ИММУНОЛОГИЧЕСКИЕ ПОДХОДЫ К ДЕТЕКЦИИ МОБ ПРИ МНОЖЕСТВЕННОЙ МИЕЛОМЕ

Л.Ю.ГРИВЦОВА

РУКОВОДИТЕЛЬ ОТДЕЛА ЛАБОРАТОРНОЙ МЕДИЦИНЫ

МРНЦ ИМ. А.Ф. ЦЫБА – ФИЛИАЛ НМИЦ РАДИОЛОГИИ МИНЗДРАВА РОССИИ

2011 г – Международная рабочая группа по ММ

иммунофенотипическая ремиссия – методы MFC
и молекулярная ремиссия, - аллель-специфичной полимеразной цепной реакции (АС-ПЦР)

NGS???

Lancet Oncol 2016; 17: e328–46

		N	MRD-negative patients	Outcomes
Paiva et al[28]	Patients with newly diagnosed multiple myeloma from GEM2000*. MRD status by MFC was determined at day 100 after ASCT	295	125 (42%)	PFS (median 71 months *vs* 37 months; p<0·001) and OS (median not reached *vs* 89 months; p=0·002) were longer in patients who were MRD-negative at day 100 after ASCT
Rawstron et al[32]	MRC IX trial of newly diagnosed multiple myeloma: intensive pathway with CTD *vs* CVAD followed by ASCT	397	246 (62%)	Median PFS for MRD-positive patients of 15·5 months *vs* 28·6 months for MRD-negative patients (p<0·001). Median OS of 59·0 months in MRD-positive patients *vs* 80·6 months in MRD-negative patients (p=0·02)
	MRC IX trial of newly diagnosed multiple myeloma: non-intensive pathway (melphalan and prednisone *vs* CTD)	245	37 (15%)	MRD-positive at end of induction associated with non-significantly inferior PFS (median 7·4 months *vs* 10·5 months, p=0·1)
Puig et al[33]	GEM2000* and GEM2005MENOS65† trials	102	52 (51%)	MRD-negative patients had longer PFS, both in intensively treated patients (median 45 months *vs* 27 months, p=0·02) and in non-intensively treated patients (not reached *vs* 27 months; p=0·002)
Sarasquete et al[39]	Patients with multiple myeloma who had achieved a complete response after transplantation	24	13 (53%)	Improved PFS for MRD-negative patients (median 27 months *vs* 10 months; p=0·05)
Paiva et al[29]	Transplant-ineligible patients with multiple myeloma who had achieved >75% reduction in the myeloma component after induction	102	31 (30%)	Achieving MRD-negativity translated into superior PFS and TTP compared with conventional complete response or stringent complete response (without clonality assessment on trephine biopsy)
Paiva et al[30]	Newly diagnosed patients with multiple myeloma from GEM2000* and GEM2005MENOS65† who achieved a complete response at day 100 after ASCT	241	154 (64%)	Presence of baseline high-risk cytogenetics and persistent MRD at day 100 after ASCT were the only independent factors that predicted unsustained complete response
Roussel et al[56]	Phase 2 study with three induction cycles followed by ASCT, consolidation, and 1-year lenalidomide maintenance	31	21 (68%)	Estimated 100% relapse-free survival at 3 years for MRD-negative patients

N=total number of patients. MRD=minimal residual disease. MFC=multiparametric flow cytometry. ASCT=autologous stem-cell transplantation. PFS=progression-free survival. OS=overall survival. CTD=cyclophosphamide, thalidomide, dexamethasone. CVAD=cyclophosphamide, vincristine, doxorubicin, dexamethasone. TTP=time to progression. *Vincristine, carmustine, melphalan, cyclophosphamide, and prednisone (VBMCP)/vincristine, carmustine, doxorubicin, dexamethasone (VBAD) induction plus ASCT. †Transplant-eligible patients: VBMCP/VBAD plus bortezomib in the last two cycles, thalidomide/dexamethasone or bortezomib/thalidomide/dexamethasone immediately after diagnosis; elderly patients: six induction cycles with bortezomib, melphalan, prednisone or bortezomib, thalidomide, prednisone.

***Table 1*: Studies using conventional flow cytometry-based assays for minimal residual disease detection**

КЛИНИЧЕСКАЯ ЗНАЧИМОСТЬ MFC ОЦЕНКИ МОБ ПРИ ММ

rk	Disease status and treatment	n	MRD negative patients	Results
Paiva et a	Patients with newly diagnosed multiple myeloma from GEM2000*. MRD status by MFC was determined at day 100 after ASCT	295	125 (42%)	PFS (median 71 months vs 37 months; p<0·001) and OS (median not reached vs 89 months; p=0·002) were longer in patients who were MRD-negative at day 100 after ASCT
Rawstron et al	MRC IX trial of newly diagnosed multiple myeloma: intensive pathway with CTD vs CVAD followed by ASCT	397	246 (62%)	Median PFS for MRD-positive patients of 15·5 months vs 28·6 months for MRD-negative patients (p<0·001). **Median OS of 59·0 months in MRD-positive patients vs 80·6 months in MRD-negative patients (p=0·02)**
	MRC IX trial of newly diagnosed multiple myeloma: non-intensive pathway (melphalan and prednisone vs CTD)	245	37 (15%)	MRD-positive at end of induction associated with non-signifi cantly inferior PFS (median 7·4 months vs 10·5 months, p=0·1)
Paiva et al	Newly diagnosed patients with multiple myeloma from GEM2000* and GEM2005MENOS65† who achieved a complete response at day 100 after ASCT	241	154 (64%)	Presence of baseline high-risk cytogenetics and persistent MRD at day 100 after ASCT were the only independent factors that predicted unsustained complete response

МИНИМАЛЬНАЯ ОСТАТОЧНАЯ БОЛЕЗНЬ ПРИ МНОЖЕСТВЕННОЙ МИЕЛОМЕ

Первично-диагностируемая ММ
MFC- 9 – исследований
PCR – 11- исследований
NGS-1 исследование

This large-cohort meta-analysis confirms that **MRD status has prognostic value** and **is a valid surrogate marker for both PFS and OS** in patients with MM, including those who had achieved a CR.
MRD is a better predictor of PFS and OS than conventional complete response

Сравнение соотношения рисков (HAZARD RATIO)
Преимущество PCR в сравнении с MFC

Minimal residual disease predicts superior survival in patients with multiple myeloma: a meta-analysis *C. Munshi*

JAMA ONCOL . 2017 JANUARY 01; 3(1): 28–35

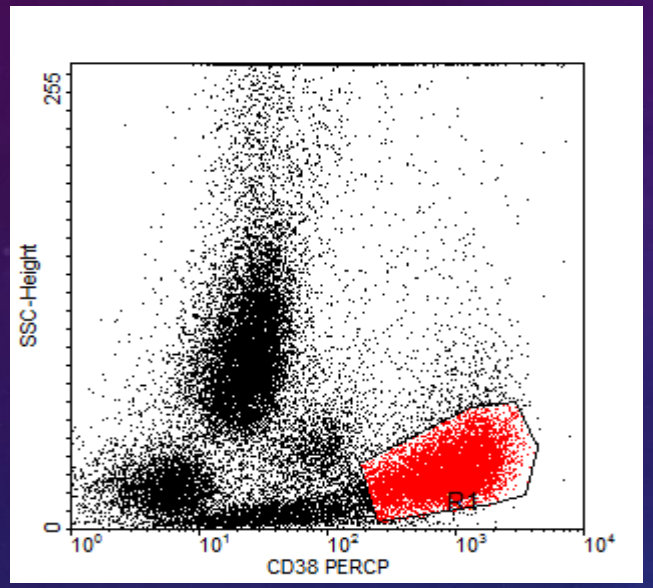
МНОГОПАРАМЕТРОВАЯ ПРОТОЧНАЯ ЦИТОМЕТРИЯ – ИНТЕГРАЛЬНАЯ ЧАСТЬ ЛАБОРАТОРНЫХ ИССЛЕДОВАНИЙ

Диагноз
Риск-стратификация
Мониторинг ответа на терапию

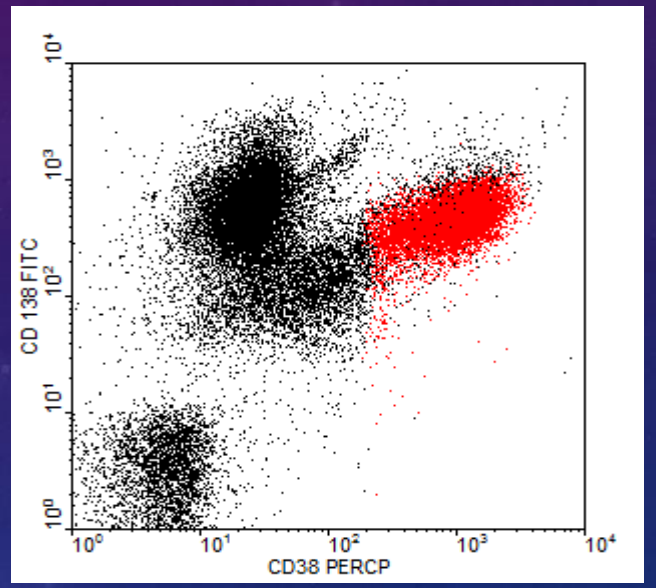
Патогенез болезни (биологические особенности прогрессии опухоли)
Особенности микроокружения опухоли
Выявление новых терапевтических мишеней

MFC - выявление плазматических клеток

СТАНДАРТНЫЕ МАРКЕРЫ ПЛАЗМОЦИТОВ



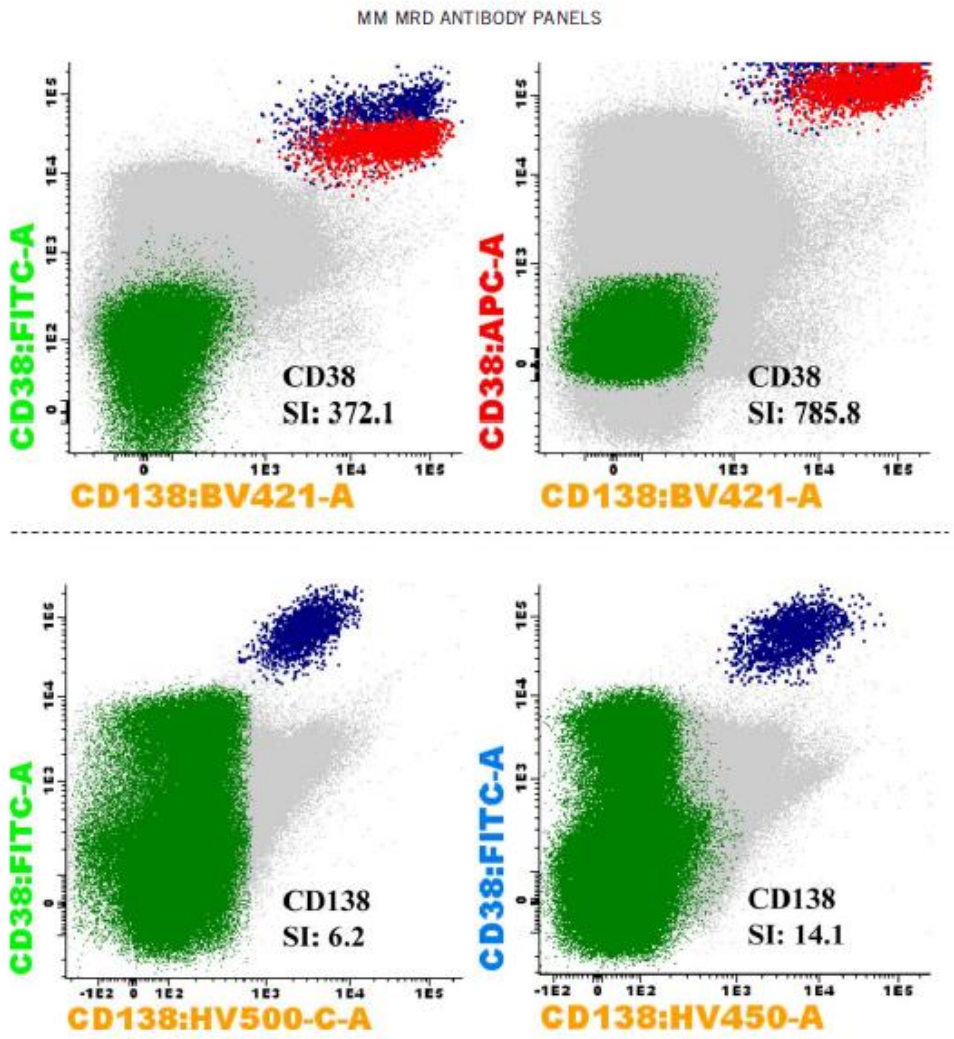
CD38/SSC



CD38/CD138

Gupta et al.,2009

ОПТИМАЛЬНЫЕ КОМБИНАЦИИ АНТИТЕЛО ФЛУОРОХРОМ



Cytometry Part B (Clinical Cytometry) 90B:61–72 (2016)

.J. Flores – Montero et al

АБЕРРАНТНОСТЬ ПЛАЗМАТИЧЕСКИХ КЛЕТОК

Table 2. List of most useful antigens for the detection of aberrant plasma cells in multiple myeloma.[5–10;20–44]

Antigen	*Normal expression profile (percentage expression on normal plasma cells)*	*Abnormal expression profile*	*Percentage of myeloma cases with abnormal expression*	*Requirement for diagnosis and monitoring*
CD19	Positive (>70%)	Negative	95%	Essential
CD56	Negative (<15%)	Strongly positive	75%	Essential
CD117	Negative (0%)	Positive	30%	Recommended
CD20	Negative (0%)	Positive	30%	Recommended
CD28	Negative/weak (<15%)	Strongly positive	15-45%	Recommended
CD27	Strongly positive (100%)	Weak or negative	40-50%	Recommended
CD81	Positive (100%)	Weak or negative	Not published	Suggested
CD200	Weakly positive	Strongly positive	Not published	Suggested

A.RAWSTRON ET AL., 2008

APPENDIX 1. MYELOMA MRD PANELS VALIDATED IN LARGE SCALE CLINICAL TRIALS

1. *Six Color panel: Source: Leeds U.K. (Myeloma XI and X and first 2000 patients for Myeloma XI)*

	FITC	PE	PerCP Cy5.5	PC7	APC	APCC750
1	CD27	CD56	CD19	CD38	CD138	CD45
2[a]	CD81	CD117	CD19	CD38	CD138	CD45

[a]Tubes 2/3 used in cases with <2 aberrant markers at diagnosis in tube 1 (e.g., CD19-CD56-CD27++). Authors de Tute and Rawstron note that the panel was developed a decade ago based on six color flow cytometers and limited fluorochrome options available at the time. With current reagent availability and flow cytometers this is not necessarily the optimal reagent combination with respect to fluorochrome, although the combination of markers remains appropriate for laboratories limited to six-color analysis.

АБЕРРАНТНОСТЬ ПЛАЗМАТИЧЕСКИХ КЛЕТОК

Table 1

Most Frequently used Markers for Detection of Myeloma Associated Phenotypes (MAP) Included in MM MRD Panels

Antigen	Aberrant pattern	% of abnormal expression cases
CD19	−	96%
CD20	dim+	17–30%
CD27	− or dim+	40–68%
CD28	+	15–45%
CD33	+	18%
CD38	dim+	80%
CD45	−	73%
CD54	dim+	60–80%
CD56	++	60–75%
CD81	− or dim+	55%
CD117	+	30–32%
CD200	+/++	≥70%
CD307	++	NA

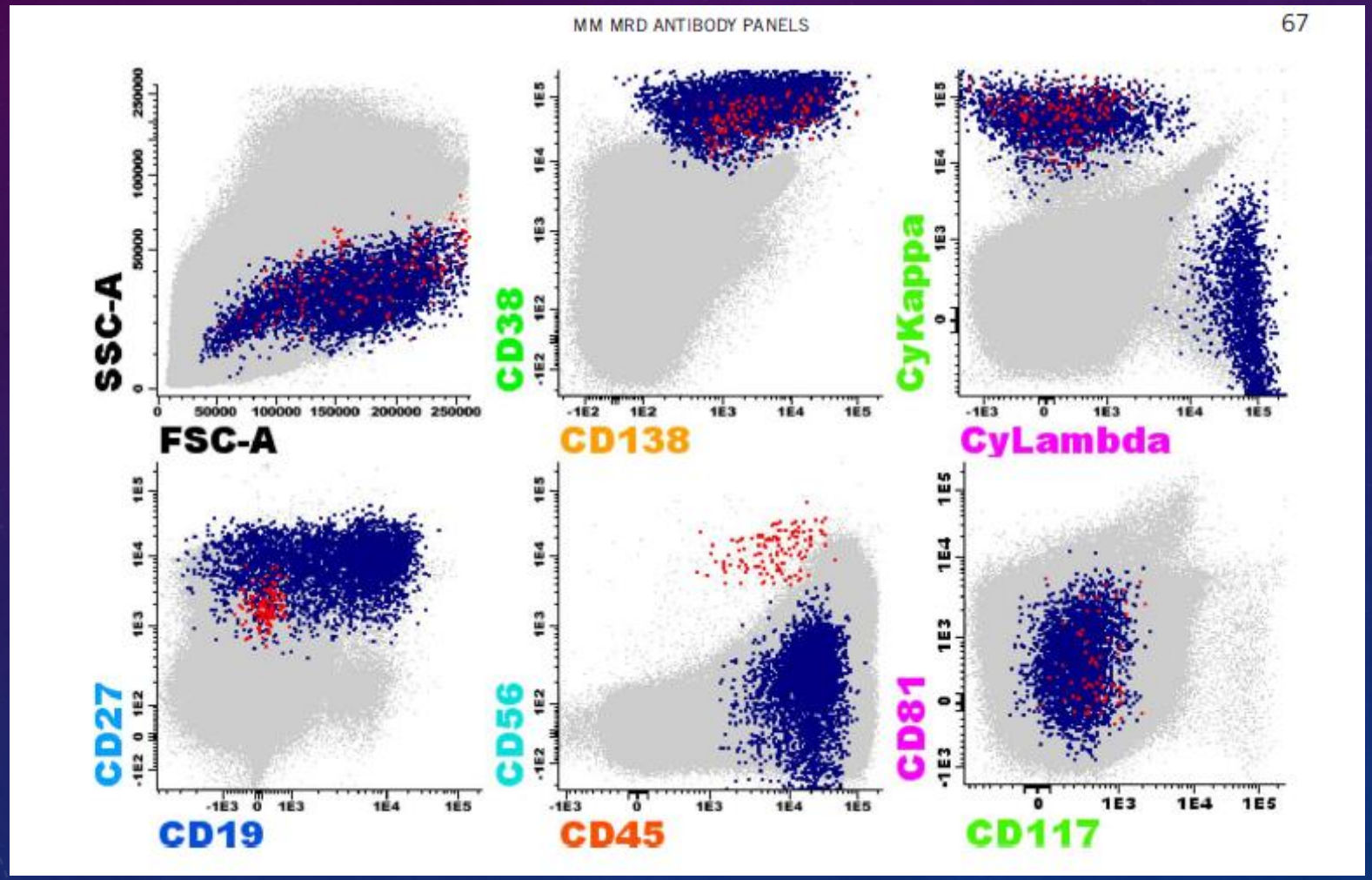
.J. Flores – Montero et al, 2016

8 ПАРАМЕТРОВАЯ ПРОТОЧНАЯ ЦИТОМЕТРИЯ

2. Eight Color Panel: EuroFlow Consortium, also used by PETHEMA

	FITC	PE	PerCP Cy5.5	PC7	APC	APCC750	V450	BV510
1	CD38 (L38, Cytognos)	CD56 (C5.9, Cytognos)	CD45 (HI30, EBioscience)	CD19 (J3-119, Beckman-Coulter)	CD117 (104D2 BD Biosciences)	CD81 (M38 Cytognos)	CD138 (MI15 BD Biosciences)	CD27 (O323 Biolegend)
2[a]	CD38 (L38, Cytognos)	CD56 (C5.9, Cytognos)	CD45 (HI30, EBioscience)	CD19 (J3-119, Beckman-Coulter)	cIgk (polyclonal, Dako)	cIgλ (polyclona Cytognos)	CD138 (MI15 BD Biosciences)	CD27 (O323 Biolegend)

8 ПАРАМЕТРОВАЯ ПРОТОЧНАЯ ЦИТОМЕТРИЯ

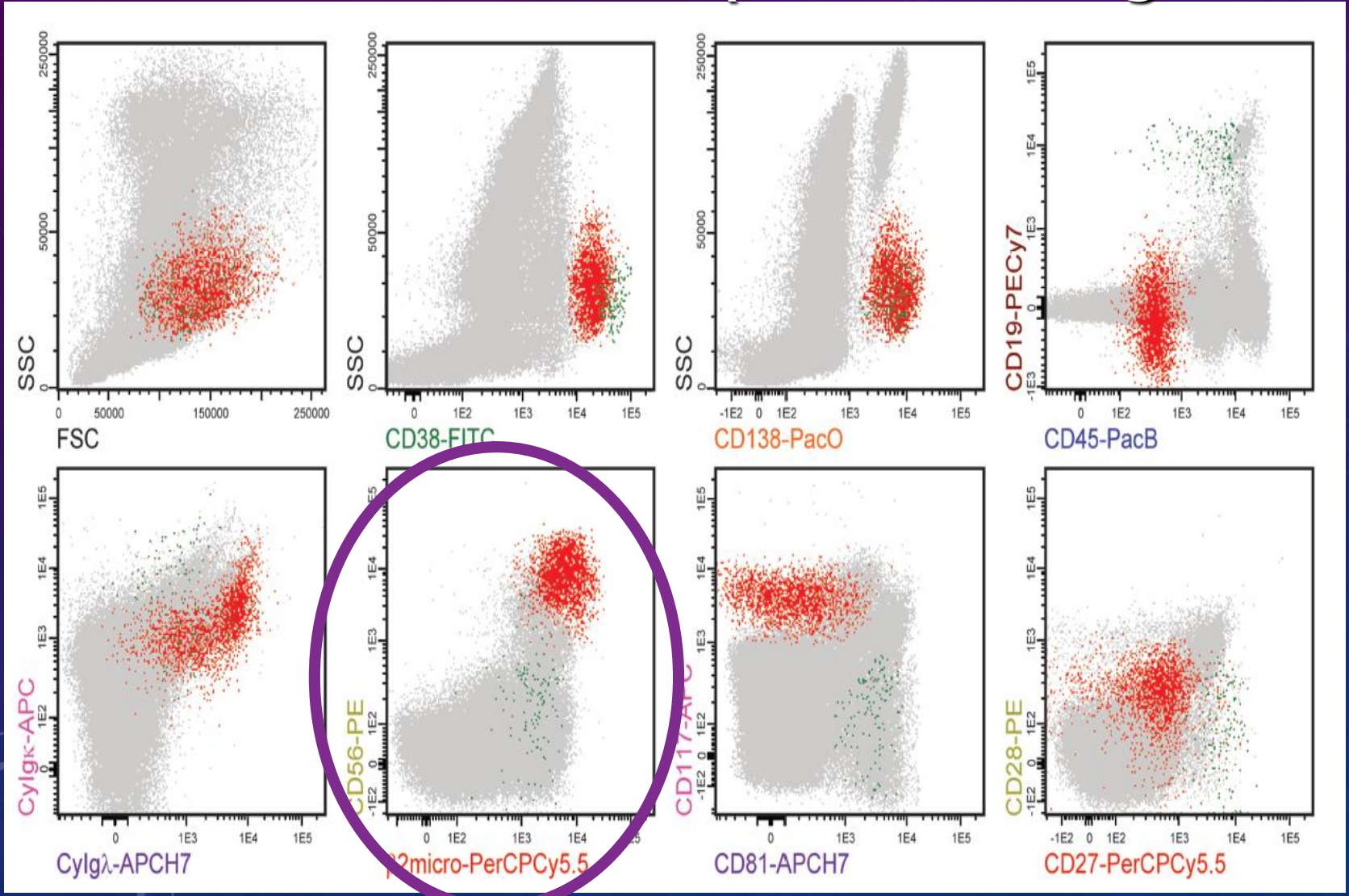


.J. Flores – Montero et al, 2016

8 ПАРАМЕТРОВАЯ ПРОТОЧНАЯ ЦИТОМЕТРИЯ

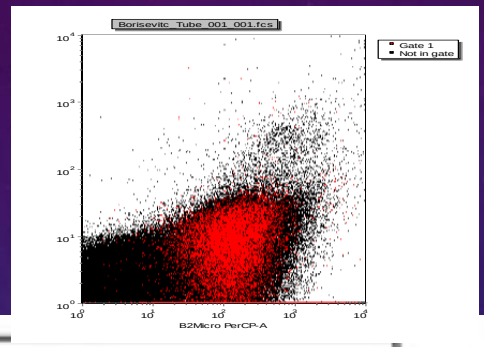
PacBlue\V450	PacOr\V500	FITC	PE	PE-cy5	Pe-cy7	APC	APC-H7
CD45	CD138	CD38	CD56	B2-micro	CD19	cylg-kappa	cylg-lambda
5 мкл	4 мкл	5 мкл	5мкл	5 мкл	5 мкл	2,5 мкл	4 мкл
CD45	CD138	CD38	CD28	CD27	CD19	CD117	CD81
5 мкл	4 мкл	5мкл	20 мкл	10 мкл	5 мкл	5 мкл	5 мкл

Сравнение нормальных (зеленые) и злокачественных (красные) плазматических клеток (JJM van Dongen et al., 2012)

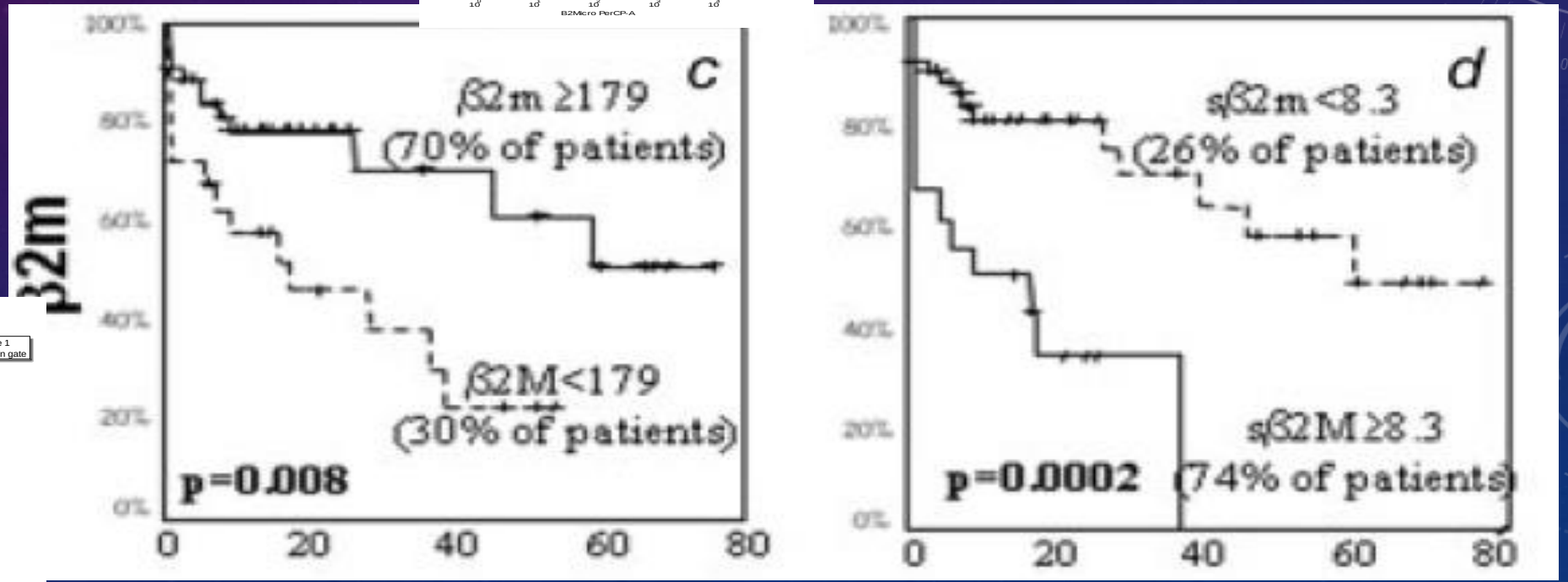


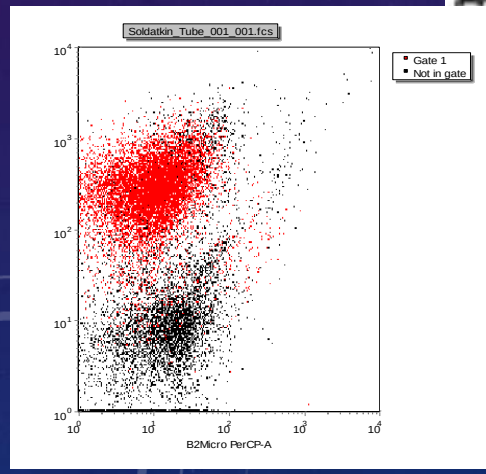
МЕМБРАННЫЙ В2-МИКРОГЛОБУЛИН

Мембранные



Сывороточные

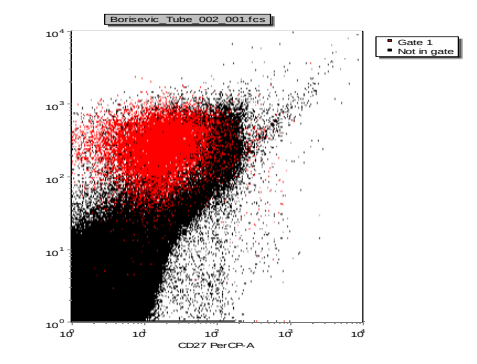




Perez-Andres M. et al., 2009

АНТИГЕНЫ CD27, CD28, CD117 – АБЕРРАНТНОСТЬ, ПРОГНОЗ

НОРМА CD27++/CD28-



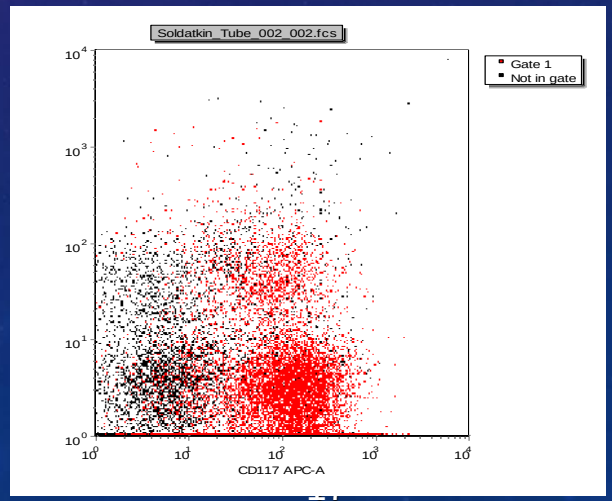
Маркер В-клеток памяти CD27 на злокачественных плазматических клетках множественной миеломы утрачивается в 49% случаев. CD27+ ММ - более благоприятный прогноз

КОМБИНАЦИЯ CD28/CD117

Низкий риск- CD117-CD28+

Промежуточный - CD117+CD28+или CD117-CD28-

Хороший прогноз – CD28-CD117+



MFS ДЕТЕКЦИЯ МОБ В СЛУЧАЕ ТАРГЕТНОЙ ТЕРАПИИ (АНТИ – CD38 МКА)

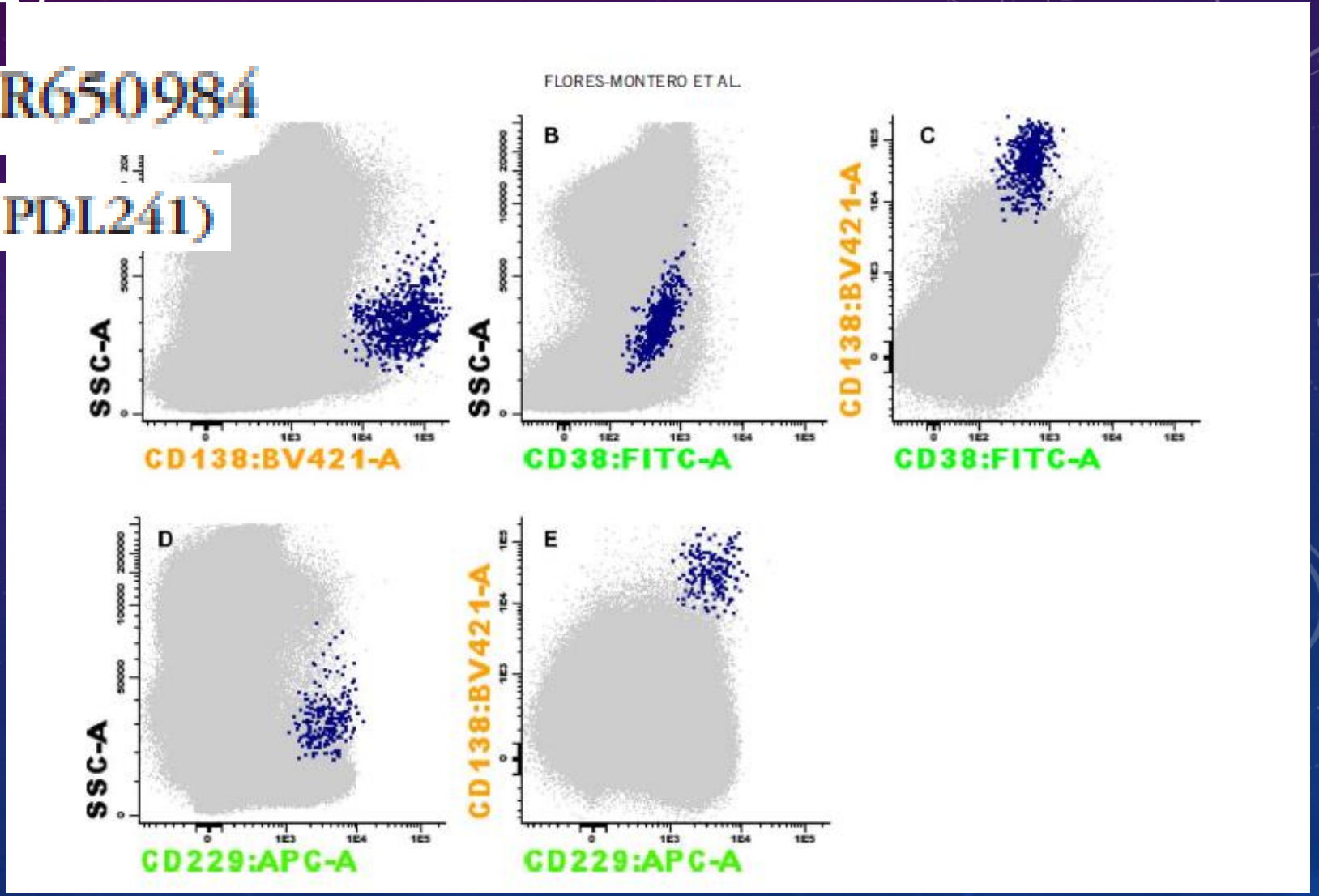
daratumumab and SAR650984

(elotuzumab/SLAMF7 and PDL241)

CD229

CD54

CD319



.J. Flores – Montero et al, 2016

Table 2
Summary List of Relevant/Validated and Relatively Recent MM Flow-MRD Panels

Study report	Tube	Combination	Main aim of study	Sensitivity
Sarasquete et al., Haematologica 2005 (61)				
	1	CD38-FITC/CD56-PE/CD19-PerCP Cy5.5/CD45-APC	Comparison with molecular techniques	10^{-4}
	2	CD138-FITC/CD28-PE/CD33-PerCP Cy5.5/CD38-APC		
	3	CD20-FITC/CD117-PE/CD138-PerCP Cy5.5/CD38-APC		
de Tute et al., Leukemia 2007 (62)				
	1	CyIgλ-FITC/CD19-PE/CyIgκ-PE Cy5/CD38-PE Cy7/CD138-APC/CD45-APC Cy7	Flow-MRD	10^{-4}
Gupta et al., Am J Clin Pathol 2009 (63)				
	1	CD19-FITC/CD56-PE/CD38-PerCP Cy5.5/CD138-APC	Flow-MRD	10^{-4}
	2	CD45-FITC/CD52-PE/CD38-PerCP Cy5.5/CD138-APC		
	3	CD20-FITC/CD117-PE/CD38-PerCP Cy5.5/CD138-APC		
Paiva et al., J Clin Oncol 2011 (10), Haematologica 2014 (64) and Puig et al, Leukemia 2014 (65)				
	1	CD38-FITC/CD56-PE/CD19-PerCP Cy5.5/CD45-APC[a]	Prognostication evaluation vs. ASO PCR	10^{-4} to 10^{-5}
	2	CD38-FITC/CD27-PE/CD45-PerCP Cy5.5/CD28-APC		
	3	β2 micro-FITC/CD81-PE/CD38-PerCP Cy5.5/CD117-APC		
Rawstron et al., J Clin Oncol 2013 (11)				
	1	CD27-FITC/CD56-PE/CD19-PerCP Cy5.5/CD38-PE Cy7/CD138-APC/CD45-APC Cy7	Prognostication	10^{-4}
	2[b]	CD81-FITC/CD117-PE		
		CD52-FITC/CD200-PE		
Robillard et al., Blood Cancer J 2013 (66) and Rousell et al., J Clin Oncol 2014 (67)				
	1	CD38-HV450/CyIgλ-FITC/CD56+CD28-PE/CD138-PE Cy5/CD19-PE Cy7/CyIgκ-APC/CD45-APC H7	Flow-MRD prognostication	10^{-5}
EuroFlow 8 (68)				
	1	CD138-BV421/CD27-BV510/CD38-FITC/CD56-PE/CD45-PerCP Cy5.5/CD19-PE Cy7/CD117-APC/CD81-APC C750	Flow-MRD	10^{-5}
	2	CD138-BV421/CD27-BV510/CD38-FITC/CD56-PE/CD45-PerCP Cy5.5/CD19-PE Cy7/CyIgκ-APC/CyIgλ-APC C750		
EuroFlow 10[c] (69)				
	1	CD138-BV421/CD27-BV510/CD117-BV605/CD38-FITC/CD56-PE/CD45-PerCP Cy5.5/CD19-PE Cy7/CyIgλ-APC/CyIgκ-APC A700/CD81-APC C750	Flow-MRD	NA

СПАСИБО ЗА ВНИМАНИЕ