HIGH SENSITIVE FLOW CYTOMETRY REMISSION BY Ig LIGHT CHAINS *RATIO* IS A POWERFUL MARKER OF OUTCOME IN MULTIPLE MYELOMA PATIENTS AFTER TANDEM AUTOLOGOUS TRANSPLANT : AN UPDATE OF IRE EXPERIENCE

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Background

The achievement of complete response (CR) significantly correlates with a better clinical outcome in multiple myeloma (MM) patients treated with autologous stem cell transplant (ASCT). The depth of response is one of the most relevant factors to predict patient's outcome, however the definition of CR through standard criteria has shown several limitations.
A solution to improve upon CR is to use more sensitive response assessment techniques enabling quantification of minimal residual disease (MRD) after treatment. Next generation flow and next generation sequencing are, to date, the more sensitive techniques capable of detecting a single residual malignant plasma cell within 1.000.000 bone marrow cells (sensitivity 10^{-6}).

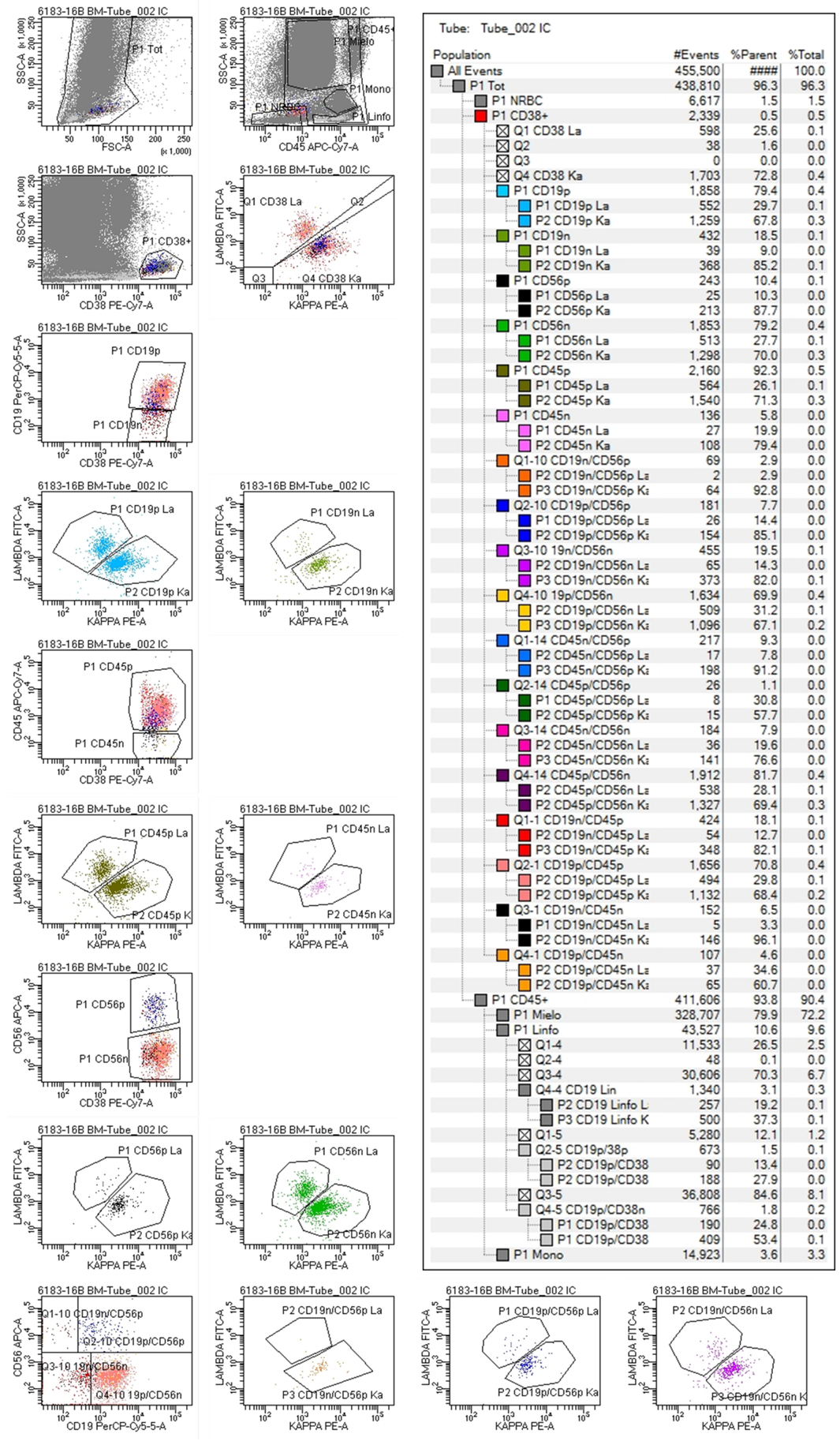
Methods

We evaluated the minimal residual disease (MRD) in 50 consecutive MM patients who underwent an up-front tandem ASCT in our center, using a single-tube six-colors flow cytometry assay (FC) based on intra-cytoplasmic immunoglobulin (cy-Ig) light chains ratio valuated on patient-specific plasma cells (PC) immune profile, in a real-life setting reaching a sensitivity up to 10^{-5}. More recently, a single-tube 8-colous approach was utilized, increasing the sensitivity up to 10^{-6}. (Fig.1)

Results

With a sensitivity up to 10^{-5}, clonal-PC were documented by FC in 36.4 % (12/33) of patients in conventional CR after second transplant. The number of flow MRD-negative patients significantly increased after induction and first ASCT, but not between first and second transplant. The 5-years progression-free survival (5ys-PFS) of flow MRD-negative patients after second transplant was significantly better than patients who remained MRD-positive considering both all patients (5ys-PFS: 70 % vs 5 %) and patients in CR according to standard criteria (5ys-PFS: 67 % vs 0 %). (Fig.2)
Utilizing a more sensitive 8-colour flow-MRD approach, with a sensitivity up to 10^{-6}, we documented a flow-MRD negativity in 45% of cases that were negative by our previous technique.
This data are in agreement with the most recent publications that, utilizing a next generation sequencing approach, confirm that MRD identifies patients with an excellent outcome in MM.

FIGURE 1. FLOW CYTOMETRY: KAPPA/LAMBDA *RATIO* ON CD38^{bright} PC SUB-POPULATIONS



Tube: Tube_002 IC

Population	#Events	%Parent	%Total
All Events	455,500	####	100.0
P1 Tot	438,810	96.3	96.3
P1 NRBC	6,617	1.5	1.5
P1 CD38+	2,339	0.5	0.5
Q1 CD38 La	598	25.6	0.1
Q2	38	1.6	0.0
Q3	0	0.0	0.0
Q4 CD38 Ka	1,703	72.8	0.4
P1 CD19p	1,858	79.4	0.4
P1 CD19p La	552	29.7	0.1
P2 CD19p Ka	1,259	67.8	0.3
P1 CD19n	432	18.5	0.1
P1 CD19n La	39	9.0	0.0
P2 CD19n Ka	368	85.2	0.1
P1 CD56p	243	10.4	0.1
P1 CD56p La	25	10.3	0.0
P2 CD56p Ka	213	87.7	0.0
P1 CD56n	1,853	79.2	0.4
P1 CD56n La	513	27.7	0.1
P2 CD56n Ka	1,298	70.0	0.3
P1 CD45p	2,160	92.3	0.5
P1 CD45p La	564	26.1	0.1
P2 CD45p Ka	1,540	71.3	0.3
P1 CD45n	136	5.8	0.0
P1 CD45n La	27	19.9	0.0
P2 CD45n Ka	108	79.4	0.0
Q1-10 CD19n/CD56p	69	2.9	0.0
P2 CD19n/CD56p La	2	2.9	0.0
P3 CD19n/CD56p Ka	64	92.8	0.0
Q2-10 CD19p/CD56p	181	7.7	0.0
P1 CD19p/CD56p La	26	14.4	0.0
P2 CD19p/CD56p Ka	154	85.1	0.0
Q3-10 19n/CD56n	455	19.5	0.1
P2 CD19n/CD56n La	65	14.3	0.0
P3 CD19n/CD56n Ka	373	82.0	0.1
Q4-10 19p/CD56n	1,634	69.9	0.4
P2 CD19p/CD56n La	509	31.2	0.1
P3 CD19p/CD56n Ka	1,096	67.1	0.2
Q1-14 CD45n/CD56p	217	9.3	0.0
P2 CD45n/CD56p La	17	7.8	0.0
P3 CD45n/CD56p Ka	198	91.2	0.0
Q2-14 CD45p/CD56p	26	1.1	0.0
P1 CD45p/CD56p La	8	30.8	0.0
P2 CD45p/CD56p Ka	15	57.7	0.0
Q3-14 CD45n/CD56n	184	7.9	0.0
P2 CD45n/CD56n La	36	19.6	0.0
P3 CD45n/CD56n Ka	141	76.6	0.0
Q4-14 CD45p/CD56n	1,912	81.7	0.4
P2 CD45p/CD56n La	538	28.1	0.1
P2 CD45p/CD56n Ka	1,327	69.4	0.3
Q1-1 CD19n/CD45p	424	18.1	0.1
P2 CD19n/CD45p La	54	12.7	0.0
P3 CD19n/CD45p Ka	348	82.1	0.1
Q2-1 CD19p/CD45p	1,656	70.8	0.4
P2 CD19p/CD45p La	494	29.8	0.1
P2 CD19p/CD45p Ka	1,132	68.4	0.2
Q3-1 CD19n/CD45n	152	6.5	0.0
P1 CD19n/CD45n La	5	3.3	0.0
P2 CD19n/CD45n Ka	146	96.1	0.0
Q4-1 CD19p/CD45n	107	4.6	0.0
P2 CD19p/CD45n La	37	34.6	0.0
P2 CD19p/CD45n Ka	65	60.7	0.0
P1 CD45+	411,606	93.8	90.4
P1 Mielo	328,707	79.9	72.2
P1 Linfo	43,527	10.6	9.6
Q1-4	11,533	26.5	2.5
Q2-4	48	0.1	0.0
Q3-4	30,606	70.3	6.7
Q4-4 CD19 Lin	1,340	3.1	0.3
P2 CD19 Linfo L	257	19.2	0.1
P3 CD19 Linfo K	500	37.3	0.1
Q1-5	5,280	12.1	1.2
Q2-5 CD19p/38p	673	1.5	0.1
P2 CD19p/CD38	90	13.4	0.0
P2 CD19p/CD38	188	27.9	0.0
Q3-5	36,808	84.6	8.1
Q4-5 CD19p/CD38n	766	1.8	0.2
P1 CD19p/CD38	190	24.8	0.0
P1 CD19p/CD38	409	53.4	0.1
P1 Mono	14,923	3.6	3.3

ABERRANT KAPPA/LAMBDA *RATIO*: <0.5 OR >4.0

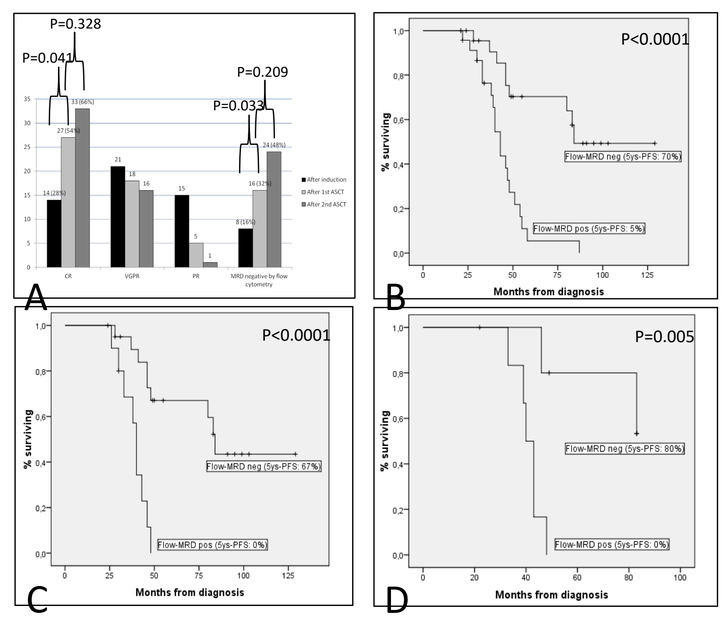


Figure 2. Clinical outcome of patients according to flow cytometry assessment. (A) Treatment response by both standard criteria and FC in all check-points of the therapeutic program. 5-years PFS curves according to MRD assessment by flow cytometry after second transplant: all patients (B); patients in CR according to standard criteria (C); patients with intermediate-high cytogenetic risk (D).

Conclusions

FC remission through cy-Ig light ratio on PC sub-populations is a sensitive, highly informative, low-cost and routinely applicable MRD assay, a powerful tool in treatment response evaluation and a crucial marker of outcome in MM. Flow-MRD, as well as next generation sequencing-MRD, both enable the identifications of patients subpopulations with highly different prognosis. High sensitive MRD should be assessed in every prospective trial and is the candidate to become a primary endpoint. Stratified therapy for MM patients according to high sensitive MRD status is at the sunrise.