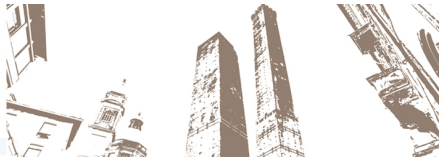
Highlights from IMW 2019

19-20 novembre 2019
Bologna
Royal Hotel Carlton

Roberto Ria

Ruolo del microambiente midollare

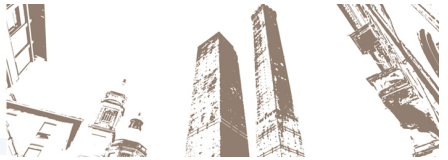
Disclosures for Roberto Ria M.D.

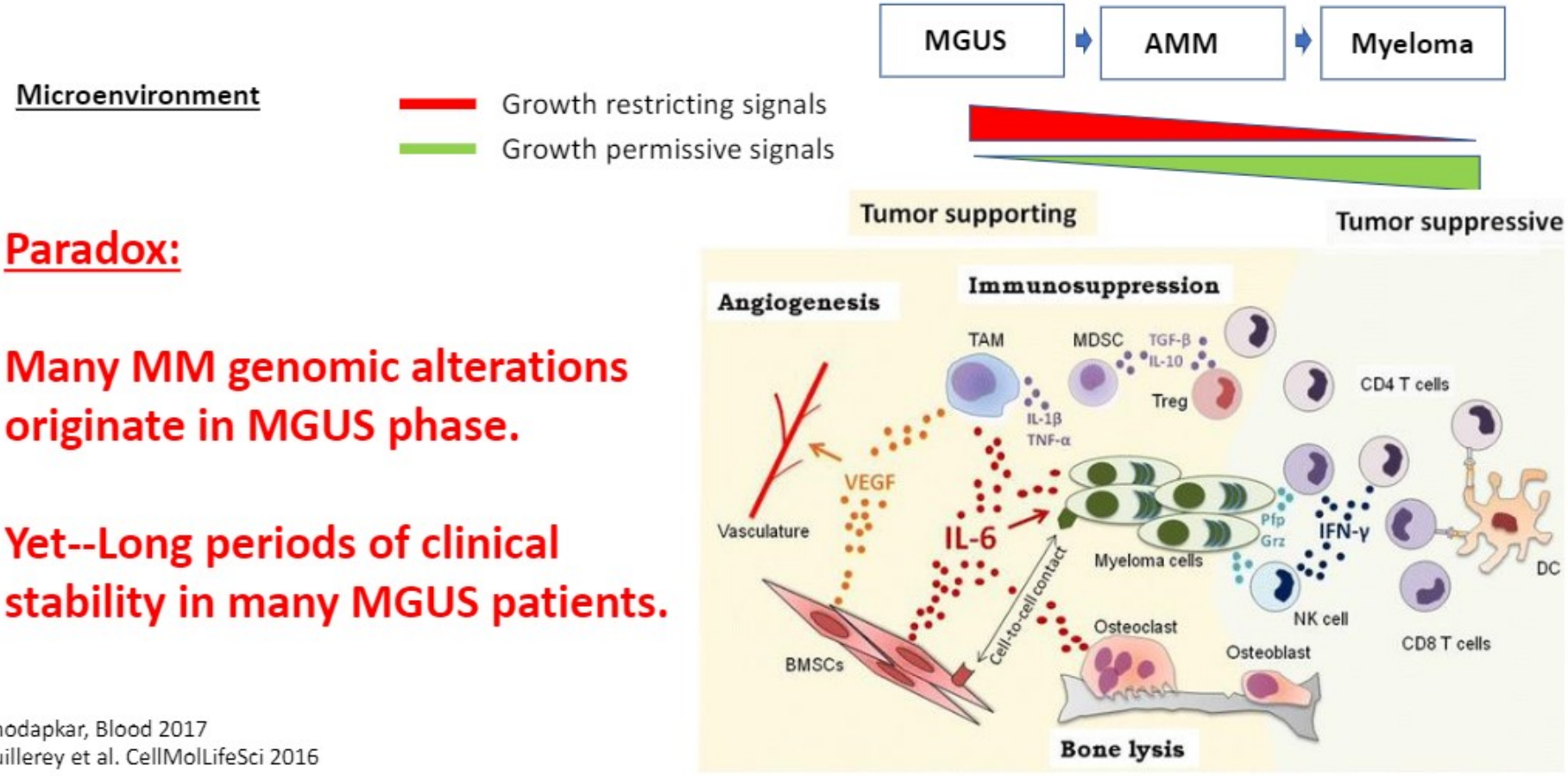


- ✓ Grant/Research Support: no disclosure.
- ✓ Speaker's Bureau: BMS, CSL Behring, Celgene, Italfarmaco, Janssen Cilag.
- ✓ Consultant: BMS, CSL Behring, Celgene, Italfarmaco, Janssen Cilag, Octapharma.
- ✓ Major Shareholder: no disclosure.
- ✓ Other: no disclosure.

I will be discussing "off-label" uses of the following medications: none

Dhodapkar
Bone marrow microenvironment and MM progression





Paradox:

Many MM genomic alterations originate in MGUS phase.

Yet--Long periods of clinical stability in many MGUS patients.

Dhodapkar, Blood 2017
Guillerey et al. CellMolLifeSci 2016

THE IMMUNE SYSTEM: Interaction between plasma cells and the host immune system

Contents lists available at ScienceDirect

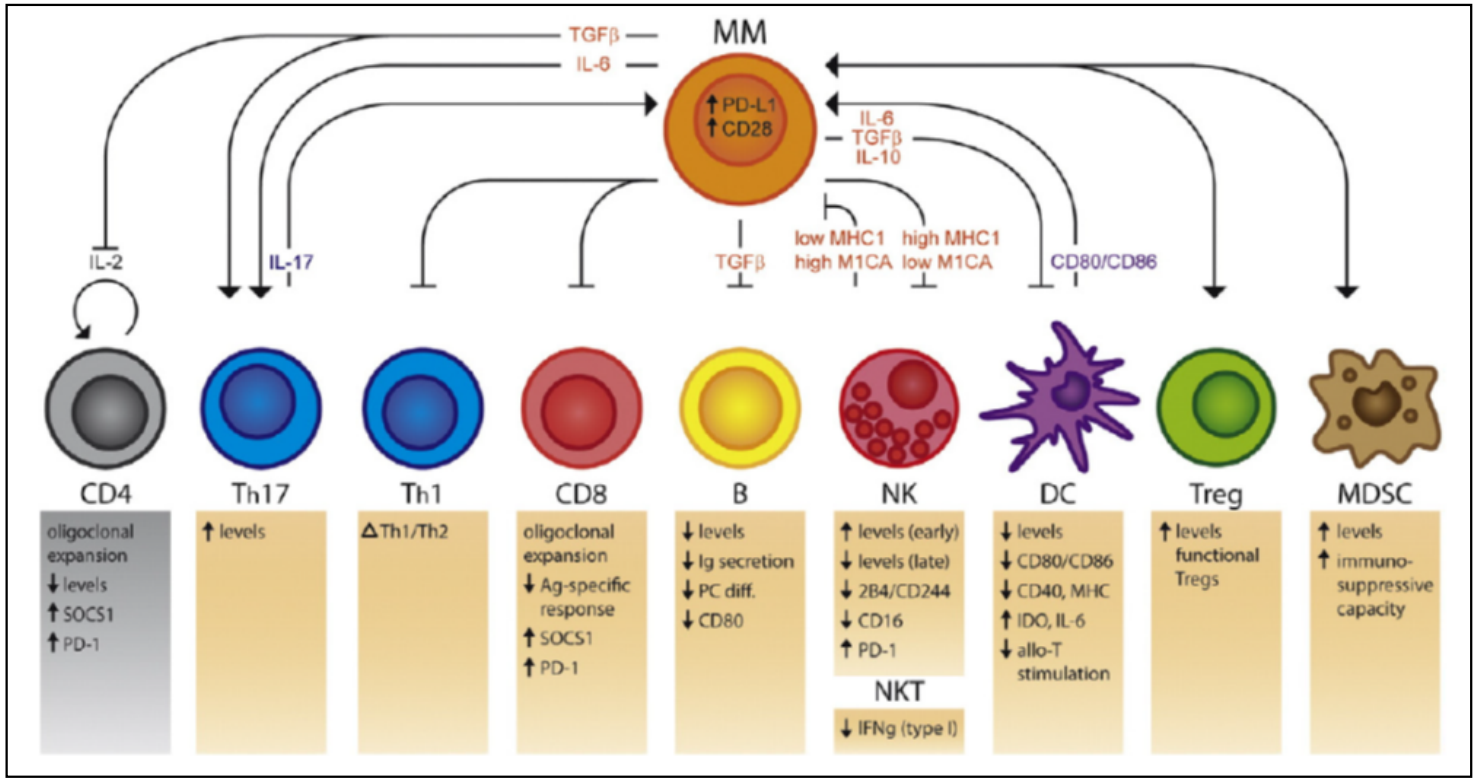
Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbacan

Review

Cellular immunotherapy in multiple myeloma: Lessons from preclinical models

M. Binsfeld [a], K. Fostier [b], J. Muller [a], F. Baron [a,c], R. Schots [b], Y. Beguin [a,c], R. Heusschen [a,*,1], J. Caers [a,c,1]



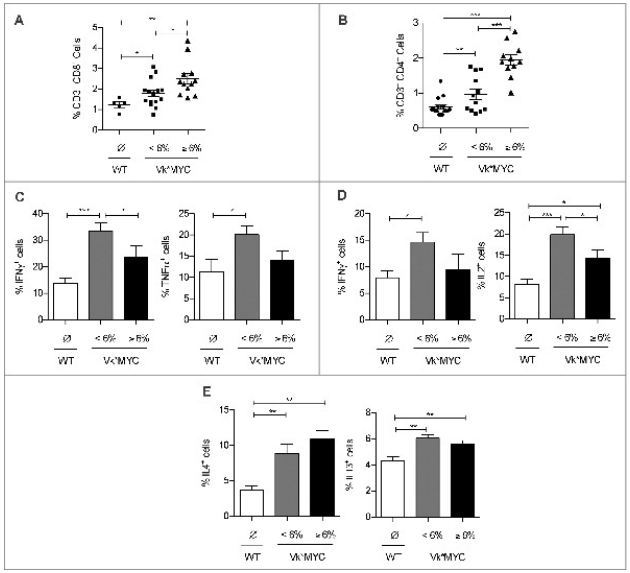
THE IMMUNE SYSTEM: Role in SMM progression to MM

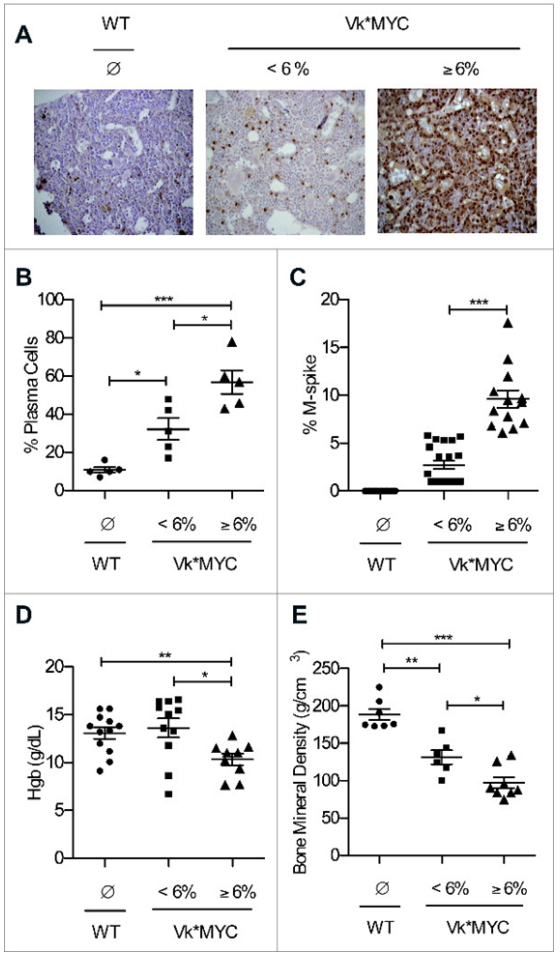
ORIGINAL RESEARCH

OncoImmunology 4:6, e1008850; June 2015; Published with license by Taylor & Francis Group, LLC

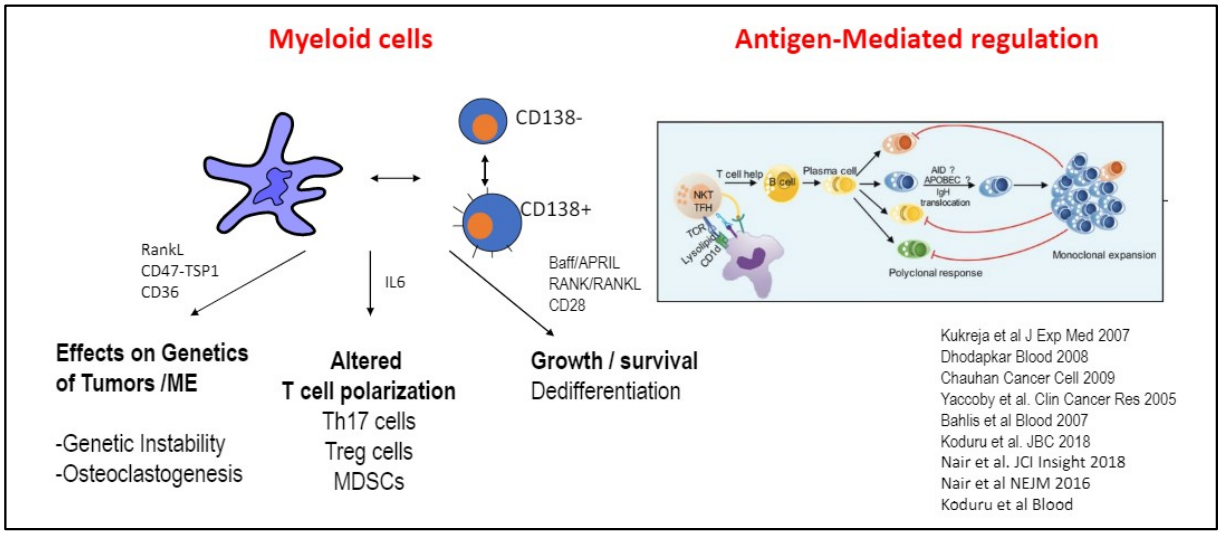
Modifications of the mouse bone marrow microenvironment favor angiogenesis and correlate with disease progression from asymptomatic to symptomatic multiple myeloma

Arianna Calcinotto[1,2], Maurilio Ponzoni[3], Roberto Ria[4], Matteo Grioni[1], Elena Cattaneo[1], Isabella Villa[5], Maria Teresa Sabrina Bertilaccio[6], Marta Chesi[7], Alessandro Rubinacci[3], Giovanni Tonon[6], P Leif Bergsagel[7], Angelo Vacca[4], and Matteo Bellone[1,*]





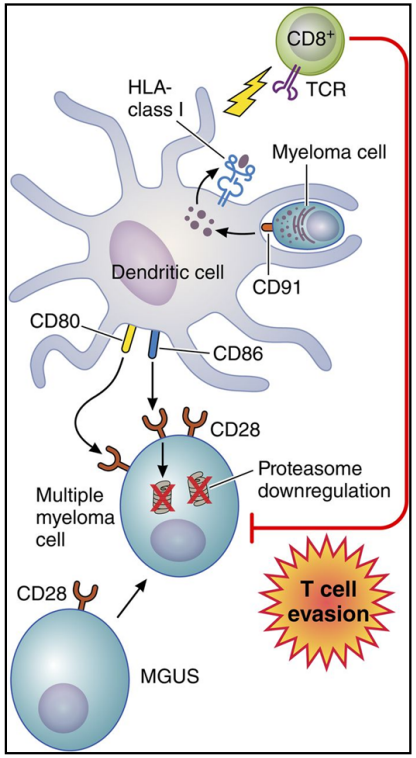
Dhodapkar
The Dark Side: microenvironment-mediated regulation and evolution in MM



LYMPHOID NEOPLASIA blood 2015 • VOLUME 126, NUMBER 12

Dendritic cells accumulate in the bone marrow of myeloma patients where they protect tumor plasma cells from CD8$^+$ T-cell killing

Patrizia Leone,[1] Simona Berardi,[1] Maria Antonia Frassanito,[1] Roberto Ria,[1] Valli De Re,[2] Sebastiano Cicco,[1] Stefano Battaglia,[1] Paolo Ditonno,[3] Franco Dammacco,[1] Angelo Vacca,[1] and Vito Racanelli[1]



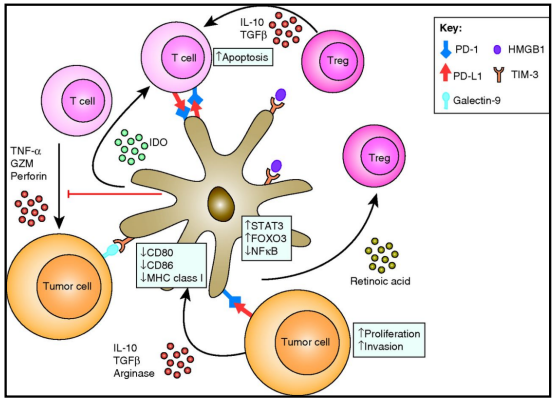
The Journal of Immunology

This information is current as of August 30, 2018.

Tumor-Infiltrating Dendritic Cells in Cancer Pathogenesis

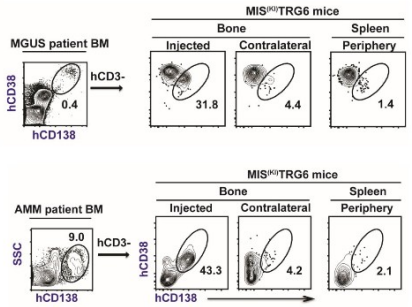
Jo Marie Tran Janco, Purushottam Lamichhane, Lavakumar Karyampudi and Keith L. Knutson

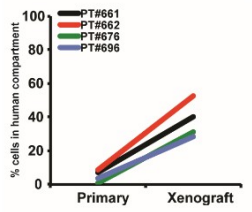
J Immunol 2015; 194:2985-2991; ;
doi: 10.4049/jimmunol.1403134
http://www.jimmunol.org/content/194/7/2985



Dhodapkar
Progressive growth of asymptomatic monoclonal gammopathies in humanized mice

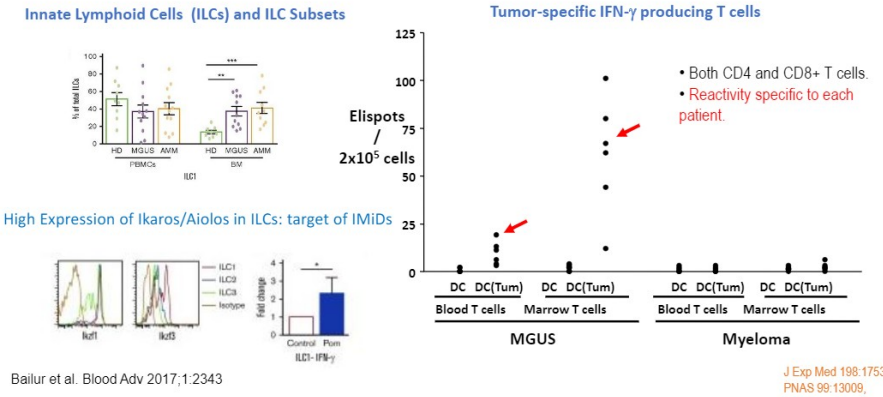
An Argument for Dominant Role of Microenvironment in Maintaining Stability In Vivo





Das et al. Nat Med 2016

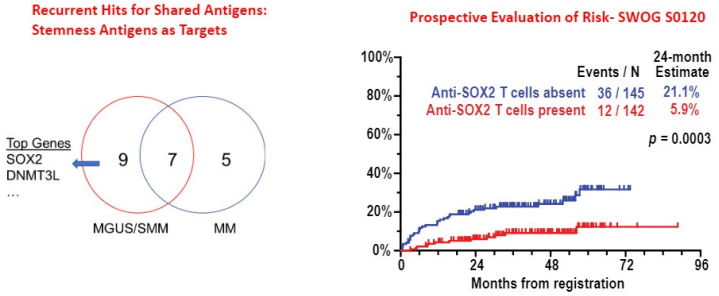
Early Alterations In Both Innate And Adaptive Immunity In The MGUS Marrow



Bailur et al. Blood Adv 2017;1:2343

J Exp Med 198:1753
PNAS 99:13009,

T Cell Immunity Against Stemness Antigens and Risk of Malignant Transformation

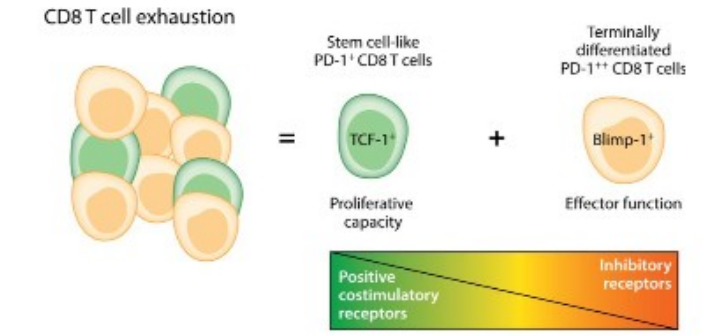


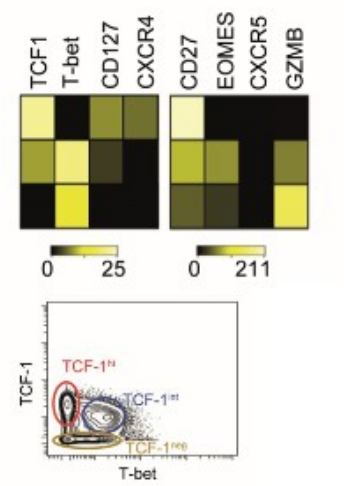
Spisek et al. J Exp Med
Dhodapkar et al, Blood

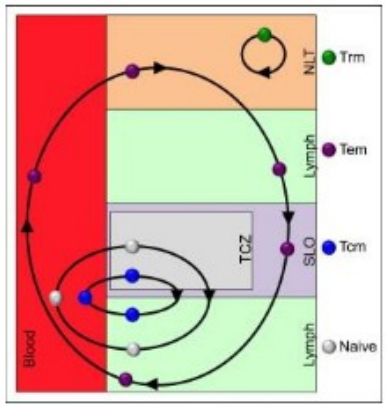
Implications of Long Premalignant Phase in Human Cancer

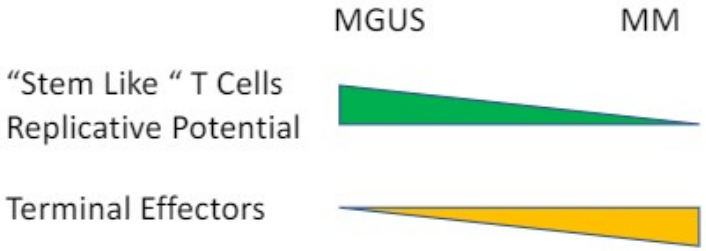
- Many of the oncogenic mutations / neoantigens originate during the precursor stages….which last many years.
- How are the memory T cells maintained for so long in spite of getting "exhausted"?
- What are the implications for immune therapies?

Dhodapkar
Attrition of “stem-like” and marrow-resident memory T cells and accumulation of terminal effector T cells from MGUS to MM



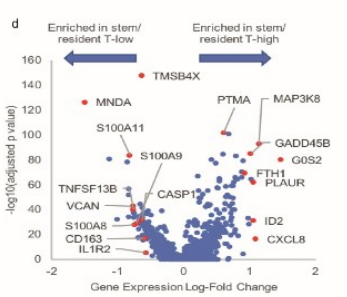




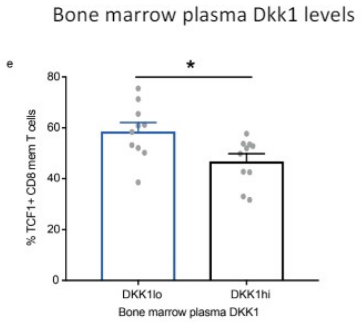


Kini Bailur et al. JCI Insight 2019
Im et al Nature 2016
Boddupalli et al. JCI
Boddupalli et al. JCI Insight

Dhodapkar
Enrichment of stem-like/marrow-resident T cells is associated with myeloid signature of TLR-mediated activation and correlates inversely with bone marrow DKk-1 levels



Gene Set ID	Description	Nominal p-value	PMID
GSE2706 (shown above)	Genes down-regulated in unstimulated vs LPS-stimulated dendritic cells	p<0.001	15995707
GSE22886	Genes down-regulated in unstimulated vs LPS-stimulated dendritic cells	p<0.001	15789058
GSE9988	Genes up-regulated in monocytes treated with anti-TREM1 and LPS vs monocytes treated with control IgG	p<0.001	18292579
GSE2706	Genes down-regulated in comparison of unstimulated vs R848-stimulated dendritic cells.	p<0.001	15995707



Kini Bailur et al. JCI Insight 2019

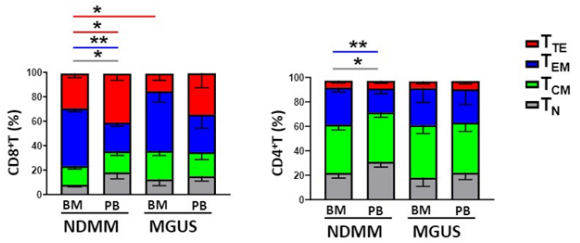
- Hierarchy of T cell exhaustion is established early in premalignancy.
- Immune response to stemness antigens as a predictor of risk of malignancy.
- Attrition of memory T cells with stem-like and tissue-residence signatures in the tumor bed with disease progression may underlie loss of immune surveillance.
- Retention or attrition of such cells in situ in turn depends on local signals in the tumor bed which change with clonal evolution and therapy.

Potential Clinical Implications:

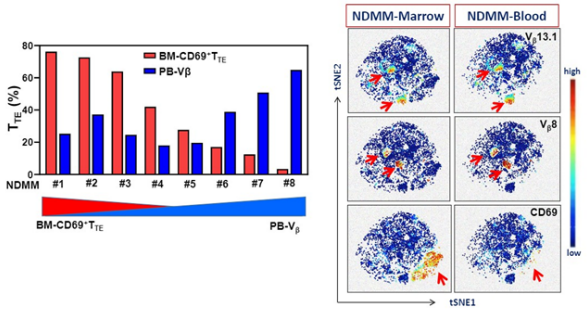
- Biology of resident and stem-like cells may determine durability / curative potential of T cell redirection in MM.
- Functional aspects of immune microenvironment may also impact durability of preventive approaches.

Bryant
T-cells

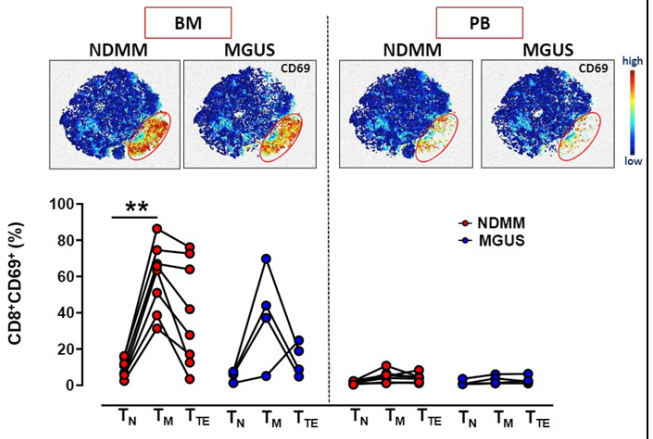
$CD8^+T_{TE}$ Accumulate in the Marrow in NDMM



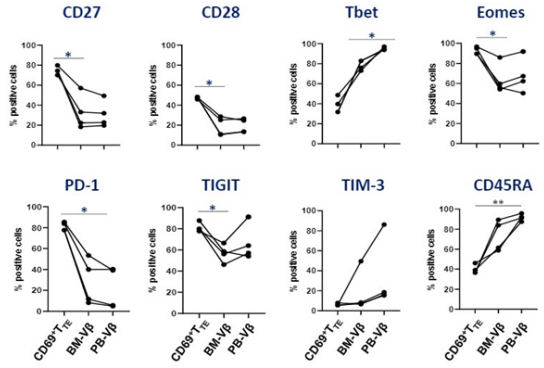
Inverse Relationship Between $CD69^+T_{TE}$ and Clonally Expanded T_{TE} in NDMM



$CD69^+T_{TE}$ Accumulate in the Marrow of NDMM



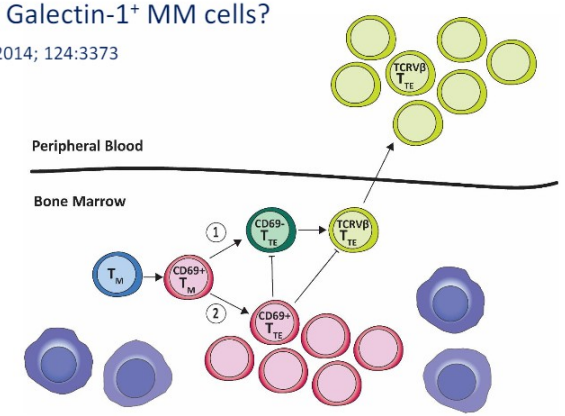
$CD69^+$ T_{TE} and Clonally Expanded T_{TE} Are Phenotypically Different



Exhausted $CD69^+$ CD8 T_{TE} cells accumulate at the expense of clonally expanded $CD8_{TE}$

- Failure of CD8 T_M to down-regulate $CD69^+$
- Role of Galectin-1^+ MM cells?

Blood. 2014; 124:3373



Clonally Exp $CD8_{TE}$
- $CD27^{low}$
- $CD28^{low}$
- $CD45RA^{hi}$
- $Eomes^{low}$
- $Tbet^{hi}$
- $PD\text{-}1^{low}$
- $TIGIT^{int}$

$CD69^+$ $CD8_{TE}$
- $CD27^{hi}$
- $CD28^{hi}$
- $CD45RA^{low}$
- $Eomes^{hi}$
- $Tbet^{low}$
- $PD\text{-}1^{hi}$
- $TIGIT^{hi}$

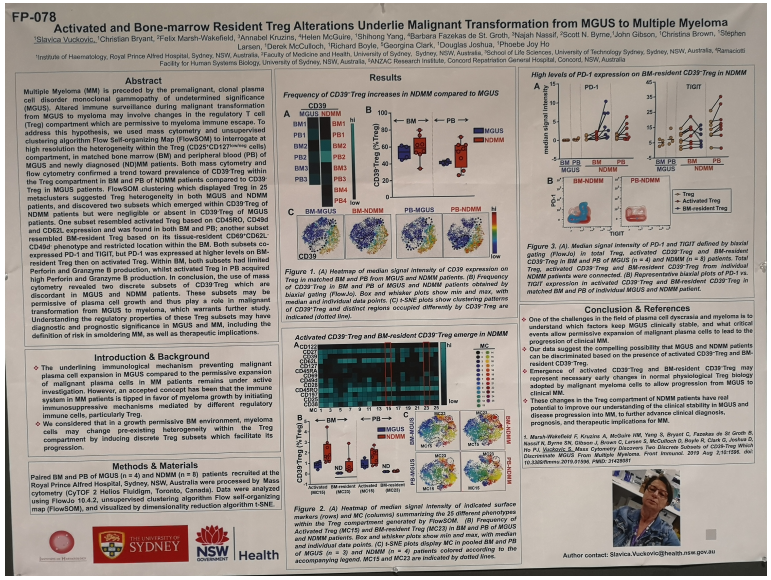
T-cells

#FP-078

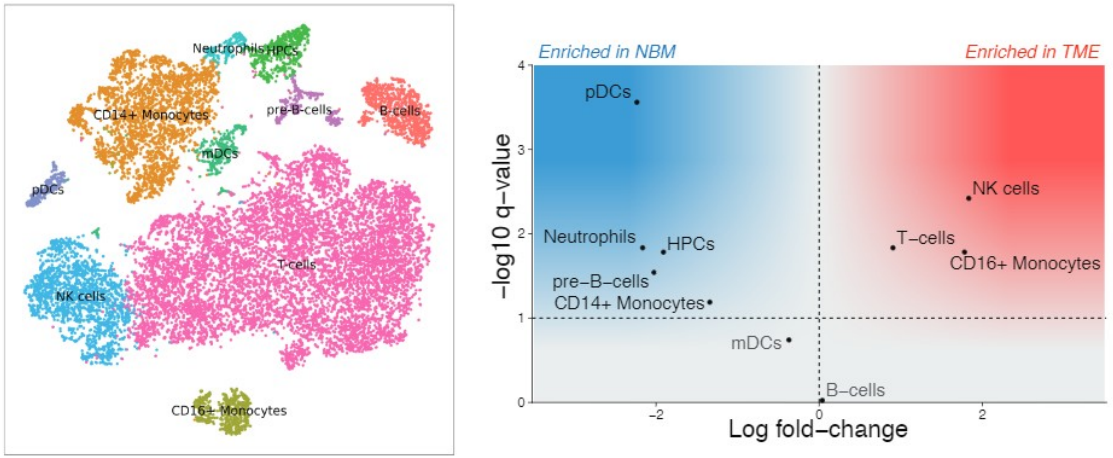
Activated and Bone-marrow Resident Treg Alterations Underlie Malignant Transformation from MGUS to Multiple Myeloma

Authors: Slavica Vuckovic et al (Australia)

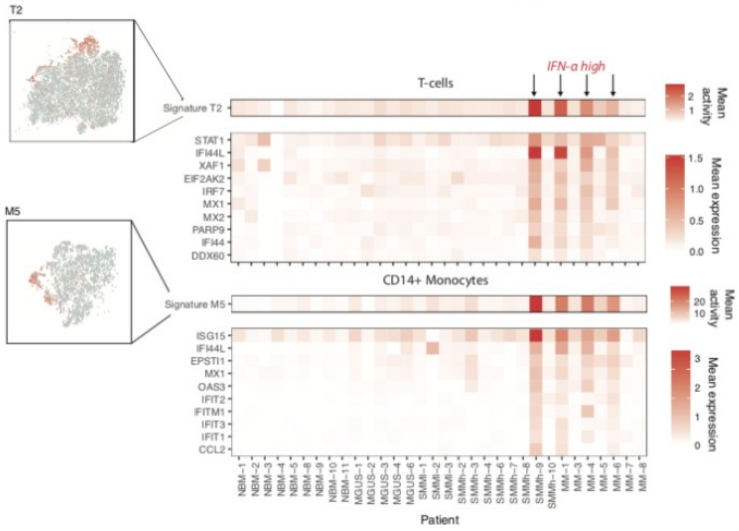
The use of mass cytometry revealed two discrete subsets of CD39-Treg which are discordant in MGUS and NDMM patients. These subsets may be permissive of plasma cell growth and thus play a role in malignant transformation from MGUS to myeloma, which warrants further study. Understanding the regulatory properties of these Treg subsets may have diagnostic and prognostic significance in MGUS and MM, including the definition of risk in smoldering MM, as well as therapeutic implications.



Compositional changes in MM bone marrow microenvironment



Individual Patients Show Upregulation of IFN type-1 Regulated Genes Across Different Cell Types



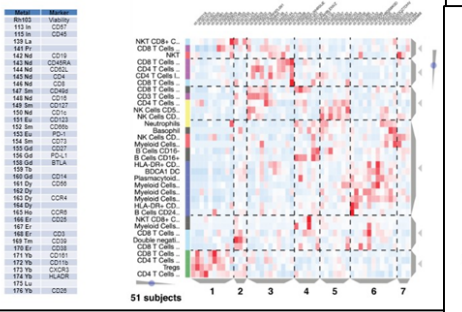
Cho
High dimensional profiling of the immune microenvironment in SMM

Immune Microenvironment in Smoldering MM

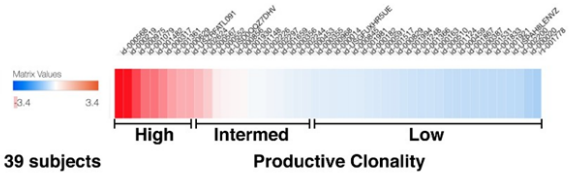
- Current risk models in SMM are based on tumor-intrinsic factors
  - M-spike/sFLC ratio, velocity, reciprocal depression
  - Cytogenetic/FISH risk factors
- Immune microenvironment of plasma cell dyscrasias is significantly altered
  - Innate *and* adaptive cellular components eg pDCs, myeloid cells, T cells (Chauhan *et al* Cancer Cell 2009; Bailur *et al*, JCI Insight 2019)
- Hypothesis: are there distinguishing features in the IME of SMM that relate to disease state/ progression?

CyTOF immunophenotyping of BMMC

- Panel of 32 markers
- Self-organizing groups of subjects identified by hierarchical clustering based on differential abundance of cell populations



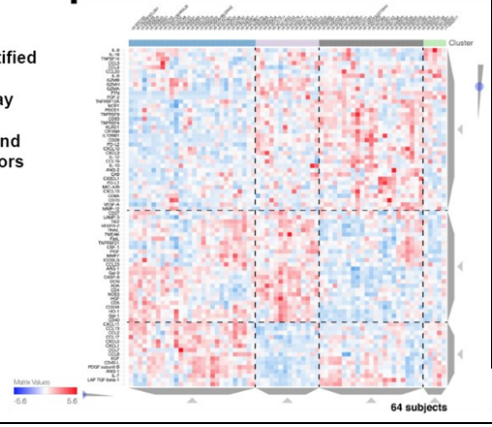
TCR sequencing of BMMC



- RNA libraries prepared from total BMMC for Adaptive Immunoseq platform
- Productive clonality quantified and mapped with Clustergrammer

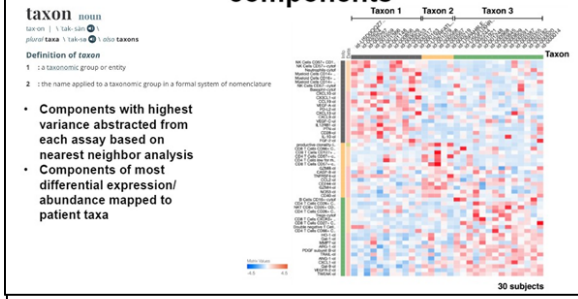
O-Link proteomics of BMP

- 92 immune analytes quantified by proximity extension assay
- Cytokines, chemokines, and soluble receptors



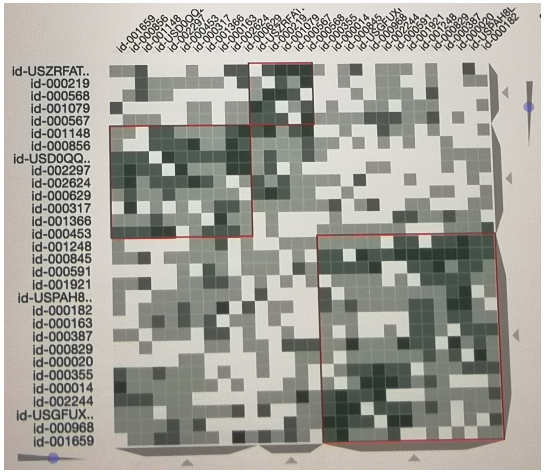
Nearest neighbor analysis: most informative components

- Components with highest variance abstracted from each assay based on nearest neighbor analysis
- Components of most differential expression/ abundance mapped to patient taxa



Cho
High dimensional profiling of the immune microenvironment in SMM

	Taxon 1	Taxon 2	Taxon 3
Cellular (CyTOF)	NK cells Monocytes Neutrophils Basophils	Effector CD4/8 T cells CD8 TEMRA	Central memory/naïve CD4/CD8 T cells Double neg T cells Treg cells CD16+ B cells NK/T cells
Proteomic (O-Link)	Angiogenesis: VEGFA/C Chemotaxis: CCL19, CXCL1, CXCL9, CXCL13, CXCL19 Inflammation: IL-10: IL-12Rbeta1 Co-stimulation (T cell): CD28 Immune checkpoint: PD-L2 Survival/proliferation: FGF-2, PTN	Apoptosis: Casp-8, Granzyme B/H, NOS-3 Co-stimulatory (APC): OX40L, CD40 Leukocyte activation: CCL2, SLAMF4 (CD244)	Apoptosis: Gal-1, HO-1,TRAIL, TWEAK Angiogenesis: ANG-1, MMP7, PDGFbeta Immunosuppression: ARG-1, Gal-9
Clonality (TCR Seq)	Intermediate	High	Low



Integrative immune analysis

- **Nearest neighbor analysis across high dimensional platforms identify distinct taxa of subjects in SMM**
- **Taxa share characteristics that may be associated with different states in the immune microenvironment**
- **Further refinement of NNA may improve precision of taxon definitions**
  - **Inclusion of tumor-specific data eg GEP**
  - **Comparison to established risk models**
  - **Prospective/retrospective analysis *with outcome data***
  - **Identification of rational targets for therapeutic intervention**

Zavidij
Single-cell RNA sequencing reveals compromised immune microenvironment in precursor stages of MM

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From MGUS to Myeloma: Dissecting the Path

To better understand changes occurring by MM progression we aim to analyze transcriptional alterations at different stages of disease:

- Examine the characteristics of
- Determine the transcriptional a
  at different stages of MM progr

BM aspirates (27 patien

MACS separation

- CD138+ cells (plasma
- CD45+ cells (hematopo

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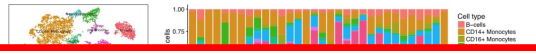
Compositional Changes in MM Bone Marrow Microenvironment



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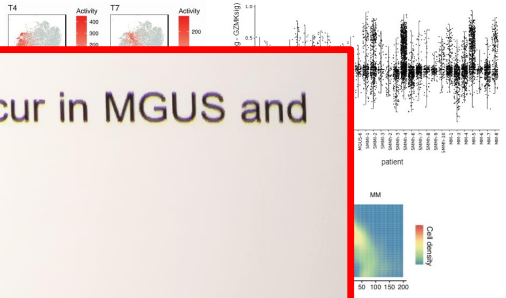
MM Patients Show Significant Skewing of Cytotoxic T Cells to the Effector State



- Changes in the tumor immune microenvironment already occur in MGUS and include:
  - upregulation of interferon type-1 signaling
  - skewed differentiation of CD8+ T cells
  - suppressive CD14+ monocytes with compromised antigen presentation

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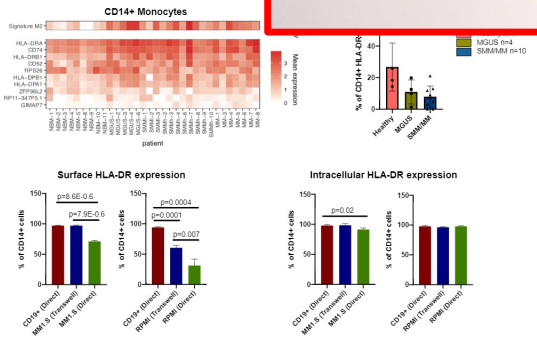
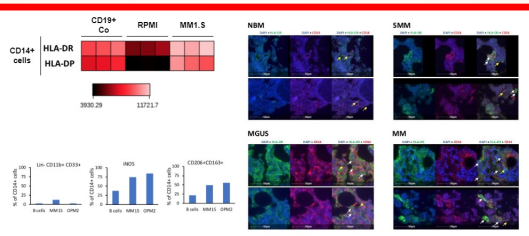
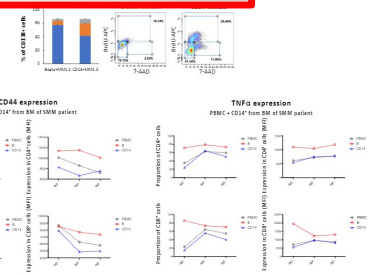
Compromised Antigen Presenta





HARVARD MEDICAL SCHOOL

f MM Cells



Mesenchymal stromal cells

#FP-062

TLR4 signaling drives mesenchymal stromal cells (MSC) commitment to promote tumor microenvironment transformation in multiple myeloma

Authors: Giallongo C., et al (Catania, Italy)

TLR4 signaling plays a key role in MSC transformation by inducing a protumor phenotype associated with a permissive microenvironment that circumvents the immune response and allows a better tumor engraftment.

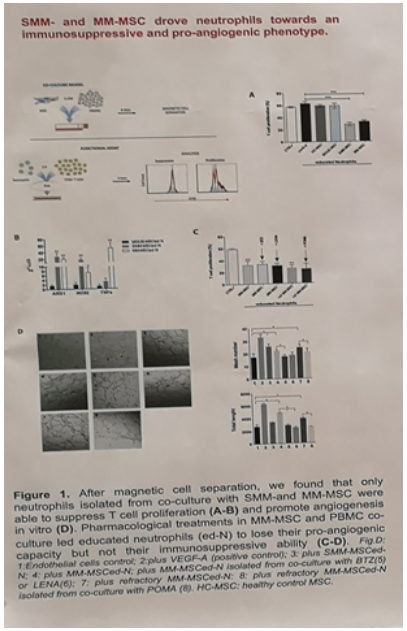


Figure 1. After magnetic cell separation, we found that only neutrophils isolated from co-culture with SMM-and MM-MSC were able to suppress T cell proliferation **(A-B)** and promote angiogenesis in vitro **(D)**. Pharmacological treatments in MM-MSC and PBMC co-culture led educated neutrophils (ed-N) to lose their pro-angiogenic capacity but not their immunosuppressive ability **(C-D)**. *Fig.D: 1:Endothelial cells control; 2:plus VEGF-A (positive control); 3: plus SMM-MSCed-N; 4: plus MM-MSCed-N; plus MM-MSCed-N isolated from co-culture with BTZ(5) or LENA(6); 7: plus refractory MM-MSCed-N; 8: plus refractory MM-MSCed-N isolated from co-culture with POMA (8). HC-MSC: healthy control MSC.*

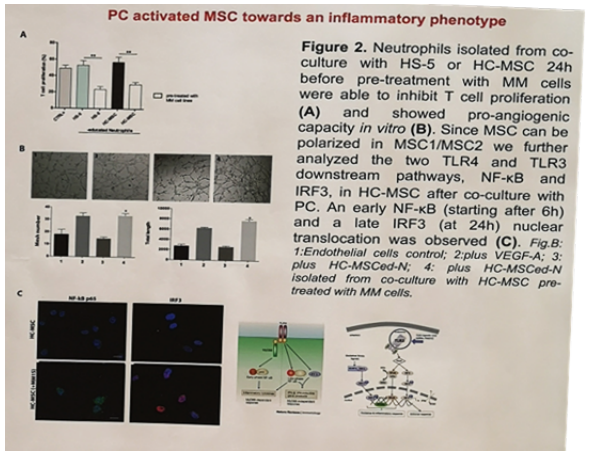


Figure 2. Neutrophils isolated from co-culture with HS-5 or HC-MSC 24h before pre-treatment with MM cells were able to inhibit T cell proliferation **(A)** and showed pro-angiogenic capacity *in vitro* **(B)**. Since MSC can be polarized in MSC1/MSC2 we further analyzed the two TLR4 and TLR3 downstream pathways, NF-κB and IRF3, in HC-MSC after co-culture with PC. An early NF-κB (starting after 6h) and a late IRF3 (at 24h) nuclear translocation was observed **(C)**. *Fig.B: 1:Endothelial cells control; 2:plus VEGF-A; 3: plus HC-MSCed-N; 4: plus HC-MSCed-N isolated from co-culture with HC-MSC pre-treated with MM cells.*

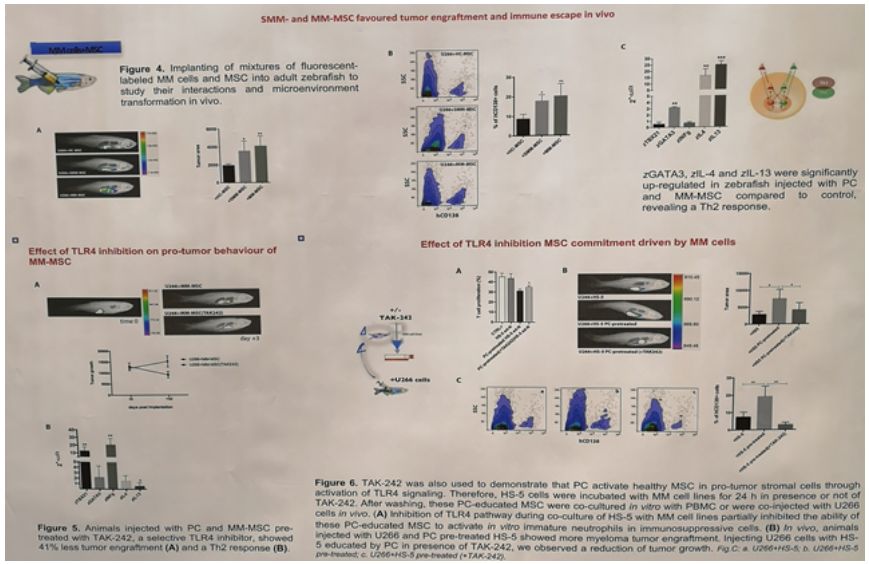


Figure 4. Implanting of mixtures of fluorescent-labeled MM cells and MSC into adult zebrafish to study their interactions and microenvironment transformation in vivo.

zGATA3, zIL-4 and zIL-13 were significantly up-regulated in zebrafish injected with PC and MM-MSC compared to control, revealing a Th2 response.

Figure 5. Animals injected with PC and MM-MSC pre-treated with TAK-242, a selective TLR4 inhibitor, showed 41% less tumor engraftment **(A)** and a Th2 response **(B)**.

Figure 6. TAK-242 was also used to demonstrate that PC activate healthy MSC in pro-tumor stromal cells through activation of TLR4 signaling. Therefore, HS-5 cells were incubated with MM cell lines for 24 h in presence or not of TAK-242. After washing, these PC-educated MSC were co-cultured *in vitro* with PBMC or were co-injected with U266 cells *in vivo*. **(A)** Inhibition of TLR4 pathway during co-culture of HS-5 with MM cell lines partially inhibited the ability of these PC-educated MSC to activate *in vitro* immature neutrophils in immunosuppressive cells. **(B)** *In vivo*, animals injected with U266 and PC pre-treated HS-5 showed more myeloma tumor engraftment. Injecting U266 cells with HS-5 educated by PC in presence of TAK-242, we observed a reduction of tumor growth. *Fig.C: a. U266+HS-5; b. U266+HS-5 pre-treated; c. U266+HS-5 pre-treated (+TAK-242).*

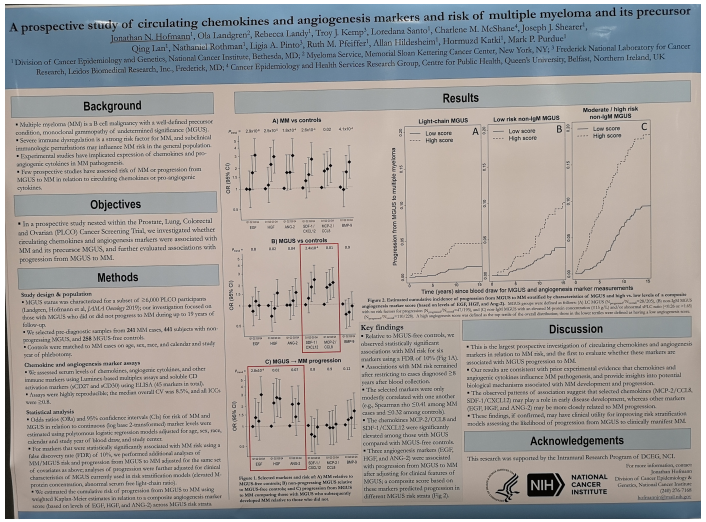
Angiogenic cytokines

#FP-064

A prospective study of circulating chemokines and angiogenesis markers and risk of multiple myeloma and its precursor

Authors: Jonathan Hofmann, et al (USA, Ireland)

Our prospective findings provide new insights into mechanisms involved in MM development and suggest that systemic angiogenesis markers could potentially improve risk stratification models for MGUS patients.



Adipocytes

#FP-058
A New 'Vicious Cycle': Bidirectional Interactions Between Myeloma Cells and Adipocytes
Authors: Heather Fairfield Campbell, Mariah Farrell, Carolyne Falank, Amel Dudakovic, Samantha Costa, Victoria DeMambro, Jessica Pettitt, Andre J. van Wijnen, Michelle McDonald, Michaela Reagan (USA, Australia)
MM-adipocytes exhibit a "senescent-like" phenotype in vitro that may explain their support of MM cells.

#FP-054
Excessive abdominal fat content indicates poor prognosis in patients with newly diagnosed multiple myeloma
Authors: Li Bao, Yutong Wang (China)
NDMM patients had higher abdominal fat content but lower adipokine levels than healthy people. Excessive subcutaneous fat might be a predictive factor for high tumor burden and poor treatment response. Visceral fat content may be correlated with high-risk cytogenetic abnormalities.

Thanks for your attention