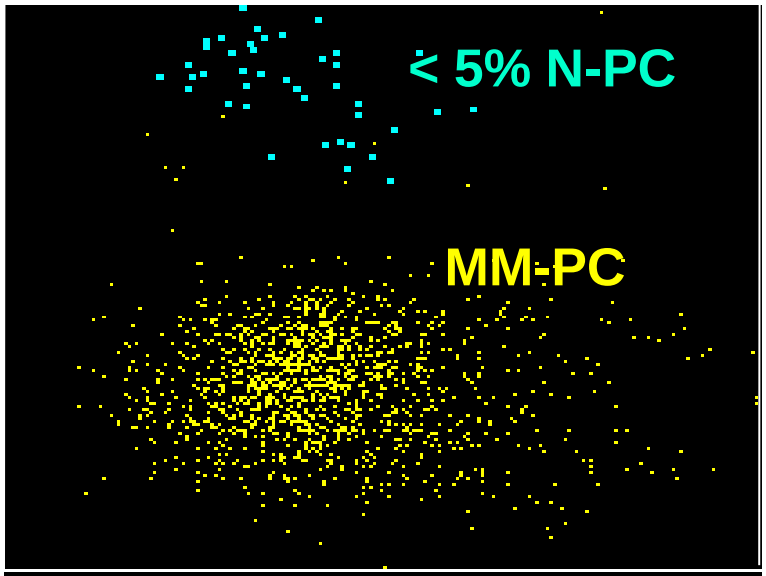
Normal versus clonal PC



FCM Immunophenotyping of PC

Differential diagnosis

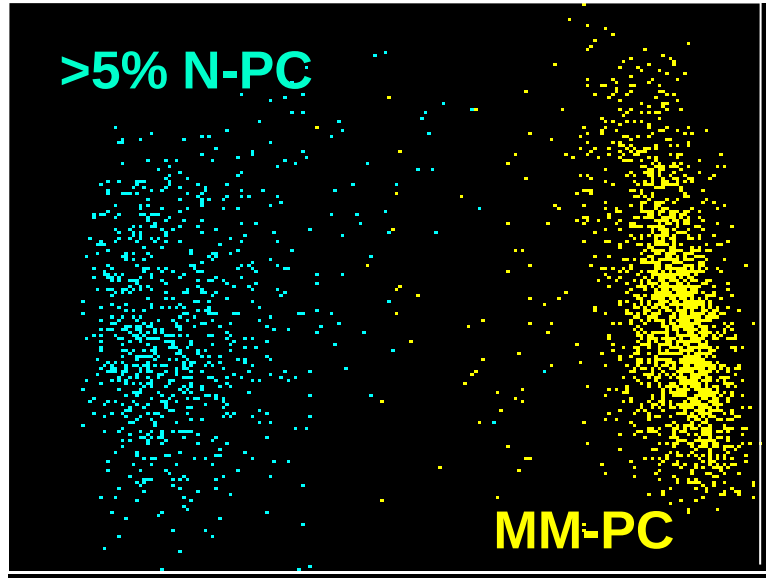
Symptomatic MM



Vast majority of cases with 100% Pathological PC

versus

MGUS/Smouldering MM



Co-existence of Pathological and Normal PC

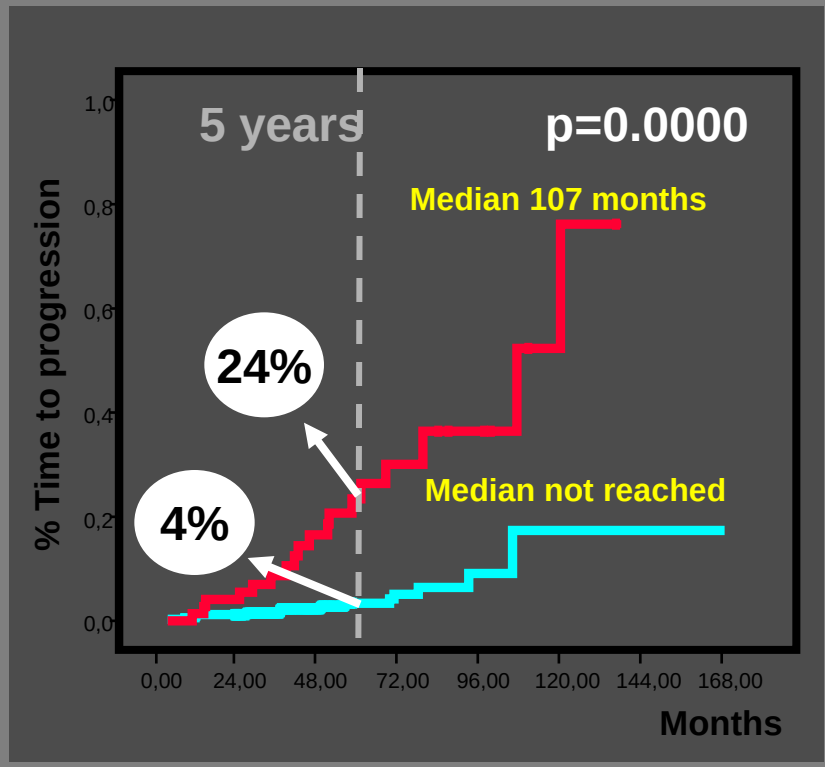
The most powerful single criteria for differential diagnosis

Ocqueteau et al, Am J Pathol 1998, 152: 1655

FCM Immunophenotyping of PC

Impact of % N-PC/BMPC in malignant transformation

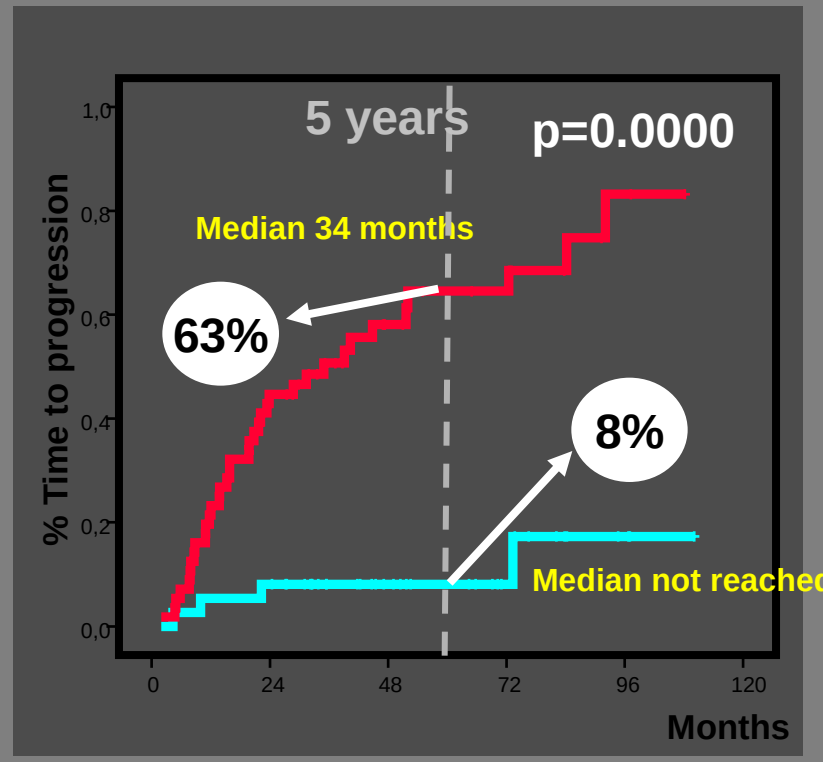
MGUS



>5% N-PC/BMPC
n= 330 (18 progr.)

≤5% N-PC/BMPC
n= 73 (15 progr.)

Smoldering MM



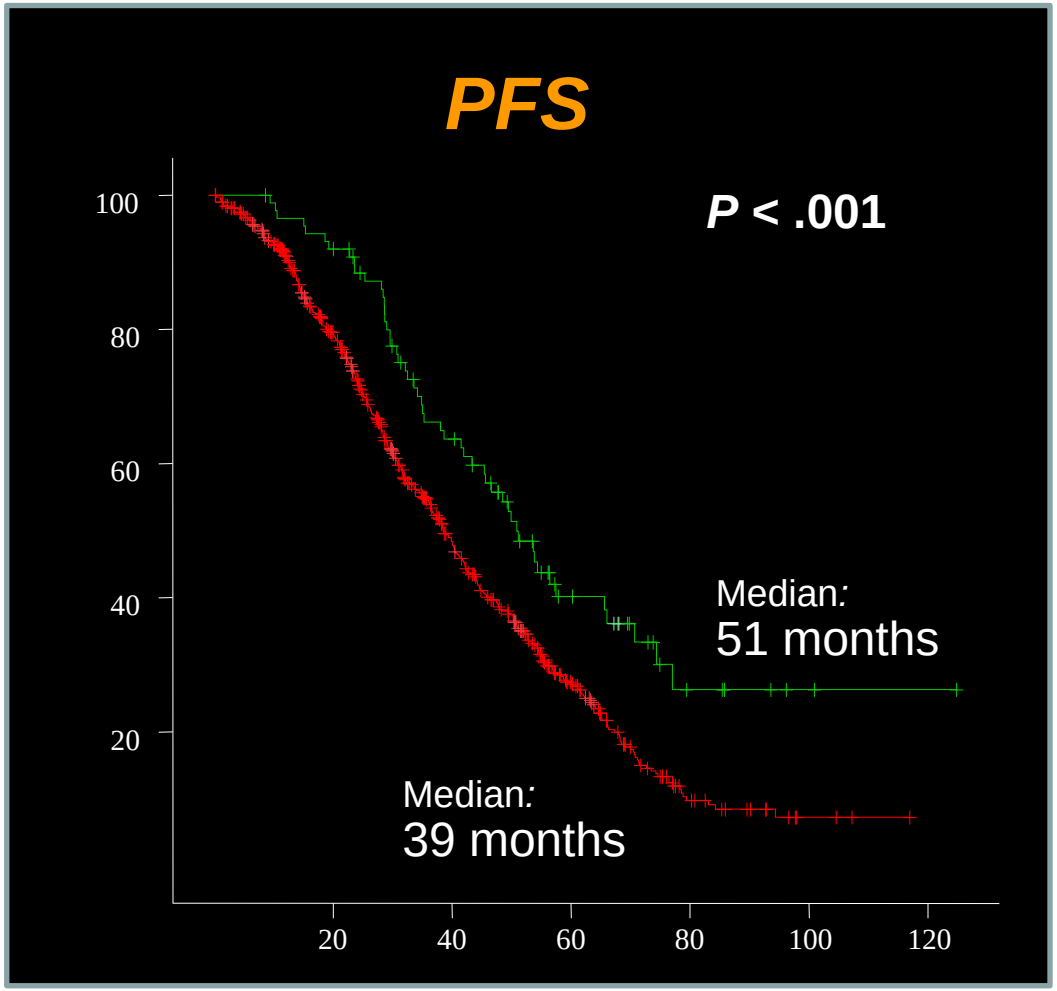
>5% N-PC/BMPC
n= 37 (4 progr.)

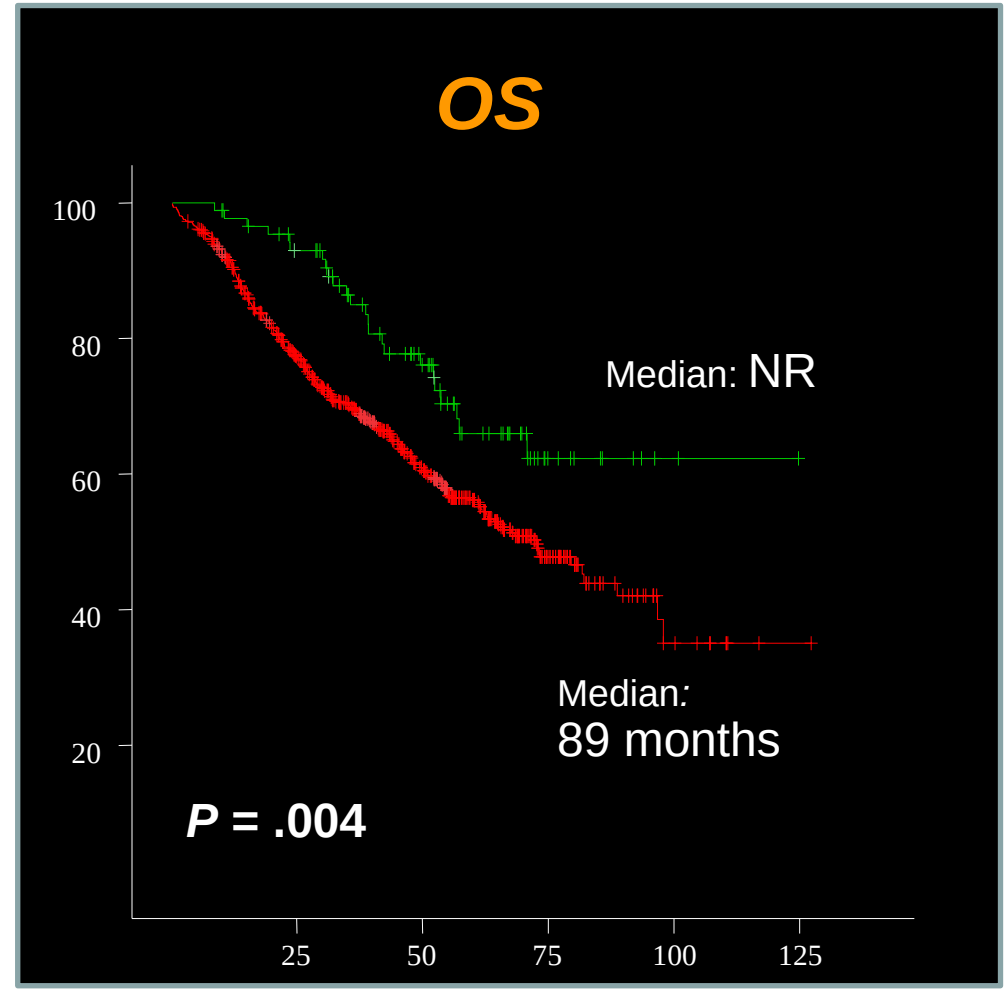
≤5% N-PC/BMPC
n= 56 (34 progr.)

Pérez-Persona et al; Blood. 2007 Oct 1;110(7):2586-92.

FCM Immunophenotyping of PC

Impact of % N-PC/BMPC in malignant transformation





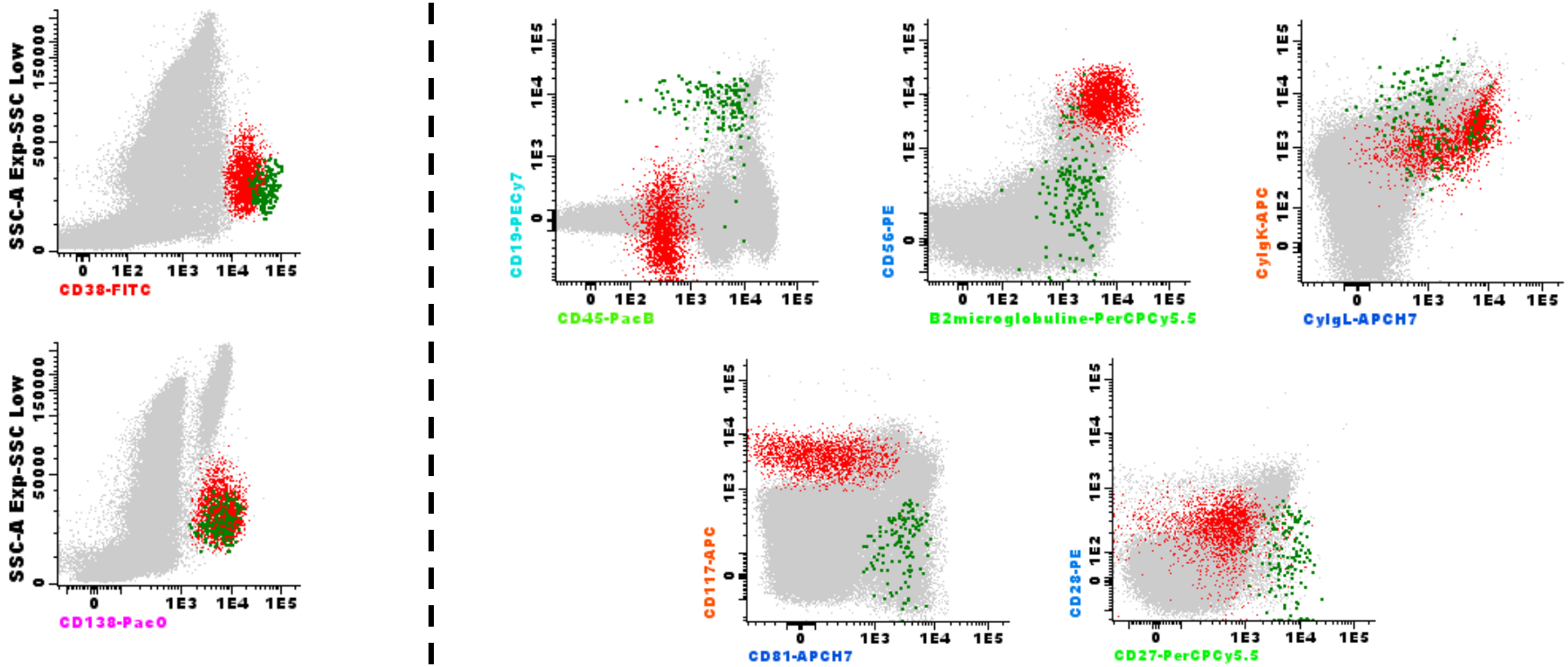
≤ 5% N-PC / BMPC (n=723)

> 5% N-PC / BMPC (n=88)

Paiva et al. Blood. 2009;114(20):4369-72

FCM Immunophenotyping of PC

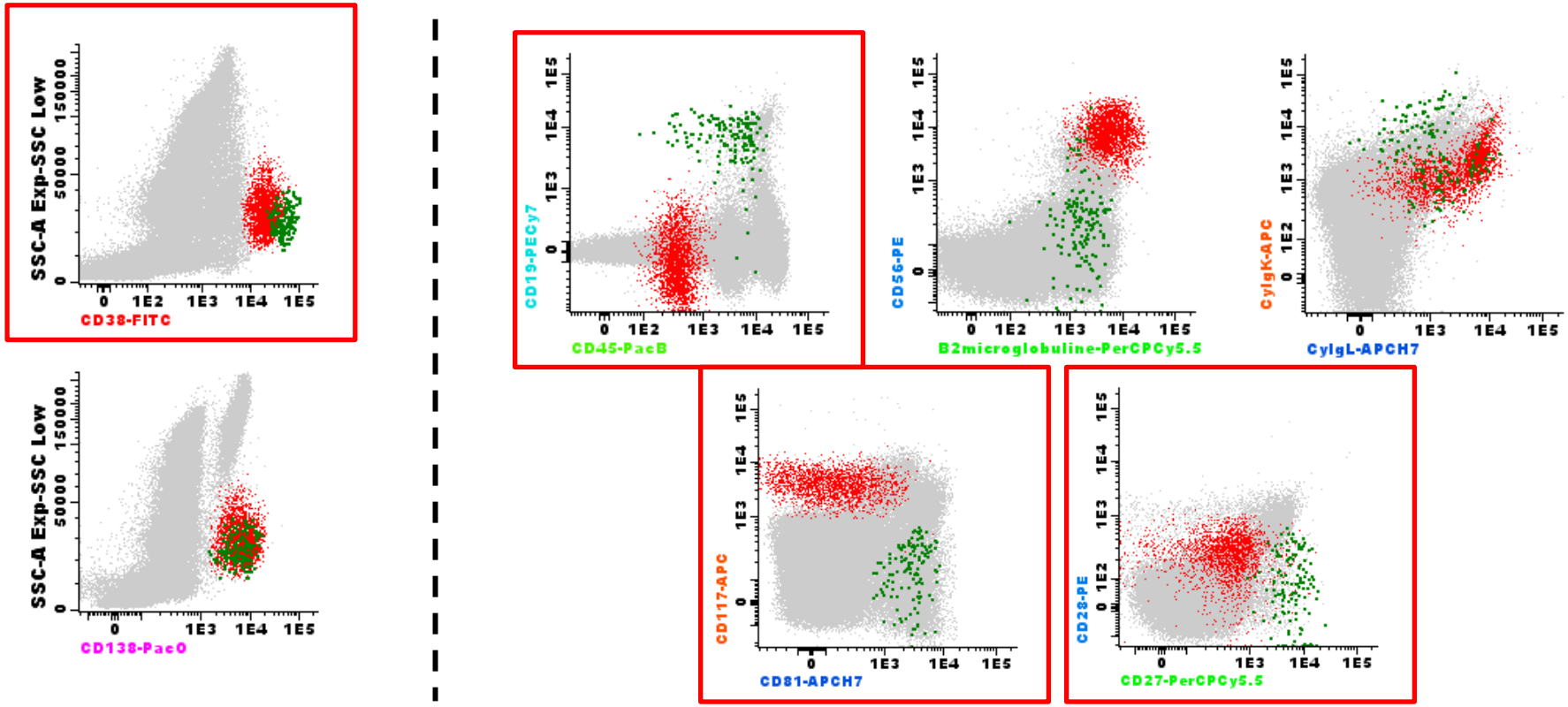
PC Immunophenotypic profile: Normal versus clonal PC



EuroFlow

FCM Immunophenotyping of PC

PC Immunophenotypic profile: Normal versus clonal PC



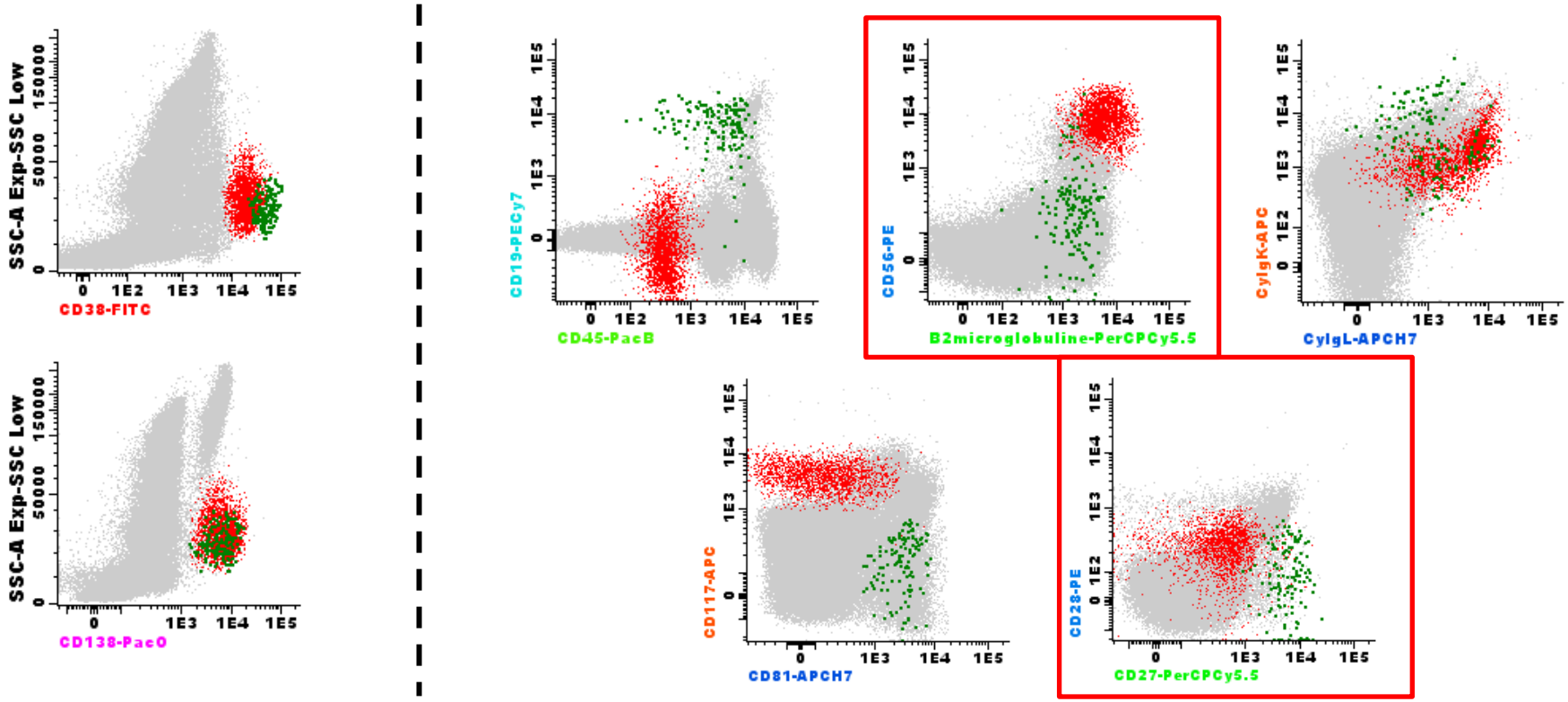
Antigen down-regulation:

$CD38^{dim}$ (~ 80%) | $CD19^{-}$ (93%) | $CD45^{-/dim}$ (83%) | $CD27^{-}$ (68%) | $CD81^{-/dim}$ (61%)

EuroFlow

FCM Immunophenotyping of PC

PC Immunophenotypic profile: Normal versus clonal PC



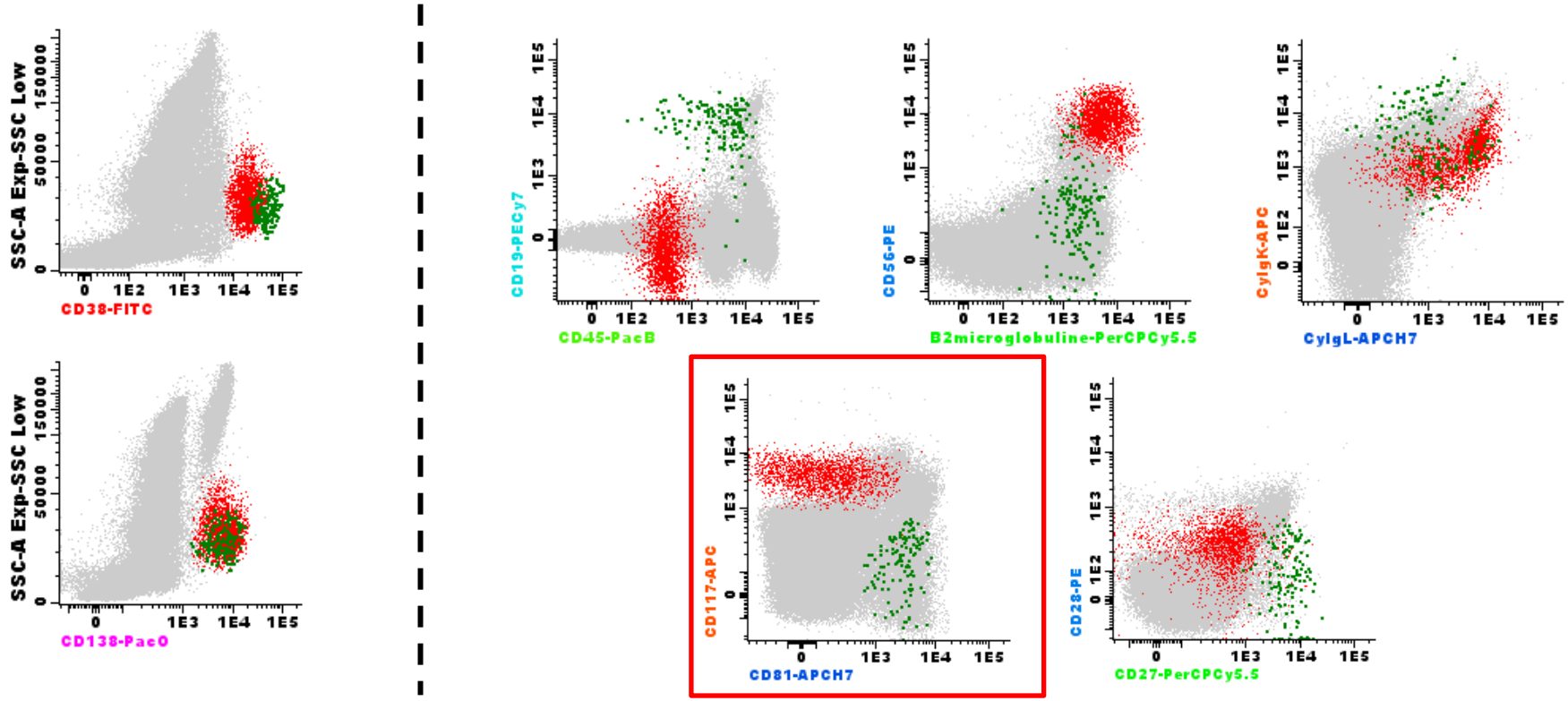
Antigen up-regulation:

$CD56^{hi}$ (57%) | $CD28^{+}$ (25%)

EuroFlow

FCM Immunophenotyping of PC

PC Immunophenotypic profile: Normal versus clonal PC



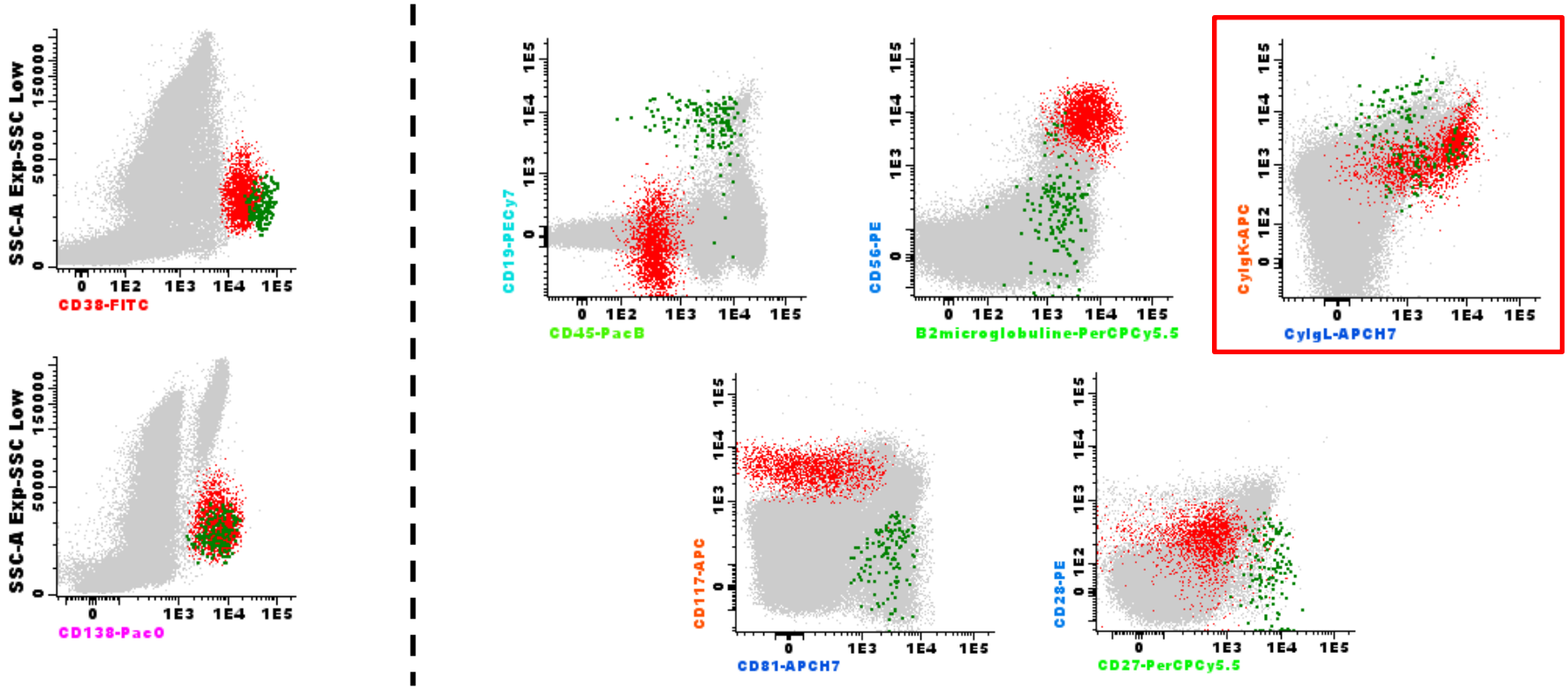
Asynchronous antigen expression:

CD117^{+} (~ 30%)

EuroFlow

FCM Immunophenotyping of PC

PC Immunophenotypic profile: Normal versus clonal PC

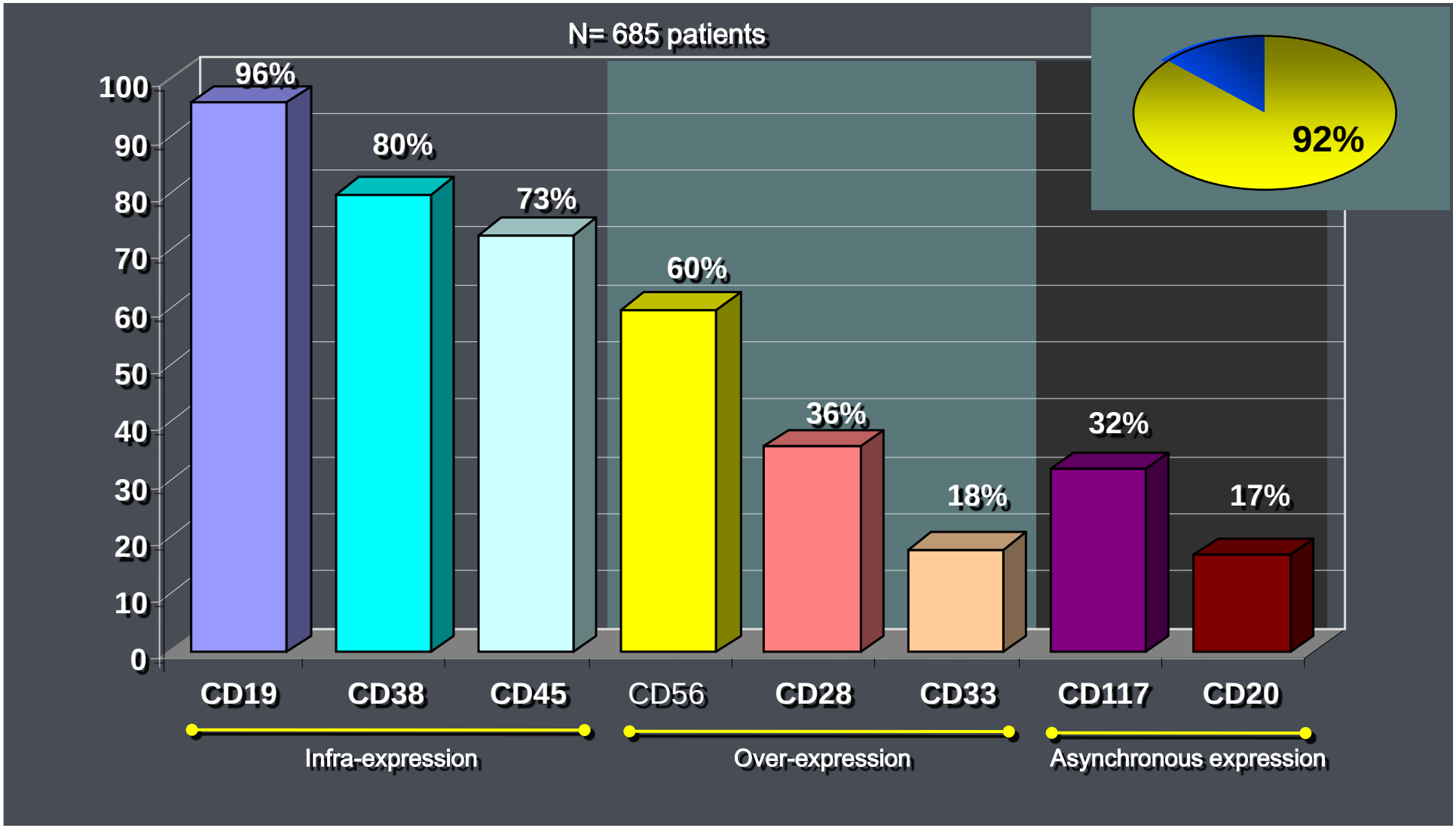


CyLight chain restriction

EuroFlow

FCM Immunophenotyping of PC

PC Immunophenotypic profile: Aberrant phenotypes

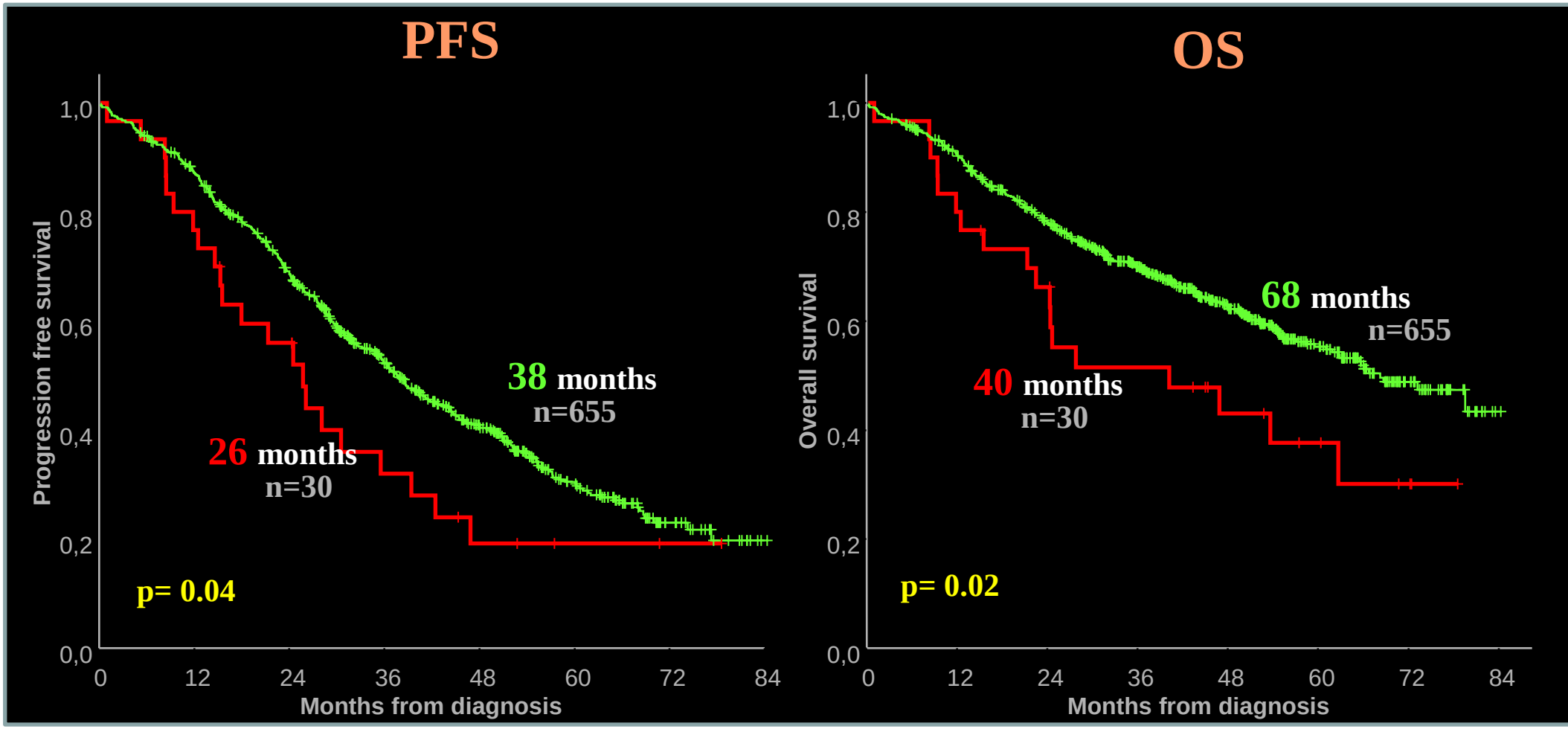


Mateo et al. J Clin Oncol; 2008;26:2737

EuroFlow

FCM Immunophenotyping of PC

PC Immunophenotypic profile: Impact of CD19 expression

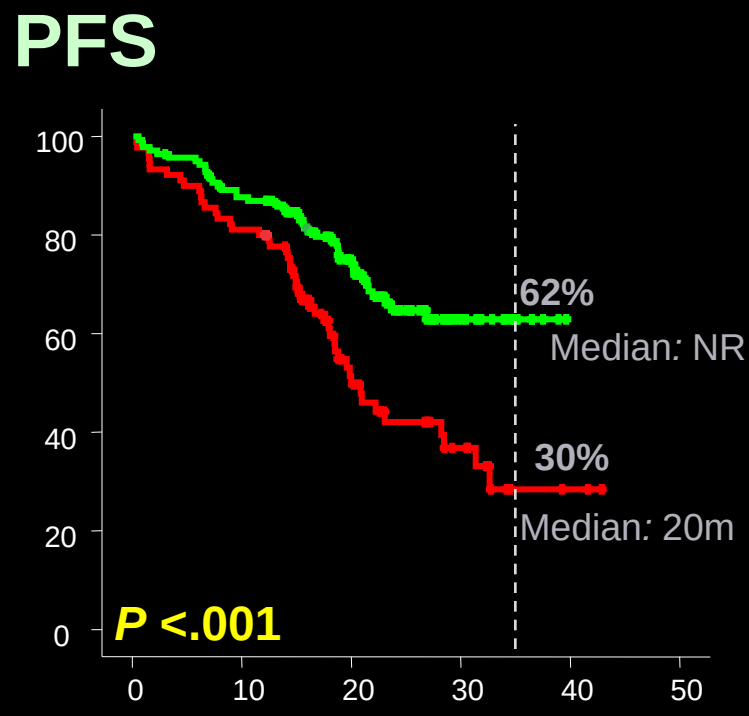


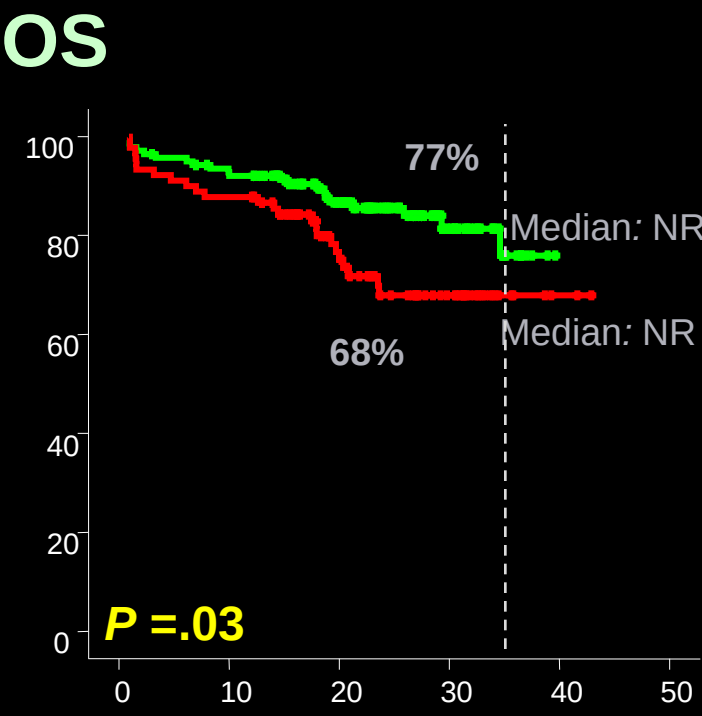
CD19 positive
CD19 negative

Mateo et al. J Clin Oncol; 2008;26:2737

FCM Immunophenotyping of PC

PC Immunophenotypic profile: Impact of CD81 expression



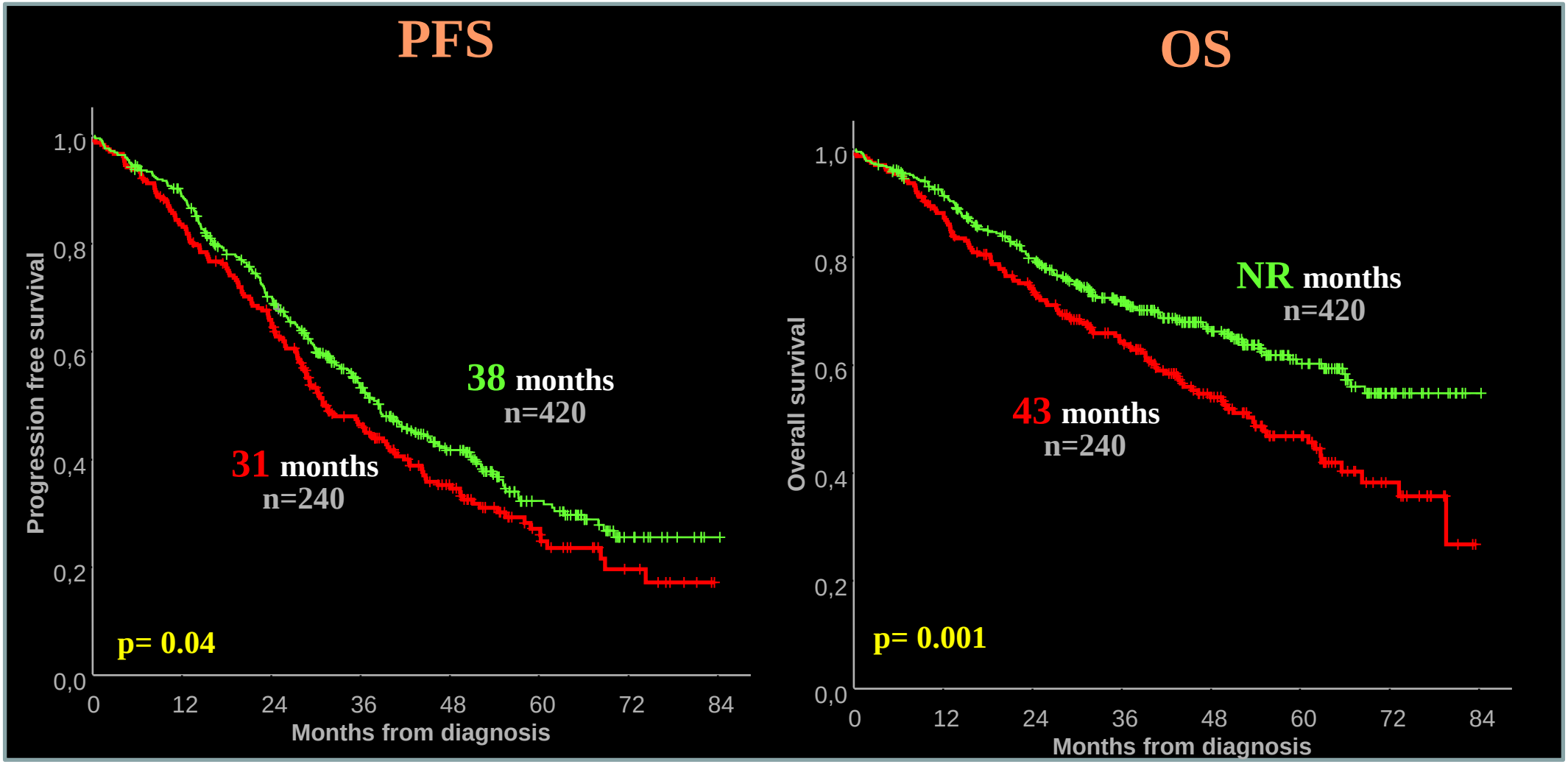


CD81 negative (n=139)
CD81 positive (n=90)

Paiva et al. Haematologica 2010; 95[suppl.2]:215, abs. 0528

FCM Immunophenotyping of PC

PC Immunophenotypic profile: Impact of CD28 expression



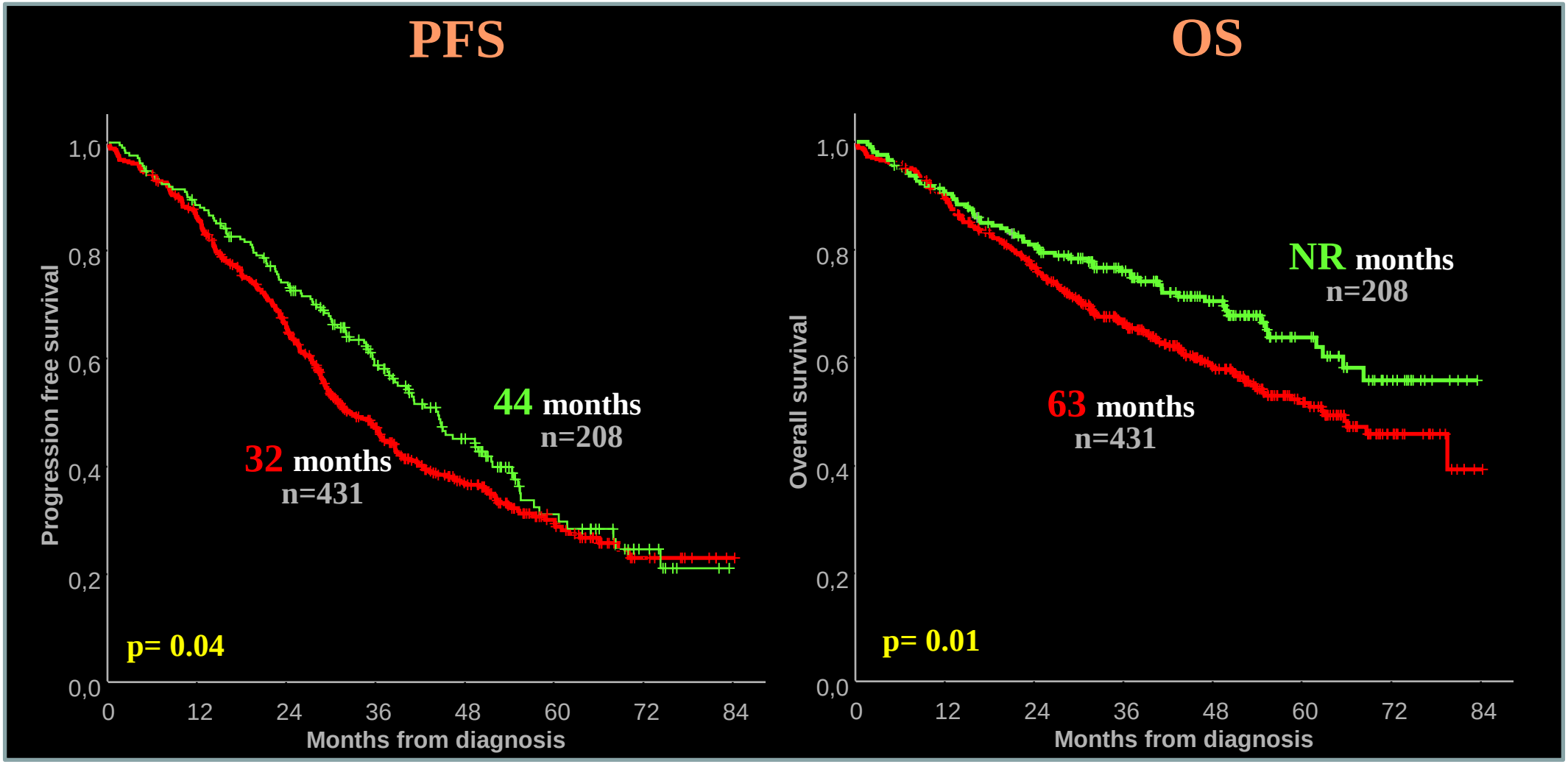
CD28 positive

CD28 negative

Mateo et al. J Clin Oncol; 2008;26:2737

FCM Immunophenotyping of PC

PC Immunophenotypic profile: Impact of CD117 expression



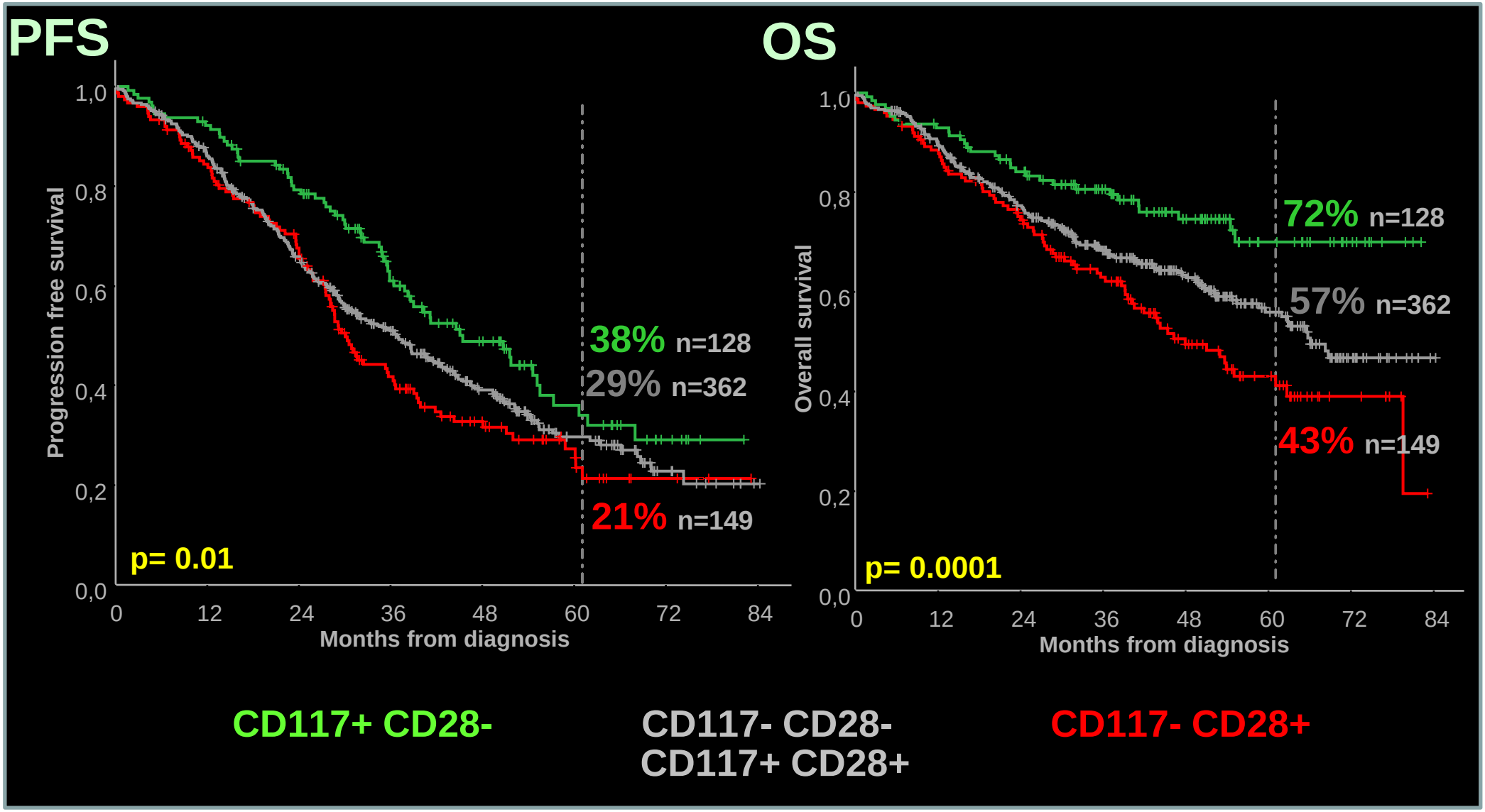
CD117 negative

CD117 positive

Mateo et al. J Clin Oncol; 2008;26:2737

FCM Immunophenotyping of PC

PC Immunophenotypic profile: Impact of CD117 expression



Mateo et al. J Clin Oncol; 2008;26:2737

FCM Immunophenotyping of PC

PC Immunophenotypic profile: Association with underlaying molecular abnormalities

	CD28		CD117	
	(-) versus (+)	p	(-) versus (+)	p
% Non-hyperdiply (FC)	**47% vs. 61%**	**0.002**	**63% vs. 32%**	**0.001**
IGH traslocations, %				
t(11;14)	13% vs. 14%	0.8	**16% vs. 7%**	**0.05**
t (4;14)	13% vs. 6%	0.09	**14% vs. 3%**	**0.02**
t (4;16)	**0.7% vs. 7%**	**0.03**	3% vs. 4%	0.6
Del(13q), %	45% vs. 39%	0.4	**48% vs. 31%**	**0.01**
Del(17p), %	**6% vs. 13%**	**0.04**	8% vs. 9%	0.6

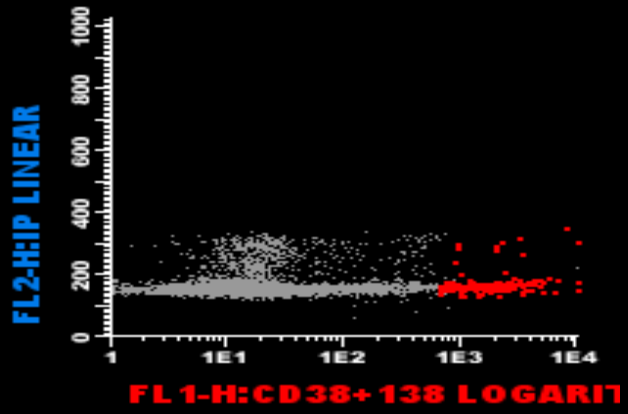
Mateo et al. J Clin Oncol; 2008;26:2737

FCM Immunophenotyping of PC

Abnormal PC ploidy and S-phase analysis

Diploid

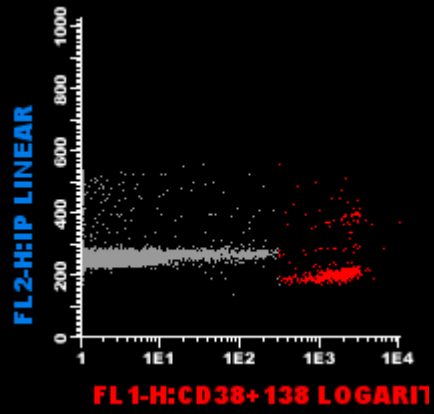
DNA index =1



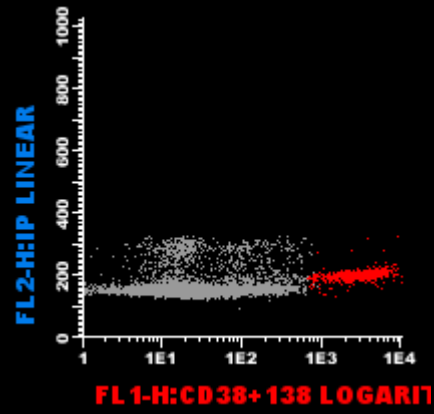
Aneuploid

DNA index ≠1

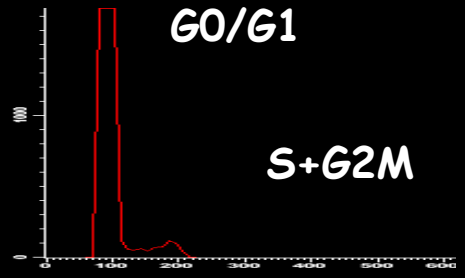
Hypodiploid



Hyperdiploid

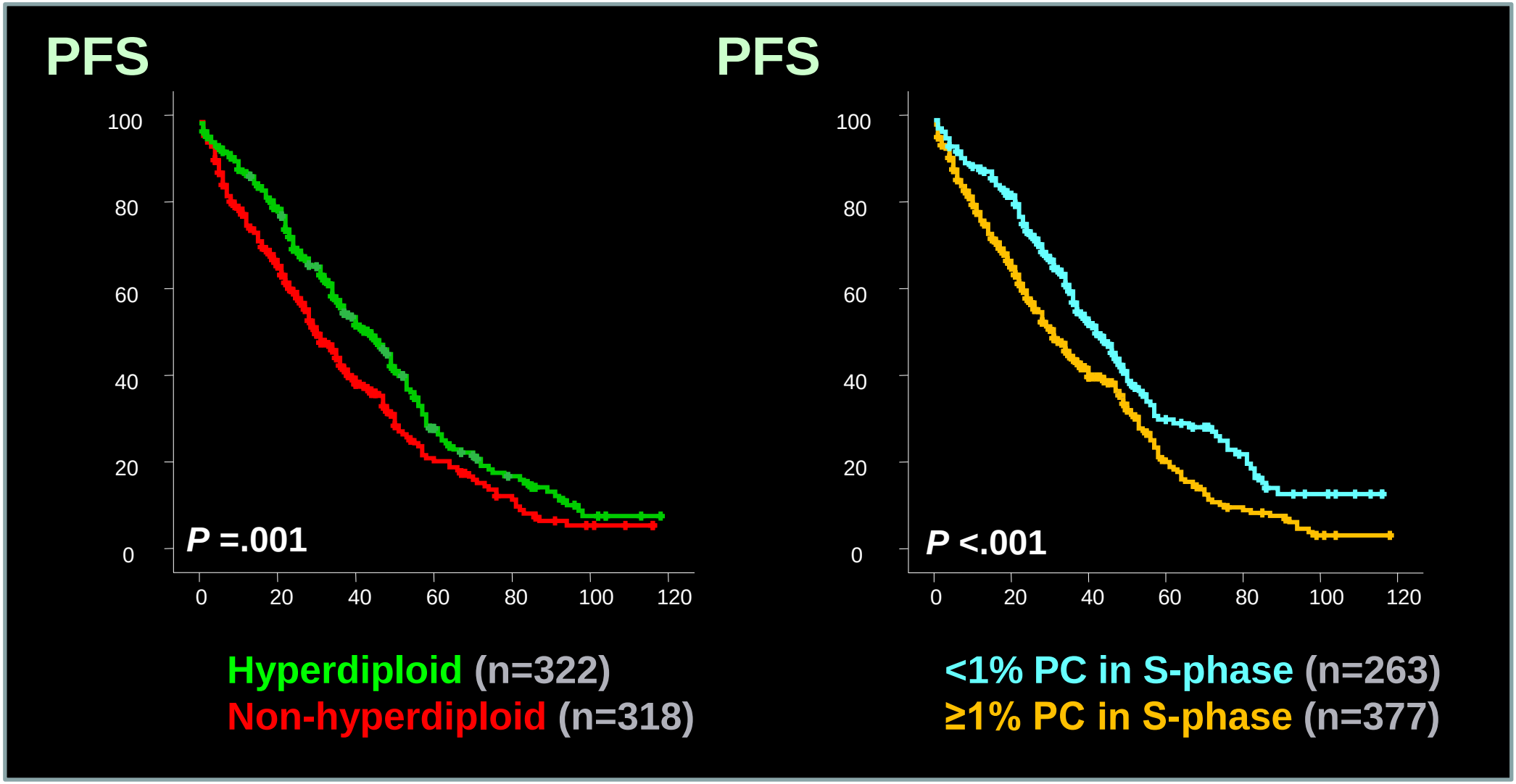


- Cell-cycle of PC:



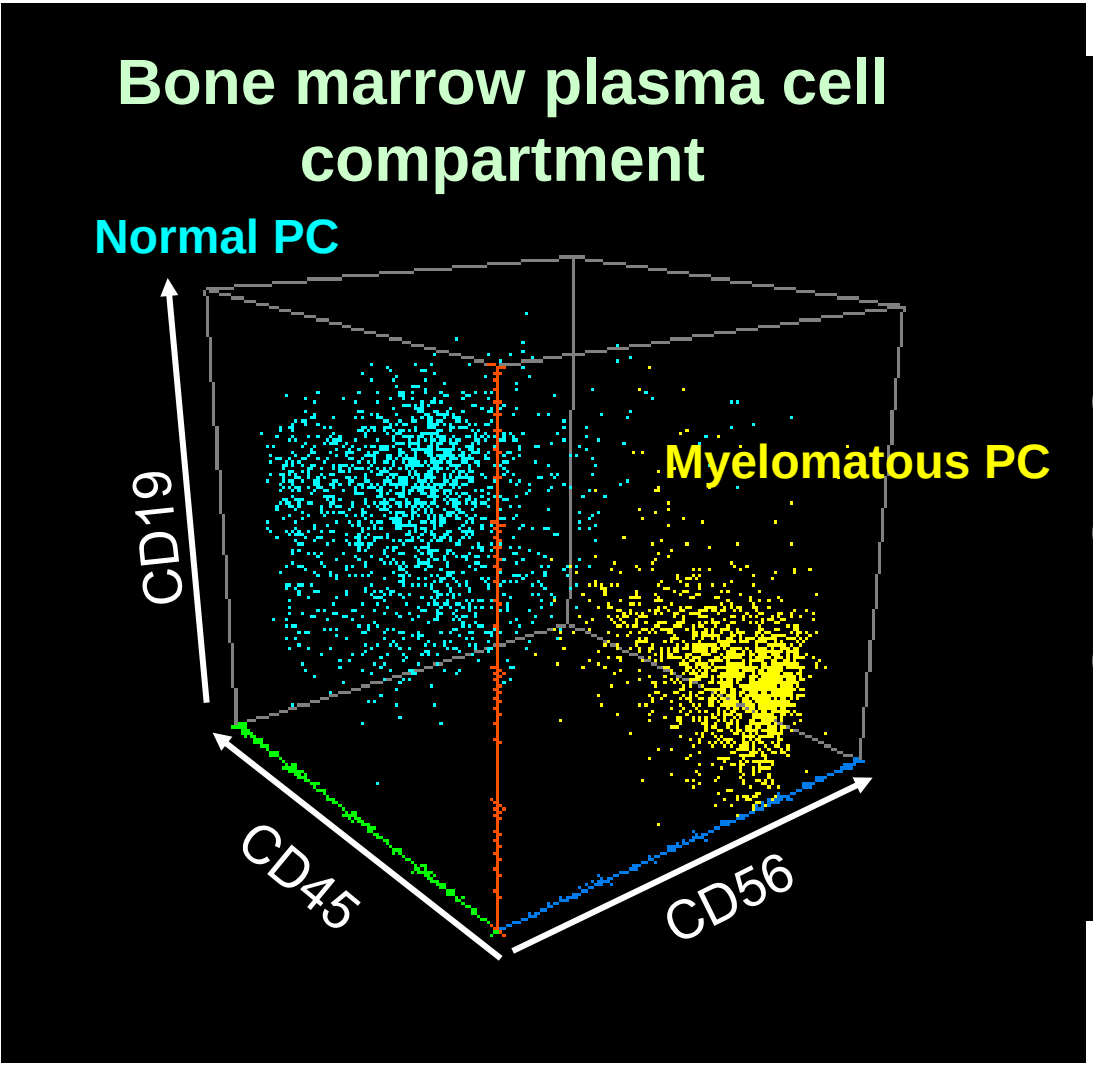
FCM Immunophenotyping of PC

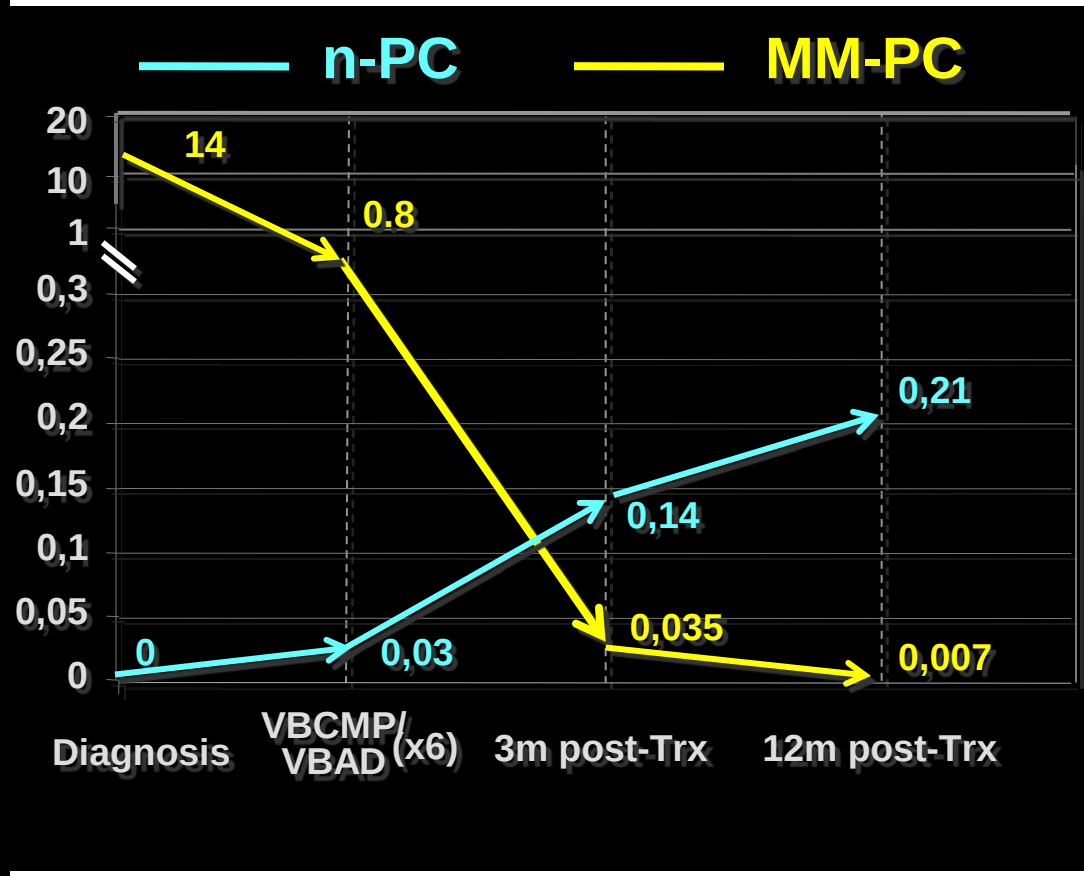
Abnormal PC ploidy and S-phase analysis. Clinical impact



FCM Immunophenotyping of PC

PC-compartment changes after treatment

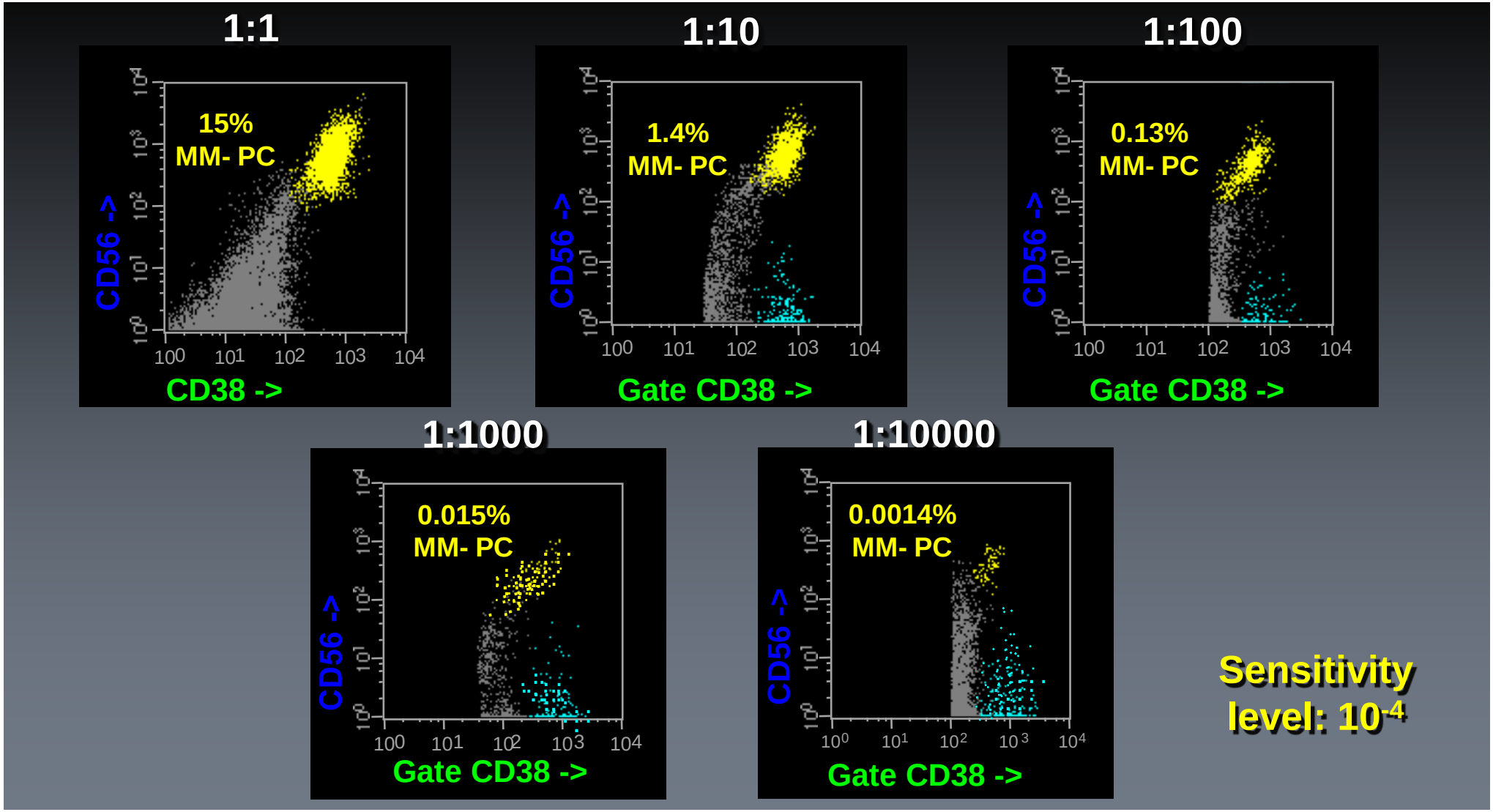




San Miguel et al Blood; 2002; 99:1853

FCM Immunophenotyping of PC

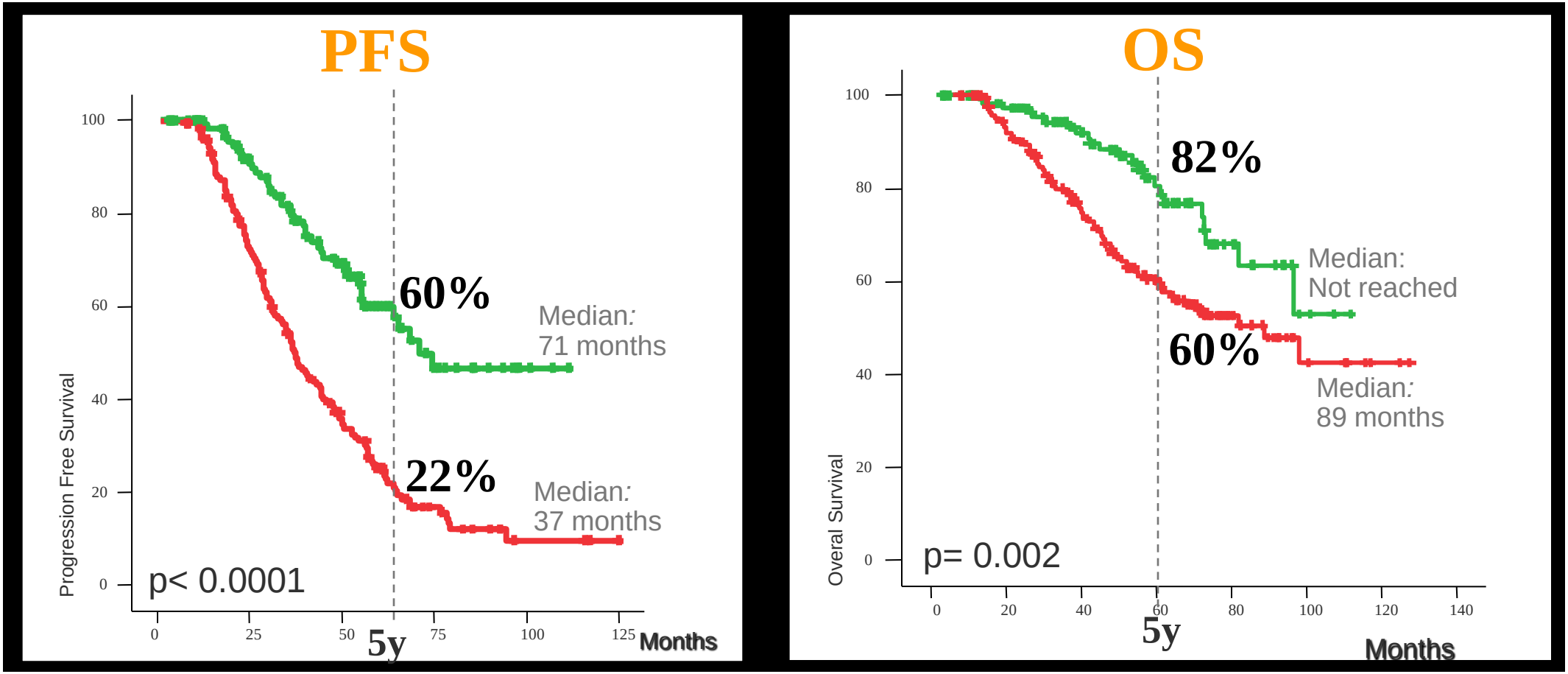
MRD monitoring. Sensitivity. 4-color FCM



San Miguel et al Blood; 2002; 99:1853

FCM Immunophenotyping of PC

MRD monitoring. Clinical impact. Survival according to MRD by Immunophenotyping in BM obtained 3 months after ASCT (n=295)



MRD negative (n=125)
MRD positive (n=170)

Paiva et al; Blood. 2008; 112: 4017

FCM Immunophenotyping of PC

The EuroFlow PCD panel

PCD panel Version	Tube	Violet laser FL channels		Blue laser FL channels				Red laser FL channels	
		PacB	AmCyan	FITC	PE	PerCP Cy5.5	PECy7	APC	APCCy7
1	1	CD45	CD19	Cylgλ	Cylgκ	CD38	CD56	CD27	
	2	CD45	CD19	β2 micro	CD81	CD38	CD117	CD28	
2	1	CD19	CD45	Cylgλ	Cylgκ	CD138	CD56	CD27	AF700 / CD38
	2	CD19	CD45	β2 micro	CD81	CD138	CD117	CD28	CD38
3	1	CD19	CD45	β2 micro	Cylgλ	CD138	CD56	Cylgκ	CD38
	2	CD19	CD45	CD27	CD81	CD138	CD117	CD28	CD38
4	1	CD19	CD45	β2 micro	Cylgλ	CD138	CD56	Cylgκ	APCH7 / CD38
	2	CD19	CD45	CD27	CD81	CD138	CD117	CD28	CD38
5	1	CD19	CD45	CD138	Cylgλ	β2 micro	CD56	Cylgκ	CD38
	2	CD19	CD45	CD138	CD81	CD27	CD117	CD28	CD38
6	1	CD45	PacO / CD138	CD38	CD56	β2 micro	CD19	Cylgκ	Cylgλ
	2	CD45	CD138	CD38	CD28	CD27	CD19	CD117	CD81

EuroFlow

FCM Immunophenotyping of PC

The EuroFlow PCD panel

Tube	PacB	PacO	FITC	PE	PerCP Cy5.5	PECy7	APC	APCH7
1	CD45	CD138	CD38	CD56	β2micro	CD19	CyIgκ	CyIgλ
2	CD45	CD138	CD38	CD28	CD27	CD19	CD117	CD81

EuroFlow

FCM Immunophenotyping of PC

The EuroFlow PCD panel

Tube	PacB	PacO	FITC	PE	PerCP Cy5.5	PECy7	APC	APCH7
1	**CD45**	**CD138**	**CD38**	CD56	β2micro	**CD19**	CyIgκ	CyIgλ
2	**CD45**	**CD138**	**CD38**	CD28	CD27	**CD19**	CD117	CD81

Backbone Markers

EuroFlow

FCM Immunophenotyping of PC

The EuroFlow PCD panel. Modular design

Tube	PacB	PacO	FITC	PE	PerCP Cy5.5	PECy7	APC	APCH7
1	CD45	CD138	CD38	CD56	β2micro	CD19	CyIgκ	CyIgλ
2	CD45	CD138	CD38	CD28	CD27	CD19	CD117	CD81

Tube 1(mandatory) :

- -Positive identification
- -Accurate quantification of PC
- -Diagnosis/assessment of clonality
- -Most frequent aberrant phenotypes
- -Potentially informative prognostic markers

EuroFlow

FCM Immunophenotyping of PC

The EuroFlow PCD panel. Modular design

Tube	PacB	PacO	FITC	PE	PerCP Cy5.5	PECy7	APC	APCH7
1	CD45	CD138	CD38	CD56	β2micro	CD19	CyIgκ	CyIgλ
2	CD45	CD138	CD38	CD28	CD27	CD19	CD117	CD81

Tube 2 (complementary):

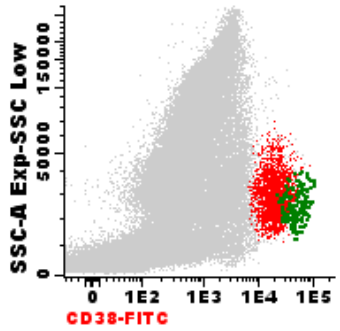
-Less frequent phenotypic aberrancyes

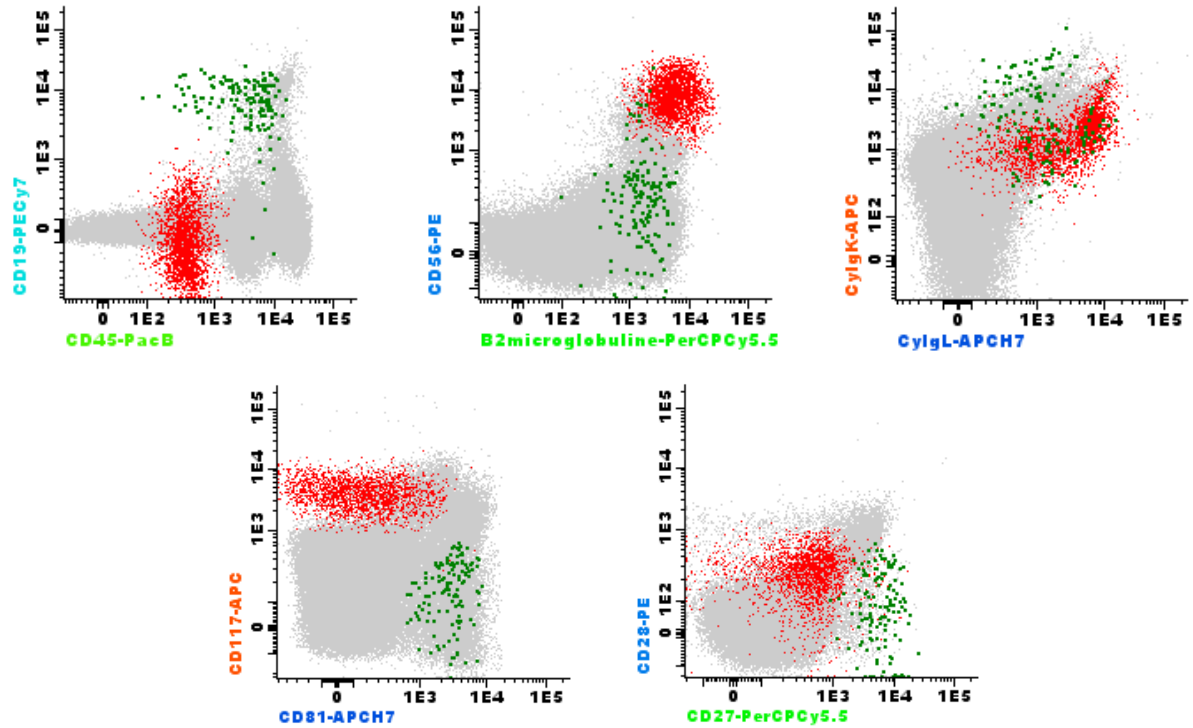
-Markers with prognostic impact

EuroFlow

FCM Immunophenotyping of PC A B

The EuroFlow PCD panel. Prospective evaluation





n=100. Clasic approach

EuroFlow

FCM Immunophenotyping of PC

The EuroFlow PCD panel. Prospective evaluation

Reference population pool

BM PC from healthy donors

n=100. Semiautomatic approach

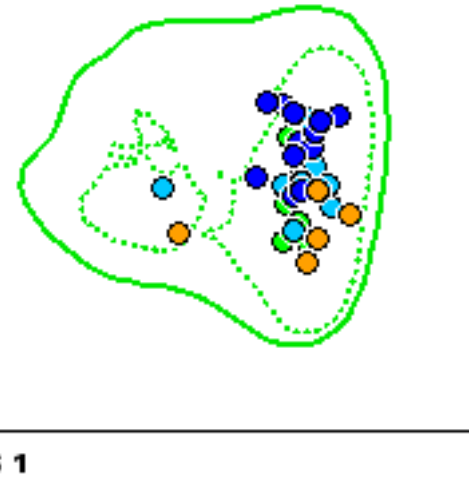
EuroFlow

FCM Immunophenotyping of PC

The EuroFlow PCD panel. Prospective evaluation

Reference population pool

BM PC from healthy, reactive and regenerative donnors



n=100. Semiautomatic approach

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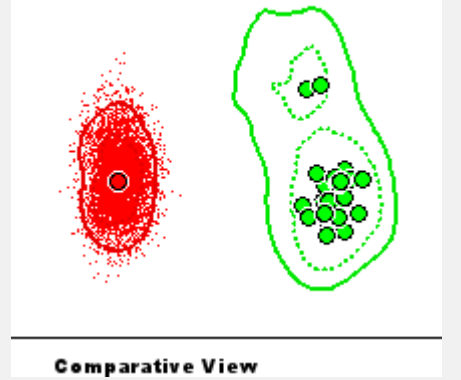
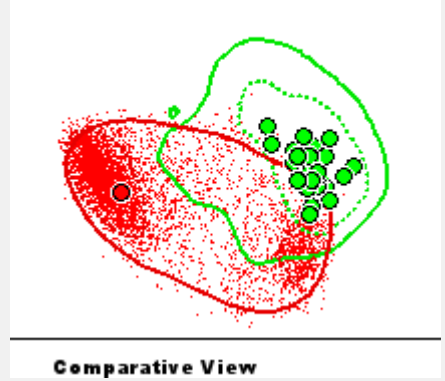
FCM Immunophenotyping of PC

The EuroFlow PCD panel. Prospective evaluation

Abnormal PC populations



CD19-PECy7	21.16
CD56-PE	18.97
CD81-APCH7	12.05
Cylgκ:APC	11.61
CD117-APC	11.33
CD27-PerCPCy5.5	8.30



CD56-PE	23.73
CD19-PECy7	21.28
CD117-APC	17.77
CD81-APCH7	9.52
CD45-PB	6.05
Cylgλ-APCH7	5.74

n=100. Semiautomatic approach

EuroFlow

Thank you

EuroFlow

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