AVANÇOS NA DETEÇÃO PRECOCE DE NEOPLASIAS HEMATOLÓGICAS: PAPEL DA CITOMETRIA DE FLUXO

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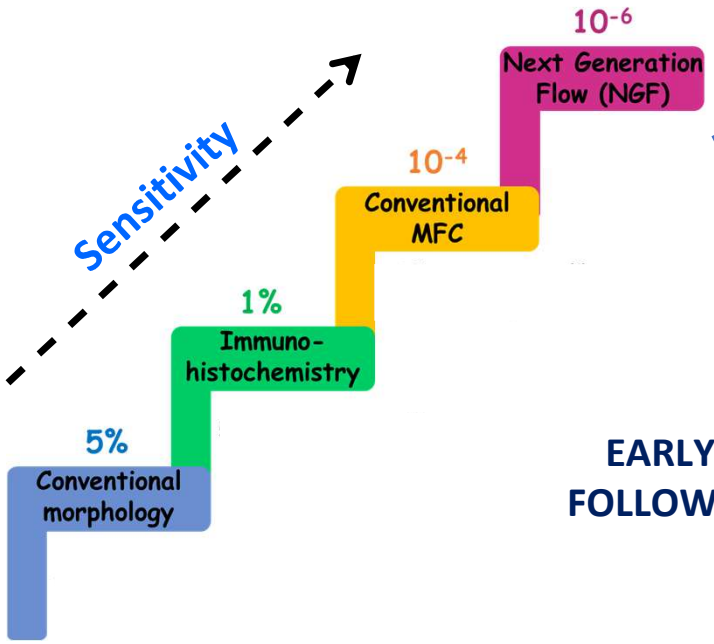
Next Generation Flow



EARLY DIAGNOSIS AND FOLLOW UP IN PERIPHERAL BLOOD

Optimization, validation, standardization

Standardized sample processing systematic acquisition of **≥ 10^7**

Automated gating and create reference databases

Matched a sample with a reference data base

(1) Paiva B. et al., Blood, 2013. (2) San Miguel JF. et al.; *Eur J Cancer*; 2006. (3) Billadeau D. et al; *Blood*; 1996. (4) Paiva B. et al.; *Leukemia*; 2011.

MBL progression to CLL

WHO 2016 CLASSIFICATION OF B-CLPD

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Mature/peripheral B cell chronic lymphoid leukemias:

- Chronic lymphocytic leukemia/Small B cell lymphocytic lymphoma
- Monoclonal B-cell lymphocytosis (CLL-like & non-CLL like)
- B-cell prolymphocytic leukemia
- Hairy cell leukemia

Mature/peripheral B-cell lymphomas:

- Splenic marginal zone lymphoma
- Splenic B-cell lymphoma/leukemia, unclassifiable (HCLv & red pulp lymphoma)
- Lymphoplasmacytic lymphoma (Waldenstrom Macroglobulinemia)
- IgM-MGUS
- Heavy chain diseases (mu, alpha and gamma)
- Extranodal marginal zone lymphoma (MALT-type)
- Nodal marginal zone lymphoma (pediatric variant)
- Follicular lymphoma (in situ follicular neoplasia, Duodenal-type & Testicular FL)
- Pediatric-type follicular lymphoma
- Large B-cell lymphoma with IRF4 rearrangement
- Primary cutaneous follicle centre lymphoma
- Mantle cell lymphoma (in situ mantle cell neoplasia)
- Diffuse large B-cell lymphoma, NOS (GC and ABC subtypes)
- Other DLBCL and related disorders
- Burkitt lymphoma
- Burkitt-like lymphoma with 11q aberration
- High-grade B cell lymphoma (with MYC and BCL2 and/or BCL6 & NOS)
- B-cell lymphoma, unclassifiable with features intermediate between DLBCL and HL

Plasma cell neoplasias:

- Non-IgM MGUS
- Plasma cell myeloma
- Solitary plasmacytoma (of bone and extraosseus)
- Monoclonal Ig deposition diseases (primary amyloidosis & light and heavy chain diseases)

5th cause of cancer worldwide
(GLOBOCAN 2018):

-509,590 new cases in 2018
-248,724 deaths in 2018

Classification of monoclonal B cell Lymphocytosis
based on clonal B-cell phenotype

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- **Low count MBL (MBL^{lo}):** **<$0.5x10^9$ clonal B-cells/L in PB of otherwise healthy subjects, typically without lymphocytosis.**
  - **MBL^{lo} is usually detected in patients screened for another (clonal) hematopoietic cell disorder and screening studies in the general population**
    - **Typical: $CD5^+$, $CD20^{lo}$, $CD79^{lo}$ and sIg^{lo}**
    - **Atypical: $CD5^+$ and $CD20^{hi}$ and/or $CD79b^{hi}$ and/or sIg^{hi}**
    - **Non CLL**
- **High count MBL (MBL^{hi}):** **Between $0.5x10^9$ and <$5x10^9$ clonal B-cells/L in PB of otherwise healthy subjects, almost always associated with lymphocytosis.**
  - **MBL^{hi} is most frequently identified in the clinical settings in a routine blood analysis (high lymphocyte counts)**

Marti et al, Br J Haematol 2005 (Rawstron et al, Cytometry B, 2010; Swerdlow et al, Blood 2016)

Prevalence of MBL in MBL in the General Population

	Europe/USA	Japonese (from Brazil	UAE	Uganda	Mexico
MBL^{lo}:	**3.5% - 14%**	**7,7 – 10%**	**5,8%**	**14%**	**9,4**
CLL-like	**85%**	**100%**	**48%**	**3%**	
Non-CLL	**15%**	**0%**	**52%**	**97%**	

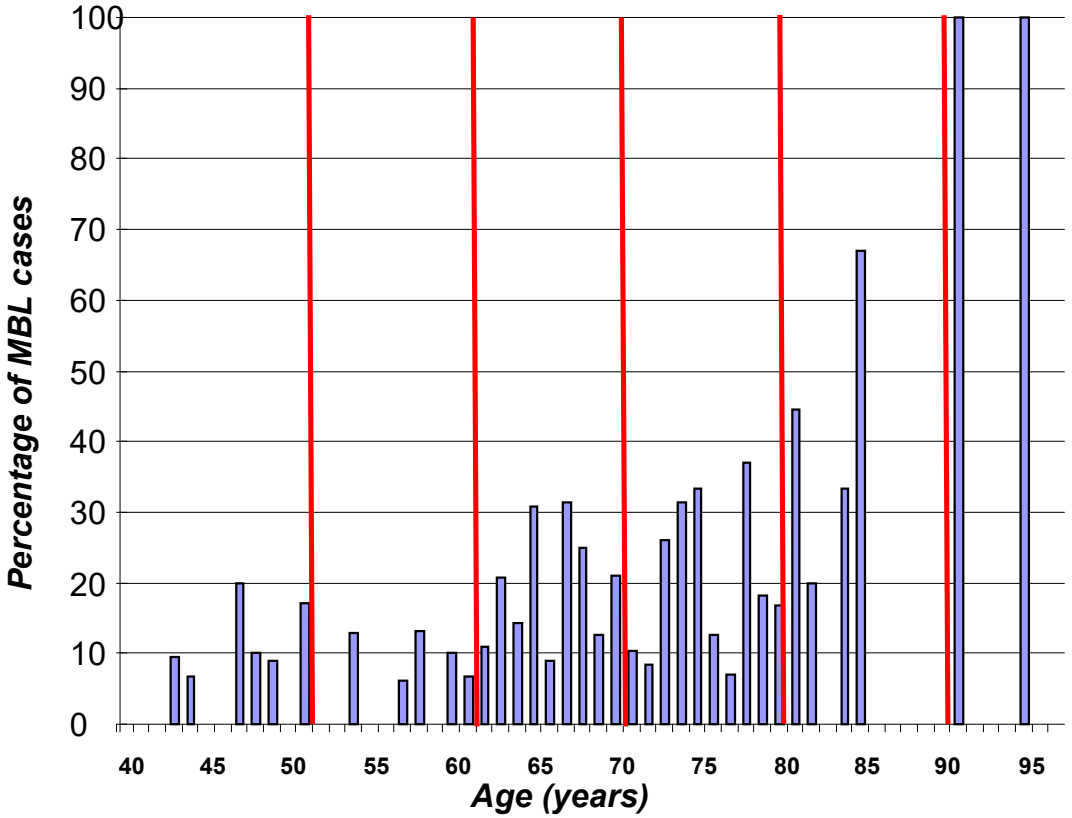
- MBL in relatives of **sporadic CLL** patients: 5%-14%
- MBL in relatives of **familial CLL** patients: 14%-18%
- MBL in patients with **lymphocytosis**: 14%

Rawstron et al, Blood 2002; Ghia et al, Blood, 2004; Rachel et al, Br J Hematol, 2007; Nieto et al, Blood, 2009; Shim et al, Blood 2014; Rawstron et al, Lancet Hematol, 2017; Rodriguez-Preciado et al, Int J Immunogen, 2017; Faria-Moss et al Haematologica 2019

Frequency of MBL in PB of healthy adults

Mean age: 70±11 years for MBL (*vs.* 61±13 years for non-MBL; p<0.05)

95/639 cases
(14.8%)



Nieto et al, Blood 2009

Low-count MBL persists after 7 years of follow-up

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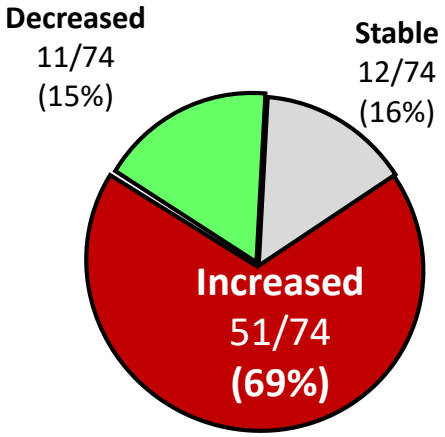
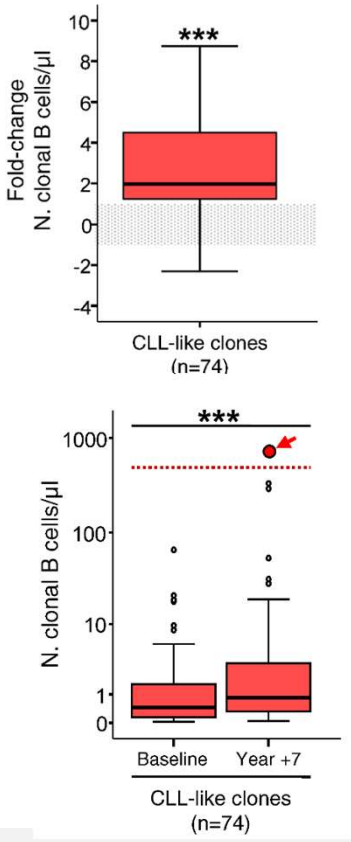
In 74/74 MBL^{lo} CLL-like clones **persisted over time**

Evolution of MBL^{lo} clone size after 7y follow-up.





In MBL^{lo} CLL-like clones persisted over time and double their numbers after 7y of follow-up with a very low rate (<2% after 7y) of progression to MBL^{hi}

Criado et al, Haematologica, 2018

CLL-like MBL clones acquire additional cytogenetic alterations after 7 years of follow up

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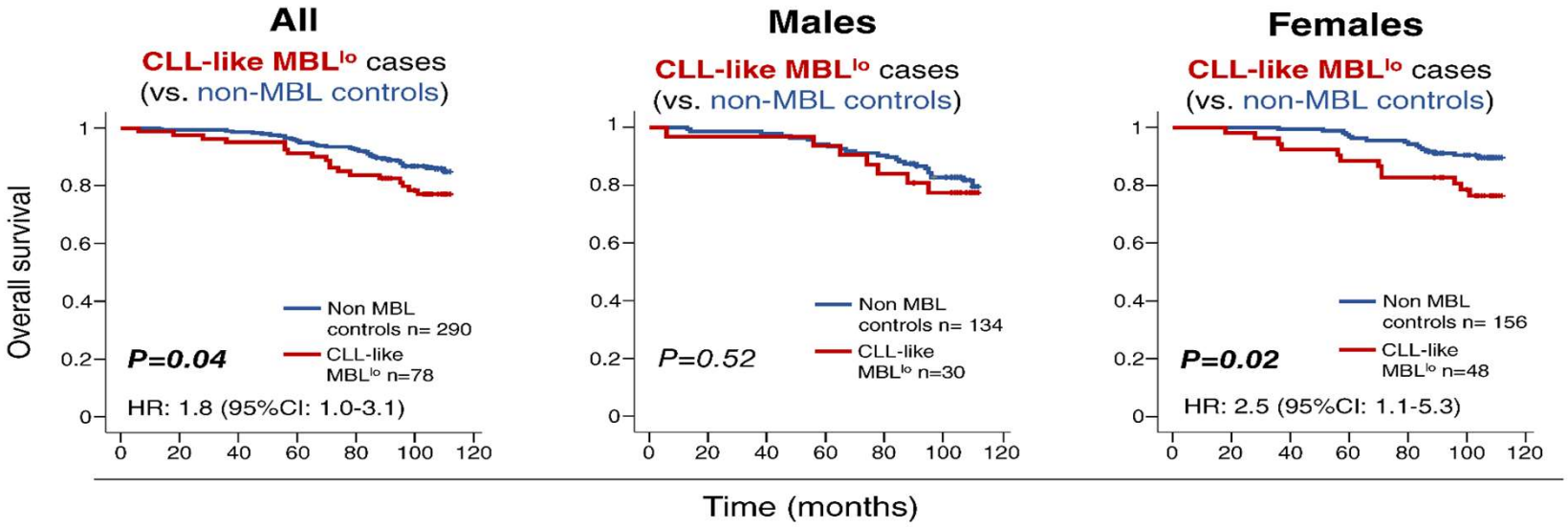
ALL CLL-like MBL^{lo} CASES	BASELINE	7y FOLLOW-UP	P-value
Altered/total cases (%)	**7/24 (29%)**	**31/50 (62%)**	0.01
del13q14(***D13S25***)	**6/20 (30%)**	**27/48 (56%)**	0.06
Trisomy 12	1/19 (5.3%)	1/49 (2%)	NS
del11q(***ATM***)	0/10 (0%)	0/48 (0%)	NA
del17p(***TP53***)	0/8 (0%)	1/48 (2.1%)	NS
del/t14q32	NA	5/23 (22%)	NA

PAIRED CLL-like MBL^{lo} SAMPLES	BASELINE	7y FOLLOW-UP	P-value
del13q14(***D13S25***)	**4/14 (29%)**	**8/14 (57%)**	0.04
Trisomy 12	1/13 (7.7%)	1/13 (7.7%)	NS
del11q(***ATM***)	0/8 (0%)	0/8 (0%)	NA
del17p(***TP53***)	0/7 (0%)	0/7 (0%)	NS

Criado et al, Haematologica, 2018

Low-count MBL in healthy subjects is associated with a shorter overall survival



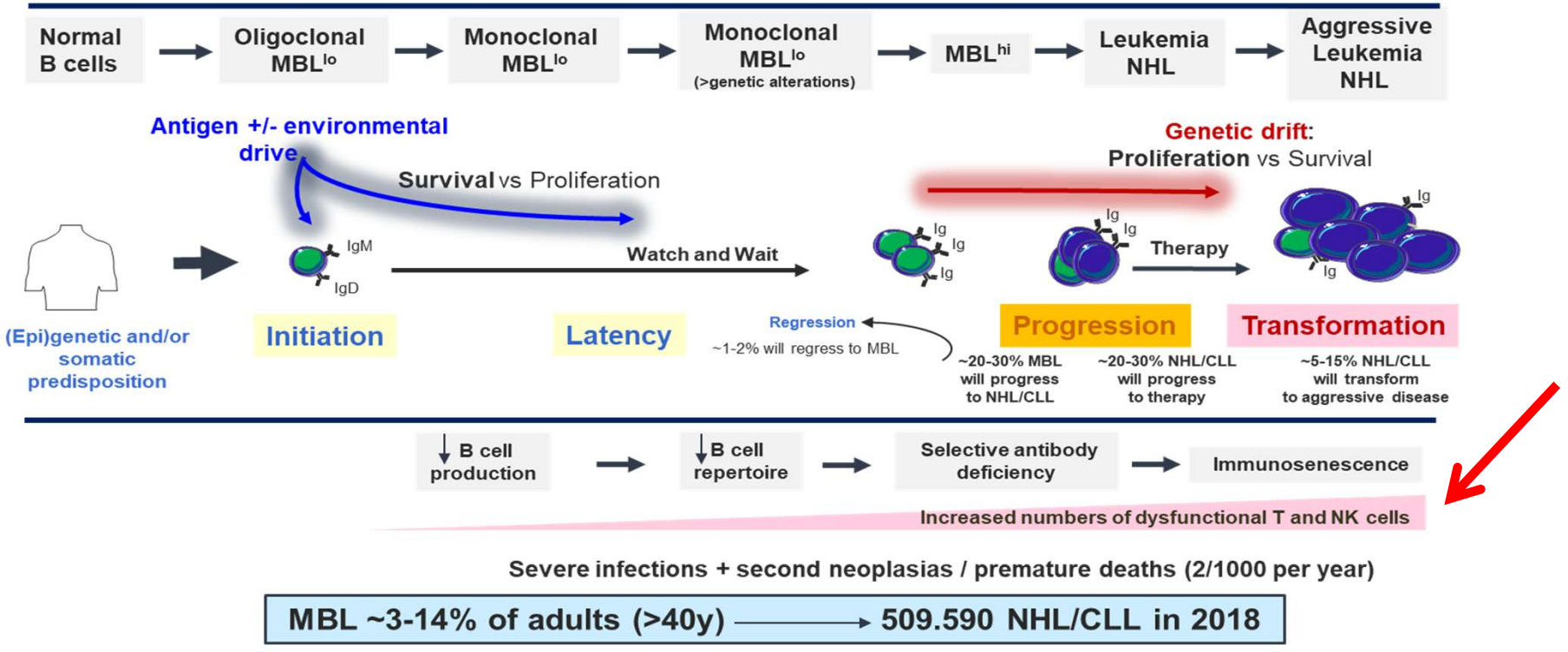
	Cardiovascular diseases	Cancer#	Infections	Other#
CLL-like MBL^{lo}	29%	36%	21%	14%
General population*	33%	26%	1.4%	39.6%

*Information obtained from INE databases.

#Infection was the direct cause of death in one individual in these groups.

Criado et al, Haematologica, 2018

Natural history of MBL is affected by environmental (antigen) and intrinsic (immune) factors



Severe infections + second neoplasias / premature deaths (2/1000 per year)

MBL ~3-14% of adults (>40y) ⟶ 509.590 NHL/CLL in 2018

Why focus on early leukaemia diagnosis based on MBL?

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- High prevalence of B cell leukaemia/lymphoma: 5th cause of cancer (509.590 cases in 2018)
- MBL is the most common early cancer presentation (3-14% of adults >40y)
- Unique early cancer model with easy access to tumor cells for unraveling oncogenesis
- Very low chance of progression (<0.1%-1% per year), but progressive immunodeficiency
- No accurate risk stratification algorithms predict progression for the general population
- Massive benefit on early identification of factors informing progression vs unlikely chance of progression and no need of care, reducing:
  - Individual psychological load
  - Social burden
  - Economic impact

Next Generation Immune Monitoring

Immune Monitoring

1. **Next generation immune monitoring**
   - Better understanding of the mode of action of immunotherapies
   - Well-defined immunophenotype of key immune cell types
   - Bone marrow vs peripheral blood
2. **Computational analysis**
3. **Integrated datasets with tumor, immunological, treatment and outcome**

CD20+ T cells in monoclonal B cell lymphocytosis and chronic lymphocytic leukemia: frequency, phenotype and association with disease progression

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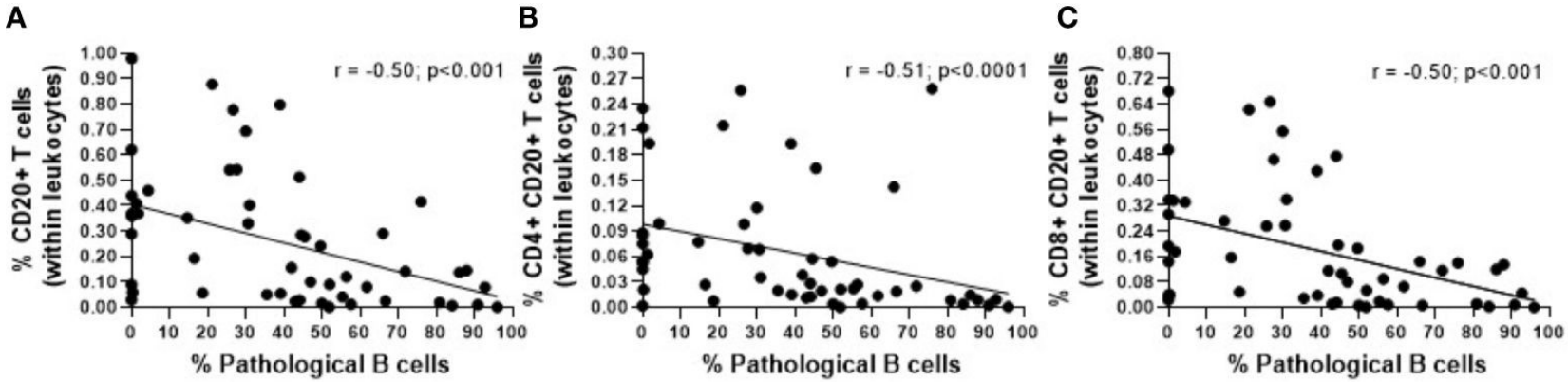
	Controls (n = 10)	LC-MBL (n = 13)	HC-MBL (n = 12)	CLL (n = 27)
CD20+ T cells No. of cells/μL % of leukocytes % after excluding neoplastic B cells	 60 ± 44 0.98 ± 0.60	 34 ± 32 0.35 ± 0.26[a] 0.35 ± 0.26[a]	 38 ± 30 0.42 ± 0.26[a] 0.58 ± 0.42	 33 ± 40 [a] 0.14 ± 0.17 [a b c] 0.43 ± 0.47 [a]
% CD20+ T cells	4.7 ± 2.0	2.6 ± 1.4	3.5 ± 2.1	2.8 ± 2.7 [a]
% CD20+ CD4+ T cells	31 ± 13	31 ± 19	22 ± 11	31 ± 20
% CD20+ CD8+ T cells	58 ± 15	65 ± 18	74 ± 14	61 ± 24
% CD20+ CD4+ CD8+ T cells	6.5 ± 11	0.88 ± 1.0	0.90 ± 1.4	0.62 ± 1.3 [a b]
% CD20+ TCR γδ+ T cells	3.0 ± 3.3	2.1 ± 1.9	2.9 ± 5.7	1.3 ± 2.0
% CD20+ TCR αβ+ CD4- CD8- TCR γδ- T cells	0.51 ± 0.74	0.94 ± 1.2	0.71 ± 1.1	5.1 ± 13

[a]p<0.05 vs controls; [b]p<0.05 vs LC-MBL; [c]p<0.05 vs HC-MBL. CLL, chronic lymphocytic leukemia; HC, high-count; LC, low-count; MBL, monoclonal B lymphocytosis.



Cristiana Rodrigues *et al*; Front. Oncol., 28 March 2024; Volume 14

CD20+ T cells in monoclonal B cell lymphocytosis and chronic lymphocytic leukemia: frequency, phenotype and association with disease progression

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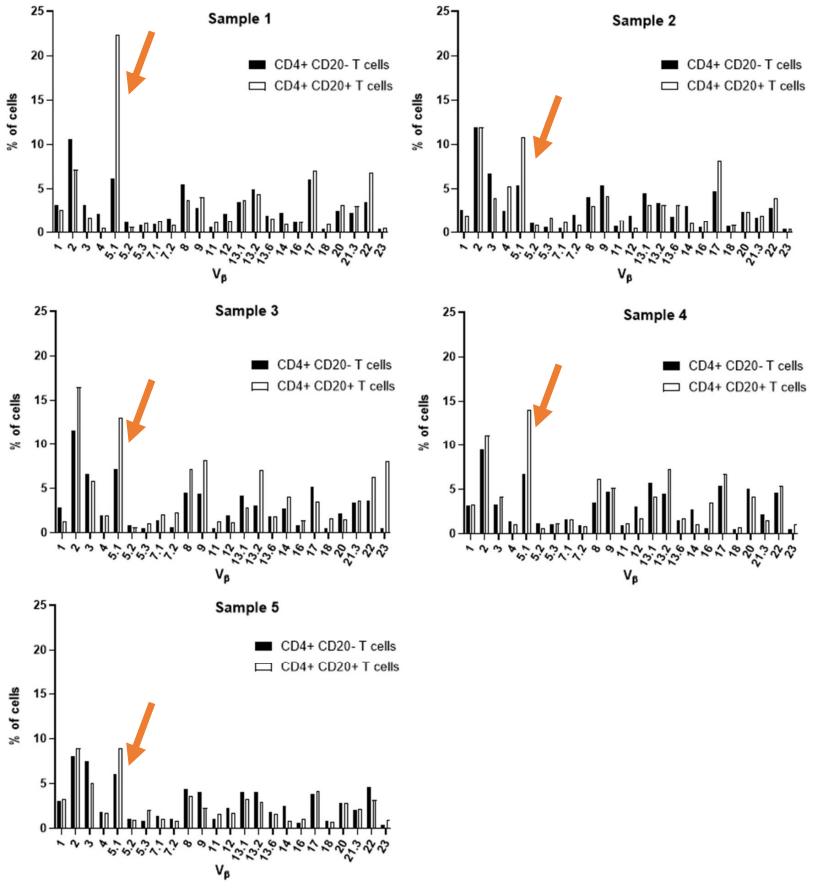
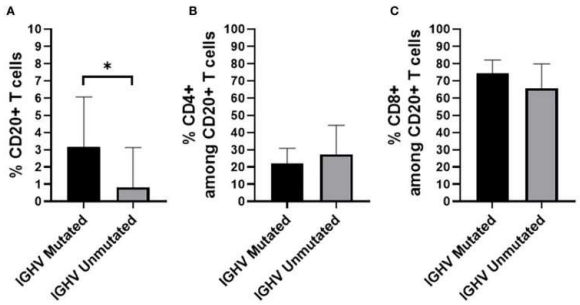
Vβ5.1 expansion in CD20+ Th cells



Increased numbers of CD20+ T cells in IGVH mutated cases



Molecular Study	HC-MBL (n = 12)	CLL (n = 27)	*P* value
TP53 gene Mutated	0/12 (0%)	2/27 (7%)	$P > 0.99$
IGHV gene SHM status Mutated	8/12 (67%)	15/27 (56%)	$P = 0.73$

CLL, chronic lymphocytic leukemia; HC, high-count; IGHV, immunoglobulin heavy chain variable region; LC, low-count; MBL, monoclonal B lymphocytosis; SHM, somatic hypermutation. Results expressed as number of cases with IGHV mutated and TP53 mutated genes from all cases analyzed in the corresponding group (and their respective percentage).

Cristiana Rodrigues *et al*; Front. Oncol., 28 March 2024; Volume 14

Quantification and Phenotypic Characterization of Peripheral Blood Vδ1 + T Cells in Chronic Lymphocytic Leukemia and Monoclonal B Cell Lymphocytosis

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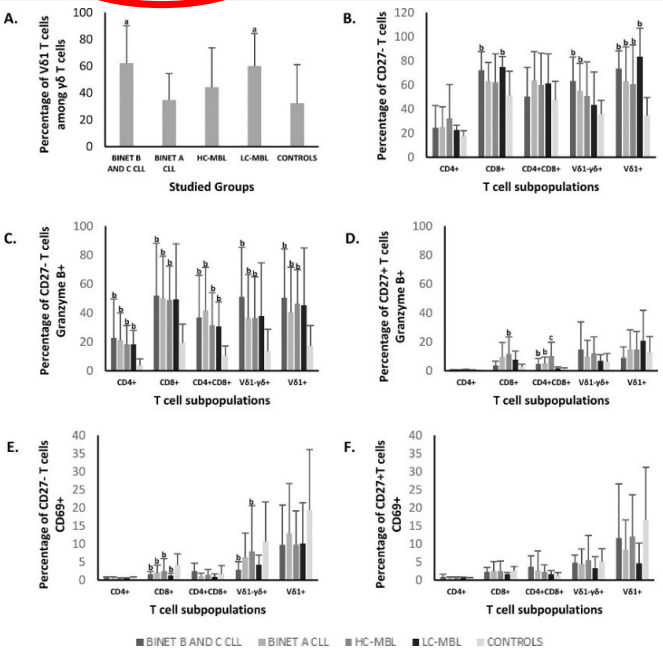
	BINET B AND C CLL (n=9)		BINET A CLL (n=26)		HC-MBL (n=10)		LC-MBL (n=5)		CONTROLS (n=8)	
	Cells/µl	%	Cells/µl	%	Cells/µl	%	Cells/µl	%	Cells/µl	%
Total γδ+ T cells	136±112	3,6±1,9	158±287	5,2±6,2	114±123	5,3±5,2	99±110	5,8±5,1	79±114	5,4±5,0
Vδ1+ T cells	88±109[d]	2,0±1,7	47±74	1,7±2,5	77±110	3,2±4,6	57±53	3,7±3,6	15±17	1,5±1,9



T cells from MBL and CLL exhibit a more cytotoxic profile (granzyme B+), particularly, Vδ1 cells

Simões C, *et al*. Cytometry Part B 2018; 00: 1–5.

MGUS progression to MM

Move from bone marrow to peripheral blood?

What we know about circulating plasmatic tumor cells (CTC)?

Circulating plasmatic tumor cells CTCs may be a powerpowerful biomarker of malignant transformation and disease aggressiveness

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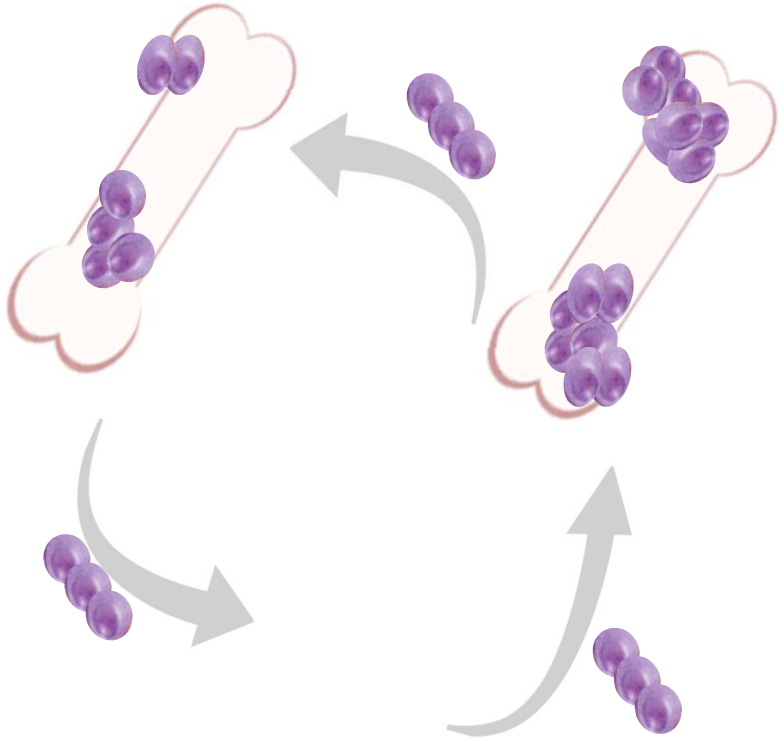

> Circulating tumor cells (CTCs) may be responsible for **MM spreading** and accordingly, their numbers in peripheral blood could be a **potential surrogate marker** for the **rate of dissemination** and overall **tumor burden** in bone marrow

Minimally-invasive characterization of MGUS and MM based on CTCs

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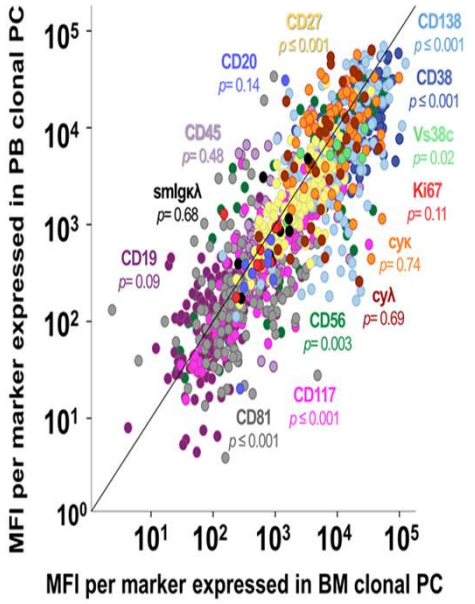
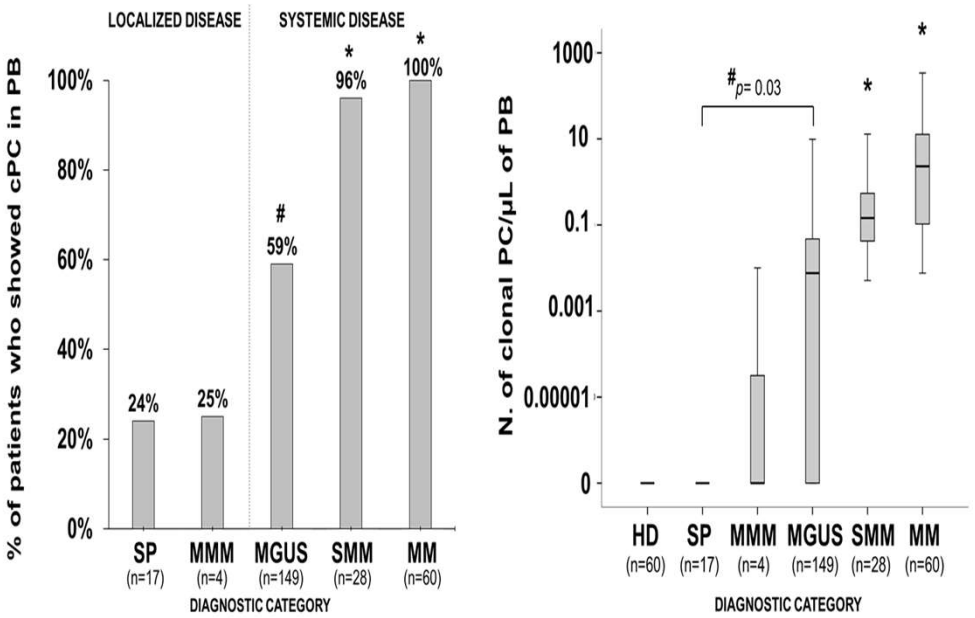
Same phenotype as BM tumor cells



CTCs detected in most MGUS and virtually all MM patients



EuroFlow

Sanoja-Flores L, et al. Blood Cancer J. 2018;8(12):117

Time to Progression According TO THE NUMBERS OF CTCs IN PB

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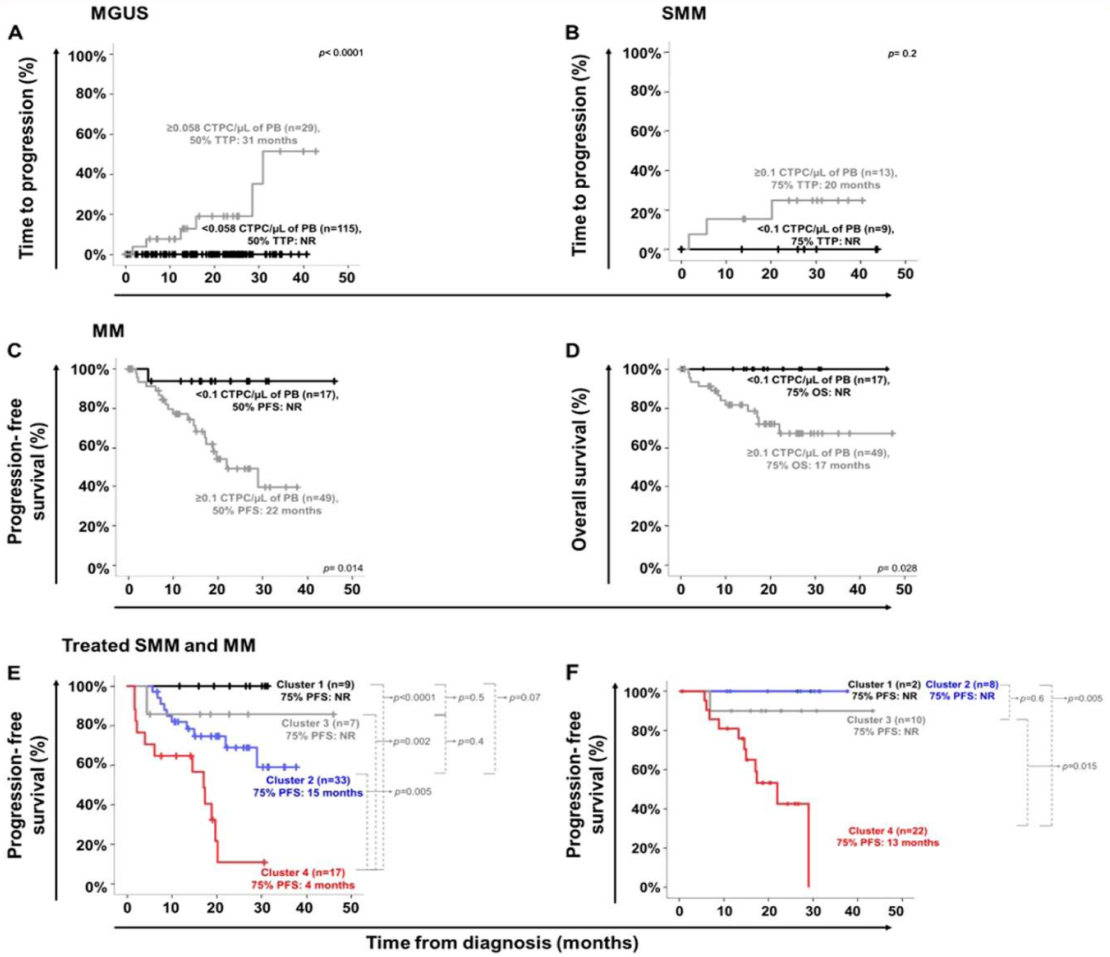

Sanoja-Flores et al. Blood Cancer Journal (2018)

CTC can replace BM PCs in the IMWG risk stratification model for SMM

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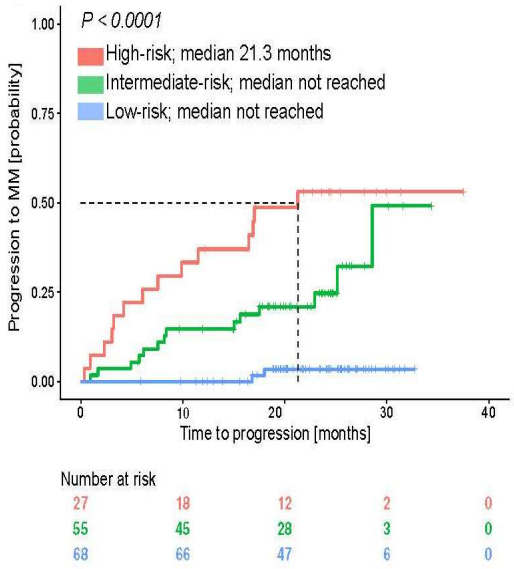
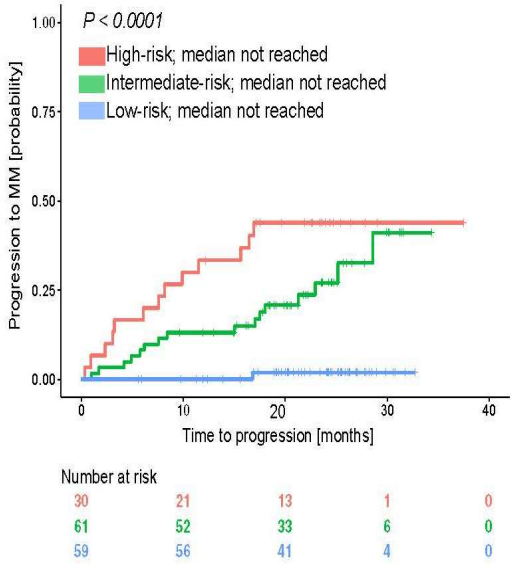
Similar performance between minimally and partially invasive models

serum free light-chain ratio >20, >2g/dL M-protein, > 20% de bone marrow plasma cells

2/20/0.015 Model (>0.015% CTCs)



2/20/20 Model (>20% BMPC)



Termini R & Žihala D, et al. Clinical Cancer Research 2022

Possible added value of dynamic risk-stratification in SMM

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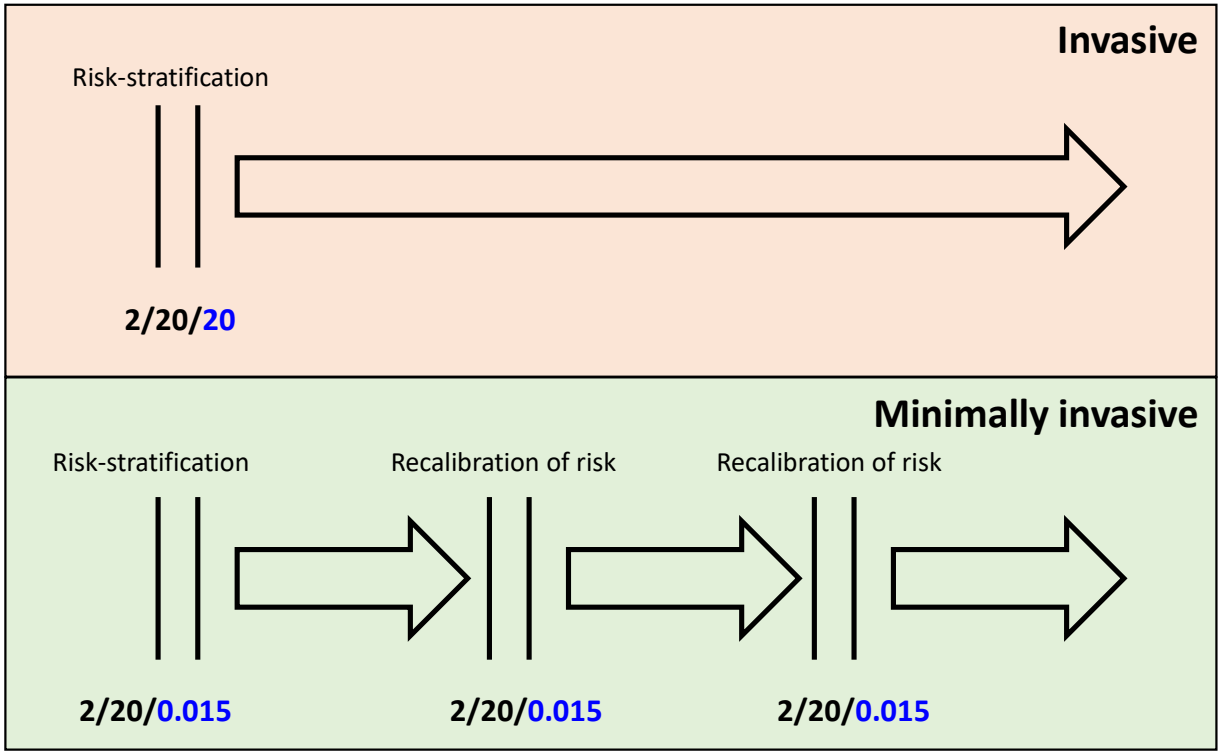
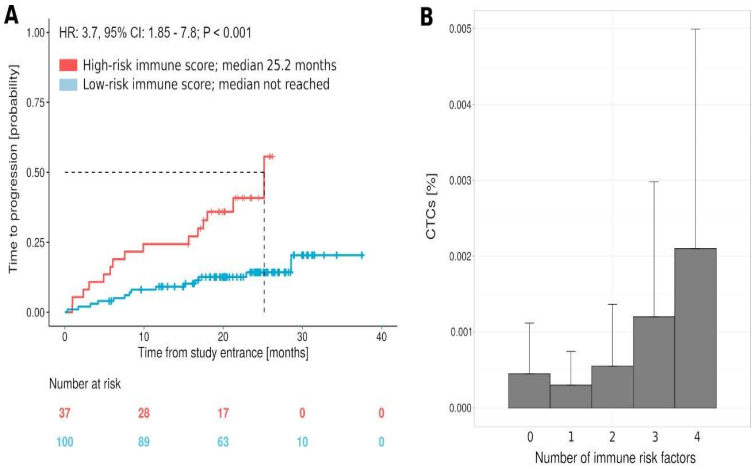
Replacing invasive by minimally invasive tumor burden



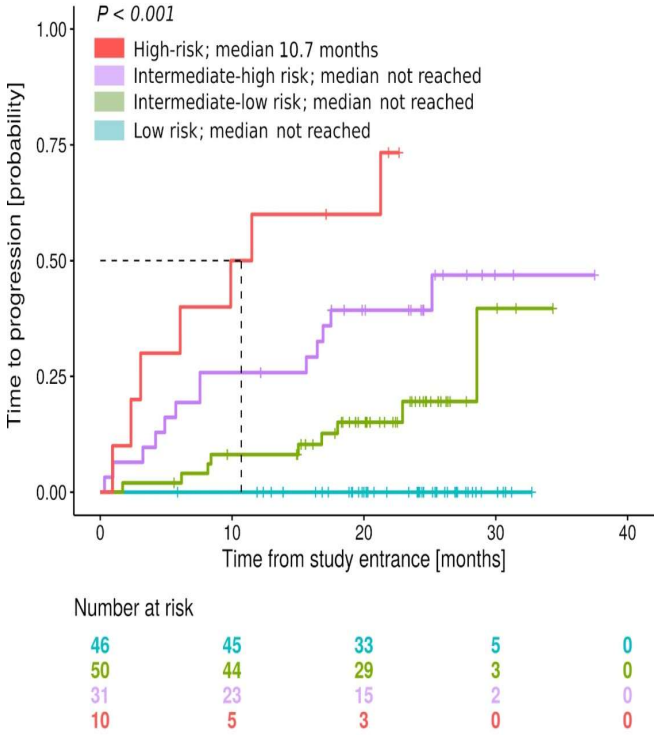
Patients **migrating to a higher risk category during follow up** had a higher progression risk compared to patients with stable/ decreased risk categorization

Visram A, et al. Blood Cancer J. 2021;11(11):186.

Minimally invasive tumor and immune risk stratification
"The iMMunocell study"



The combination of the 20/2/0.015 model with an immune risk-score based on the percentages of **SLAN+ and SLAN- non-classical monocytes, CD69+HLADR+ cytotoxic NK cells, and CD4+CXCR3+ stem central memory T cells**, allowed patient' stratification into low, intermediate-low, intermediate-high and high-risk disease with 0%, 20%, 39% and 73% rates of progression at two years.



Termini R & Žihala D, et al. Clinical Cancer Research 2022

Diagnosis: : CTC are one of the most relevant prognostic factors in MM

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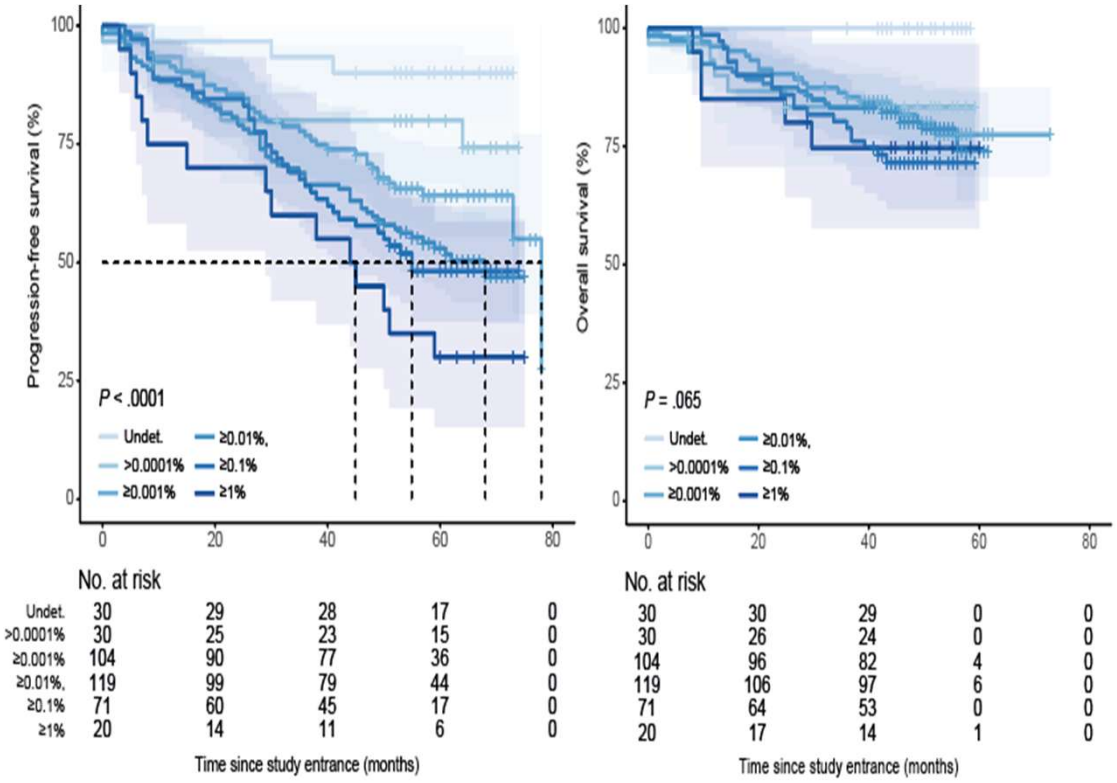
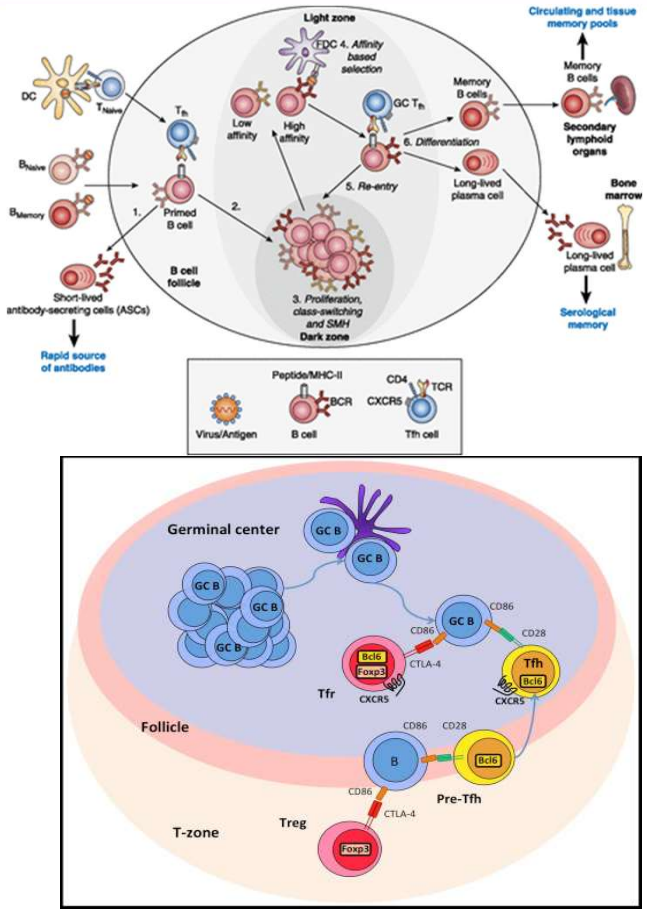
Undetectable CTCs defines a unique patient subgroup (8% in NDTE)



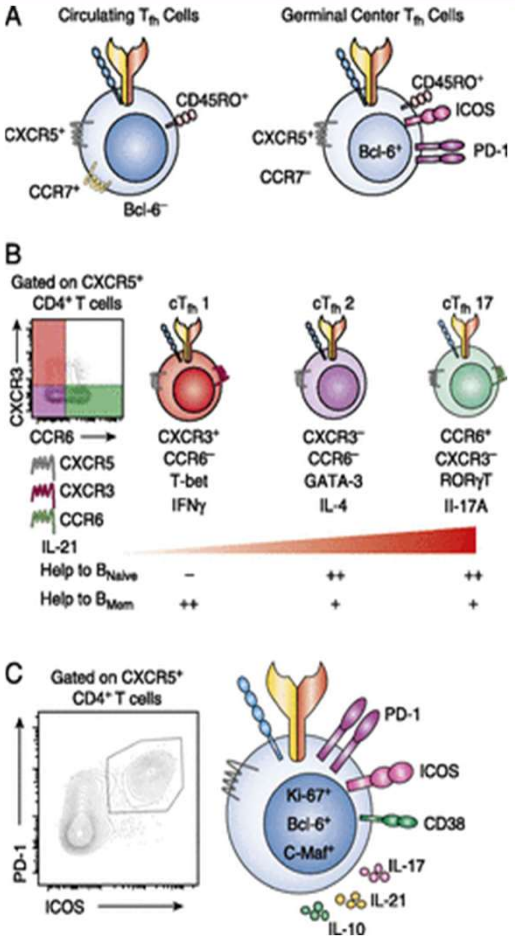
Garces JJ, et al. J Clin Oncol. 2022:JCO2101365. doi: 10.1200/JCO.21.01365.

Characterization of Tumor microenvironment:
immune monitoring

Role of Follicular-like and regulatory T cells in monoclonal gammopathies



Michelle A. Linterman and Alice E. Denton. *Immunity*. 2014.



Marios Koutsakos et al. *J Immunol*. 2019

Role of Bone Marrow Follicular-like and Treg cells in Monoclonal Gammopathies

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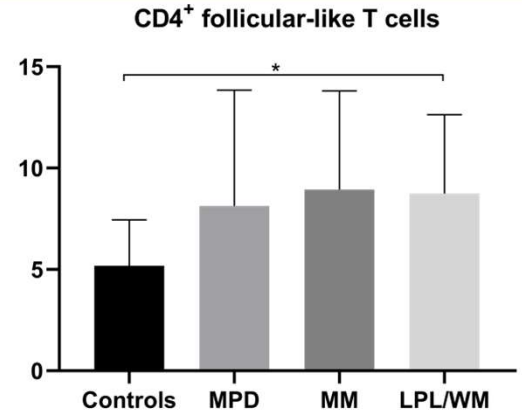
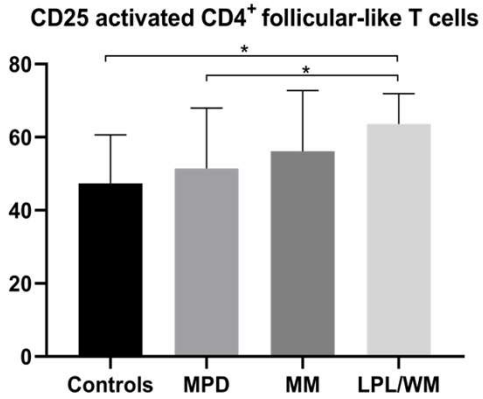
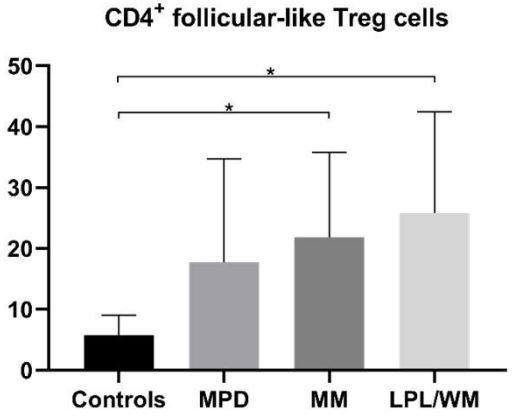
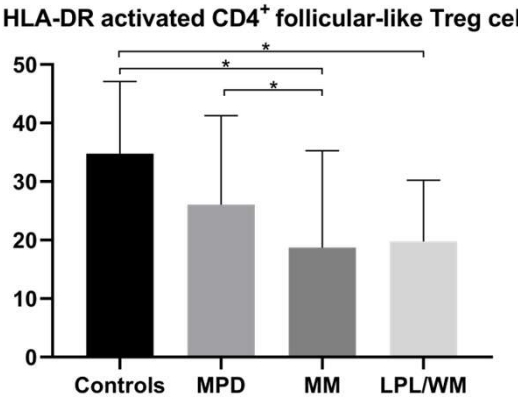


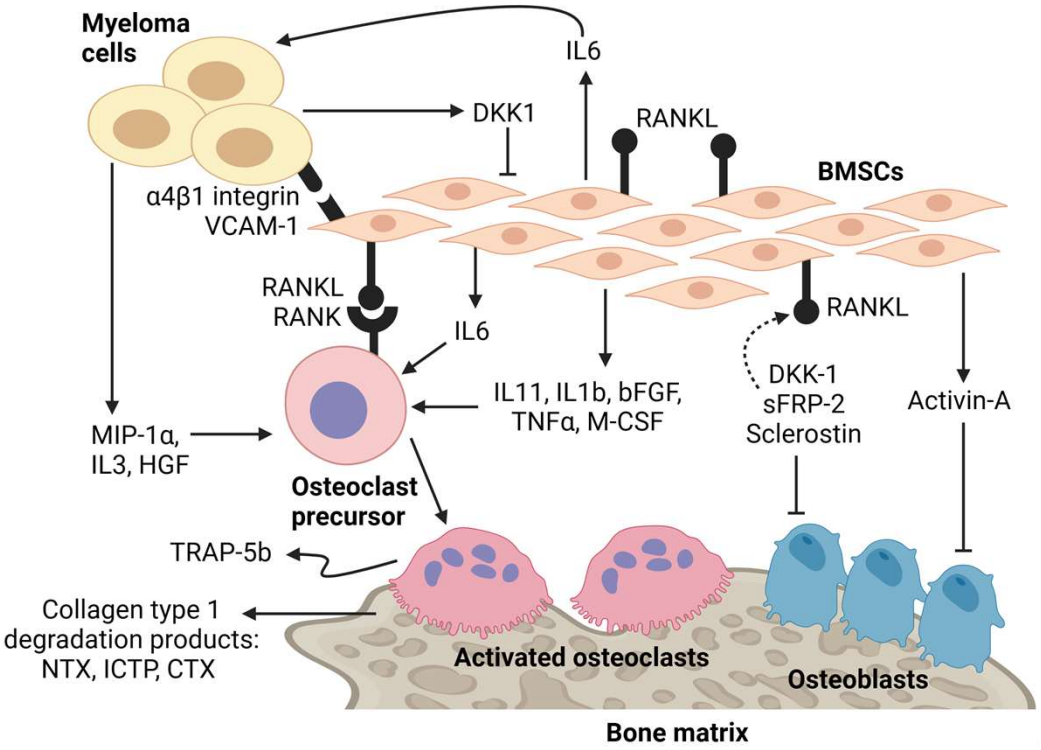






Ana Silva *et al*, Clinical Cytometry 2022: Jan: 102 (1). 70-72.

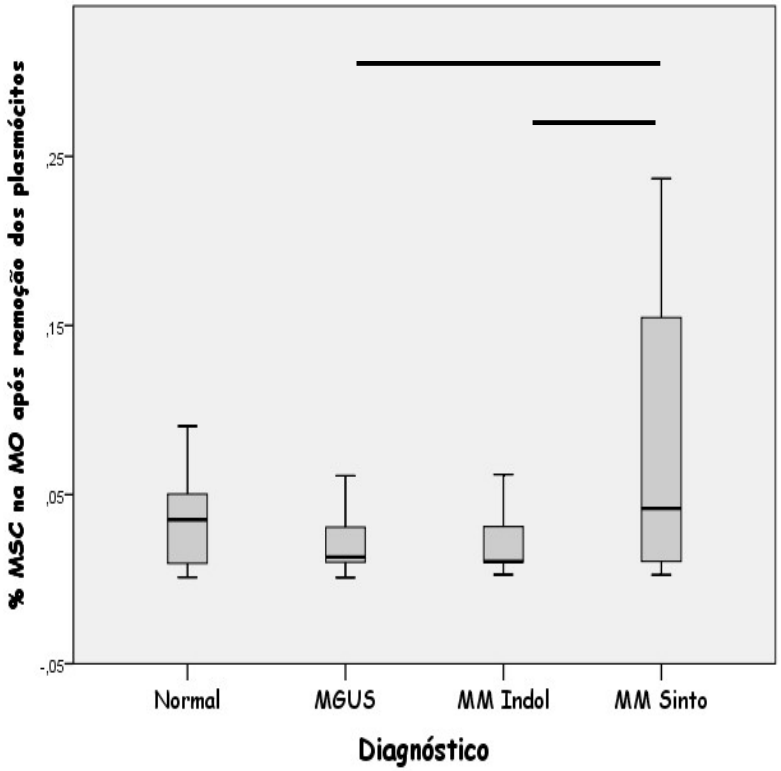
Role of Mesenchymal stromal cells in Monoclonal Gammopathies



*Adapted from Lentzsch, et al.[7] and Mukkamalla, et al.[8]Created with BioRender.com.

Role of Mesenchymal stromal cells in Monoclonal Gammopathies

XXIV JORNADAS CIENTÍFICAS DE ANÁLISES CLÍNICAS E DE GENÉTICA HUMANA



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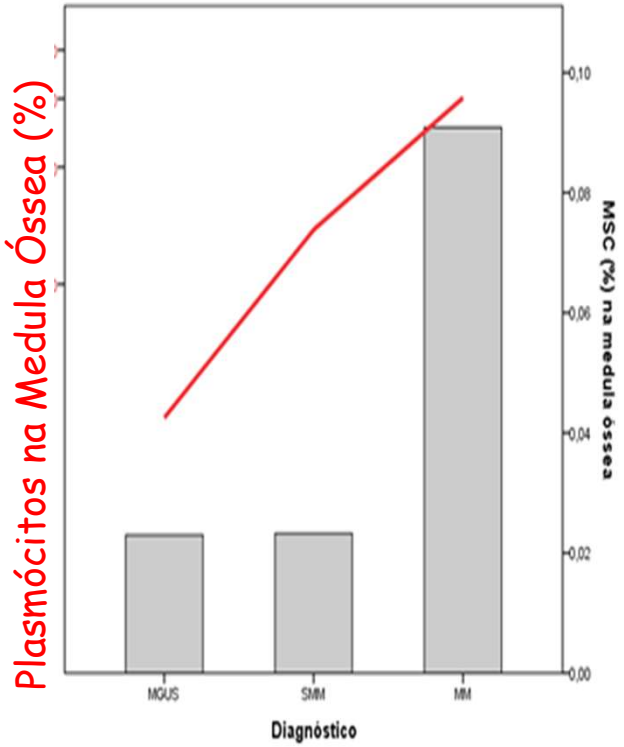
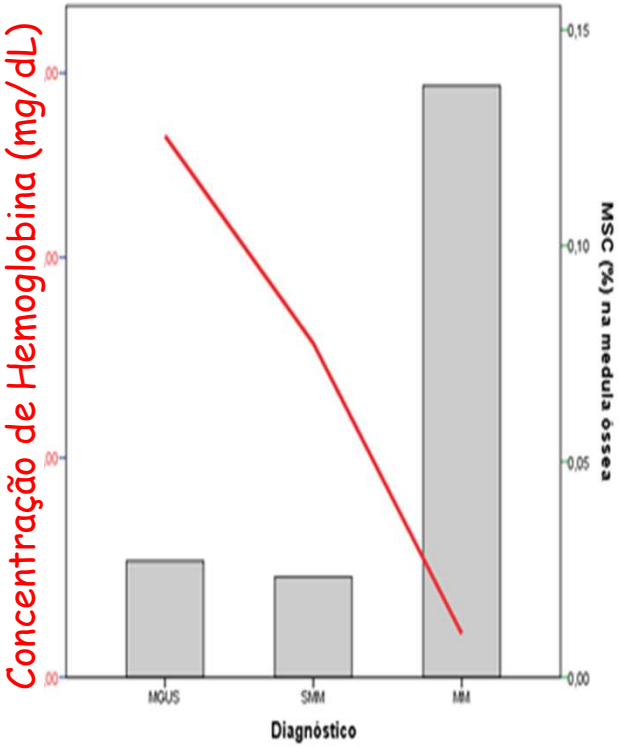




Unpublished results.

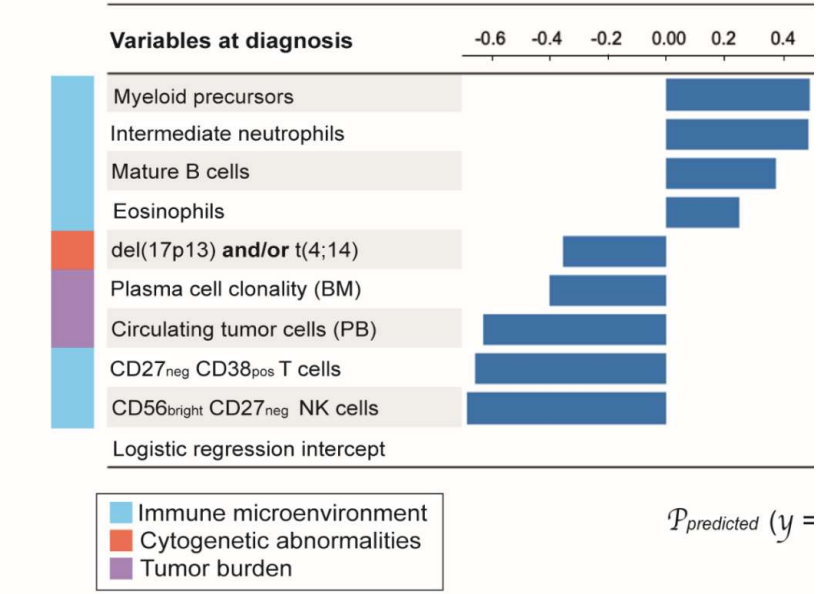
Next generation immune monitoring

- Better understanding of the mode of action of immunotherapies
- Well-defined immunophenotype of key immune cell types

Integrated datasets with tumor, immunological, treatment and outcome

Can we predict MRD outcomes before initiating treatment?

A machine learning model using tumor and immune biomarkers



Predicts in 71% of patients

Guerrero C, et al. Clin Cancer Res. 2022;28(12):2598-2609.

Acknowledgments

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Obrigado