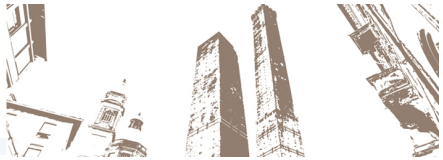
Highlights from IMW 2019

19-20 novembre 2019
Bologna
Royal Hotel Carlton

Aldo M. Roccaro, MD, PhD

Come e quando valutare l'MRD nel sangue periferico



Conflict of Interest Disclosure:
Aldo M Roccaro

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	Janssen
Research Funding:	Fondazione AIRC
	European Hematology Association
	European Union (ERA-NET Transcan2)
	AstraZeneca

Rational for testing MRD in MM using peripheral blood samples

- ✓ Avoid serial assessments requiring repeated BM aspirate sampling
- ✓ Negative MRD may be obtained as a result of dilution of BM with blood and/or the patchy distribution of transformed plasma cells

Are malignant plasma cells detectable within a peripheral blood specimen?

Flow cytometry
NGS
Mass spectrometry

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Detection of Myeloma Cells in the Peripheral Blood by Flow Cytometry

Thomas E. Witzig, Teresa K. Kimlinger, Gregory J, Ahmann, Jerry A. Katzmann, and Philip R. Greipp

Division of Internal Medicine and Hematology (T.E.W., P.R.G.) and Department of Laboratory Medicine and Pathology (T.K.K., G.J.A., J.A.K.), Mayo Clinic and Mayo Foundation, Rochester, Minnesota

Cytometry (Communications in Clinical Cytometry) 26:113–120 (1996)

AIM: to identify circulating plasma cells from whole blood specimens, using

- ✓ two-color FC (CD38; CD45)
- ✓ three-color FC (CD38; CD45; κ; λ)

Witzig et al, Cytometry, 1996

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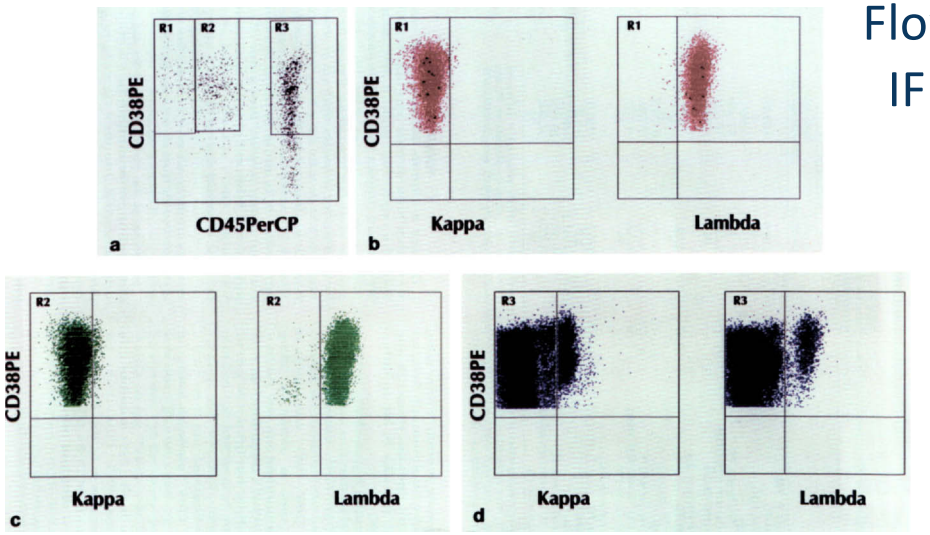

Fig. 4. Flow cytometry dot-plots of the mononuclear cells from a patient with documented monoclonal λ plasma cells by immunofluorescence microscopy. **a:** CD45PerCP vs. CD38PE. Gate 1 contains $CD38^{bright}$ CD45− cells; Gate 2, $CD38^{bright}CD45^{dim}$ cells; and Gate 3, $CD38^{bright}CD45+$ cells. **b, c,** and **d:** The kappa/lambda analysis on the cells in gates R1, R2, and R3, respectively. Monoclonal lambda cells are found predominantly in the $CD38^{bright}CD45-$ and $CD38^{bright}CD45^{dim}$ cell populations.

Flow cytometry of mononuclear cells from a patient with IF microscopy- documented monoclonal λ plasma cells

$CD38^{bright}/CD45^{neg}/\lambda$

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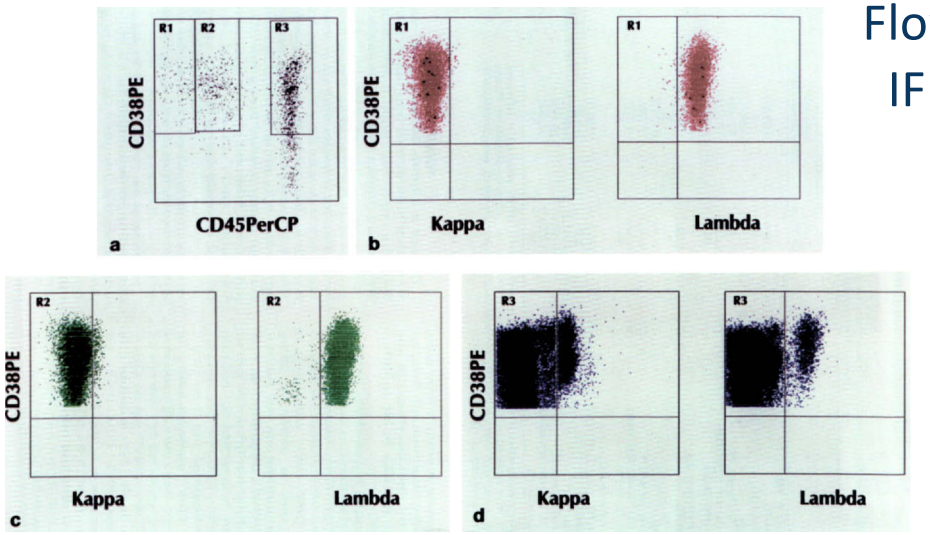

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$CD38^{bright}/CD45^{dim}/\lambda$

Out of the IF microscopy positive cases

two-color: detection 94%

Three-color: detection 77%

Whole Blood Flow Cytometry

Ab anti-CD38 and –CD45

Witzig et al, Cytometry, 1996

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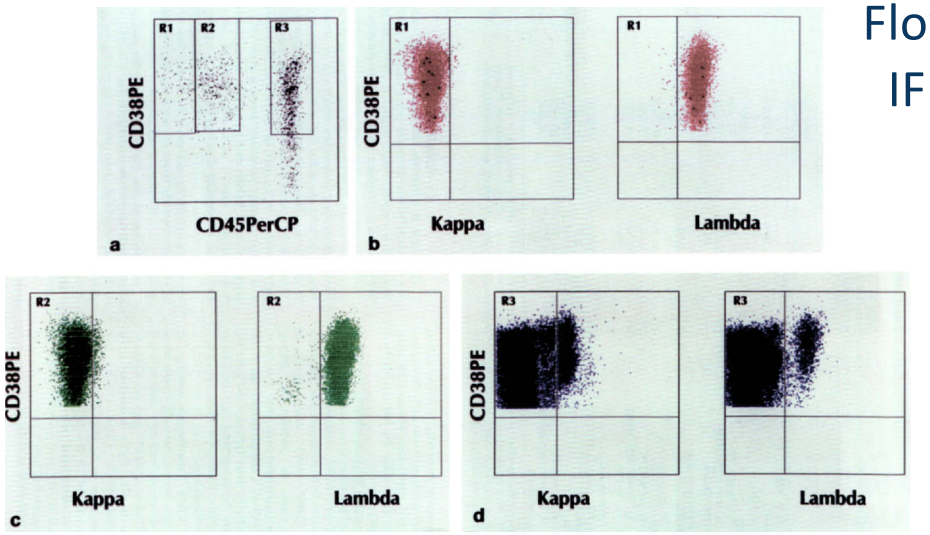

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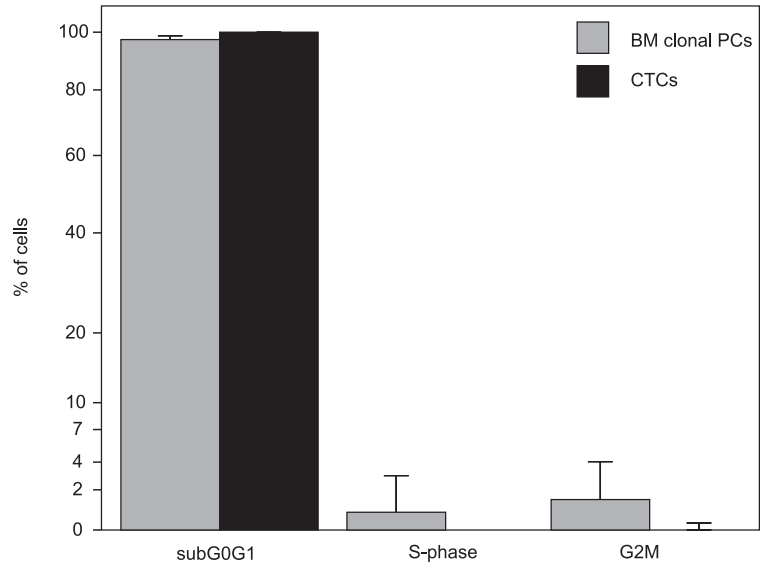
Whole Blood Flow Cytometry

Ab anti-CD38 and –CD45

Practical and reliable method to detect and quantify circulating malignant myeloma plasma cells

Witzig et al, Cytometry, 1996

CTCs represent a unique subset of the whole BM clonal plasma cells compartment



circulating neoplastic PCs vs matched BM

✓ mostly quiescent: arrested in the subG0-G1
lower S-phase
lower G2/M

Paiva B, et al. Blood 2016

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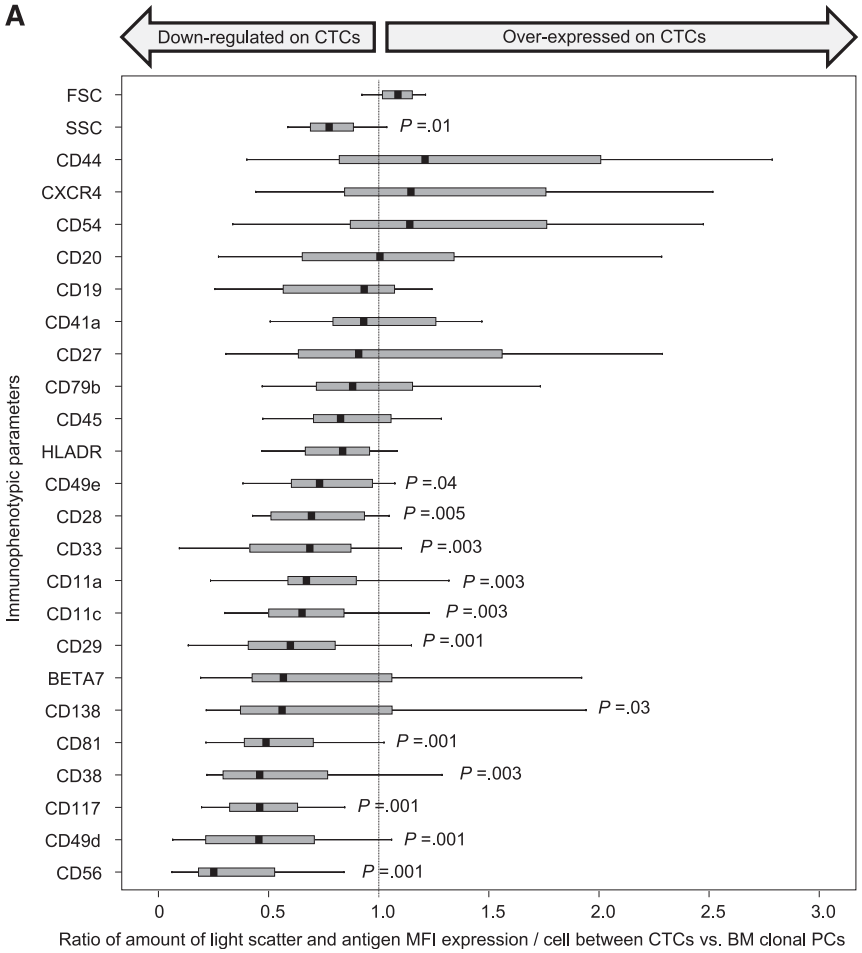

downregulation ($P < .05$) of:

- ✓ integrins (CD11a/CD11c/CD29/CD49d/CD49e)
- ✓ adhesion (CD33/CD56/CD117/CD138)
- ✓ activation molecules (CD28/CD38/CD81)

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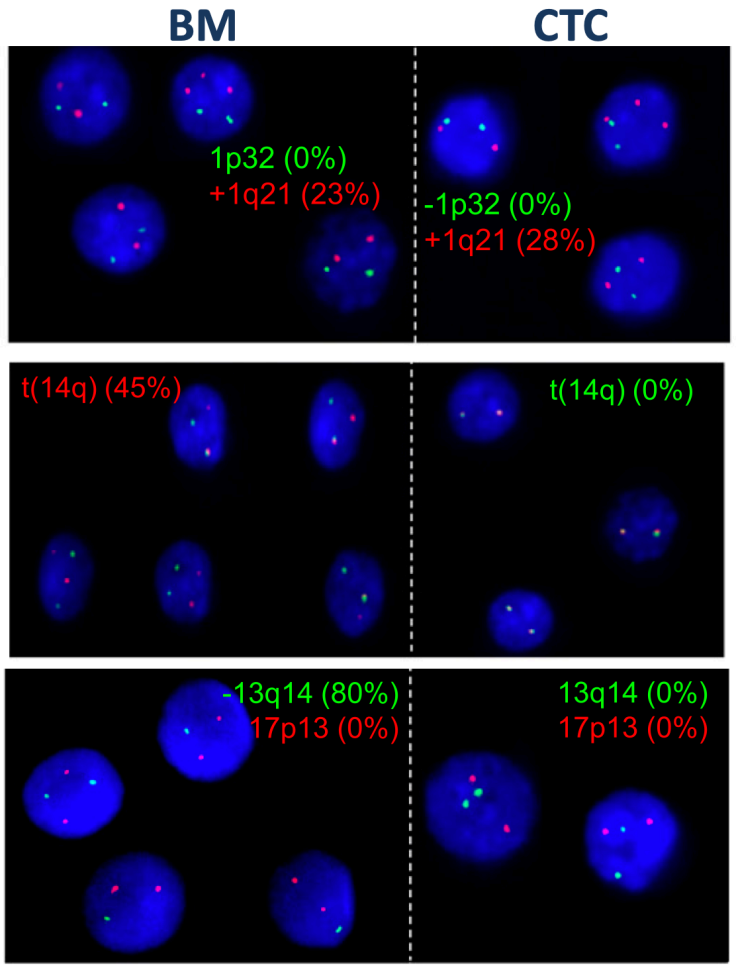

circulating neoplastic PCs vs matched BM

✓ different cytogenetic profile

Paiva B, et al. Blood 2016

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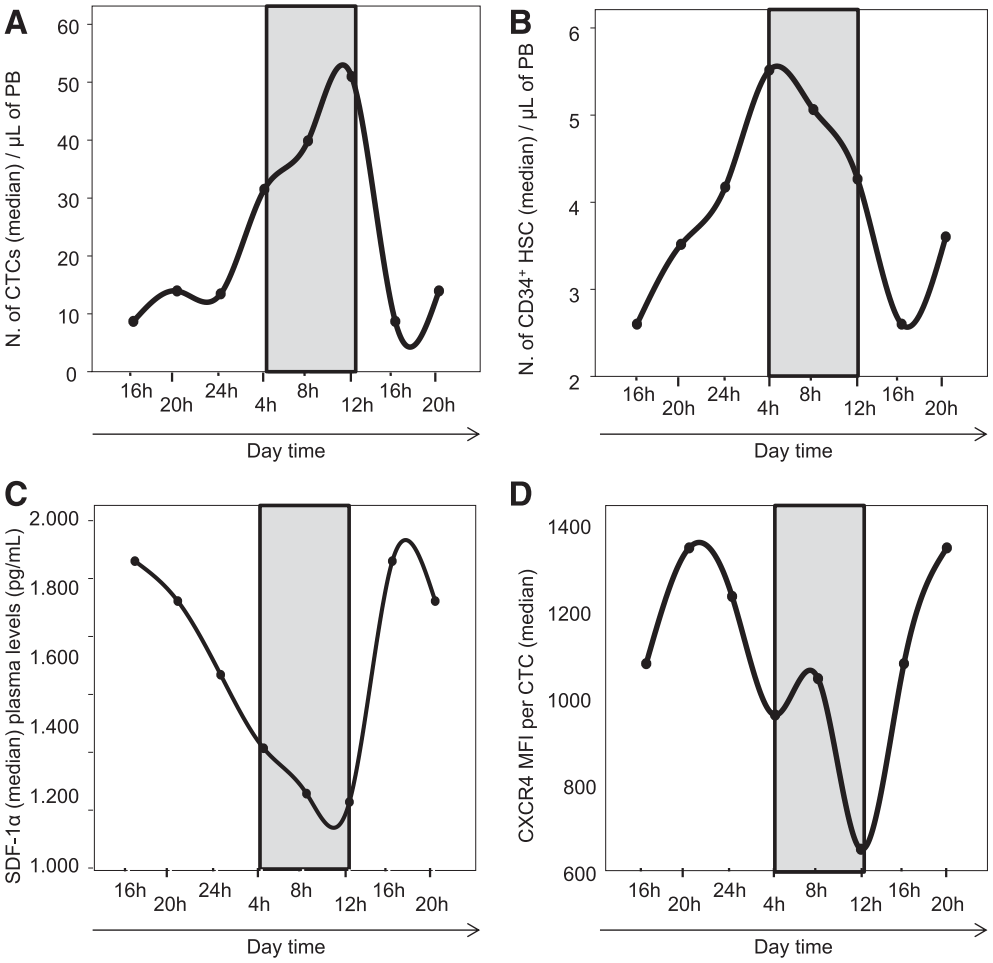
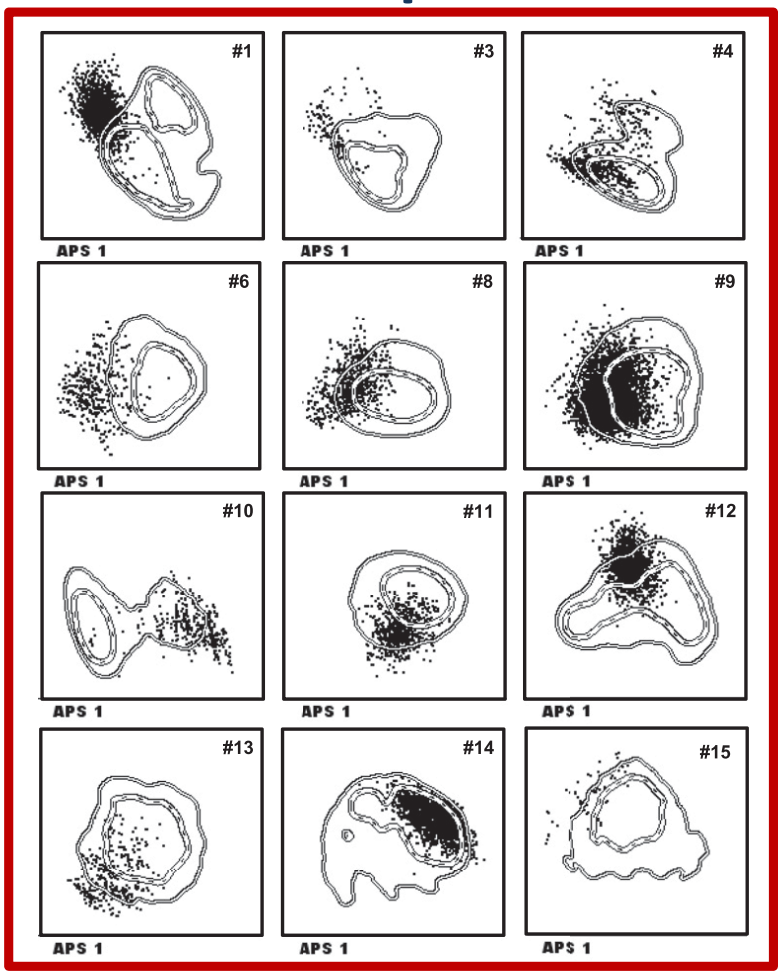


CTCs: circadian distribution

- ✓ similar pattern to CD34 cells
- ✓ opposite to SDF-1 plasma levels and CXCR4 surface expression on clonal PCs
- ✓ CTCs may egress to PB to colonize/metastasize other sites in the BM during the patients' resting period

Paiva B, et al. Blood 2016

CTCs represent a unique subset of the whole BM clonal PC compartment



iPEP of BM clonal PCs is represented by 1 and 2 SD lines paired CTCs are represented by black dots.

- ✓ CTCs clustered in a uniquely restricted area of that occupied by BM clonal PCs: 12/15

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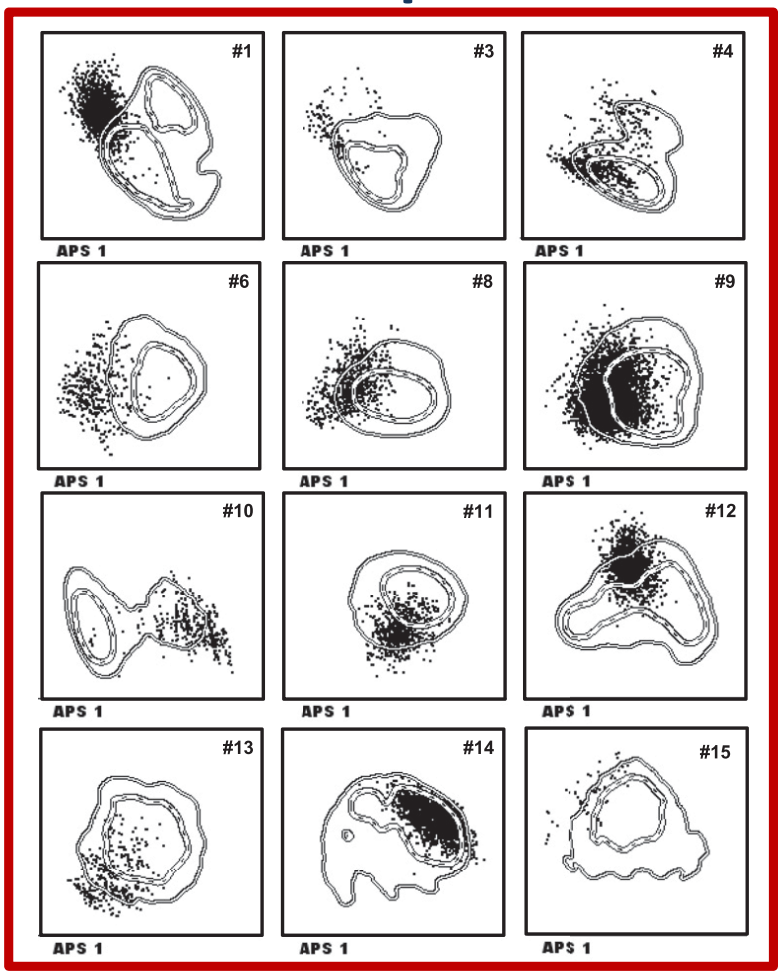
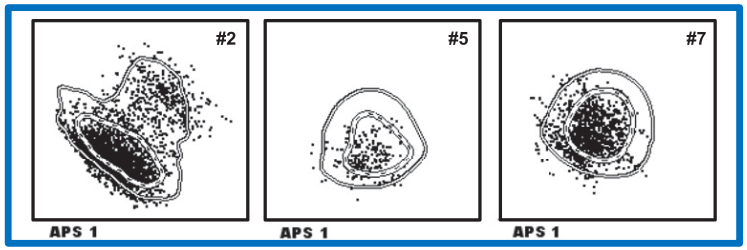


iPEP of BM clonal PCs is represented by 1 and 2 SD lines paired CTCs are represented by black dots.

- ✓ CTCs clustered in a uniquely restricted area of that occupied by BM clonal PCs: 12/15
- ✓ CTCs were spread throughout the whole BM clonal PC compartment: 3/15



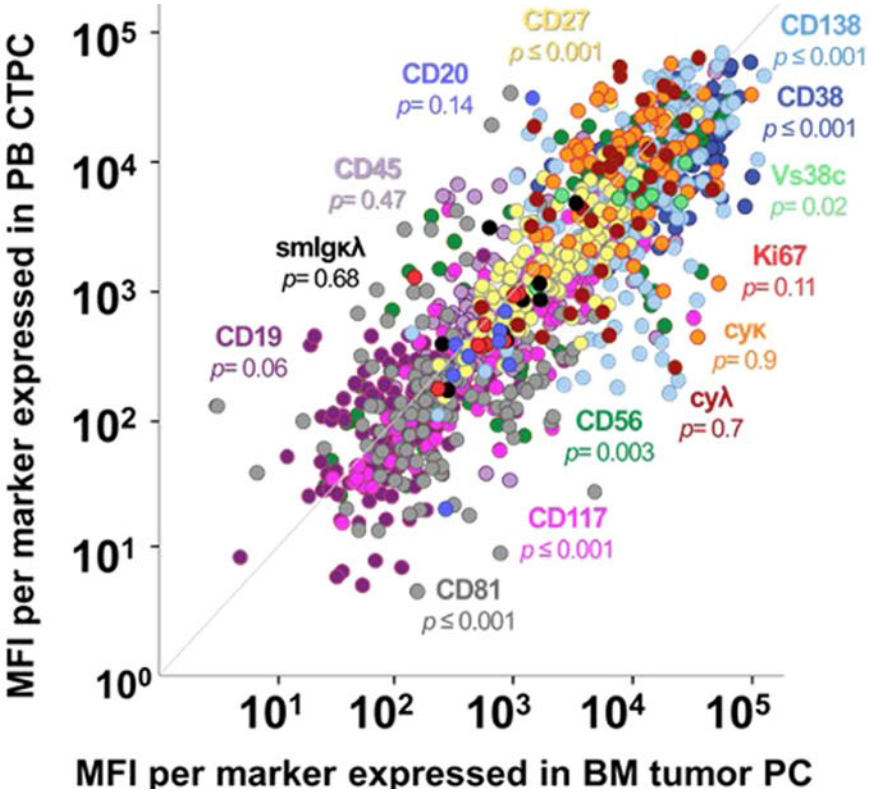
Paiva B, et al. Blood 2016

CTCs represent a unique subset of the whole BM clonal plasma cells compartment

Detailed characterization of multiple myeloma circulating tumor cells shows unique phenotypic, cytogenetic, functional, and circadian distribution profile

Paiva B, et al. Blood 2016

Correlation between the immunophenotype of tumor PCs in paired PB and BM samples from newly diagnosed MM



PB-CTPCs vs BM-TPCs: similar immunophenotypic profile

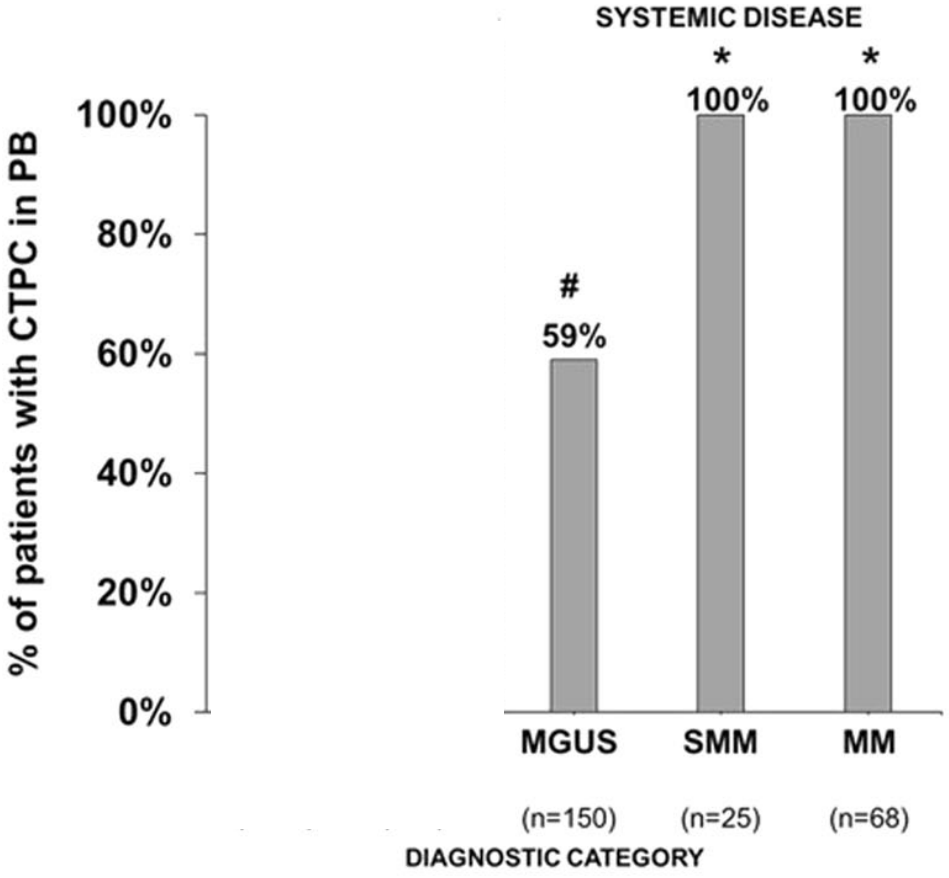
PB-CTPCs significantly lower ($p < 0.05$) of CD38, CD138, CD81, CD56, CD27, and Vs38c maturation-associated markers, CD117

more immature and less proliferative immunophenotype for paired PB vs. BM

PB-CTPCs: peripheral blood-circulating tumor plasma cells
BM-TPCs: bone marrow-tumor plasma cells

Flores-Montero J, et al. Leukemia 2017

Detection of tumor plasma cells within the peripheral blood of patients with plasma cell neoplasias - NGF



Presence of CTPC in PB is associated with systemic disease (i.e., MGUS, SMM and MM) vs localized disease (i.e., macrofocal MM, solitary plasmacytoma)

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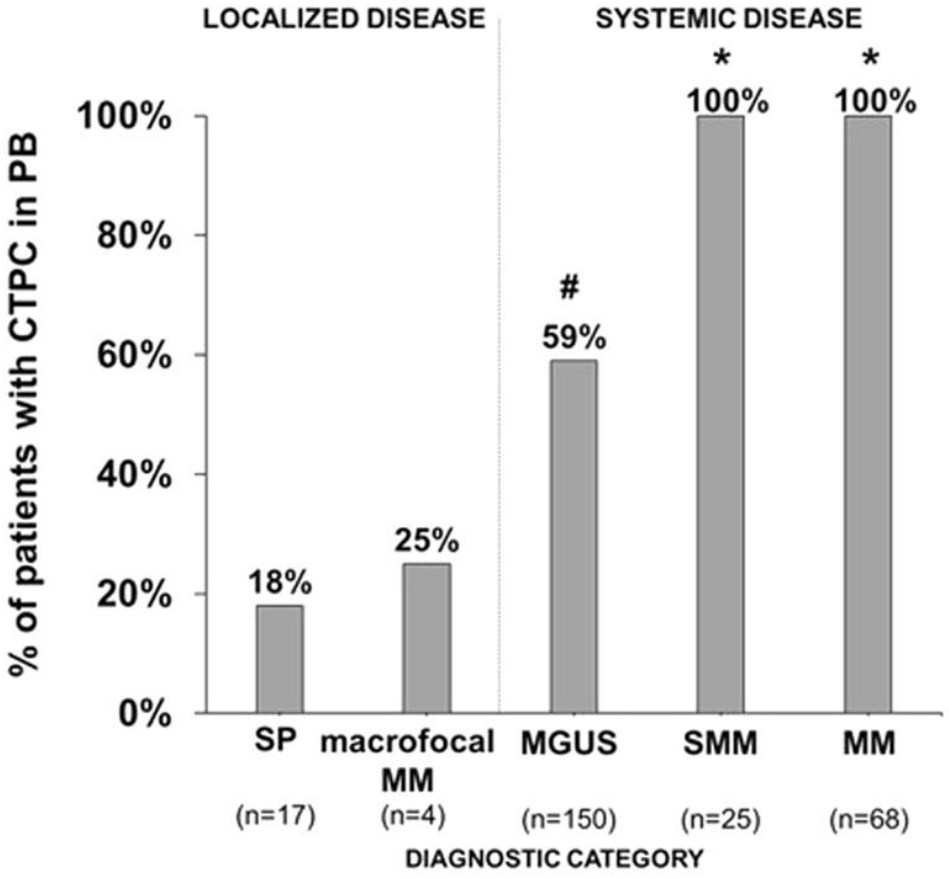

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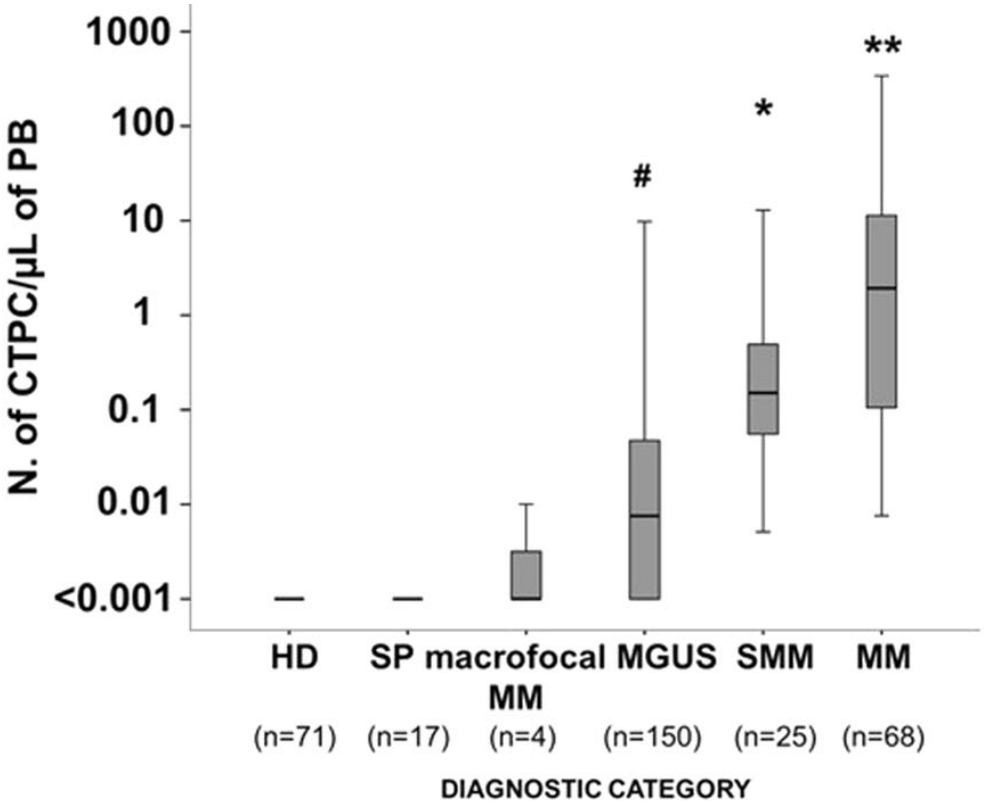

›verall number of PB CTPC as assessed by NGF also increased progressively from SP and nacrofocal MM to MGUS, SMM, and MM

Flores-Montero J, et al. Leukemia 2017

Detection of tumor plasma cells within the peripheral blood of patients with plasma cell neoplasias - NGF

All treated MM patients who showed CTPC after therapy always showed MRD + of paired BM samples (data not shown)

persistence/presence of CTPC in MM patients who had undergone therapy, might be used as a surrogate marker of BM MRD-positivity

Preliminary results

Further validation needed

Flores-Montero J, et al. Leukemia 2017

Are malignant plasma cells detectable within a peripheral blood specimen?

Flow cytometry

NGS

Mass spectrometry

Detection of tumor plasma cells within the peripheral blood of patients with plasma cell neoplasias evaluating ctDNA

- ✓ Circulating tumor DNA: promising non-invasive tool, for monitoring response to therapy
- ✓ Clonotypic V(D)J rearrangement: monitoring MM ctDNA after treatment initiation
  Non-responders/progressors: detectable ctDNA at times of high tumor burden compared with less than half of responders

Kumar S, et al. Lancet Oncol, 2010; Buedts L, et al. Haematologica, 2016
Wan JCM, et al. Nar Rev Cancer, 2017; Oberle A, et al. Haematologica 2017; Perrot A, et al, Blood, 2018

MRD by NGS Clonotypic V(D)J rearrangement: peripheral blood vs matches BM samples

Mazzotti C, et al, Blood Advances, 2018

Myeloma MRD by deep sequencing from circulating tumor DNA does not correlate with results obtained in the bone marrow

Table 1. Comparison of MRD status obtained in plasma and in bone marrow

	Plasma MRD positive	Plasma MRD negative	Total
Bone marrow MRD positive	8	18	26
Bone marrow MRD negative	1	10	11
Total	9 (PPV_{plasma} = 89%)	28 (NPV_{plasma} = 36%)	37

NPV_{plasma}, negative predictive value of MRD assessed from plasma sample; PPV_{plasma}, positive predictive value of MRD assessed from plasma sample.

- ✓ At the time of MRD (n. 37): 49% (18/37) consistency between paired plasma and BM

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- ✓ Most frequent discrepancy: undetectable MRD in plasma, which was BM positive (18/26)
- ✓ MRD assessment of ctDNA: only 36% negative predictive value; 89% positive predictive value
- ✓ No quantitative correlation between plasma and BM was found, including when MRD was positive in both samples

Mazzotti C, et al, Blood Advances, 2018

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Only 1 discrepant case in which MRD was plasma-positive and BM-negative

A) Extramedullary (EM) relapse → PET/CT negative for EM relapse (at the time of MRD)

B) False-negative result in BM →
- Not PB-diluted
- Patchy nature of the disesase within the BM
- Possibly, additional BM sample analysis may have revealted MRD positivity withn the BM

Mazzotti C, et al, Blood Advances, 2018

Myeloma MRD by deep sequencing from circulating tumor DNA does not correlate with results obtained in the bone marrow

Only 39% of patients with less than a VGPR displayed detectable ctDNA

ctDNA was undetectable in 69% of patients with MRD detected within the BM

ctDNA may not serve as a sufficient analyte for MRD monitoring

Oberle C,et al. Haematologica, 2017

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Flow cytometry

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Peripheral blood approached by using mass spectrometry

mass spectrometry

targets the (M- protein) similar to conventional electrophoresis

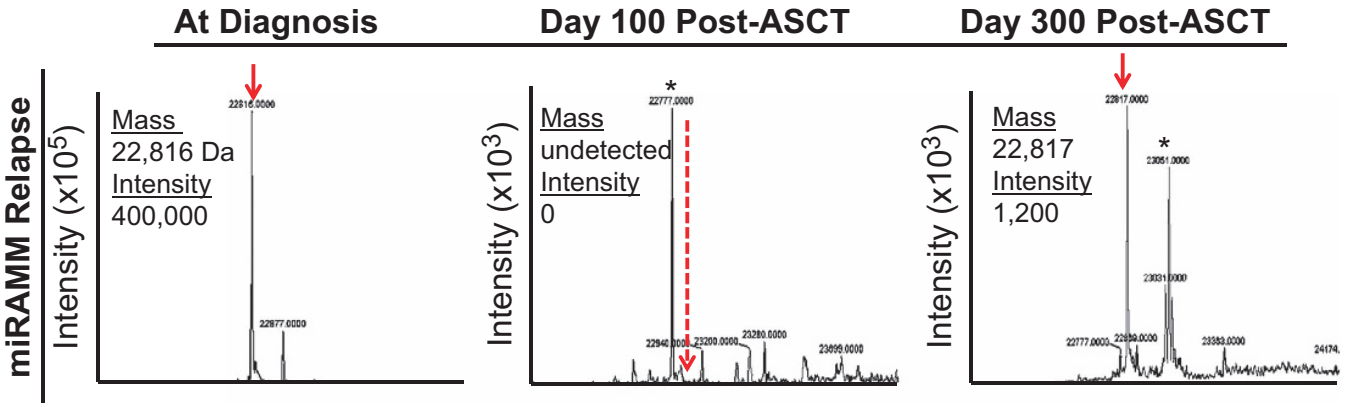
looks for the unique mass of the unique amino acid sequencing on each monoclonal Ig

much higher sensitivity

Peripheral blood approached by using mass spectrometry

miRAMM: monoclonal immunoglobulin rapid accurate mass measurement

(sCR, post ASCT)



miRAMM relapsed

Mills JR, et al. Blood Cancer J, 2017

Peripheral blood approached by using mass spectrometry

miRAMM: monoclonal immunoglobulin rapid accurate mass measurement

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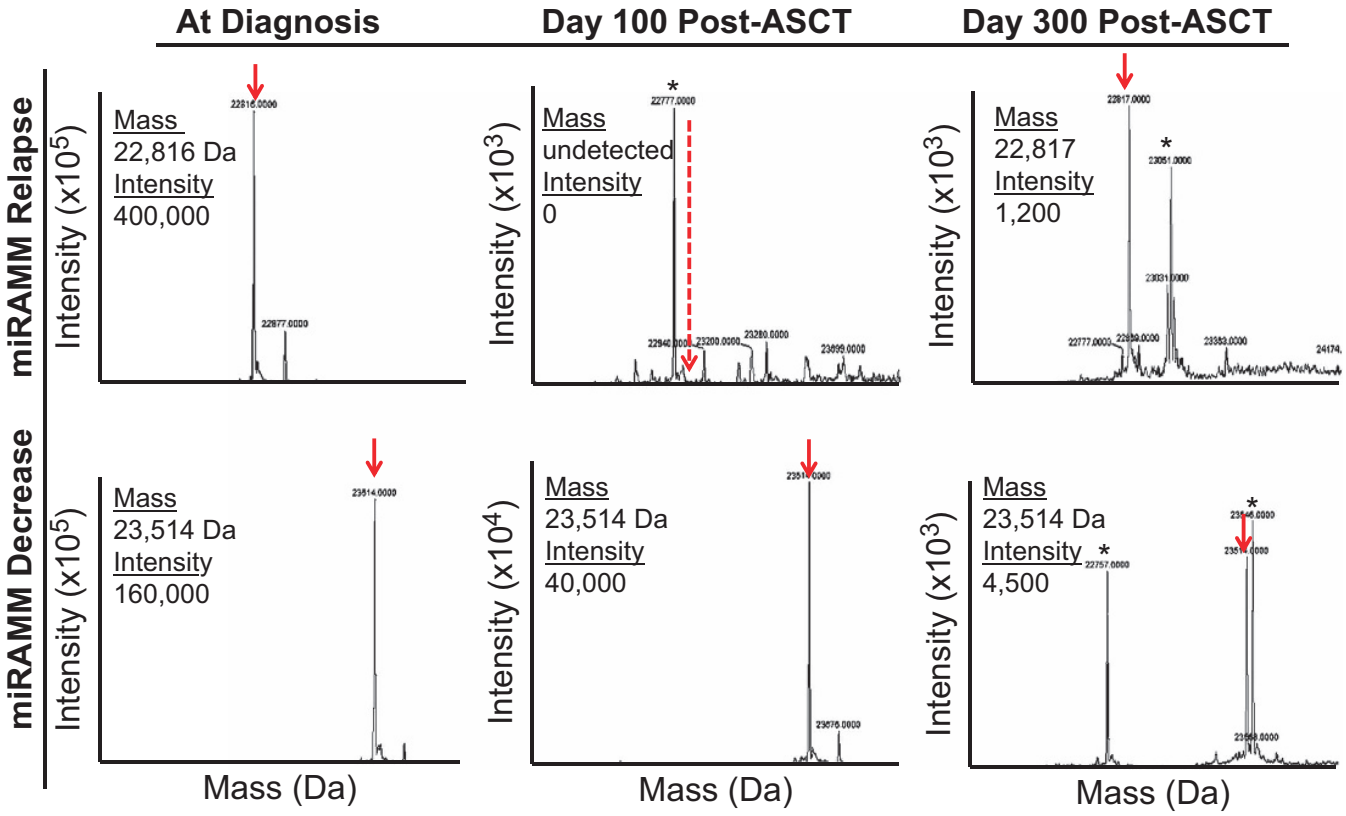


miRAMM relapsed

deepening miRAMM response

Mills JR, et al. Blood Cancer J, 2017

Peripheral blood approached by using mass spectrometry

Eveillar M, et al. ASCO 2019, #e19525

Matrix-**A**ssisted **L**aser **D**esorption**/I**onization **- T**ime-**O**f-**F**light mass spectrometer

MALDI-TOF in PB vs flow cytometry BM-based

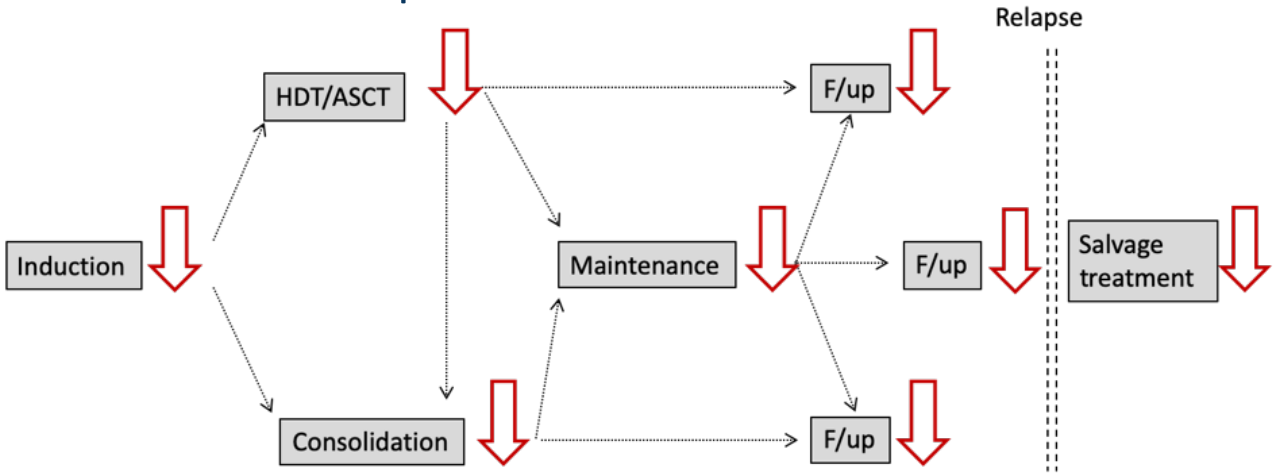
MRD results were concordant for 44/71 (62%)
patients (8+/+, 36 -/- respectively)
27 were discordant (10 +/-, 17-/+)

When to evaluate MRD?

With courtesy and permission of Dr. B. Paiva
IMW 2019, Boston, MA, USA

MRD evaluation is prognostic at any time point. Consider evaluating to:

- ✓ Define quality of CR
- ✓ Evaluate efficacy of subsequent treatment after CR (eg. Consolidation)
- ✓ Identify high risk patients
- ✓ Help on treatment decisions



Paiva B, et al. Blood, 2015

When to evaluate MRD?

With courtesy and permission of Dr. B. Paiva
IMW 2019, Boston, MA, USA

Bone marrow-based MRD

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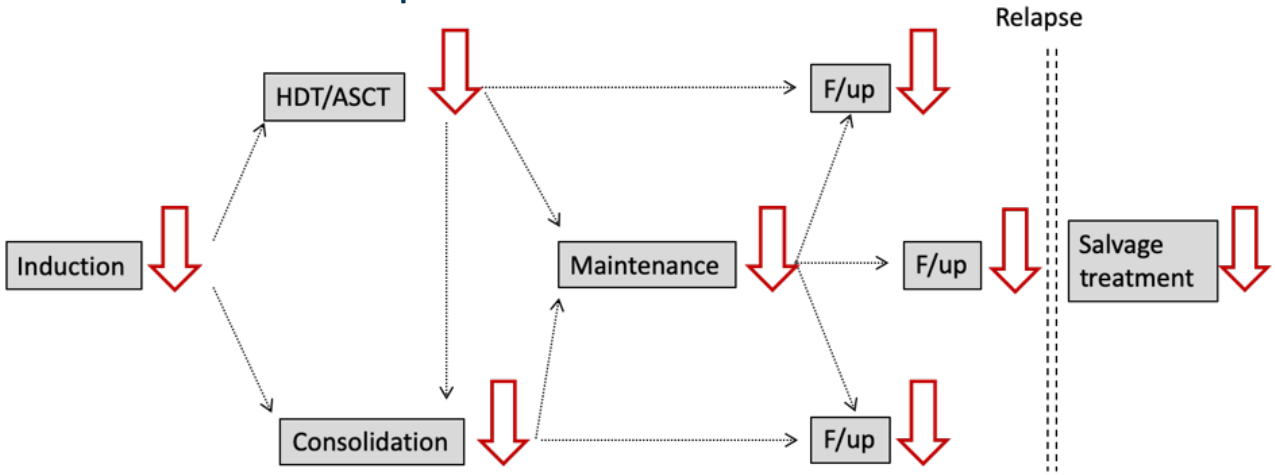

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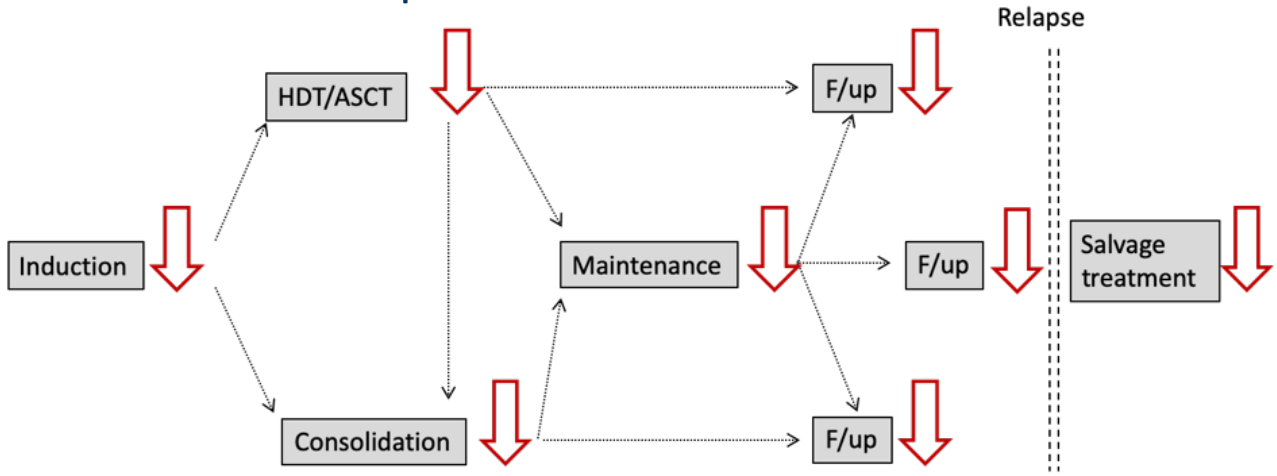

Peripheral blood-based MRD: TBD

Paiva B, et al. Blood, 2015

Take-home Points

- ✓ Existing recommendations for MRD testing (i.e., to be performed for VGPR in addition to CR) are all related to the BM-based evaluation.
- ✓ One-time, blind BM aspiration biopsy is not able to provide information of the cancer on its entirety and can introduce bias and false negatives. A peripheral blood approach could potentially overcome these problems.
- ✓ ctDNA may not serve as a sufficient analyte for MRD monitoring.
- ✓ CTCs: possible source for monitoring MRD by NGF.
- ✓ Mass-spectrometry-based assay are being taken into consideration.