DICHIARAZIONE

Relatore: Angelo Vacca

Come da nuova regolamentazione della Commissione Nazionale per la Formazione Continua del Ministero della Salute, è richiesta la trasparenza delle fonti di finanziamento e dei rapporti con soggetti portatori di interessi commerciali in campo sanitario.

- Posizione di dipendente in aziende con interessi commerciali in campo sanitario (**NIENTE DA DICHIARARE / NOME AZIENDA**)
- Consulenza ad aziende con interessi commerciali in campo sanitario (**GSK, CsI-Behring/ Takeda/ Novartis Oncology**)
- Fondi per la ricerca da aziende con interessi commerciali in campo sanitario (**Takeda IgRT, AstraZeneca**)
- Partecipazione ad Advisory Board **(CsI-Behring)**
- Titolarità di brevetti in compartecipazione ad aziende con interessi commerciali in campo sanitario (**NIENTE DA DICHIARARE / NOME AZIENDA**)
- Partecipazioni azionarie in aziende con interessi commerciali in campo sanitario (**NIENTE DA DICHIARARE / NOME AZIENDA**)
- Altro

3rd Cuneo City ImmunoTherapy Conference (CCITC)

Immunotherapy in Hematological Malignancies 2023

CUNEO
May 18-20, 2023
Rondò dei talenti, Cuneo

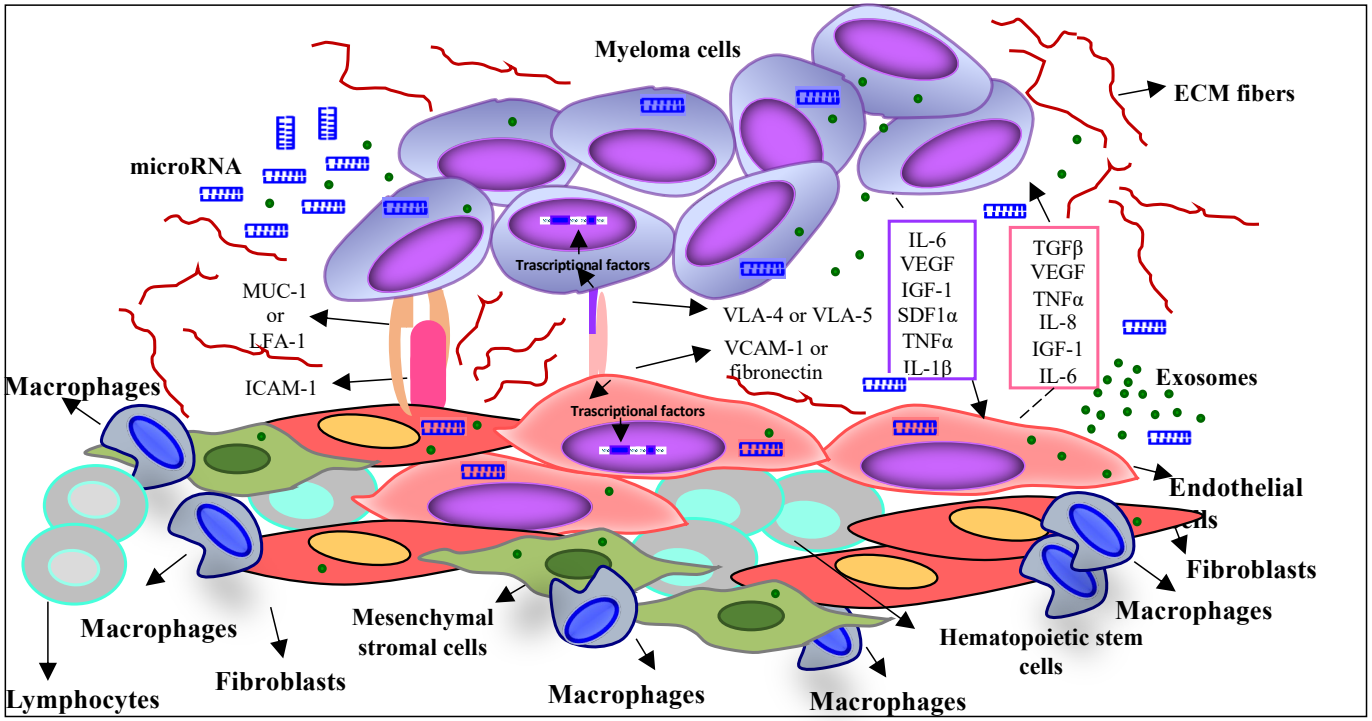
Angelo Vacca

The tumor microenvironment in MM: hurdles or opportunities for immunotherapy?

Organized by Prof. Massimo Massaia, SC Ematologia AO S.Croce e Carle, Cuneo, Italy
and Centro Interdipartimentale di Ricerca in Biologia Molecolare (CIRBM), Torino, Italy

Bone marrow microenvironment in multiple myeloma

NON-CELLULAR (ECM fibers, soluble factors) **AND CELLULAR COMPARTMENT** (hematopoietic and non-hematopoietic cells)

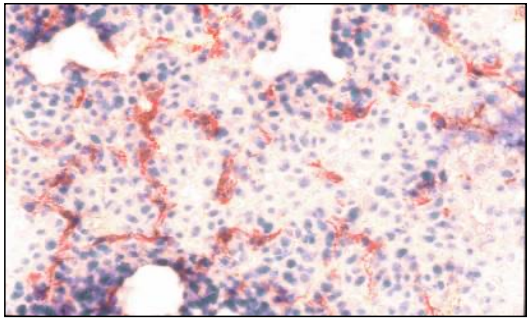


THE MICROENVIRONMENT IN MYELOMA FAVORS TUMOR CELL SURVIVAL, PROLIFERATION, IMMUNOSURVEILLANCE ESCAPE AND DRUG RESISTANCE

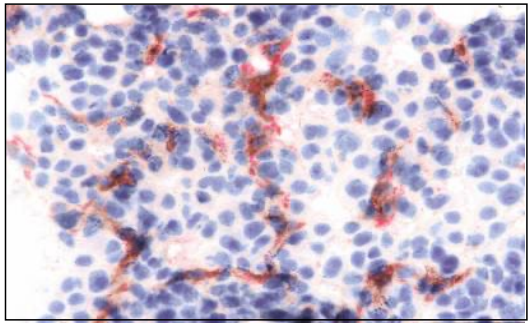
Solimando et al, Cancers 2022

Bone marrow angiogenesis in patients with active multiple myeloma

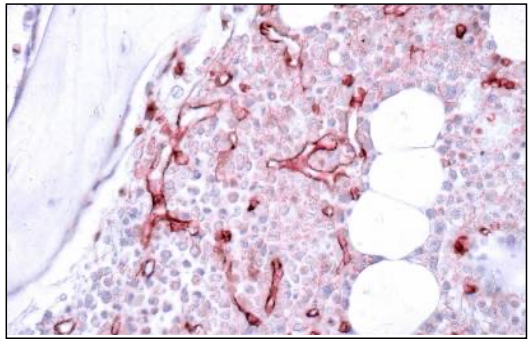
Megafield



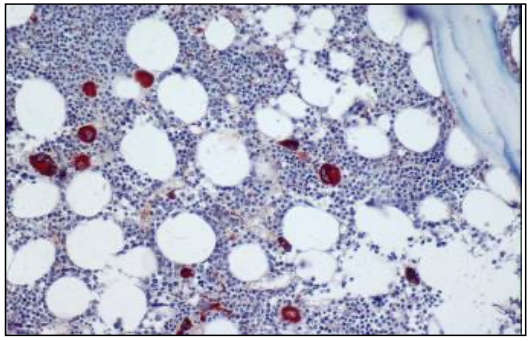
Single or clustered endothelial cells



Vessel arborizations

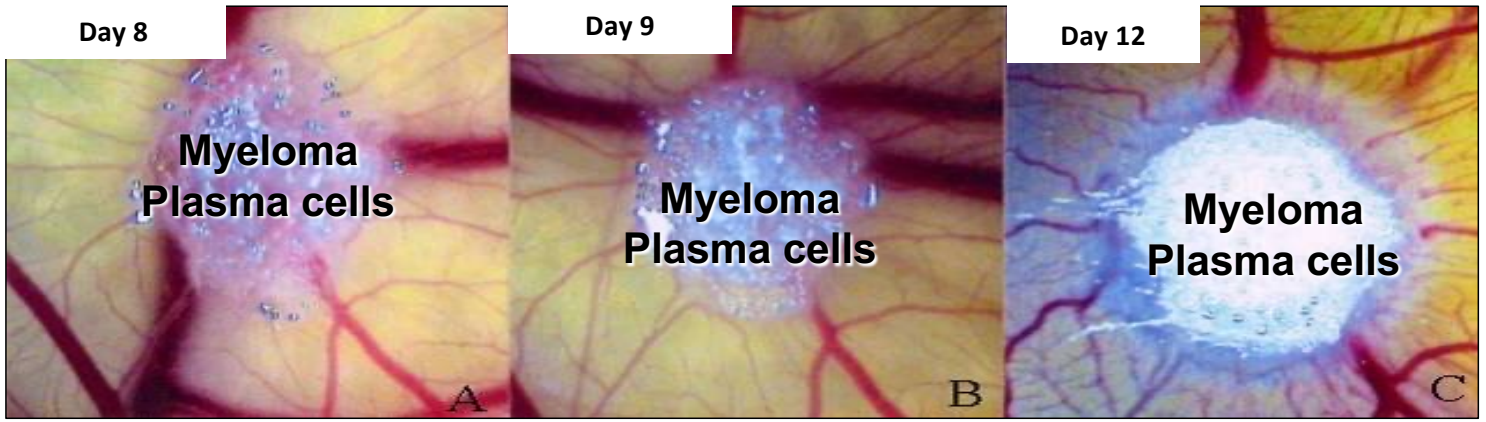


MGUS: no vessels

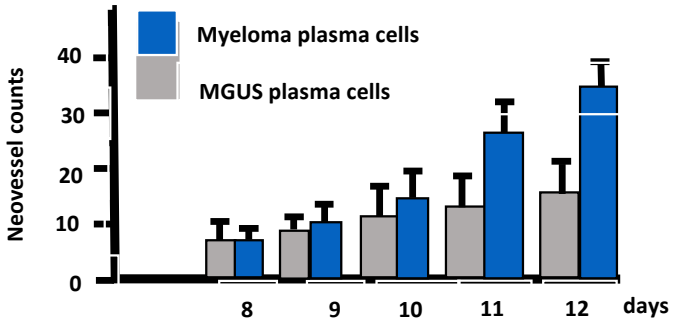


Vacca et al, Br J Haematol 1994

Time-course of angiogenesis induction by myeloma plasma cells in the *in vivo* CAM-sponge assay

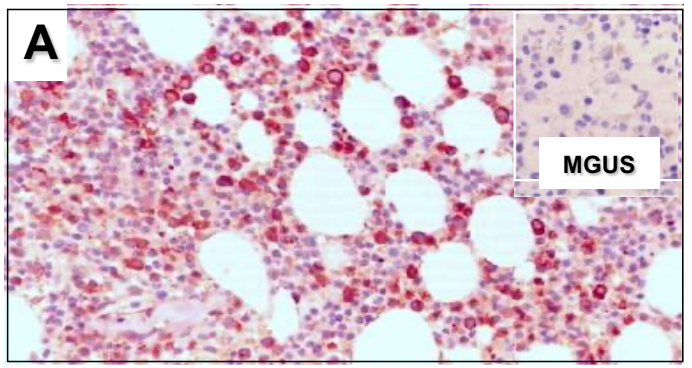


Prof. Domenico Ribatti



Vacca & Ribatti, Leukemia 2006; Ribatti – Vacca, Leukemia 2007

VEGF-A+ myeloma plasma cells



Università degli Studi di Perugia

Comitato per la Vita "Daniele Chianelli"

A.O. Perugia

Prof. C. Martelli and his group, Perugia

THE JOURNAL OF BIOLOGICAL CHEMISTRY
© 2005 by The American Society for Biochemistry and Molecular Biology, Inc.
Vol. 280, No. 28, Issue of July 15, pp. 26467–26476, 2005
Printed in U.S.A.

Characterization of Mitochondrial and Extra-mitochondrial Oxygen Consuming Reactions in Human Hematopoietic Stem Cells

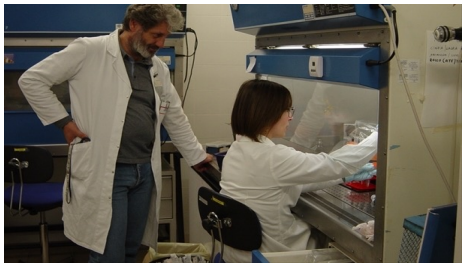
NOVEL EVIDENCE OF THE OCCURRENCE OF NAD(P)H OXIDASE ACTIVITY*

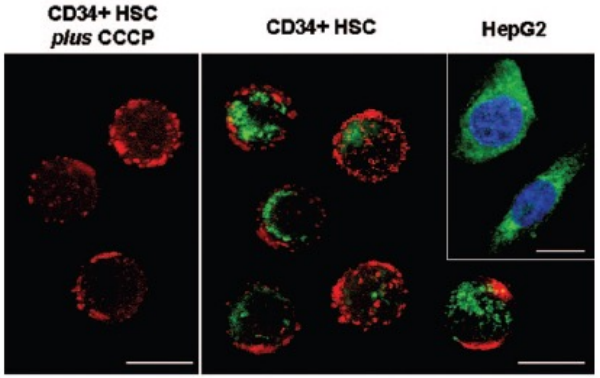
Received for publication, January 3, 2005, and in revised form, April 29, 2005
Published, JBC Papers in Press, May 9, 2005, DOI 10.1074/jbc.M500047200

Claudia Piccoli‡, Roberto Ria§, Rosella Scrima‡, Olga Cela‡, Annamaria D'Aprile‡, Domenico Boffoli‡, Franca Falzetti§, Antonio Tabilio§¶, and Nazzareno Capitanio‡¶‖

From the ‡Department of Biomedical Science, University of Foggia, Foggia, Italy 71100 and the §Department of Clinical and Experimental Medicine, Hematology and Clinical Immunology Section, University of Perugia, Perugia, Italy 06100

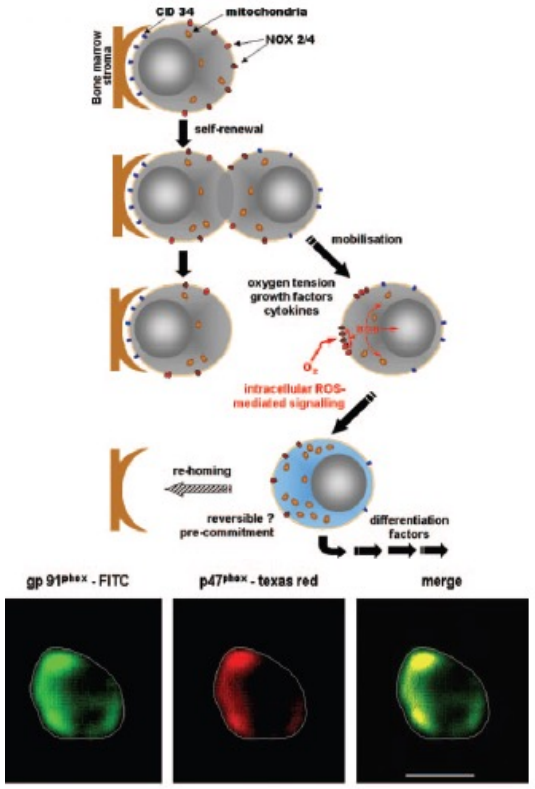
Prof. R. Ria, Dr. A. Ferrucci, my lab





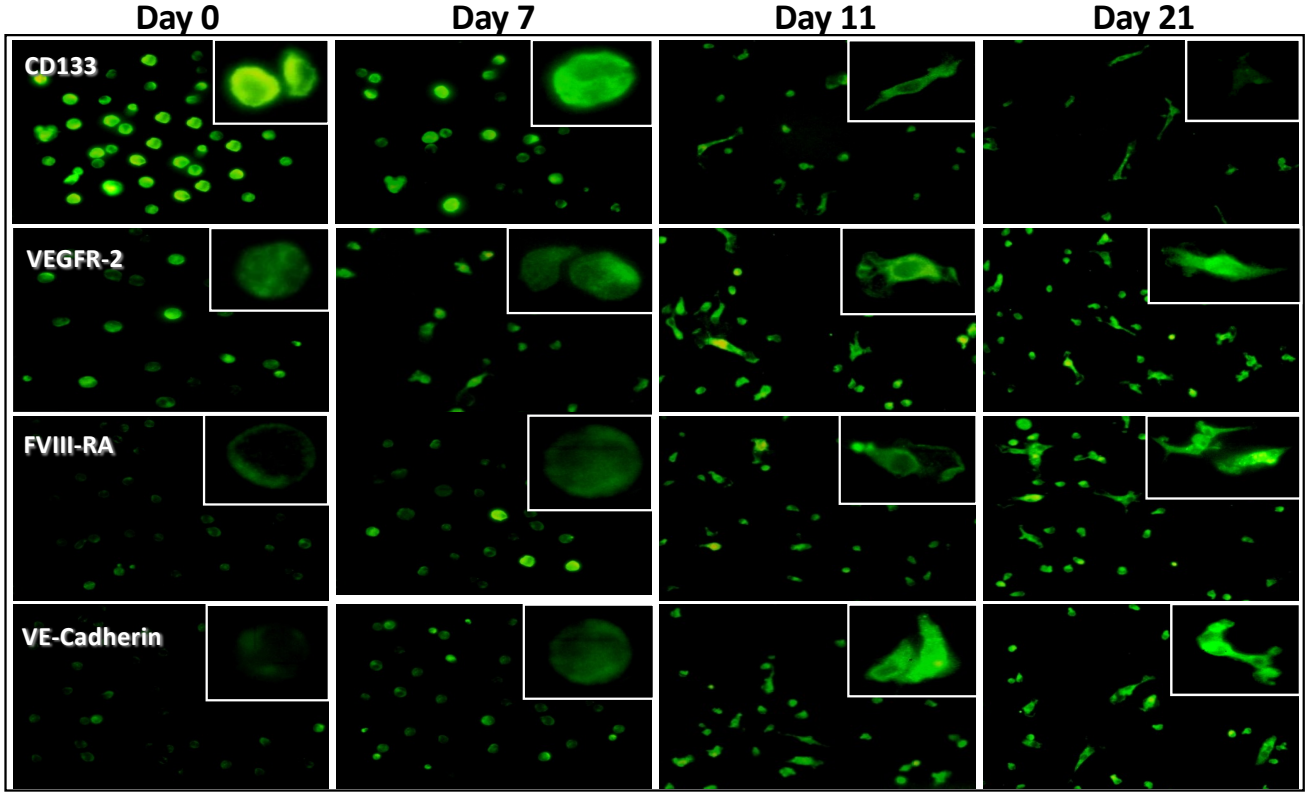
PREMIO ANTONIO TABILIO

dedicato alla produzione scientifica di
un giovane ricercatore in campo ematologico



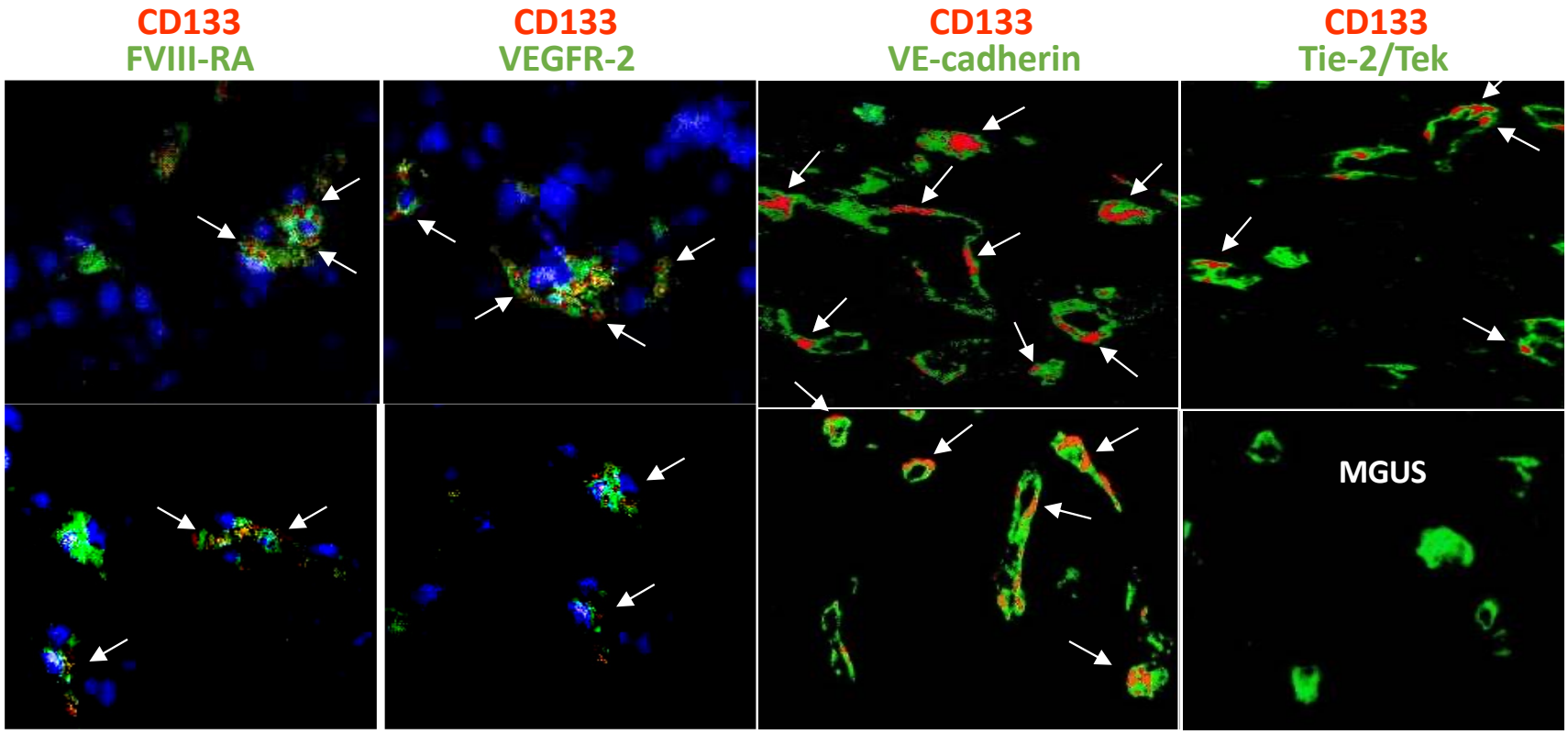
Vasculogenesis in patients with MM: differentiation of mobilized $CD34^+CD133^+$ hematopoietic precursors into mature endothelial cells

VEGF + FGF-2 + IGF on fibronectin



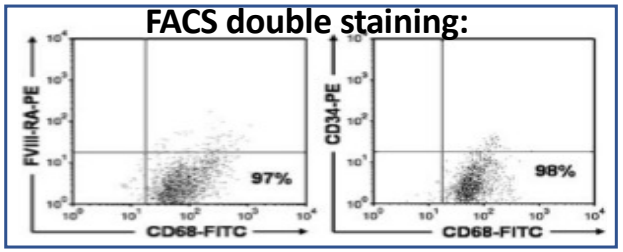
Ria R. et al, Clin Cancer Res 2008

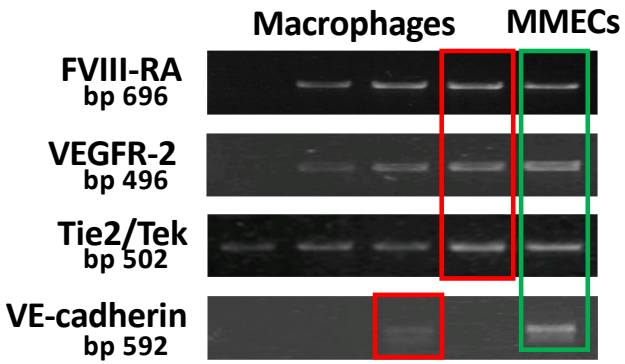
Incorporation of CD133+ hematopoietic precursors into the neovessel walls of myeloma patients



Tumor associated macrophages in multiple myeloma mimic endothelial cells

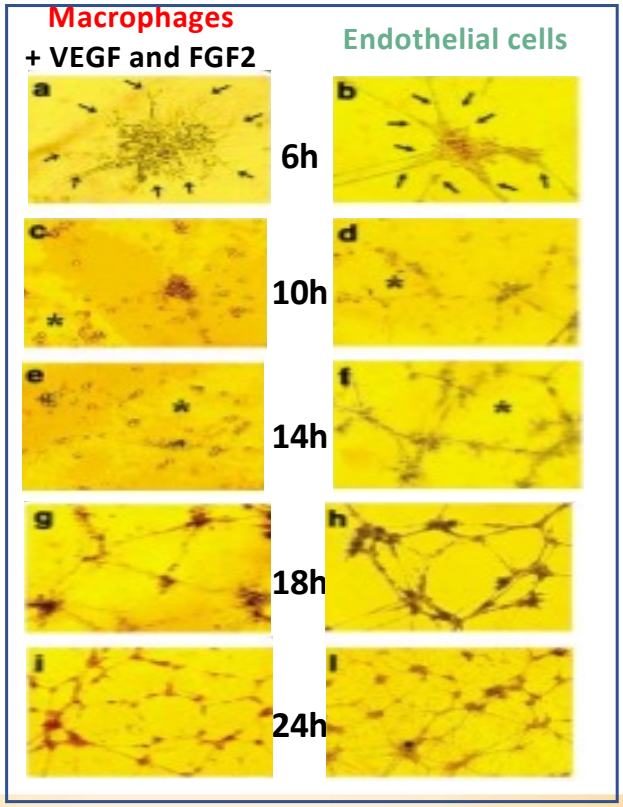
FVIII-RA/ CD68 or CD34/ CD68





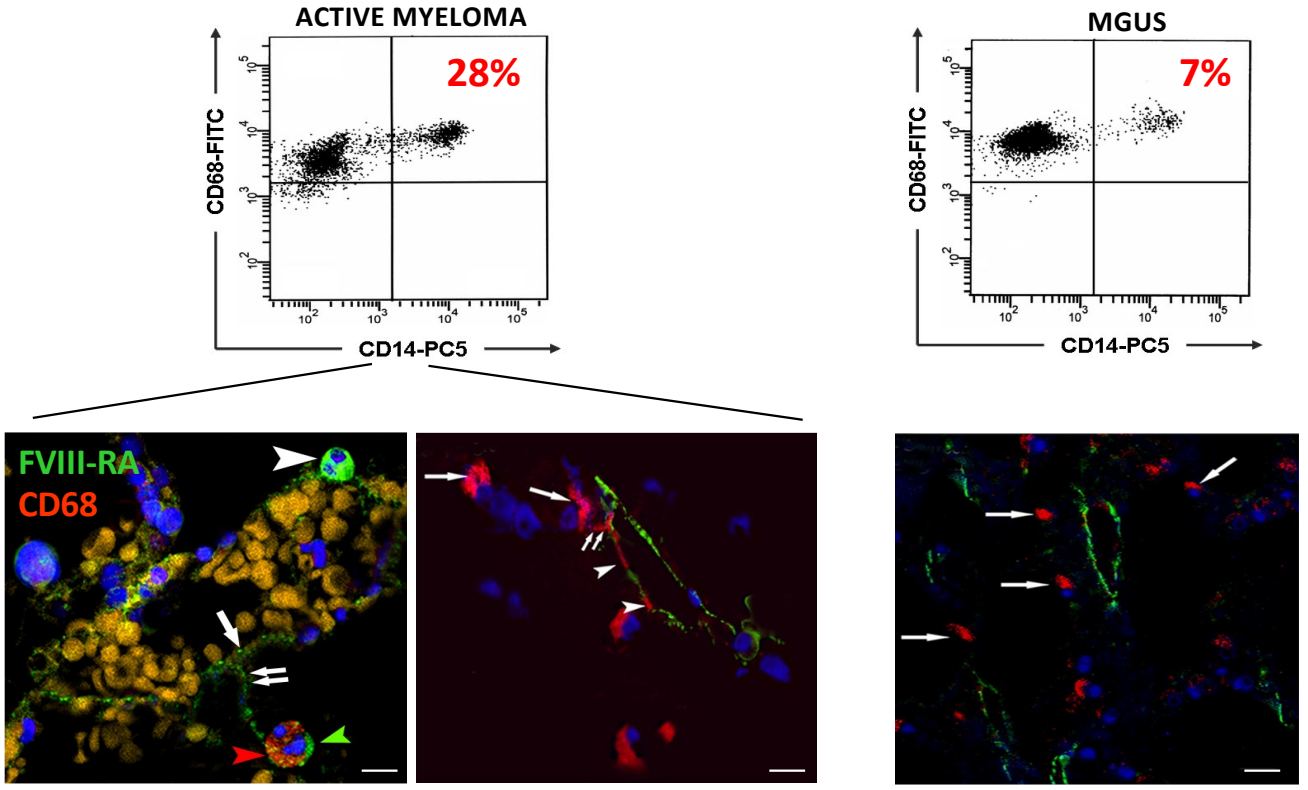
Scavelli et al., Oncogene 2008

Capillarogenic activity of macrophages and endothelial cells



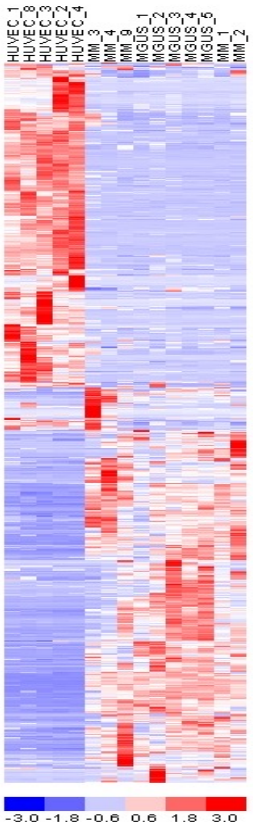
MACROPHAGES CONTRIBUTE TO BUILD NEOVESSELS IN ACTIVE MM THROUGH VASCULOGENIC MIMICRY

Myeloma macrophages cooperate with endothelial cells in building the neovessel wall in myeloma

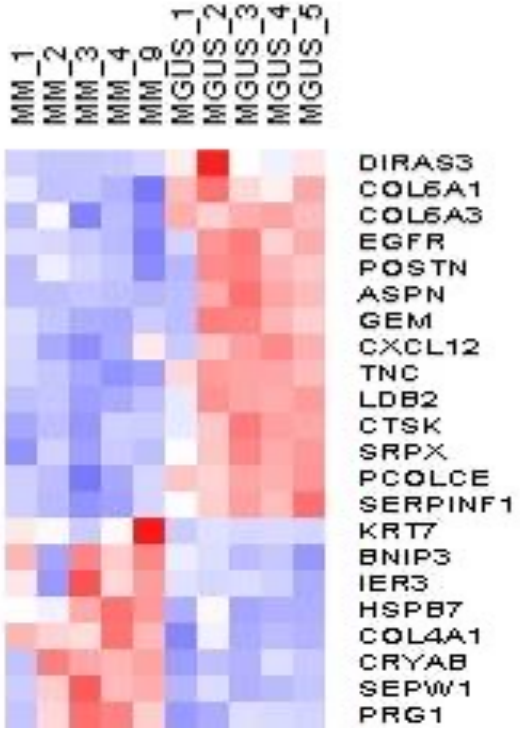


EC-LIKE MACROPHAGES AND MACROPHAGES FORM 'MOSAIC' VESSELS IN BONE MARROW OF PATIENTS WITH ACTIVE MYELOMA BUT NOT IN THOSE WITH MGUS

Unsupervised analysis
MMECs *vs* MGECs



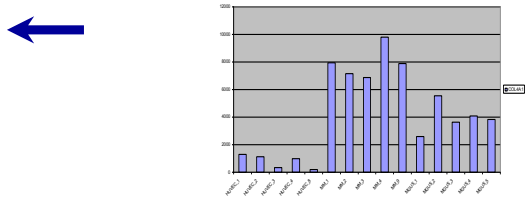
Supervised analysis
MMECs *vs* MGECs



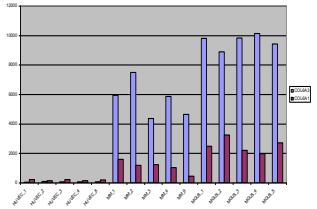
Searching genes specifically distinguishing
MM *vs* MGUS endothelial cells

22 genes

down

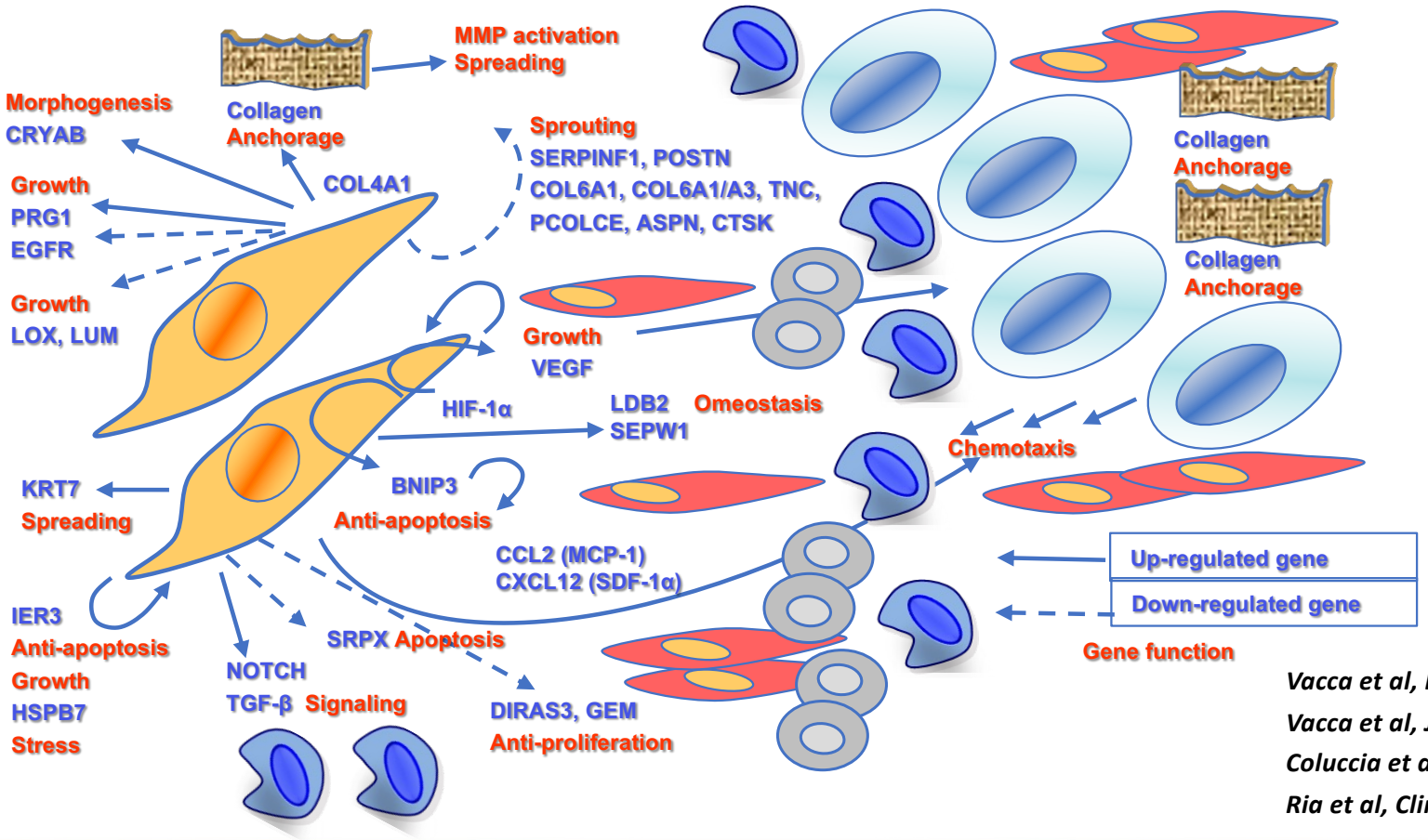


up



Ria et al, Clin. Cancer Res. 2009

Genes expressed by myeloma endothelial cells support homing and survival of plasma cells and microenvironment cells



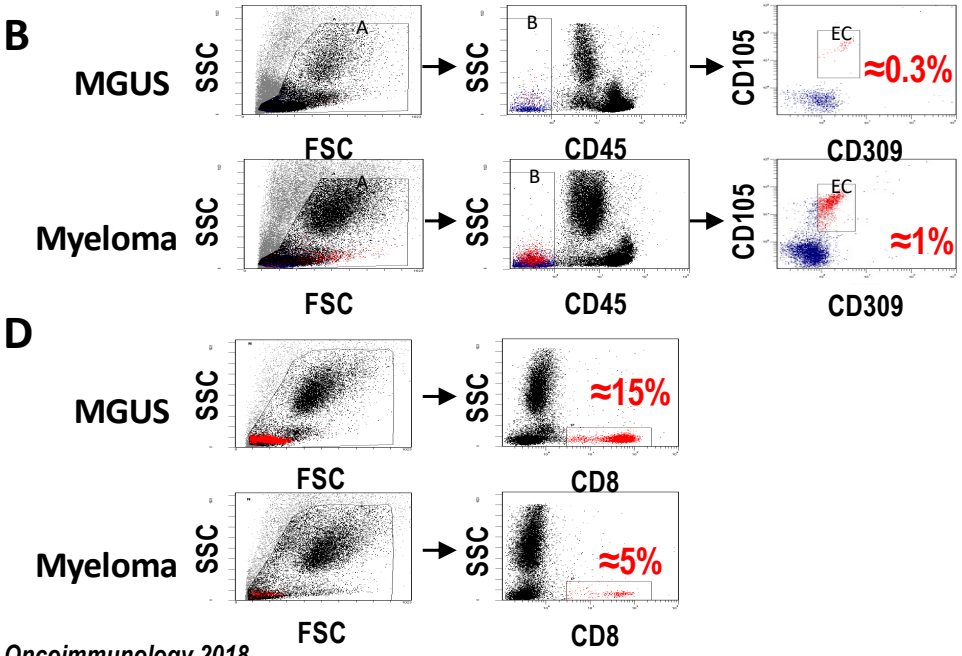
Vacca et al, Blood 2003

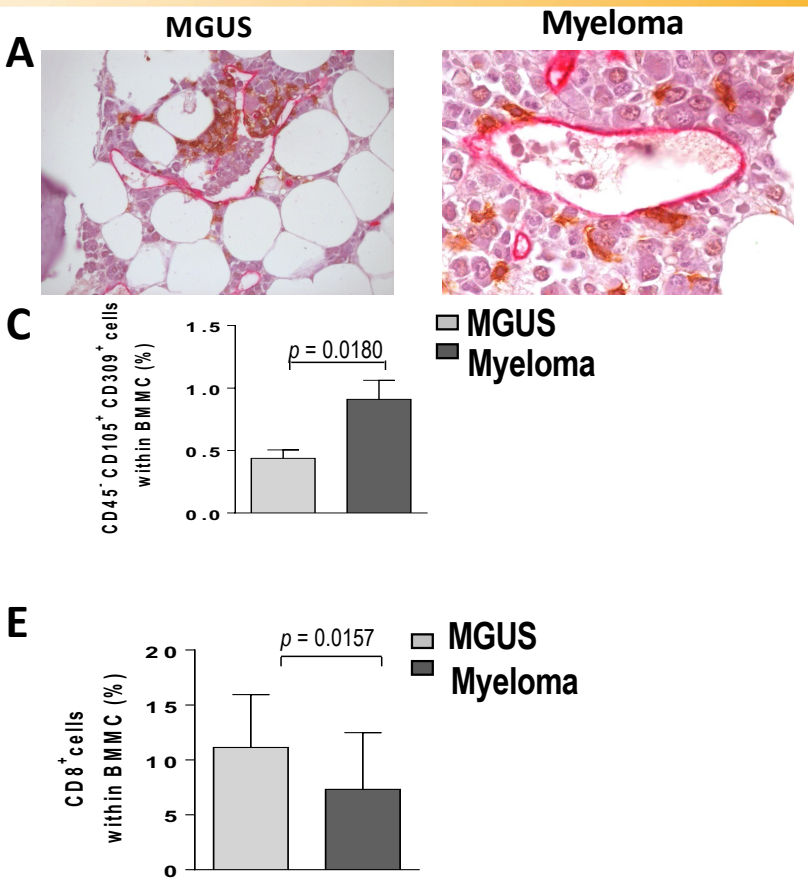
Vacca et al, JCO 2005

Coluccia et al, Blood 2008

Ria et al, Clin. Cancer Res 2009

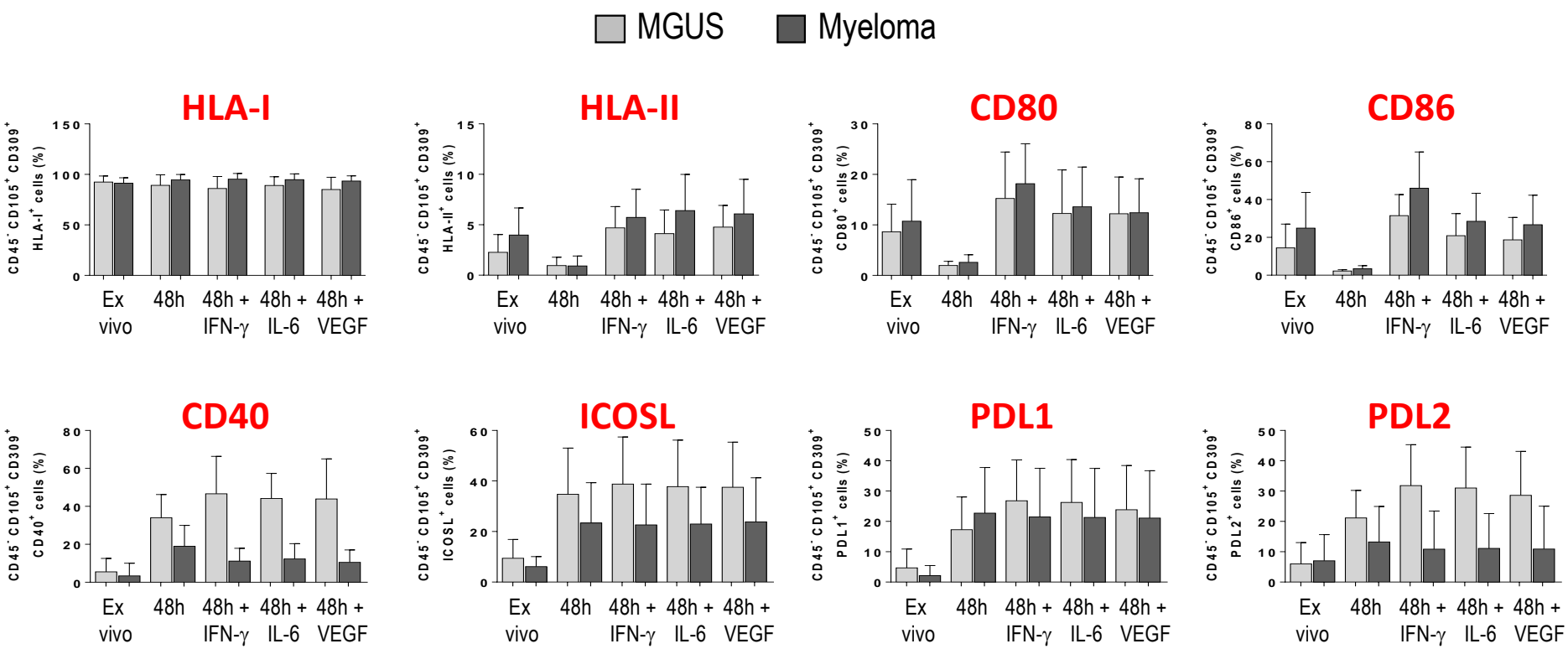
Interactions between endothelial cells and T cells in myeloma microenvironment



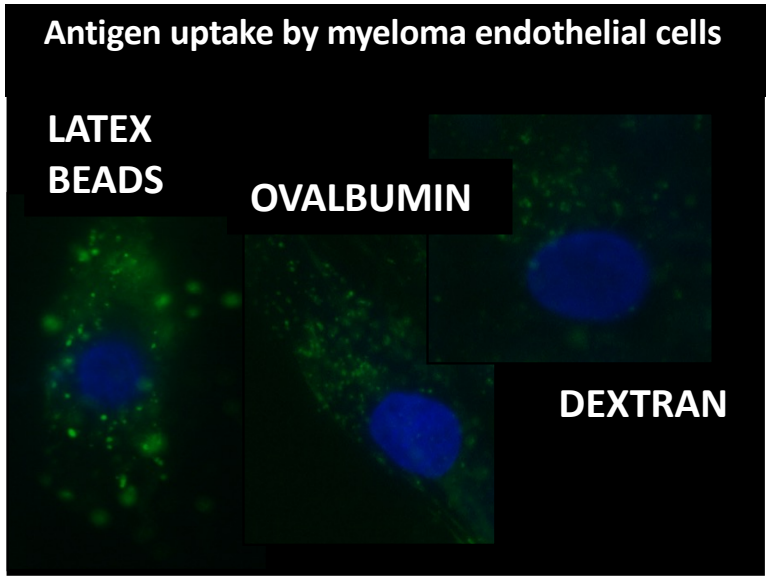


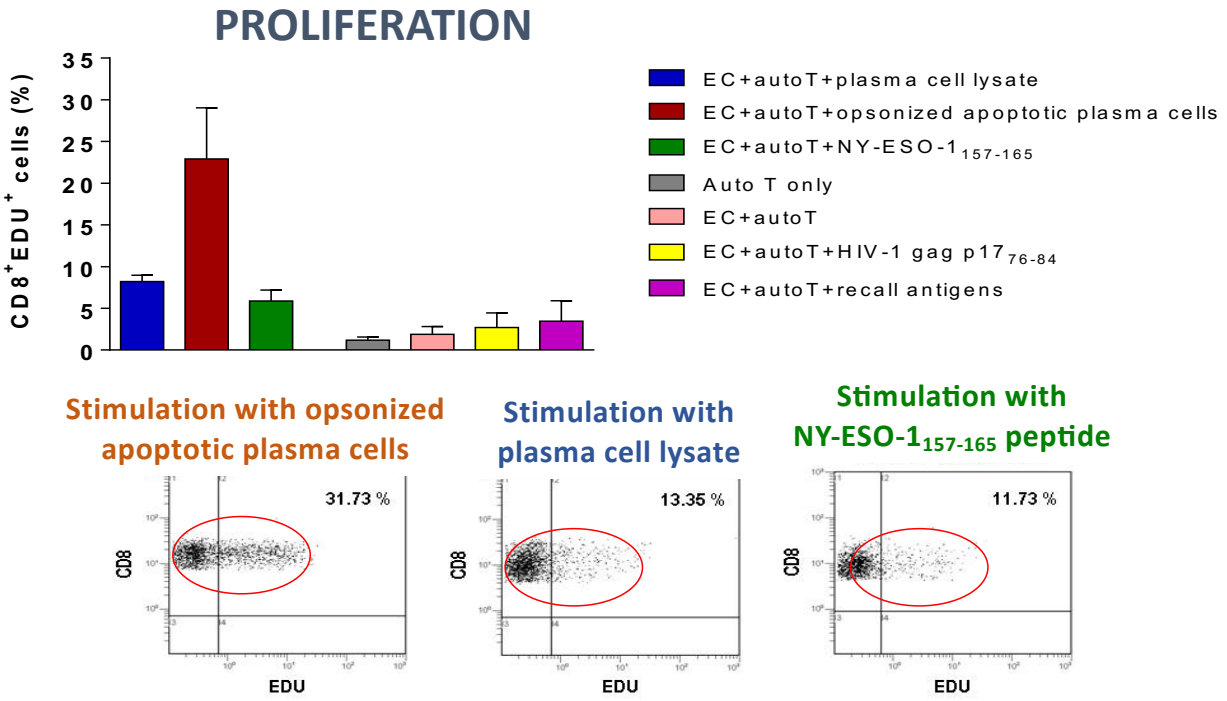
Leone et al. Oncoimmunology 2018

Phenotype of bone marrow endothelial cells in active myeloma



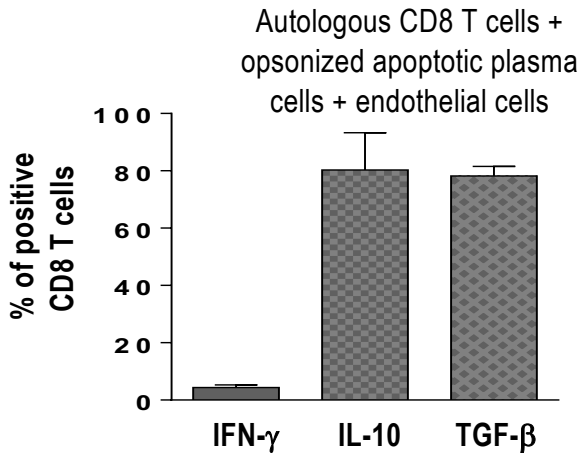
Ability of bone marrow endothelial cells to stimulate autologous (myeloma-restricted) CD8+ T cells (from bone marrow) (1)

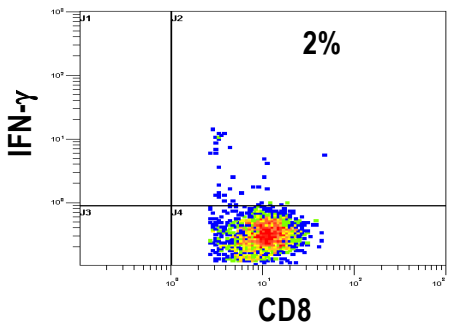


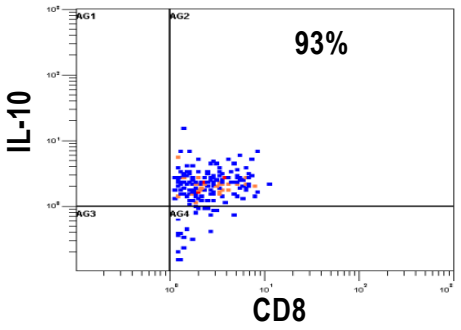


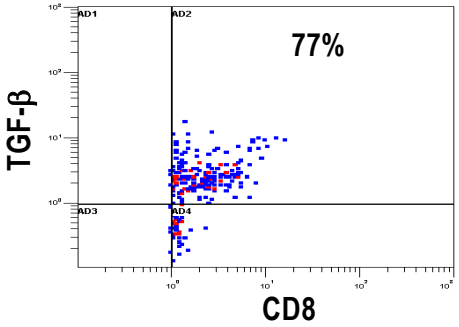
Ability of bone marrow endothelial cells to stimulate autologous (myeloma-restricted) CD8+ T cells (from bone marrow) (2)

CYTOKINE PRODUCTION BY CD8+ T CELLS



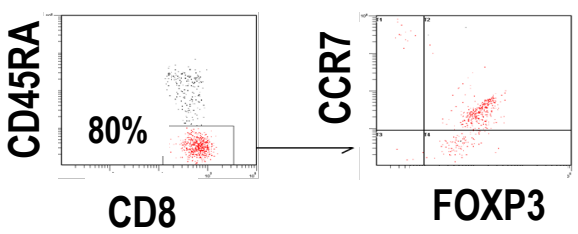




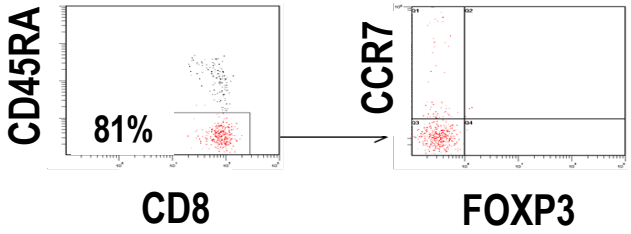


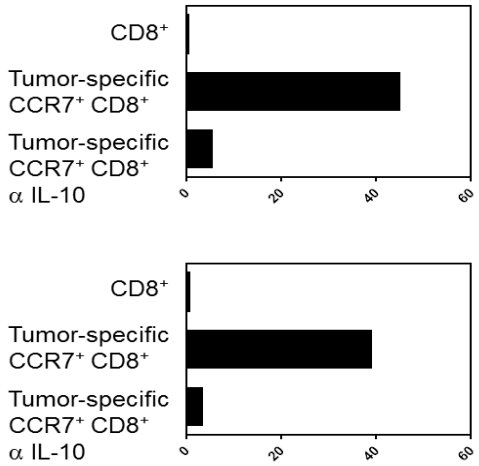
Antigen-specific suppressor capacity of endothelial cell-reactive CCR7^{+}CD8^{+} T cells (4 experiments)

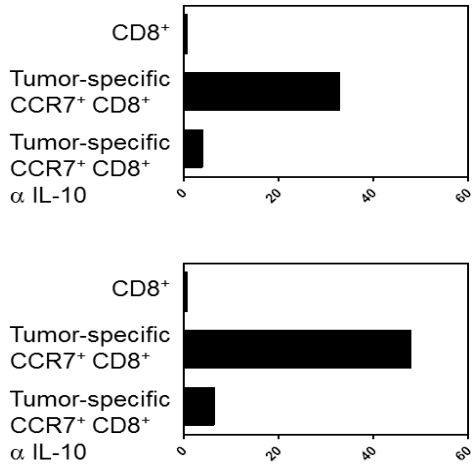
Stimulated with endothelial cells



Stimulated with dendritic cells







% inhibition tumor-specific CCR7^{-}CD8^{+} T cell proliferation

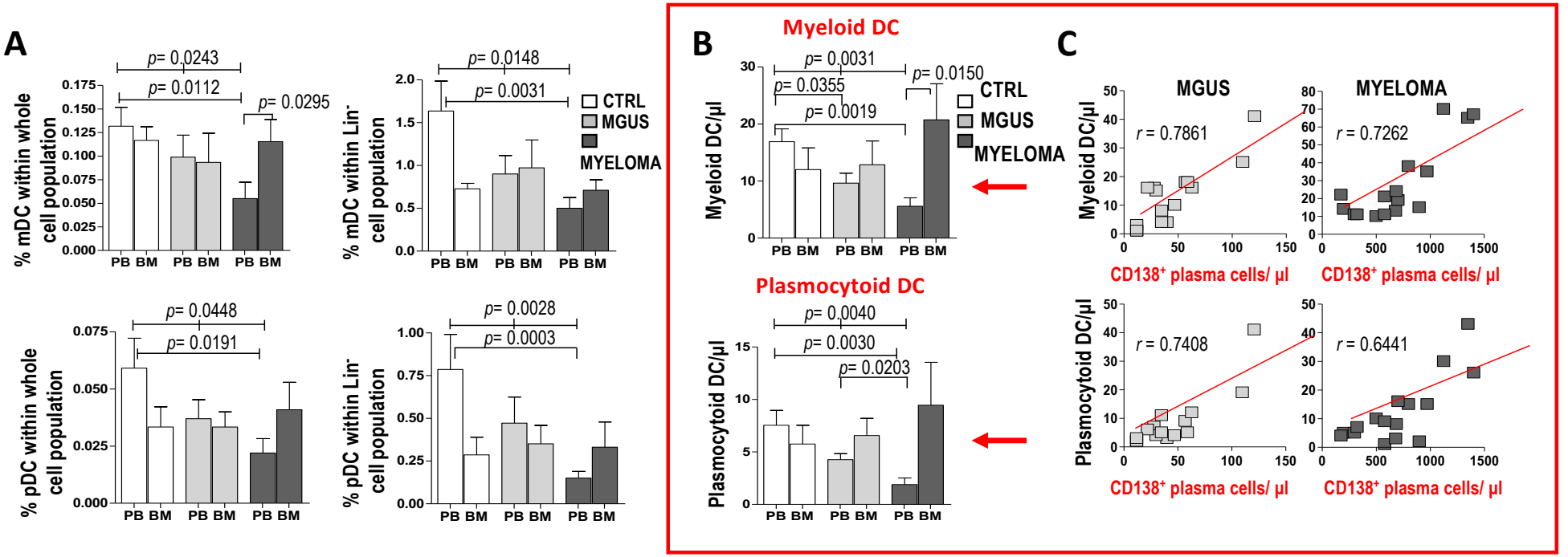
% inhibition tumor-specific CCR7^{-}CD8^{+} T proliferation

CONCLUSIONS

Tumor-specific effector memory $CD8^+$ T cells in the bone marrow of patients with multiple myeloma are inefficient because of the concomitant presence of endothelial cell-reactive tumor-specific central memory $CD8^+$ T cells producing considerable amounts of IL-10 and TGF-β.

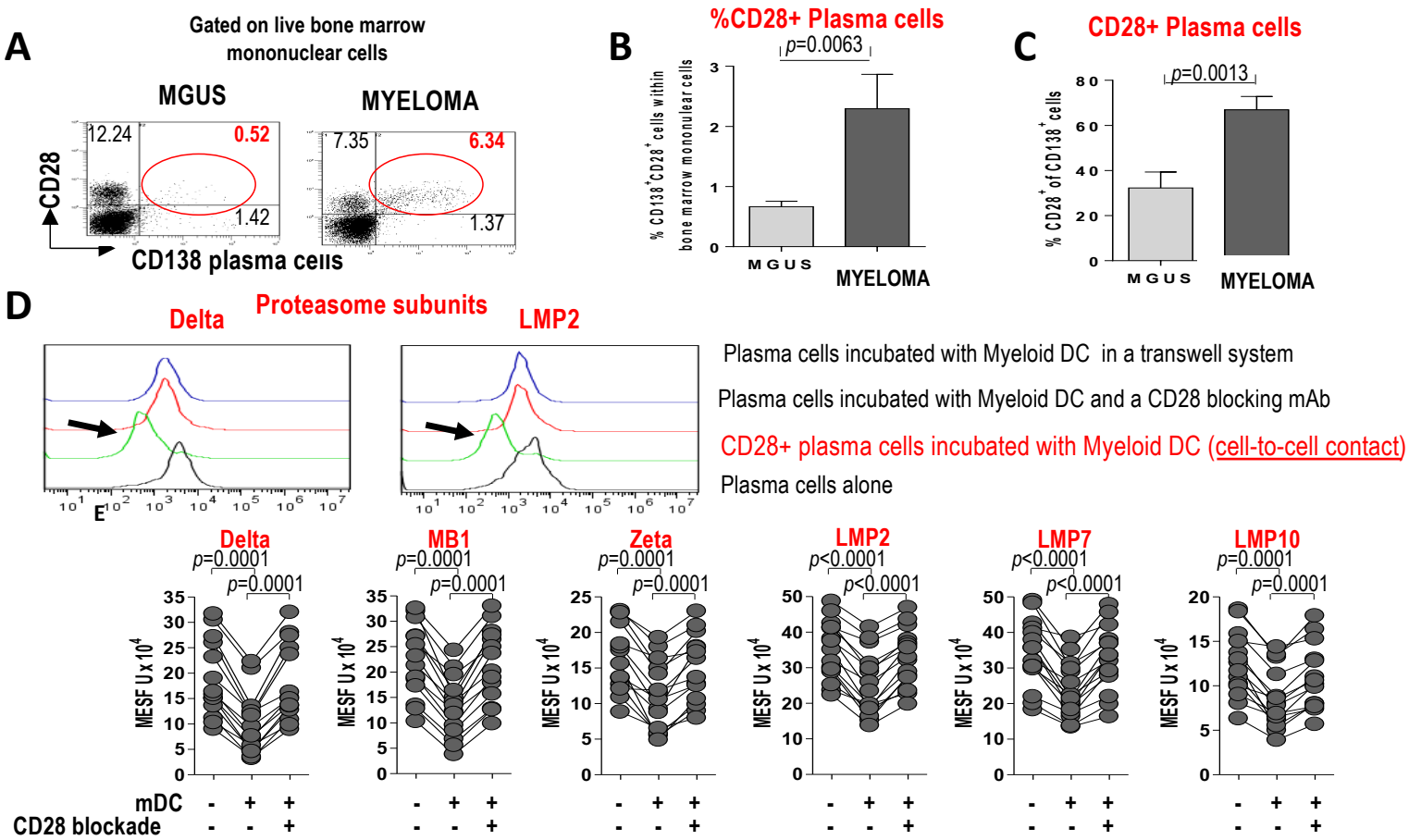
ANGIOGENESIS IS IMMUNOSUPPRESSIVE IN PATIENTS WITH MULTIPLE MYELOMA

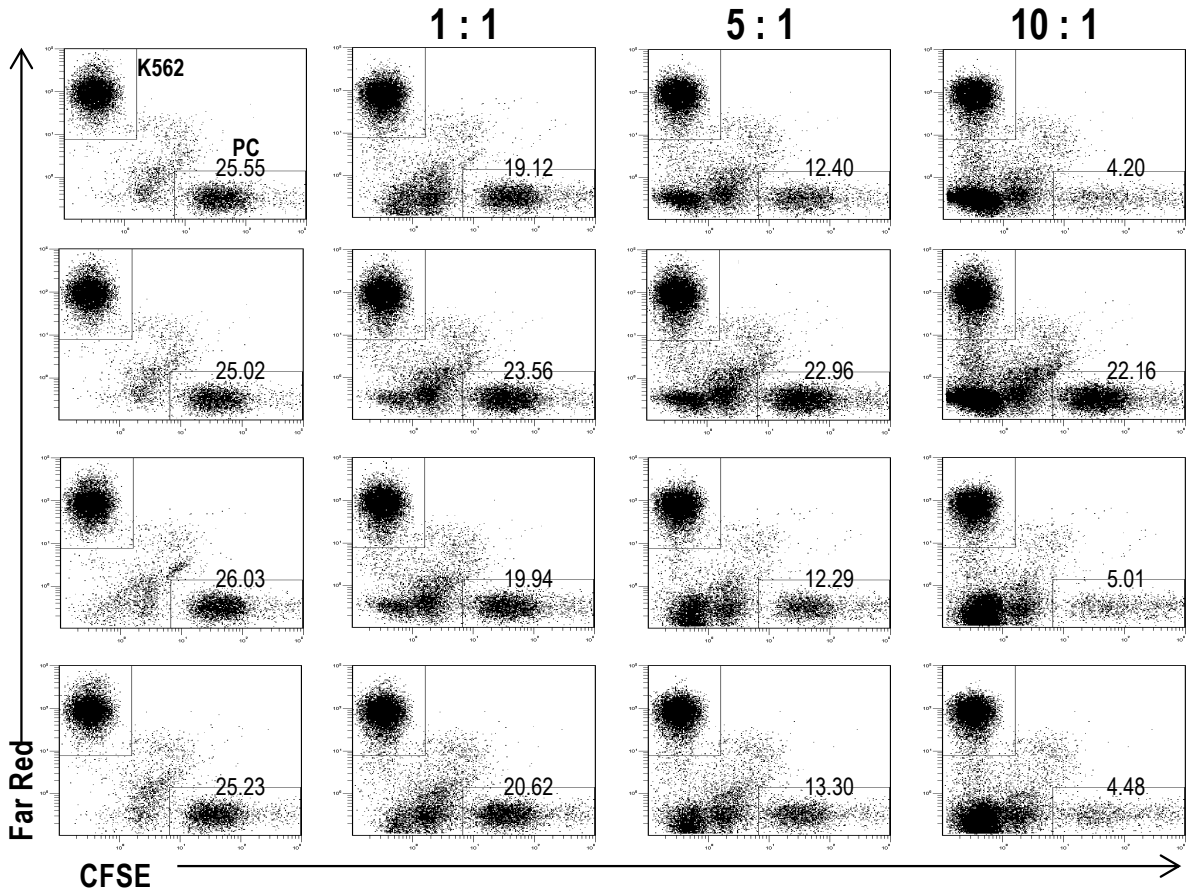
Frequency of DCs in whole blood and marrow samples



Leone et al, Blood 2015

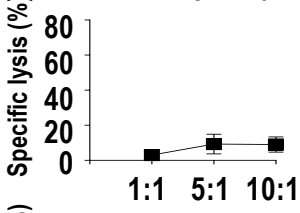
CD28+ plasma cells and their T cell evasion



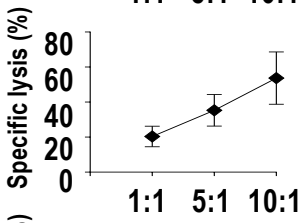


Effect of DCs on tumor plasma cell susceptibility to CD8+ T cell–mediated killing

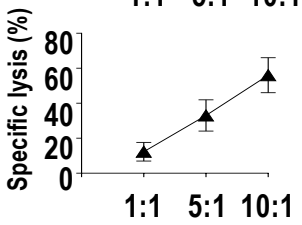
No Plasma cells pre-incubation



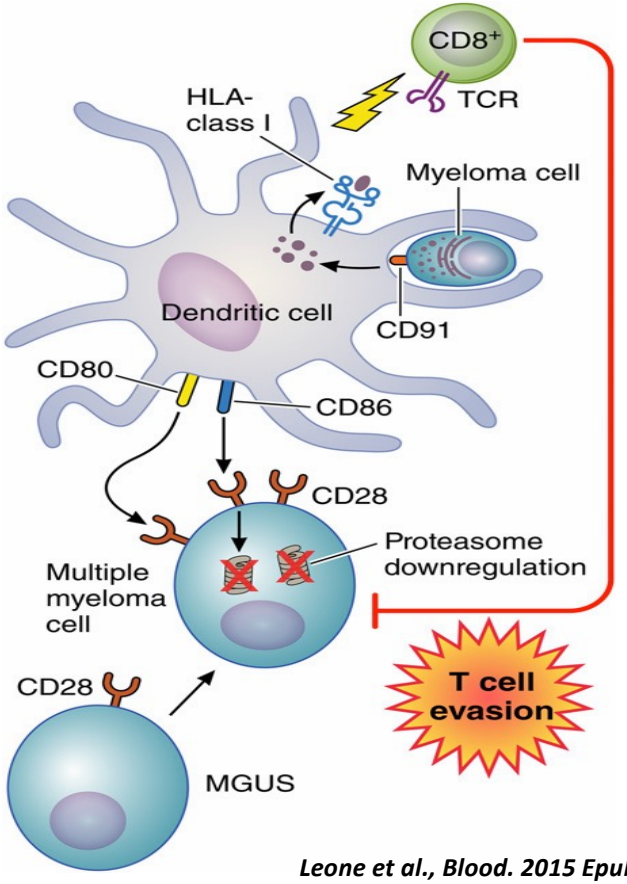
CD28+ plasma cells pre-incubation with Myeloid DC (cell-to-cell contact)



CD28+ plasma cells pre-incubation with Myeloid DC (transwell)



CD28+ plasma cells pre-incubation with Myeloid DC + CD28 blocking



Leone et al., Blood. 2015 Epub 2015 Jul 16.

Comment on Leone et al, page 1443

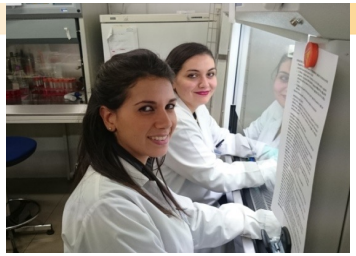
Myeloma escape from
immunity: an "inside" job

Aaron P. Rapoport UNIVERSITY OF MARYLAND SCHOOL OF MEDICINE

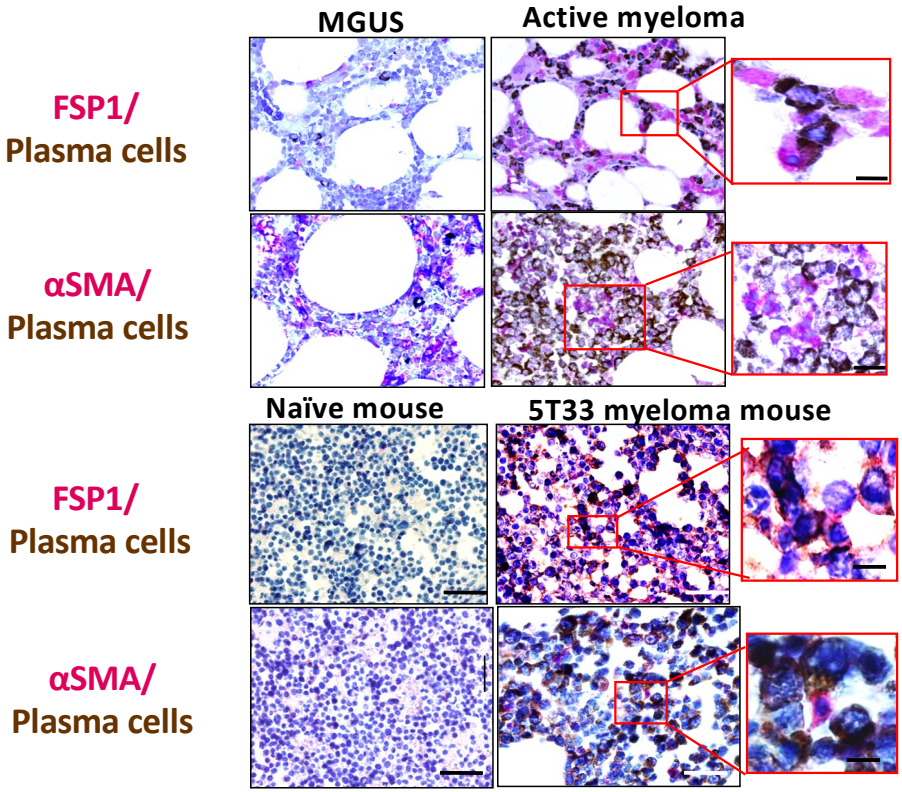
In this issue of Blood, Leone et al describe a novel mechanism mediated by bone marrow dendritic cells (DCs) that impairs T-cell recognition and killing of myeloma cells.

DCs protect tumor plasma cells from CD8+ T cell killing

Drs. M.A. Frassanito, V. Desantis, L. Di Marzo, I. Saltarella, A. Lamanuzzi, my lab

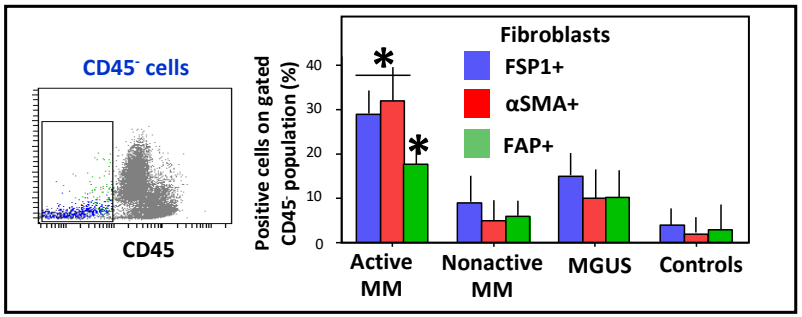


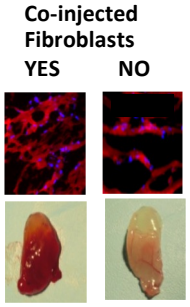
Fibroblasts increase in bone marrow of myeloma patients and mice; and are always in close contact with plasma cells



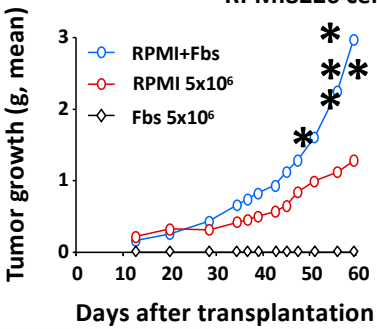
Frassanito et al. Leukemia 2014

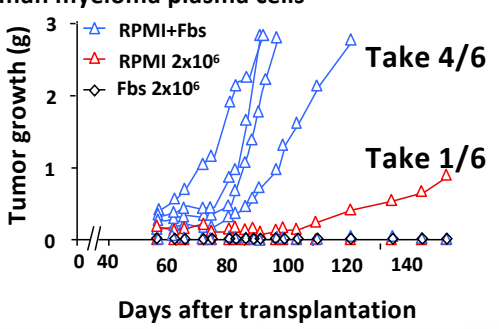
Fibroblasts induce myeloma initiation and progression



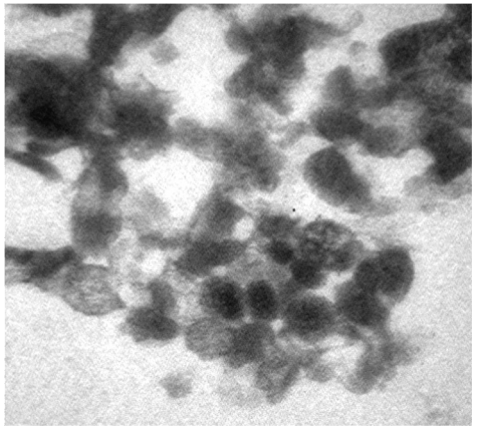


RPMI8226 cells are human myeloma plasma cells

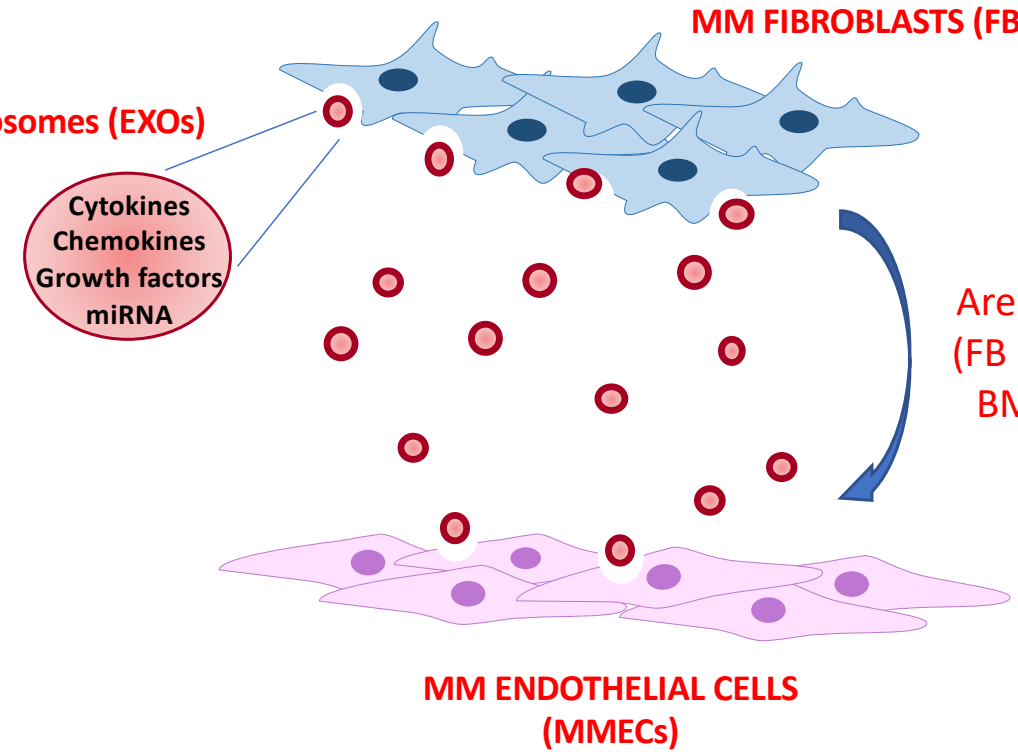




FBs EXOs ON
TRANSMISSION ELECTRON MICROSCOPY



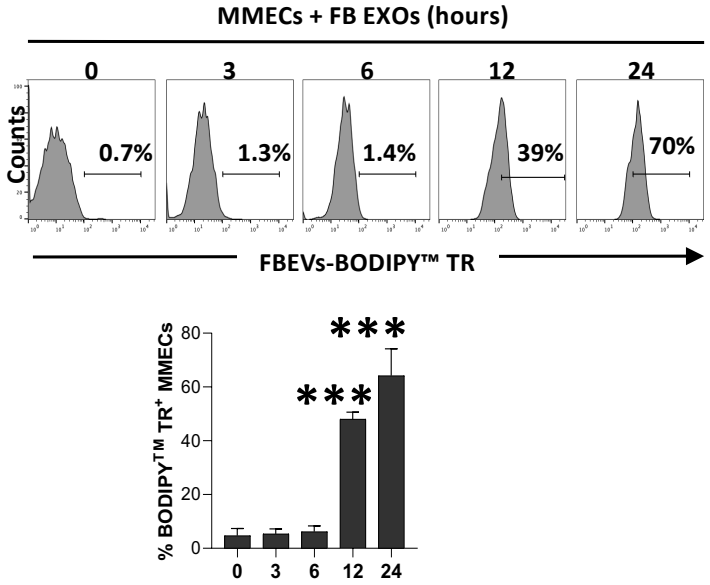
Saltarella et al. 2023 submitted



Are FB-derived EXOs (FB EXOs) involved in BM angiogenesis?

FB-derived exosomes (FB EXOs) promote an early uptake-independent overangiogenic effect in MMECs

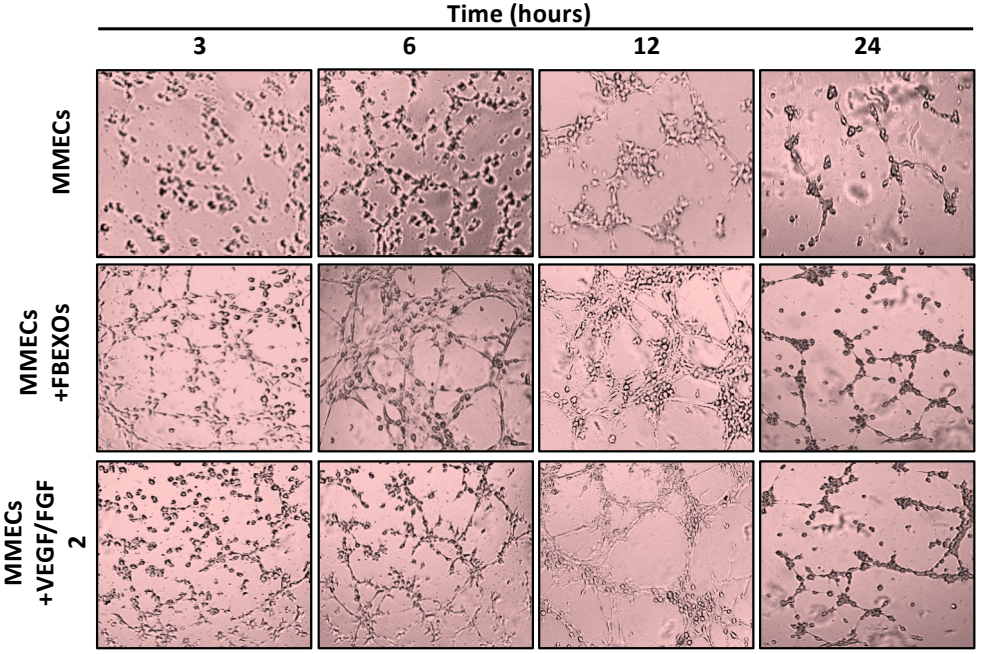
Uptake of Bodipy TR ceramide-labelled EXOs



Uptake of FB EXOs takes longer than 6 hours

Saltarella et al. 2023 submitted

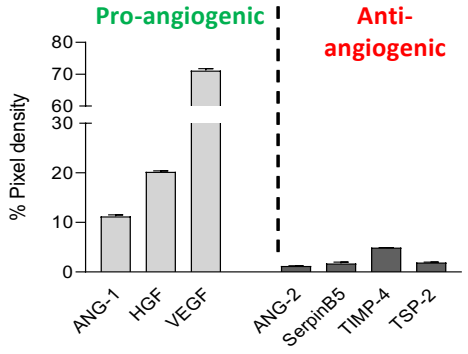
Angiogenesis on Matrigel



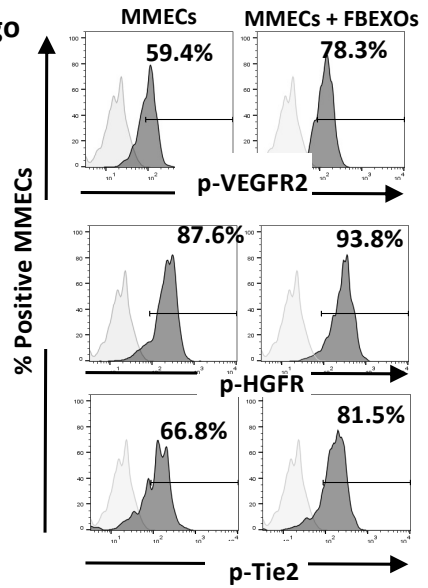
FB EXOs trigger the *in vitro* angiogenesis by MMECs at 3 and 6 hours before internalization

FB-derived exosomes (FB EXOs) contain angiogenic cytokines and activate MMECs

Angiogenesis array of FB EXOs cargo

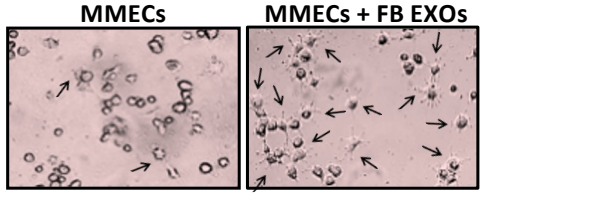


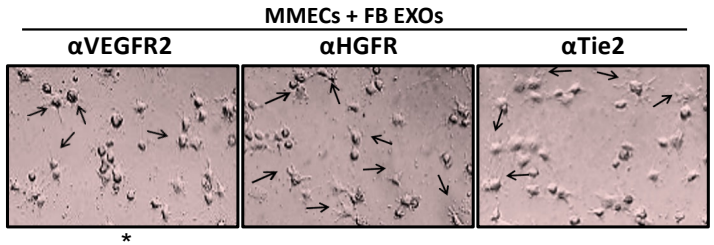
Flow cytometry analysis at 1 hour FB EXOs:MMECs coculture

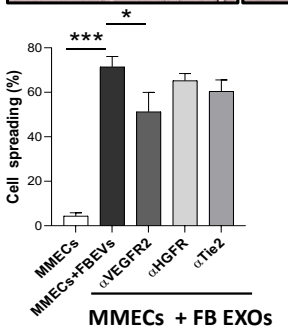


FBEXs contain VEGF, HGF, and ANG-1 and activate their receptors on MMECs at 1 hour of MMECs:FB EXO coculture

***In vitro* cell spreading at 1 hour FB EXOs:MMECs coculture**





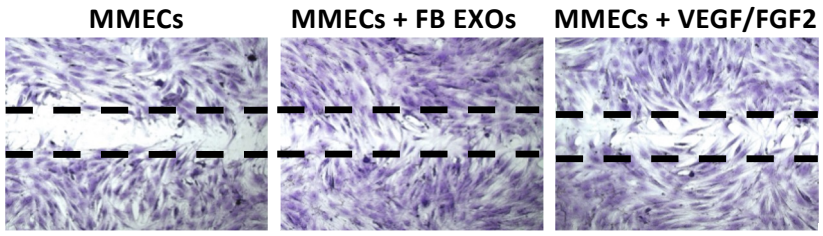


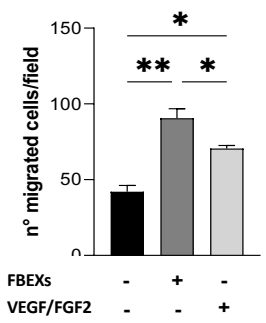
Blockade of VEGFR2 significantly reduce FB EXOs-induced MMECs spreading

FB EXOs foster an early uptake-independent angiogenic effect in a cytokine-mediated fashion

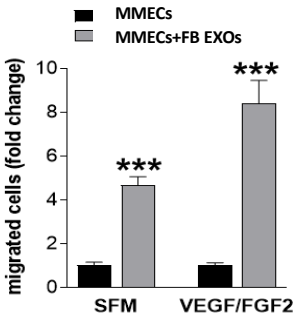
FB-derived exosomes (FB EXOs) induce a late angiogenic response after their uptake

Cell migration after 24 hours of FB EXOs:MMECs coculture

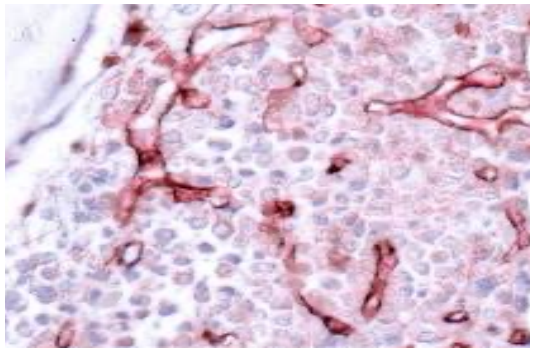




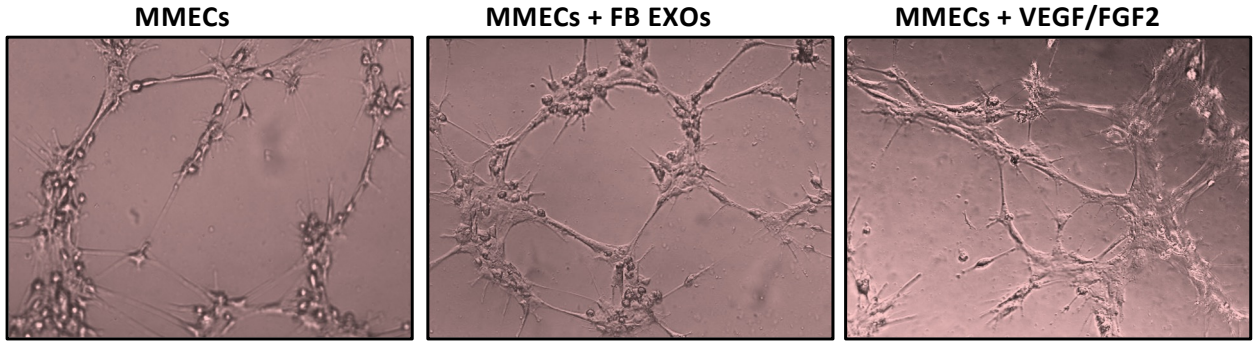
Chemotaxis after 24 hours of FB EXOs:MMECs coculture



Patient's biopsy



***In vitro* angiogenesis after 24 hours of FB EXOs:MMECs coculture**



Modulation of intracellular pathways at 1 and 24 hours of FB EXOs : MMECs coculture

6 hours

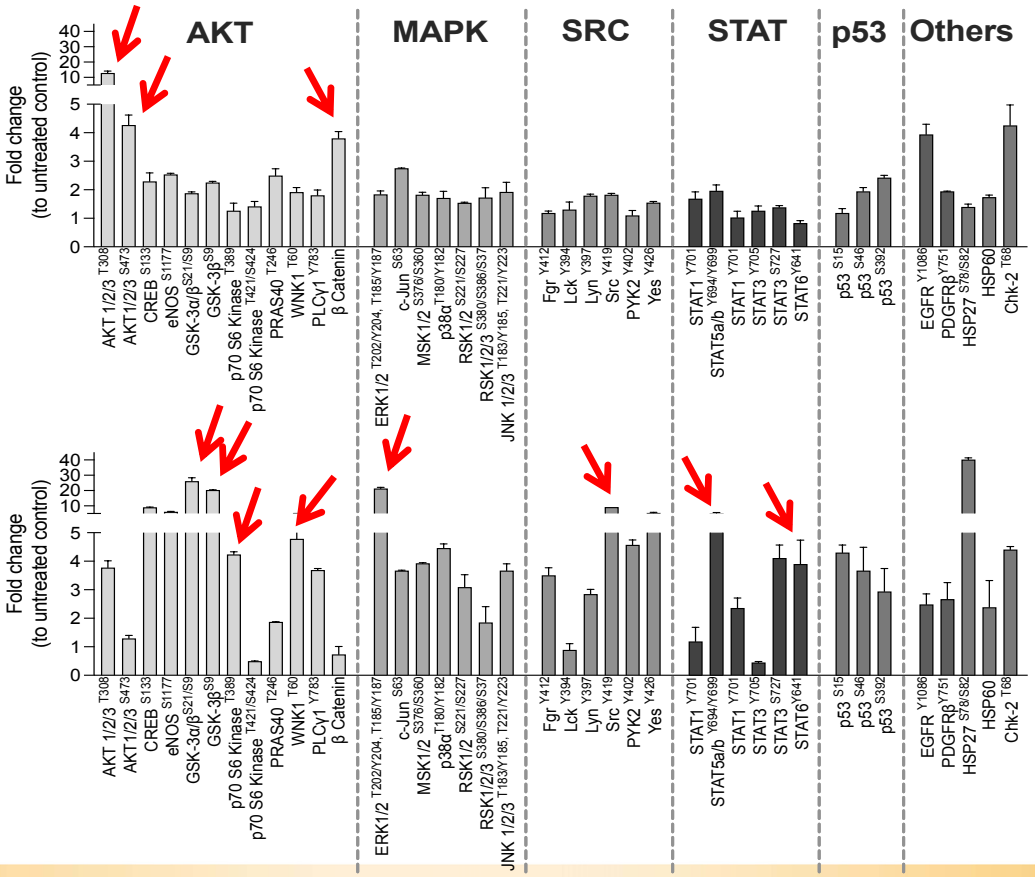
Activation of mTORC2, AKT, and Wnt/β-catenin pathway

24 hours

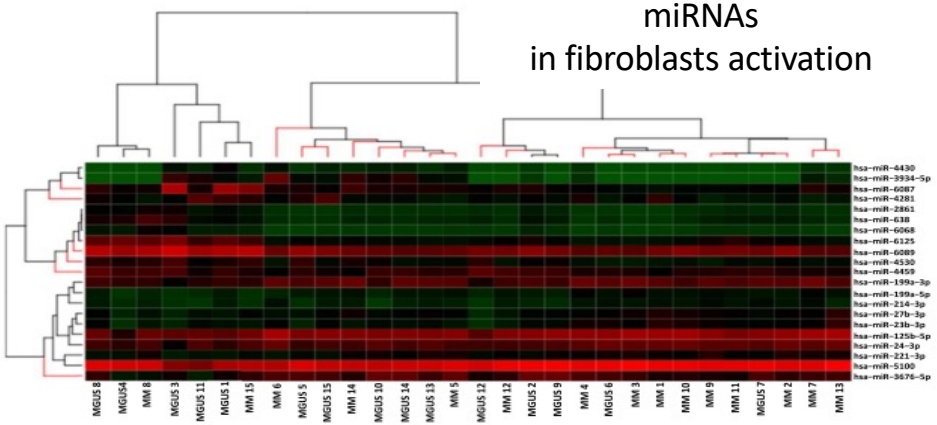
Activation of mTORC1 and MAPK pathways and of their downstream targets

Saltarella et al. 2023, submitted

Phospho-kinase array of MMECs co-cultured with FB EXOs



Different miRNAs expression profile in fibroblasts from Myeloma *vs.* MGUS patients



miRNAs
in fibroblasts activation

Twenty-six differentially expressed miRNAs were identified, 9 were up-regulated and 17 down-regulated:

qRT-PCR

The top miRNAs UP REGULATED are:

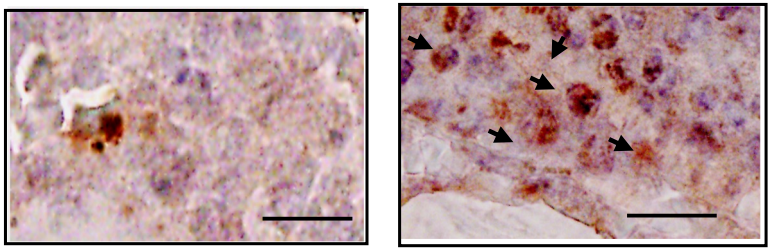
- **hsa-miR-23b-3p – fold change: 0.351**
- **hsa-miR-27 – fold change: 0.366**
- **hsa-miR-125 - fold change: 0.431**
- **hsa-miR-214 – fold change: 0.342**
- hsa-miR-199a-5p – fold change: 0.33

Frassanito et al. J. Pathol. 2019

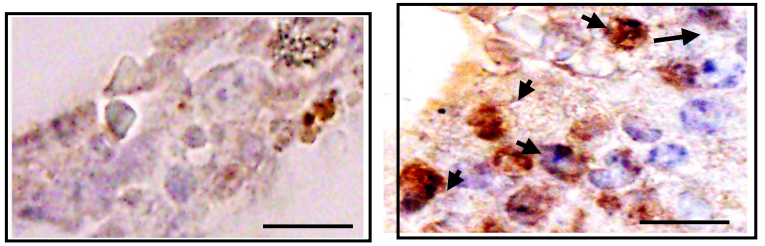
Upregulation of miR-27 and miR-214 in fibroblasts of patients with myeloma *vs.* MGUS

MGUS **1st diagnosed MM**

miR-27/FAP



miR-214/FAP

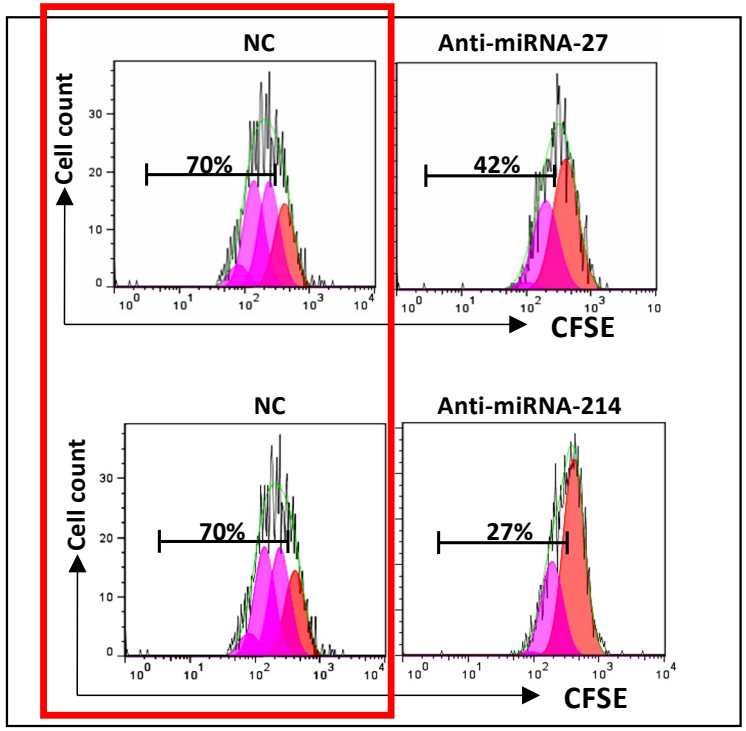


In situ hybridization

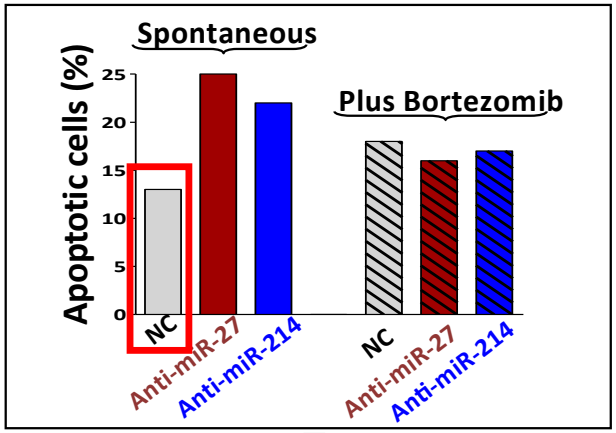
Fibroblasts co-expression of FAP (brown) and the miRNA (blue) gives dark-brown dots

Effect of miRNA-27 and miRNA-214 inhibition on proliferation and apoptosis of myeloma fibroblasts

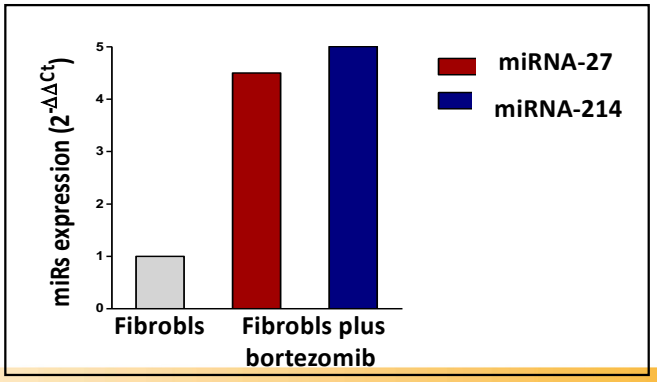
Cell proliferation



Apoptosis

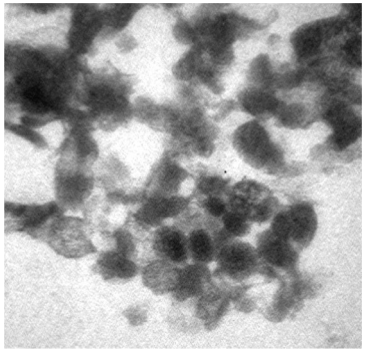


Bortezomib induces miRNA-27 and miRNA-214 expression

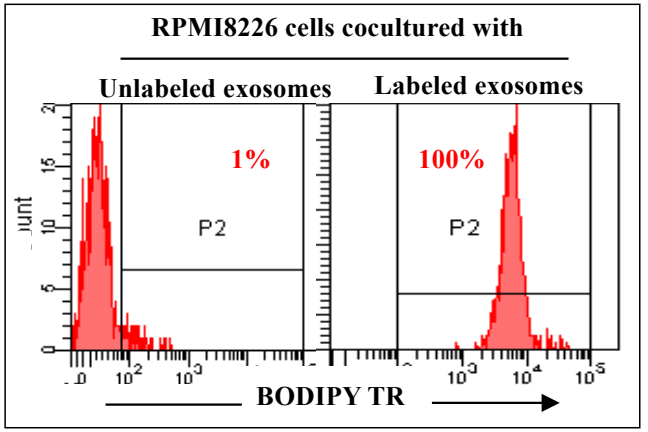


Fibroblasts-derived exosomes (FB EXOs) are fully uptaken by myeloma cells

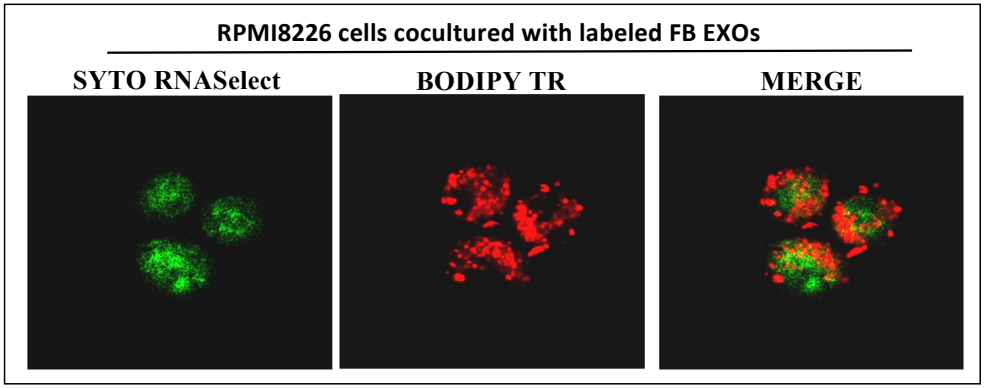
FBs EXOs ON TRANSMISSION ELECTRON MICROSCOPY



FLOW CYTOMETRY ANALYSIS OF EXOSOMES UPTAKE BY RPMI8226 PLASMA CELLS



DUAL IMMUNOFLUORESCENCE CONFOCAL LASER SCANNING MICROSCOPY IMAGES OF FB EXOs UPTAKE BY RPMI8226 PLASMA CELLS

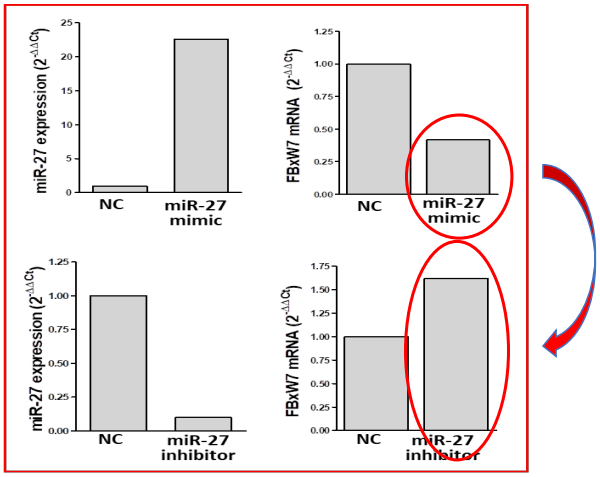


Frassanito et al. J. Pathol. 2019

miRNA-27 and miRNA-214 gene targets

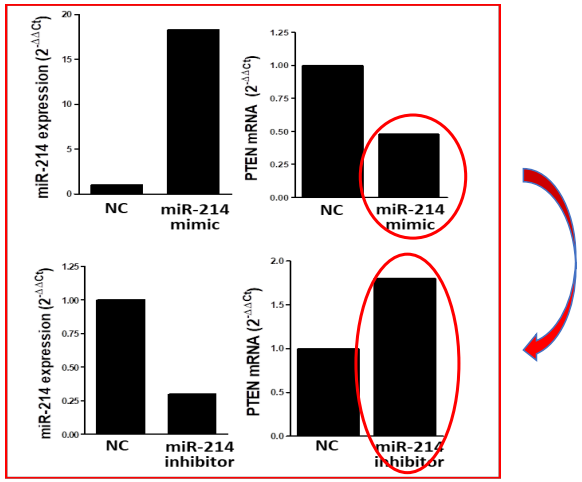
(by MIRANDA and TargetScan)

FBxW7



FBxW7 is a component of SCF complex: it binds specific protein substrates, i.e. Notch, Cyclin E, Mcl-1, for ubiquitylation and degradation

PTEN



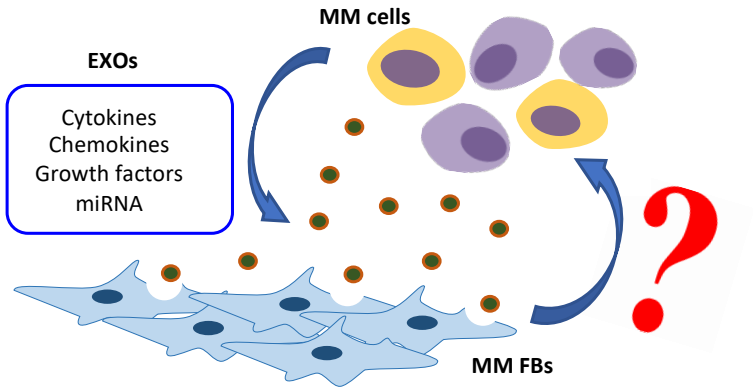
PTEN is the main negative regulator of PI3K/AKT pathway

FBxW7 and PTEN pathways are involved in cell proliferation and apoptosis

Frassanito et al. J. Pathol. 2019

Do myeloma FB EXOs express the same up-regulated miRNAs of myeloma FBs?

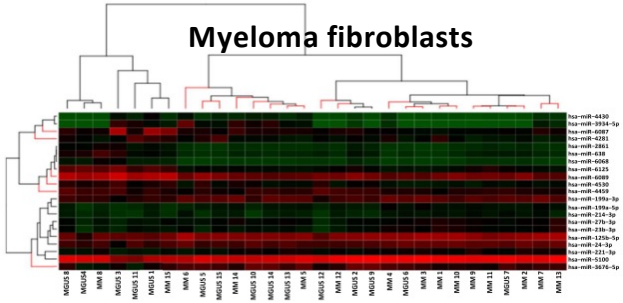
…….Yes! They do!



Are myeloma FB EXOs involved in myeloma cells proliferation and anti-apoptosis?

…….Yes! They are!

The overexpressed miRNAs in FB EXOs overlap the aberrant miRNA profile of fibroblasts in MM patients

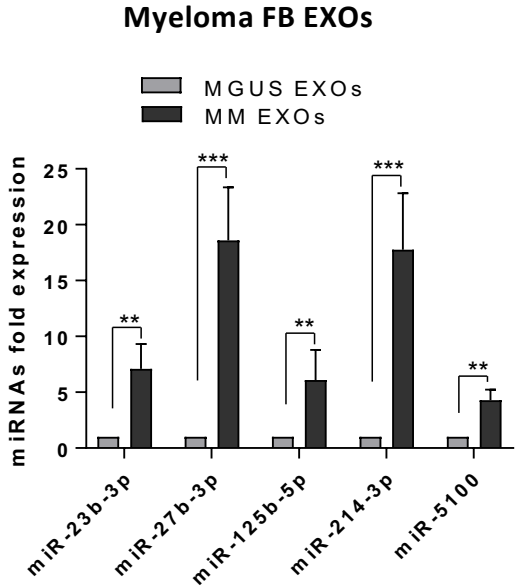


qRT-PCR

The top miRNAs UP REGULATED are:

- hsa-miR-23b-3p – fold change: 0.351
- **hsa-miR-27 – fold change: 0.366**
- hsa-miR-125 - fold change: 0.431
- **hsa-miR-214 – fold change: 0.342**
- hsa-miR-5100 – fold change: 0.33

Frassanito et al. J. Pathol. 2019

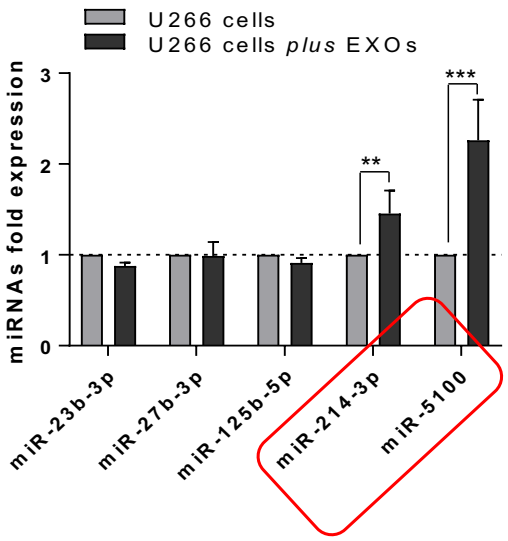


qRT-PCR studies reveal higher expression of miR-23, -27, -125, -214 and -5100 in MM FB EXOs than MGUS FB EXOs.

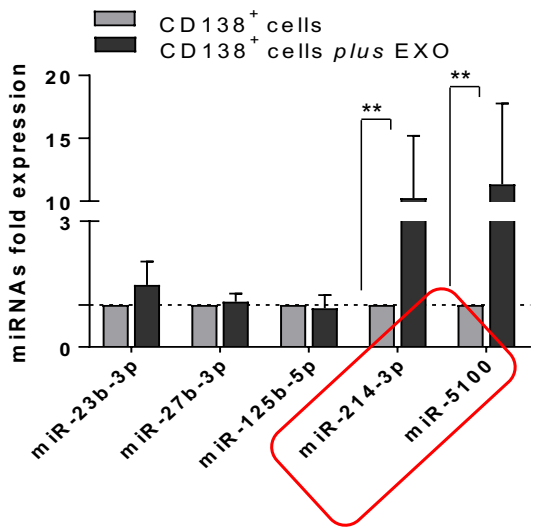
MM cells do uptake FB EXOs but selectively overexpress only miR-214 and miR-5100

(but not miR-23, miR-27, nor miR-125)

U266 cell line



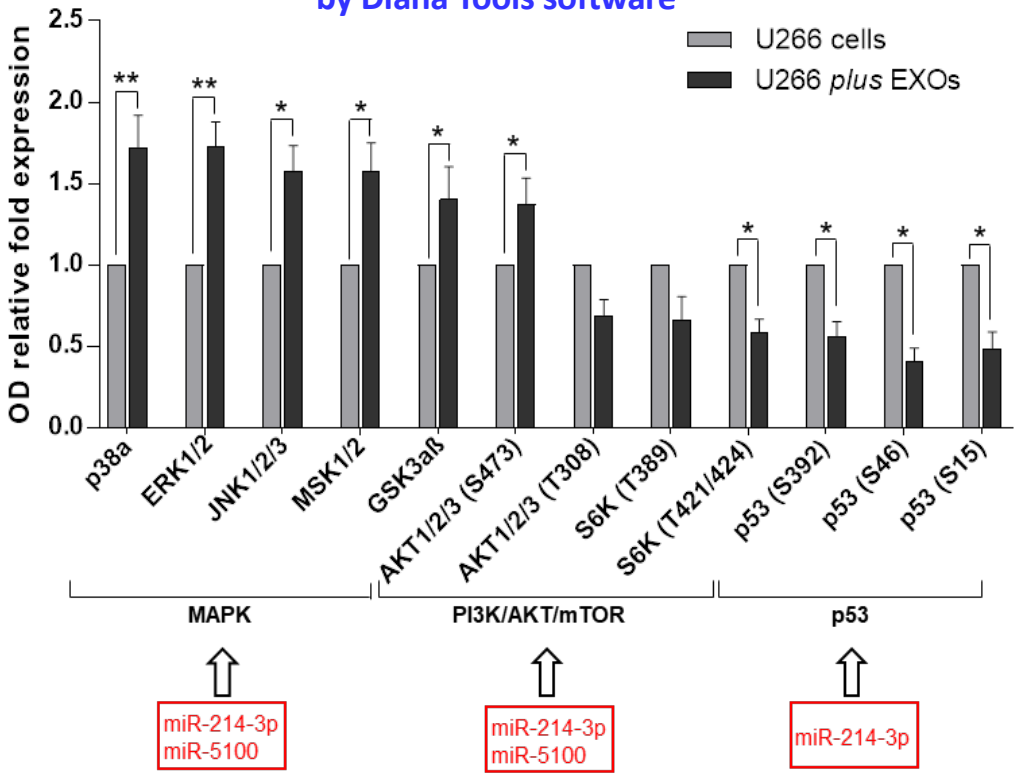
Fresh CD138+ plasma cells



Saltarella et al., J. Pathol. 2022

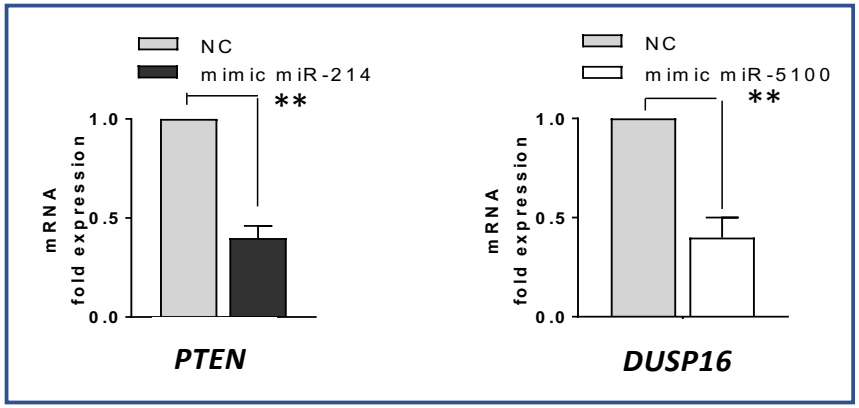
FB EXOs modulate intracellular pathways via miRNA-214 and miRNA-5100

by Diana Tools software

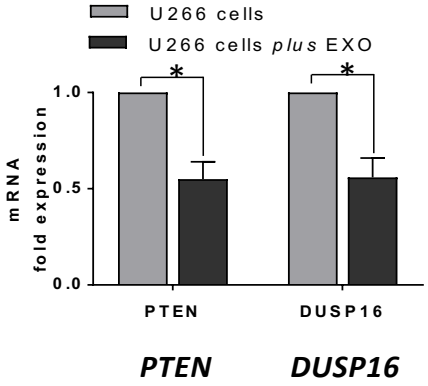


miRNA-214 and miRNA-5100 target genes indicate that FB EXOs enhance MM cell proliferation…

PTEN and *DUSP16* are target genes of miR-214 and miR-5100, respectively (DIANA Tools analysis)



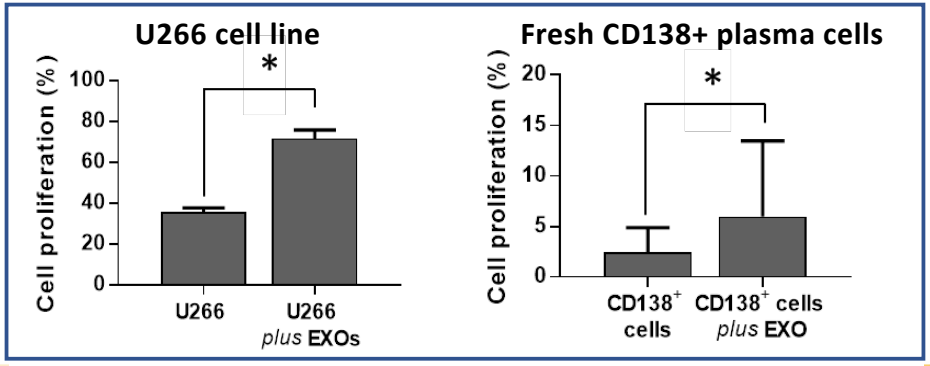
MM fibroblasts EXOs act as mimic probes



Target genes of miR-214 and miR-5100 enhance cell proliferation….

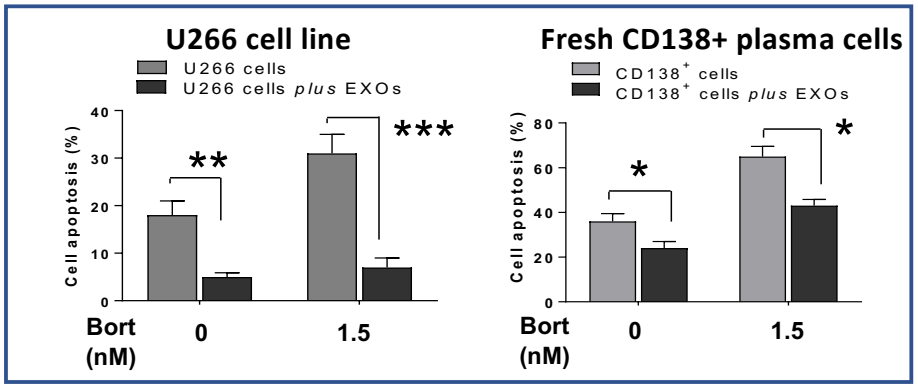
Saltarella et al., J. Pathol. 2022

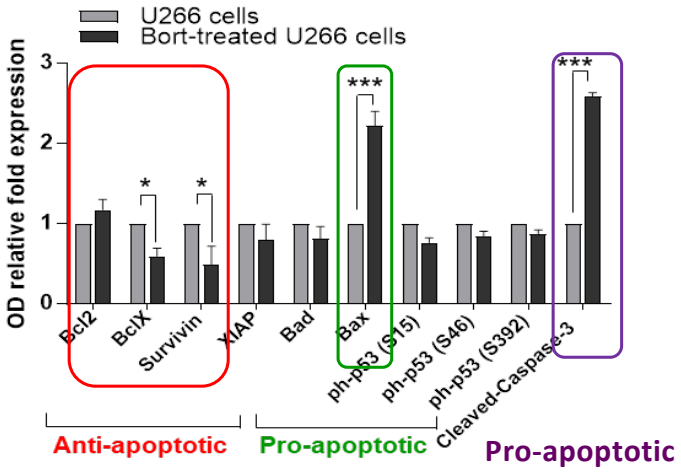
Enhancement of MM cell proliferation

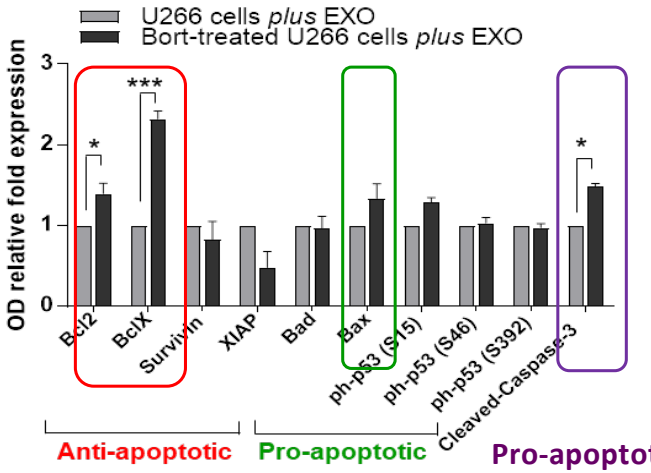


Bort-induced apoptosis of MM cells

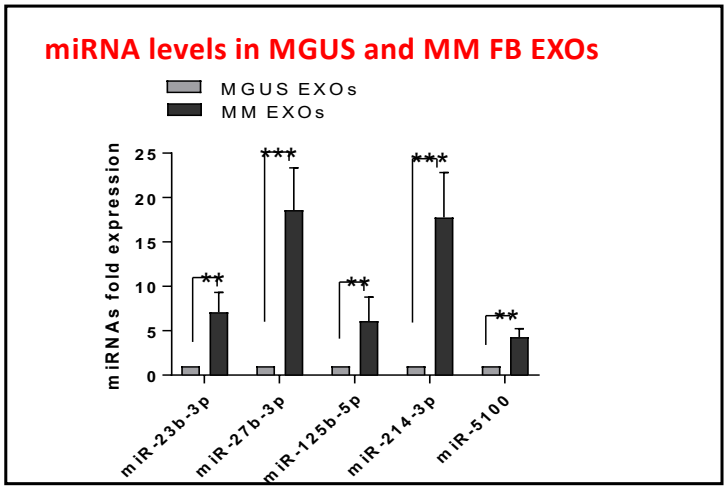
……and inhibit apoptosis



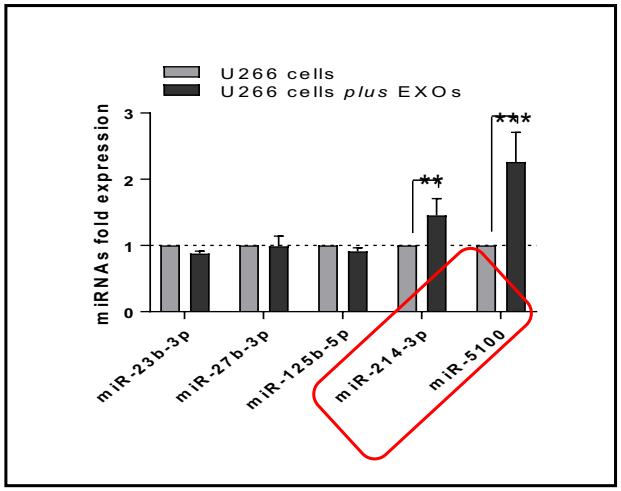




miRNA levels in MGUS and MM FB EXOs

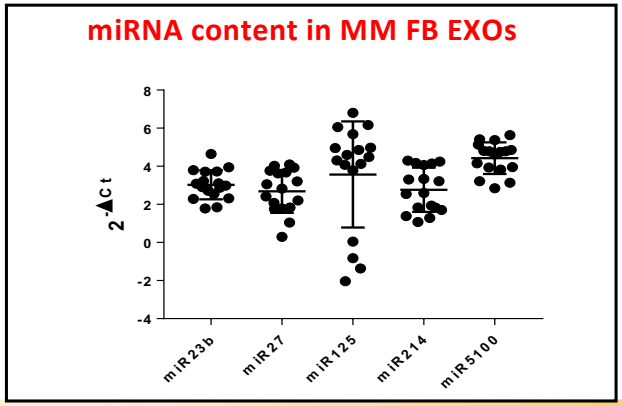


miRNAs transfered in MM cells

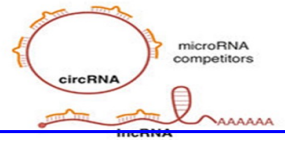


Why do MM cells selectively uptake only miR-214 and miR-5100?

miRNA content in MM FB EXOs



Putative role of lncRNA



ONCOLOGY LETTERS 19: 595-605, 2020

Research progress on the interactions between long non-coding RNAs and microRNAs in human cancer (Review)

BINYU SUN*, CHUNXIA LIU*, HAO LI, LU ZHANG, GANG LUO, SICHENG LIANG and MUHAN LÜ

Leukemia
https://doi.org/10.1038/s41375-020-01034-y

ARTICLE

Multiple myeloma gammopathies

The long non-coding RNA CRNDE regulates growth of multiple myeloma cells via an effect on IL6 signalling

Antoine David[1] · Simone Zocchi[1] · Alexis Talbot[1,2] · Caroline Choisy[1] · Ashley Ohnona[1] · Julien Lion[3] · Wendy Cuccuini[4] · Jean Soulier[4,5] · Bertrand Arnulf[1,2] · Jean-Christophe Bories[1] · Michele Goodhardt[1] · David Garrick[1]

Yin et al. BMC Medical Genomics (2020) 13:84
https://doi.org/10.1186/s12920-020-00741-w

BMC Medical Genomics

RESEARCH ARTICLE Open Access

Construction and analysis of a lncRNA-miRNA-mRNA network based on competitive endogenous RNA reveal functional lncRNAs in oral cancer

Junhao Yin[1,2], Xiaoli Zeng[1,2], Zexin Ai[1,2], Miao Yu[1,2], Yang'ou Wu[1,2] and Shengjiao Li[1,2*]

frontiers in Oncology

MINI REVIEW
published: 25 July 2019
doi: 10.3389/fonc.2019.00669

Crosstalk Between Long Non-coding RNAs, Micro-RNAs and mRNAs: Deciphering Molecular Mechanisms of Master Regulators in Cancer

Eduardo López-Urrutia[1], Lilia P. Bustamante Montes[2], Diego Ladrón de Guevara Cervantes[2], Carlos Pérez-Plasencia[1,2] and Alma D. Campos-Parra[2]*

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

ELSEVIER

Review

Potential regulatory role of lncRNA-miRNA-mRNA axis in osteosarcoma

Jin-yan Wang[1], Yan Yang[1], Yajun Ma[1], Fen Wang, Aili Xue, Jing Zhu, Hui Yang, Qi Chen, Meili Chen, Lingling Ye, Hao Wu, Quan'an Zhang*

Department of Oncology, The Affiliated Jiangning Hospital with Nanjing Medical University, Nanjing 210000, Jiangsu, PR China

Olgun et al. BMC Genomics (2018) 19:650
https://doi.org/10.1186/s12864-018-5006-1

BMC Genomics

RESEARCH ARTICLE Open Access

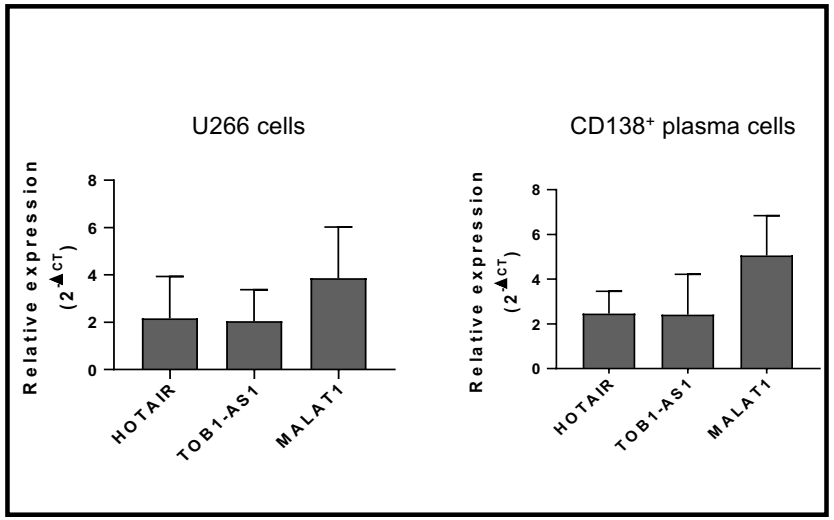
Discovering lncRNA mediated sponge interactions in breast cancer molecular subtypes

Gulden Olgun[1], Ozgur Sahin[2] and Oznur Tastan[3*]

Expression of long non-coding RNAs in MM cells

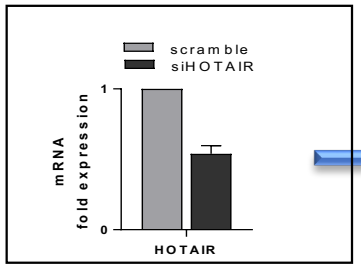
lncRNA	miRNA target	Ref
HOTAIR	miRNA-23	T Yang *et al.*, Gene. 2018
TOB1-AS1	miRNA-23 and miRNA-27	WJ Shangguan *et al.*, Exp Ther Med. 2019
MALAT1	miRNA-125	H Xie *et al.*, J Cancer. 2017

MM cells express lncRNAs HOTAIR, TOB1-AS1, MALAT1

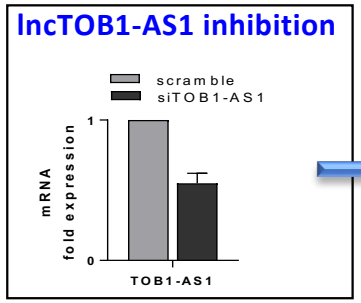


Saltarella et al., Cancers 2022

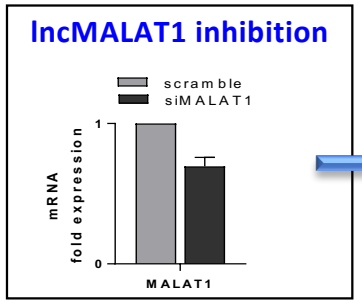
lncHOTAIR inhibition



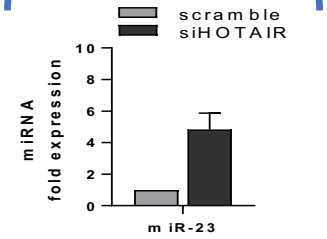
lncTOB1-AS1 inhibition



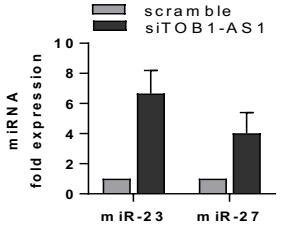
lncMALAT1 inhibition



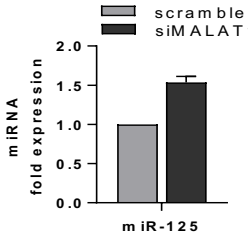
U266 cells *plus* EXO



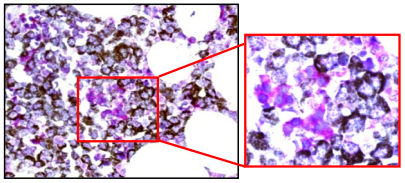
miR-23 overexpression



miR-23 and miR-27 overexpression



miR-125 overexpression



CONCLUSIONS

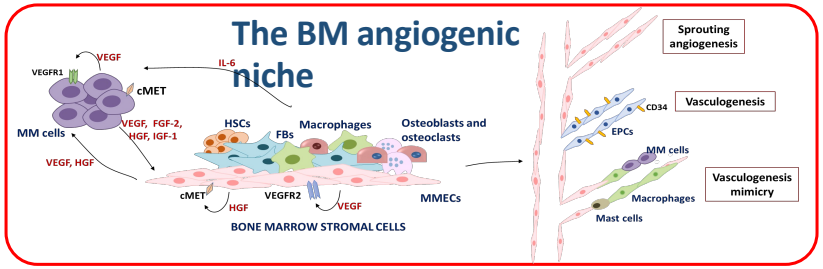
- Myeloma FBs create a supportive niche for plasma cell proliferation, anti-apoptosis and drug resistance;

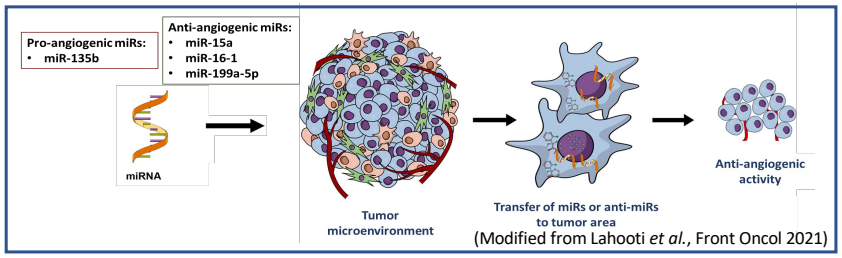
- FBs express an aberrant miRNA profile in myeloma patients;

- FBs-derived EXOs selectively transfer miR-214 and miR-5100 into MM cells modulating the MAPK, β-catenin/Wnt, mTOR, p53 pathways that enhance cell proliferation and reduce spontaneous and bortezomib-induced apoptosis;

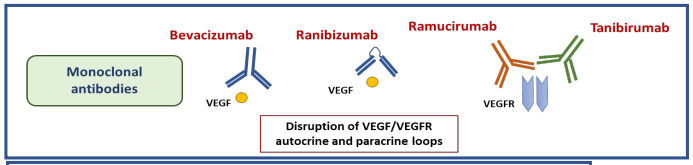
- The selective miRNAs transfer into plasma cells is due to expression of specific lncRNAs by these cells.

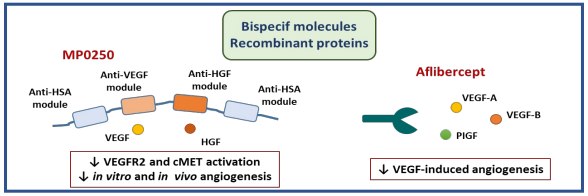
Anti-angiogenic activity of anti-myeloma drugs

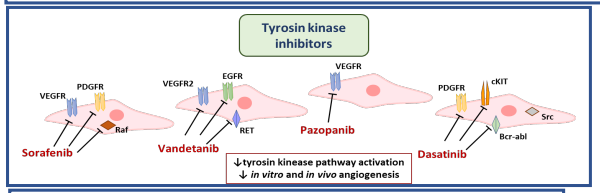


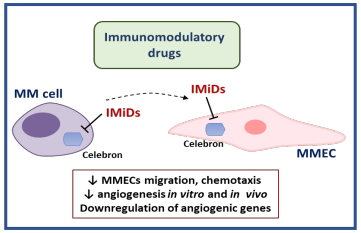


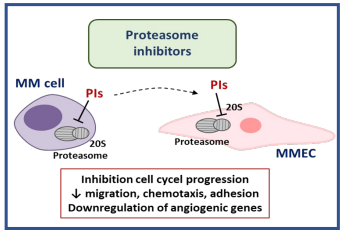
(Modified from Lahooti *et al.*, Front Oncol 2021)

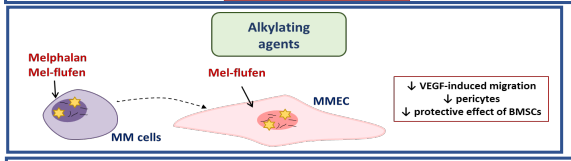


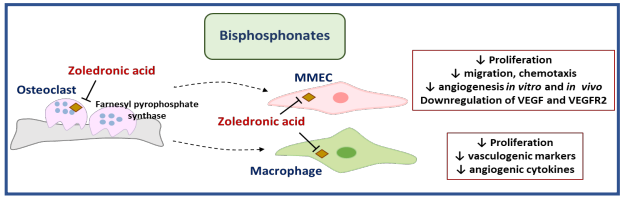


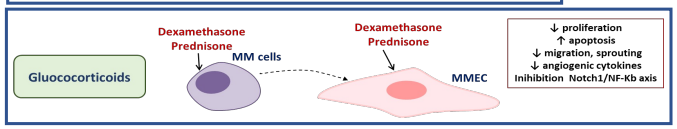












Saltarella et al., Cancers 2023

3rd Cuneo City ImmunoTherapy Conference (CCITC)

UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO

Immunotherapy in Hematological Malignancies 2023

Internal Medicine Unit «Guido Baccelli»:

Head A. Vacca, MD, PhD

Lab Angiogenesis and Vascular Biology

M.A. Frassanito, PhD

V. Desantis, PhD

I. Saltarella, PhD

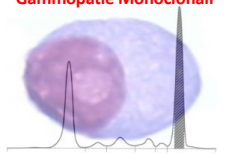
A. Lamanuzzi, PhD

Patients (treatment, trials)

R. Ria, MD

A.G. Solimando, MD, PhD

Unità per lo Studio e la Terapia delle Gammopatie Monoclonali



Collaborations

K. Vanderkerken - Myeloma Center Brussels, Vrije Universiteit Brussel, Belgium

H. Einsele, MD - University of Würzburg, Germany

G. Martinelli - Direttore Scientifico dell'Istituto Romagnolo per lo Studio dei Tumori "Dino Amadori"- IRST S.r.l. Irccs

M. Bellone - Unità Clinica di Immunologia, Reumatologia, Allergologia e Malattie rare dell'IRCCS Ospedale San Raffaele, Milano

RESEARCH FOR INNOVATION

Regione Puglia

AIRC

Associazione Italiana per la Ricerca sul Cancro (AIRC, Milan)

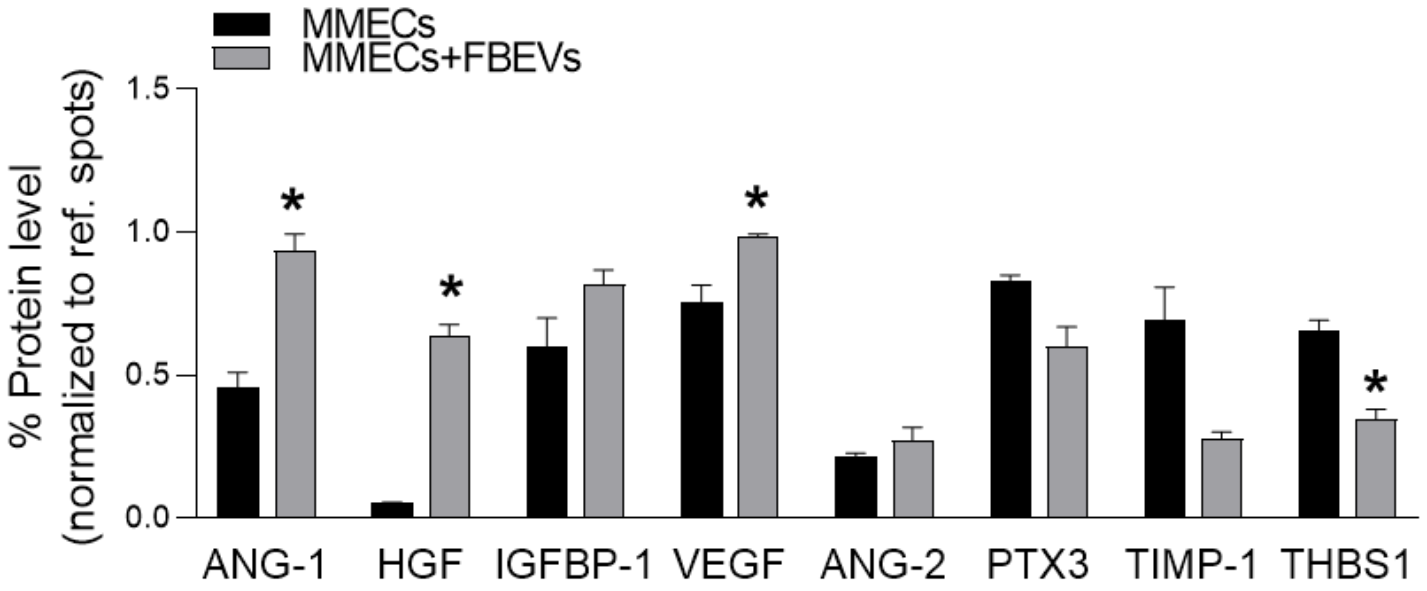
CITEL TELEMEDICINE RESEARCH CENTER

CUNEO, **MAY 18-20, 2023**
RONDÒ DEI TALENTI

Immunotherapy in Hematological Malignancies 2023

FB extracellular vescicles induce the secretion of angiogenic factors that sustain angiogenic loops in MM

Angiogenesis array of MMECs co-cultured with FBEVs for 24 hours



(Lamanuzzi A *et al.* Biomedicines, submitted)