University of Bari Aldo Moro

Department of Biomedical Sciences and Human Oncology
Section of Internal Medicine and Clinical Oncology
University of Bari Medical School, Bari, Italy

Myeloma Microenvironment:

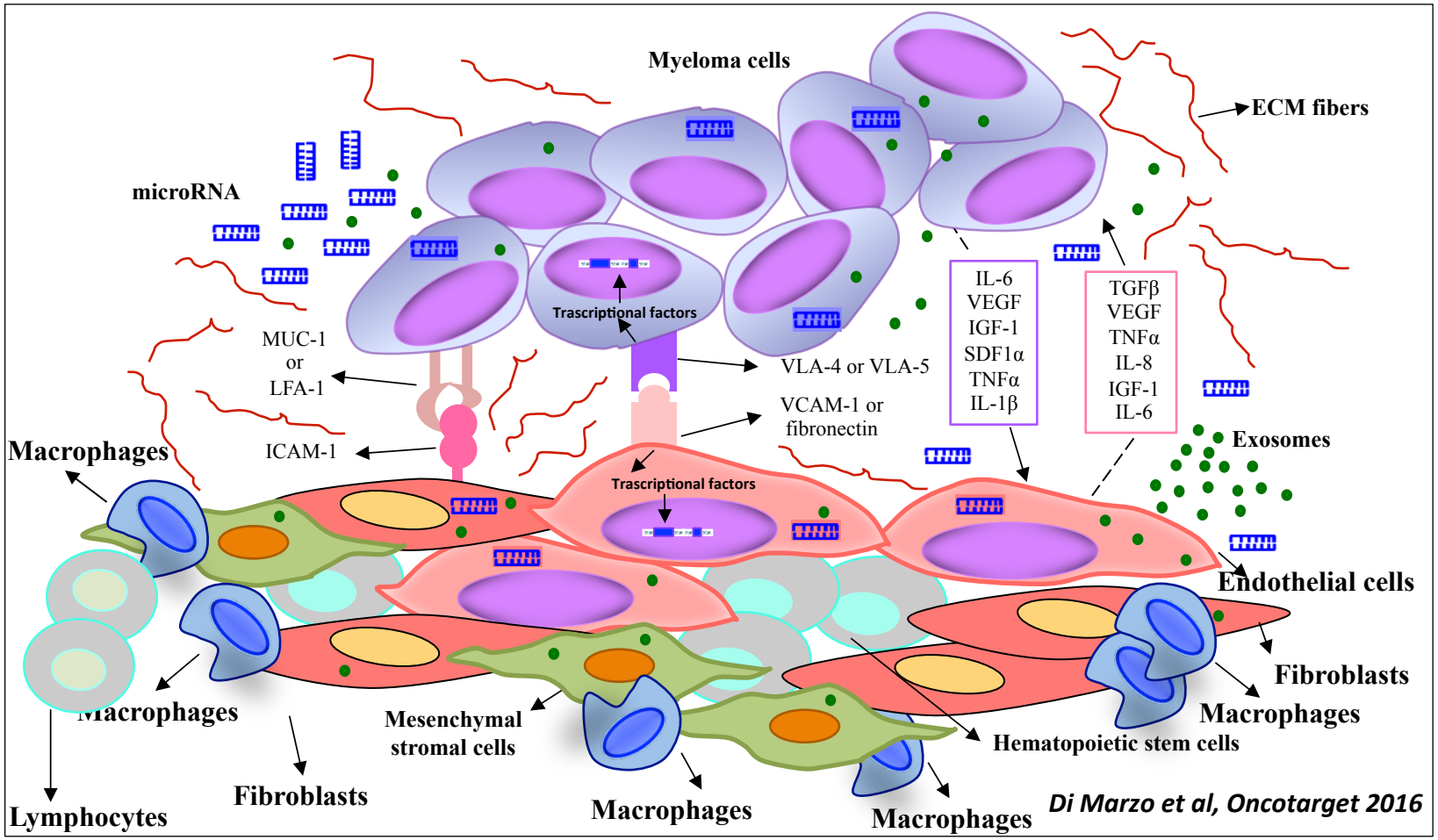
New perspectives to overcome drug resistance

Prof. Angelo Vacca

1st CCITC – Immunotherapy in Hematological Malignancies 2018
Cuneo 17-19 May, 2018

Bone marrow microenvironment in multiple myeloma

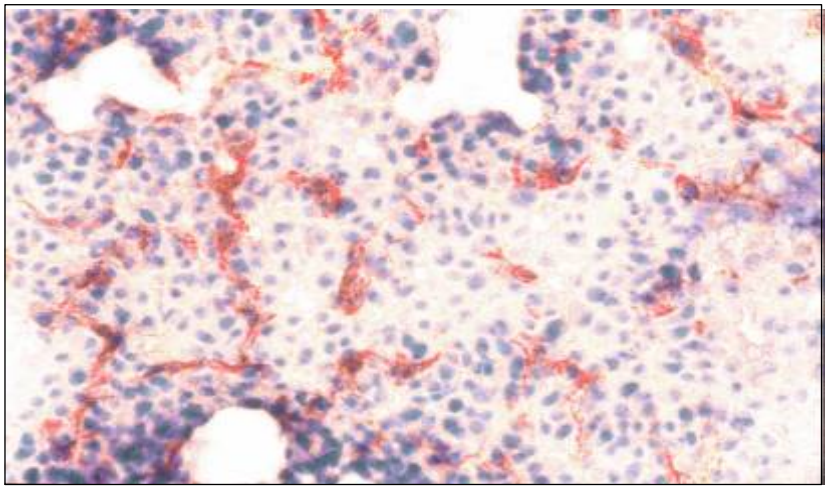
NON-CELLULAR (ECM fibers, soluble factors)
AND
CELLULAR COMPARTMENT (hematopoietic and non-hematopoietic cells)



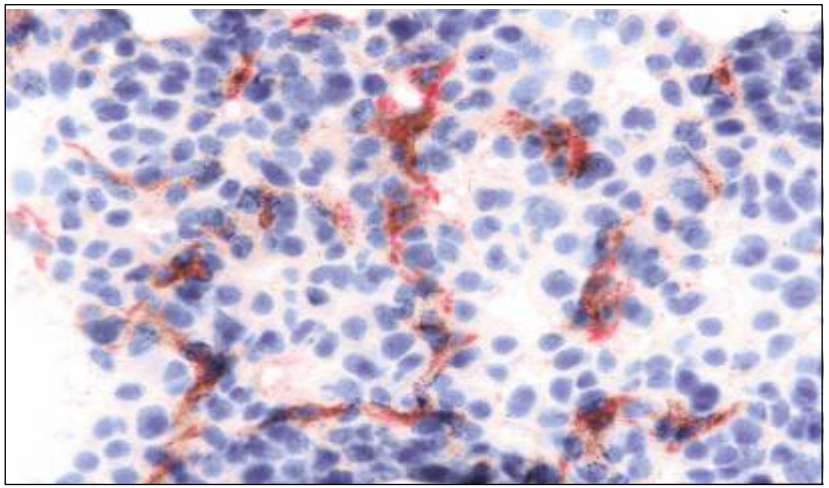
THE MICROENVIRONMENT IN MYELOMA FAVORS TUMOR CELL SURVIVAL, PROLIFERATION, IMMUNOSURVEILLANCE ESCAPE AND DRUG RESISTANCE

Bone marrow angiogenesis in patients with active multiple myeloma

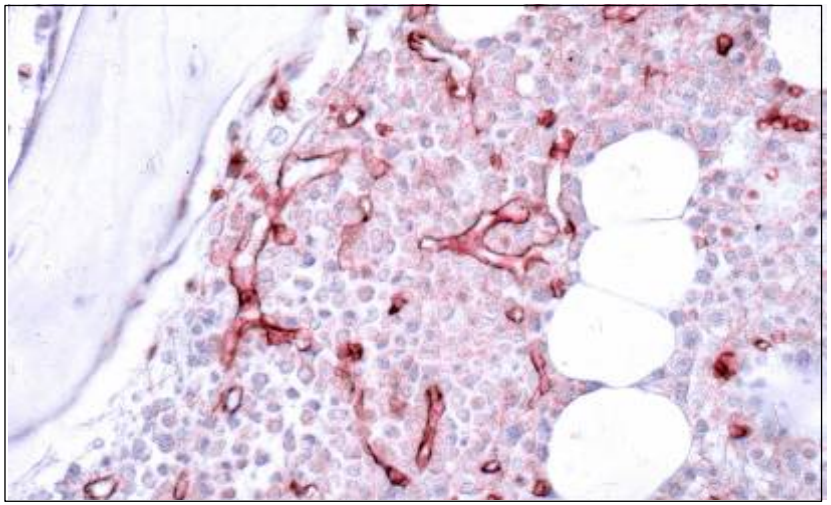
Megafield



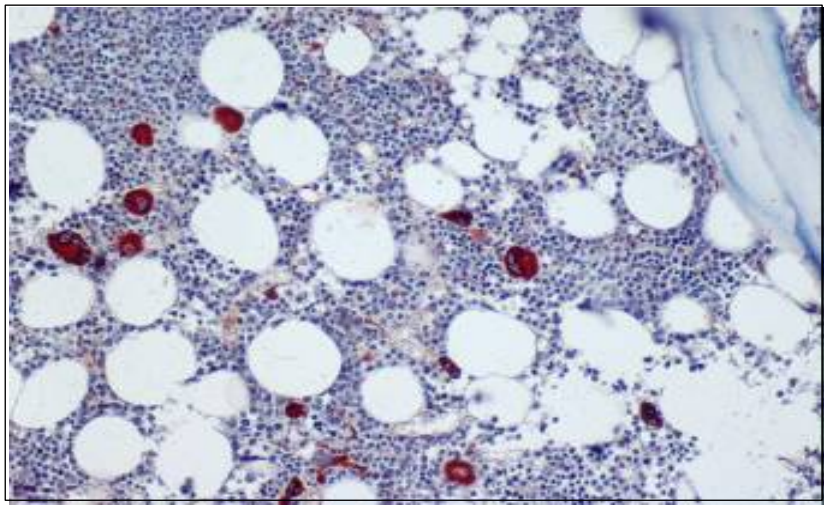
Single or clustered endothelial cells



Vessel arborizations



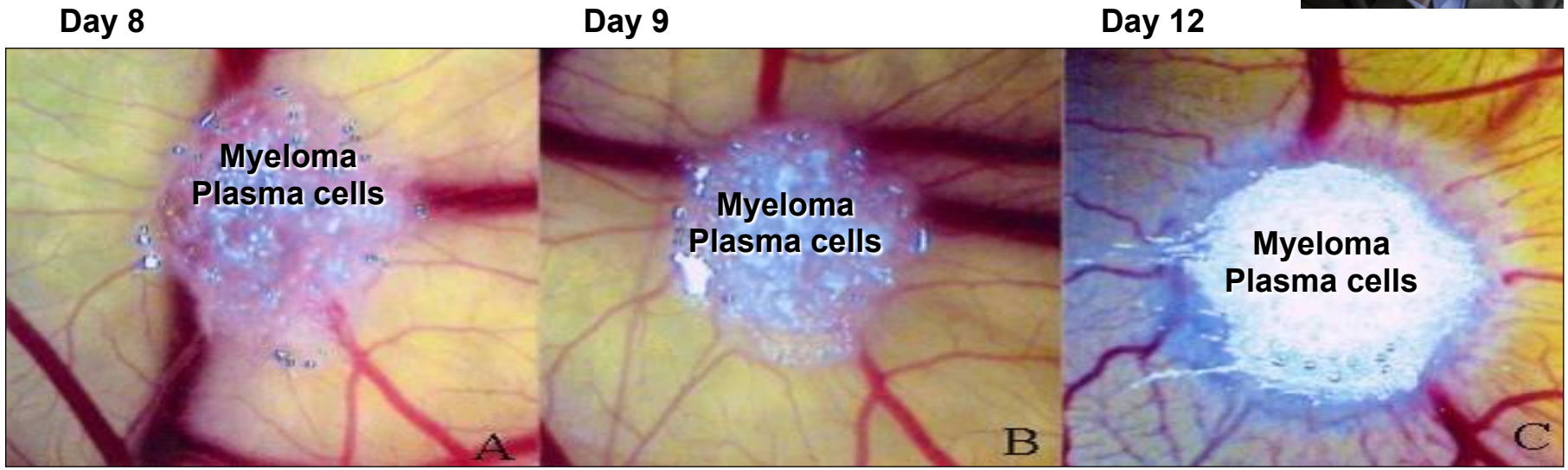
MGUS: no vessels

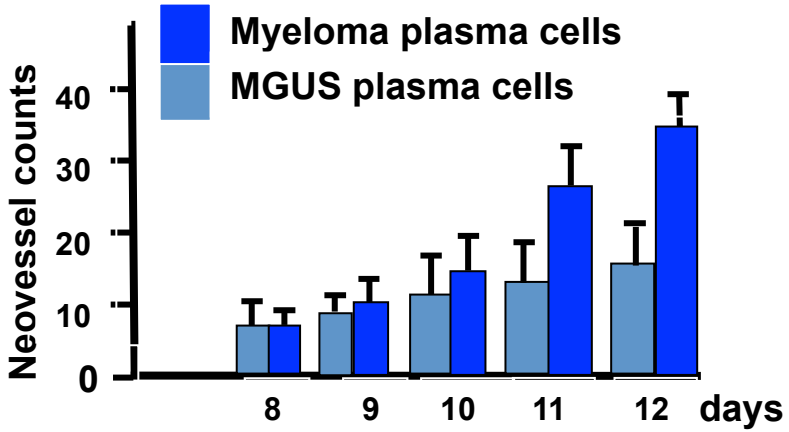


Vacca et al, Br J Haematol 1994

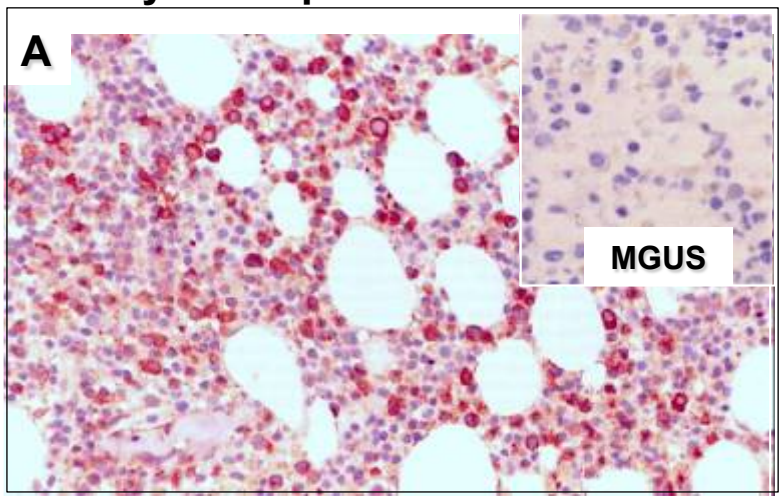
Time-course of angiogenesis induction by myeloma plasma cells in the *in vivo* CAM-sponge assay

Prof. Domenico Ribatti





VEGF-A+ myeloma plasma cells



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

Prof. C. Martelli and his group, Perugia

Università degli Studi di Perugia

Comitato per la Vita "Daniele Chianelli"

A.O. Perugia

PREMIO ANTONIO TABILIO

dedicato alla produzione scientifica di un giovane ricercatore in campo ematologico

THE JOURNAL OF BIOLOGICAL CHEMISTRY
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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‡¶‖

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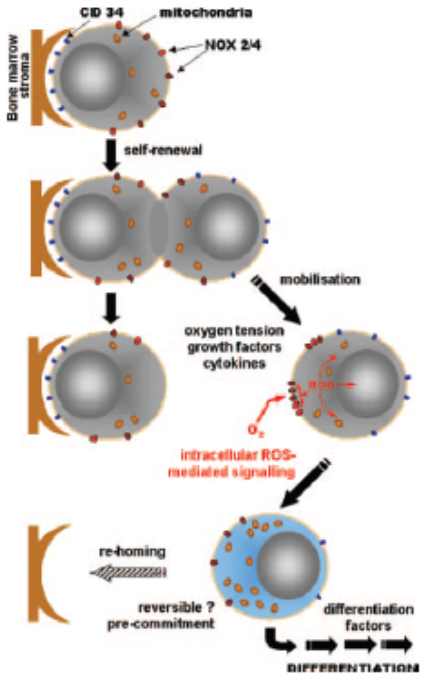
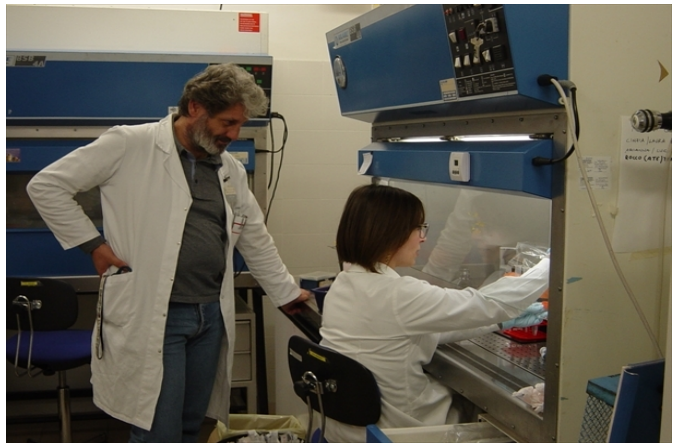
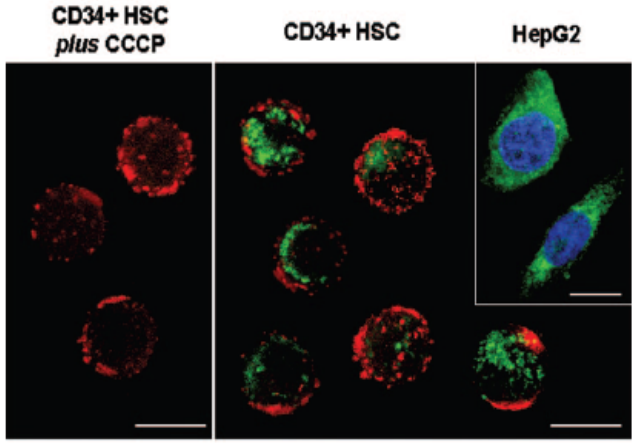
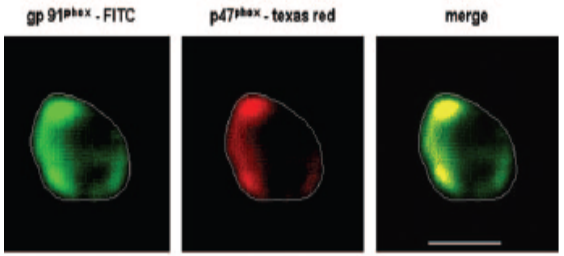


Prof. R. Ria, Dr. A. Ferrucci, my lab

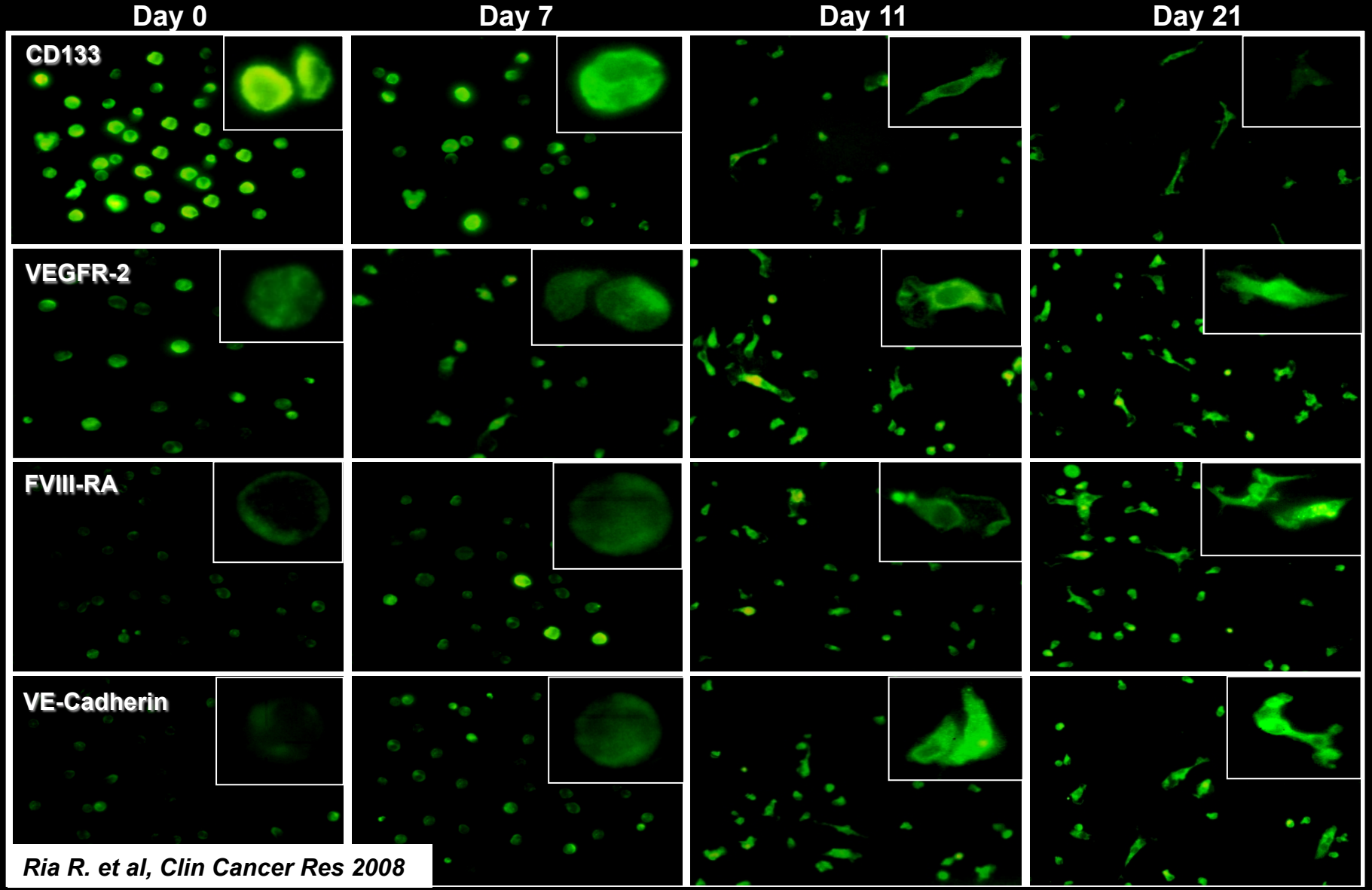






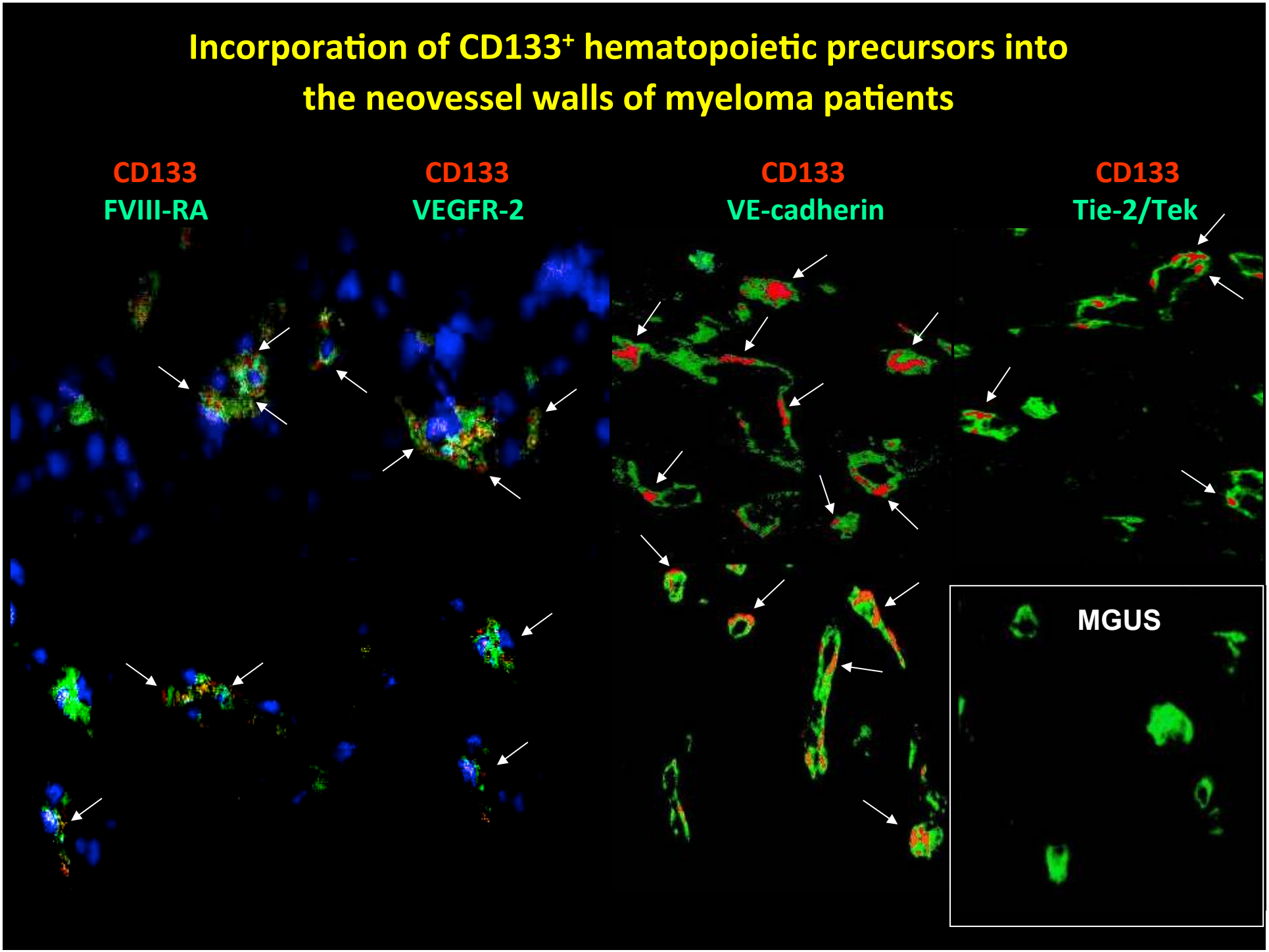
Vasculogenesis in patients with multiple myeloma: differentiation of mobilized $CD34^{+}CD133^{+}$ hematopoietic precursors into mature endothelial cells

VEGF + FGF-2 + IGF on fibronectin



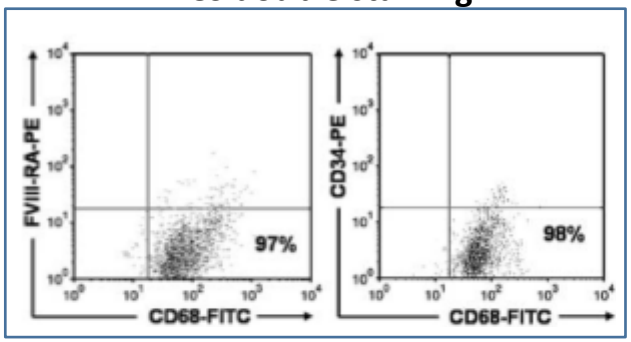
Ria R. et al, Clin Cancer Res 2008

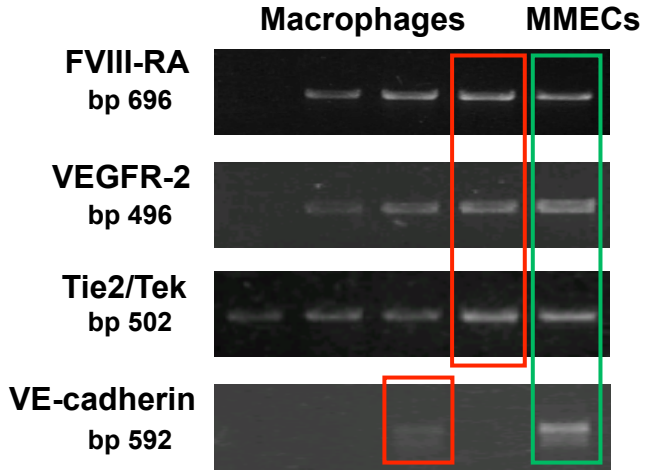
Incorporation of CD133^{+} hematopoietic precursors into the neovessel walls of myeloma patients



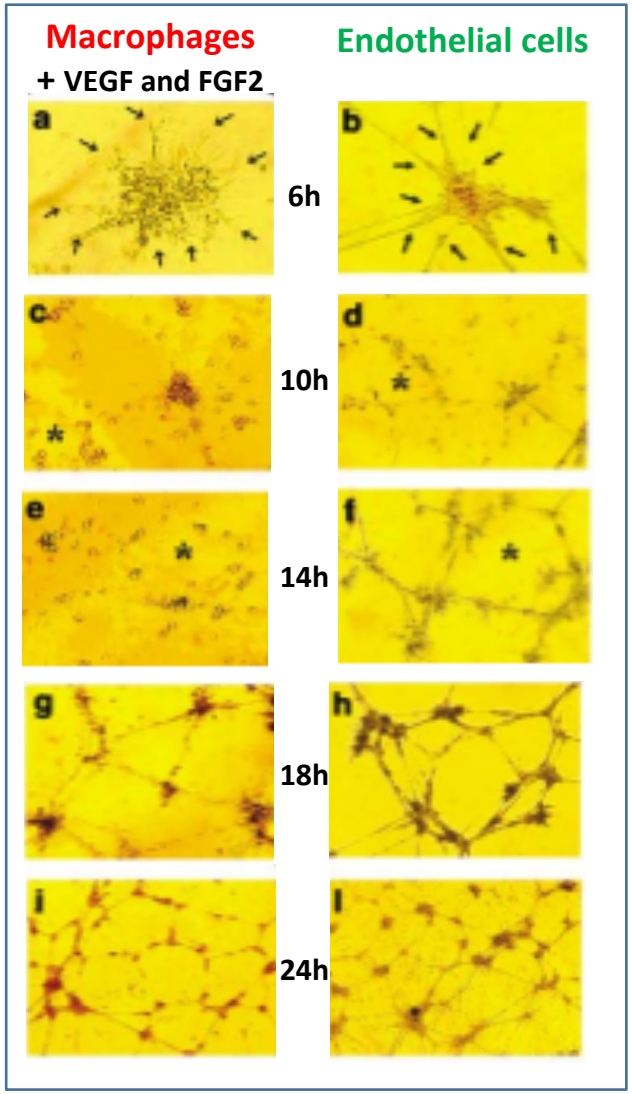
Tumor associated macrophages in multiple myeloma mimic endothelial cells

FVIII-RA/ CD68 or CD34/ CD68
FACS double staining:





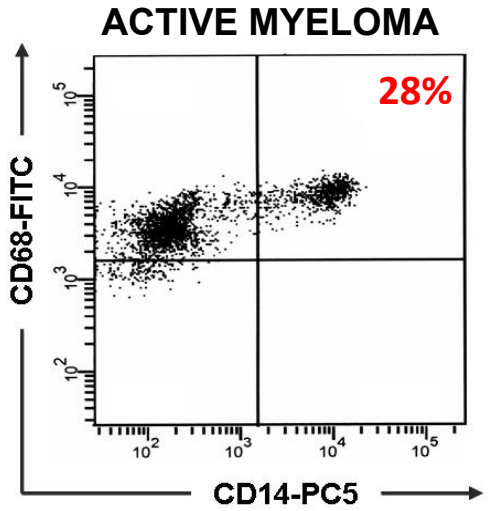
Capillarogenic activity of macrophages and endothelial cells

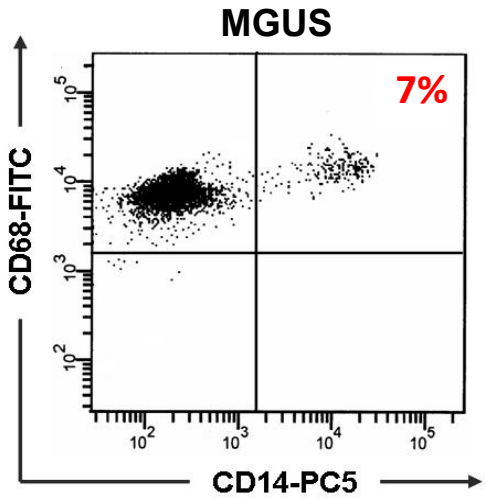


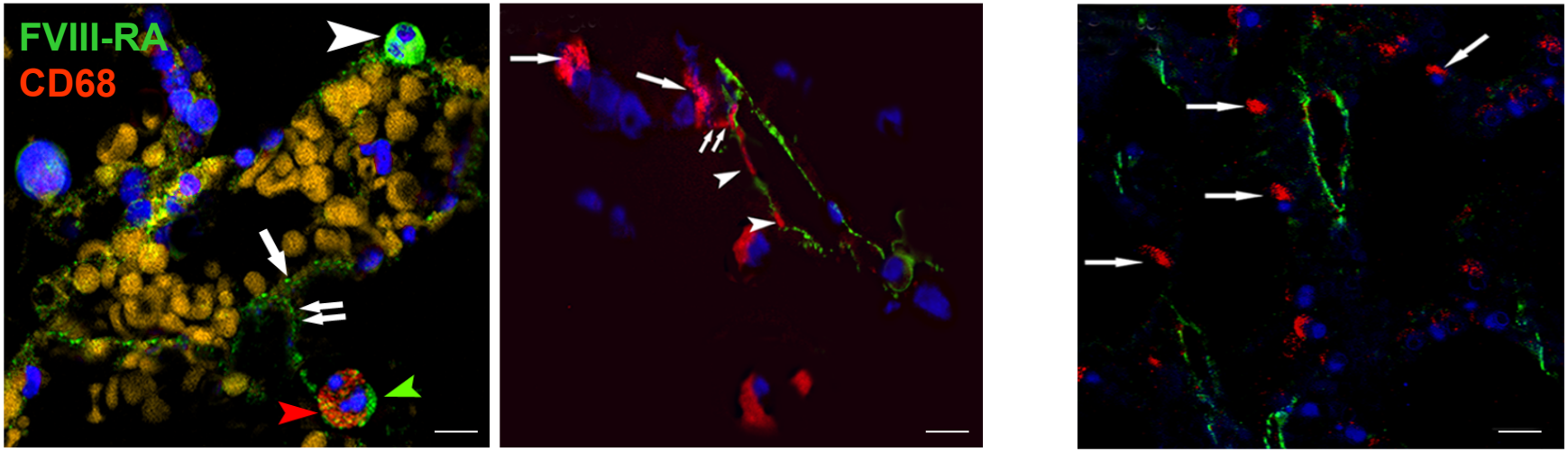
MACROPHAGES CONTRIBUTE TO BUILD NEOVESSELS IN ACTIVE MM THROUGH VASCULOGENIC MIMICRY

Scavelli et al, Oncogene 2008

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

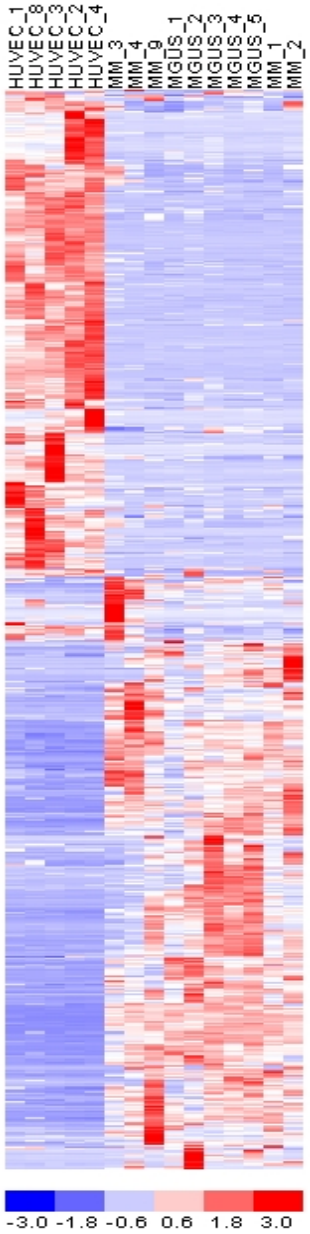




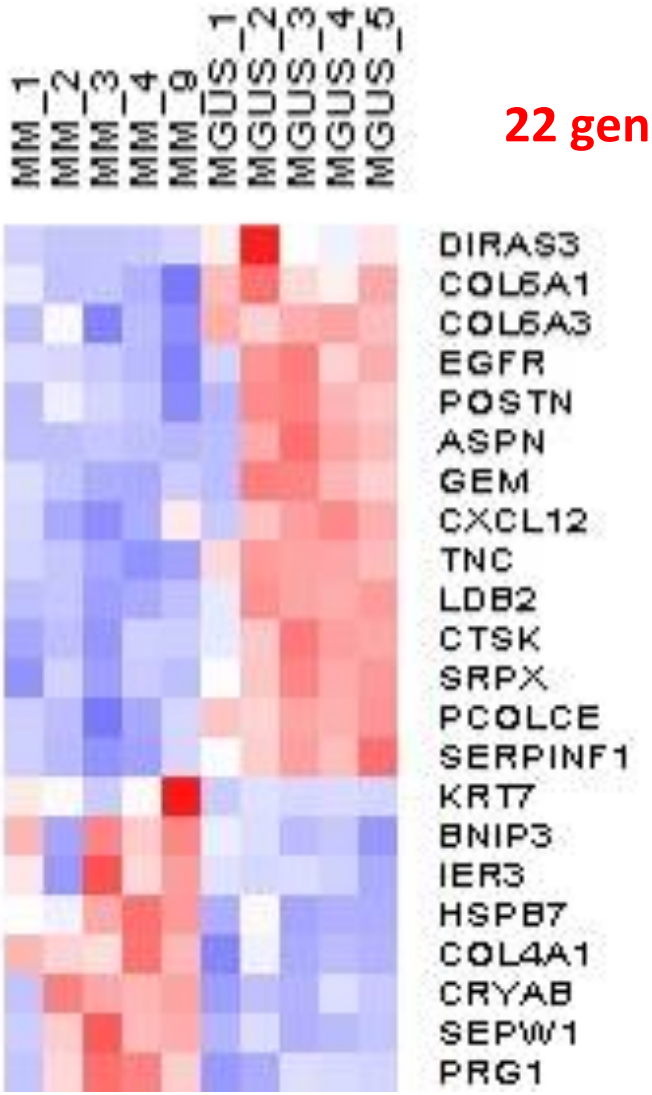


EC-LIKE MACROPHAGES AND MACROPHAGES FORM 'MOSAIC' VESSELS IN BONE MARROW OF PATIENTS WITH ACTIVE MYELOMA BUT NOT IN THOSE WITH MGUS

Unsupervised analysis
MMECs *vs* MGECs



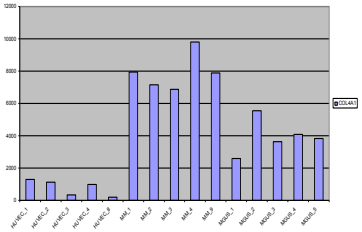
Supervised analysis
MMECs *vs* MGECs



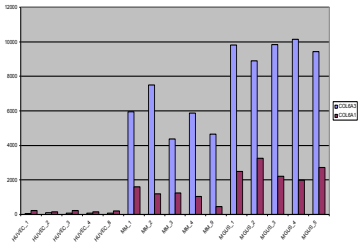
Searching genes specifically distinguishing MM *vs* MGUS endothelial cells

22 genes

down

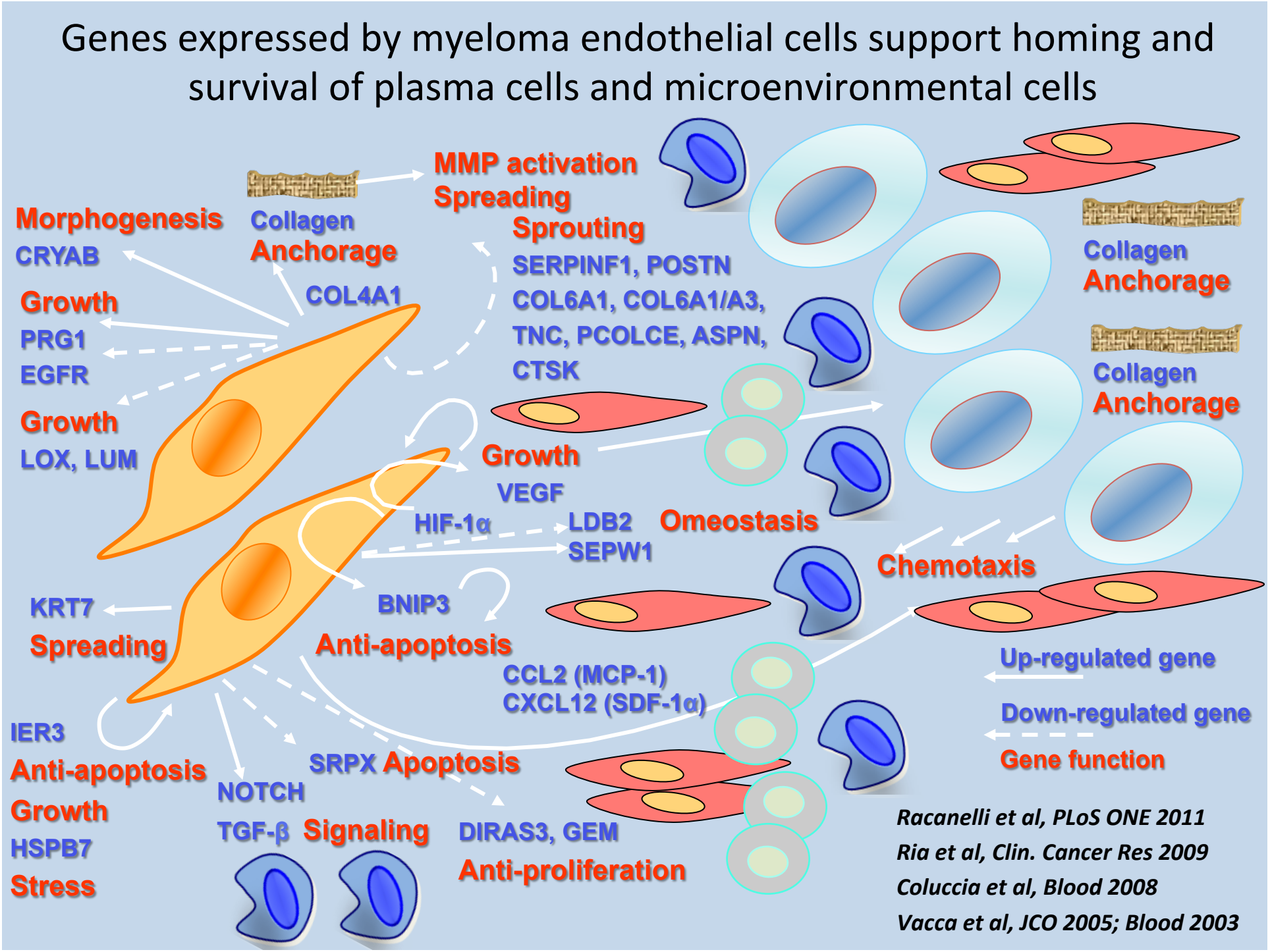


up



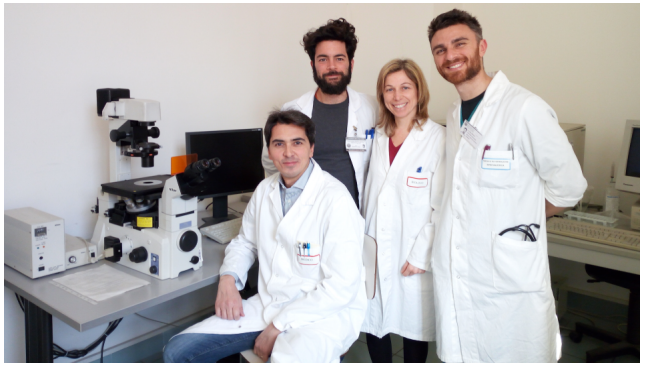
Ria et al, Clin. Cancer Res. 2009

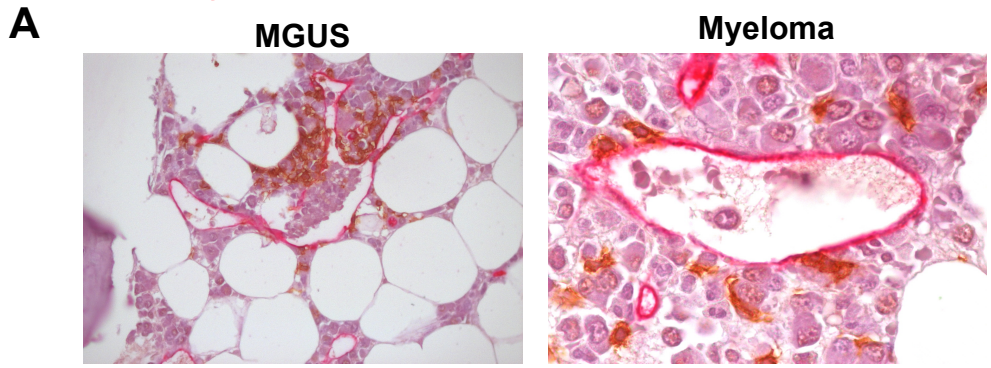
Genes expressed by myeloma endothelial cells support homing and survival of plasma cells and microenvironmental cells

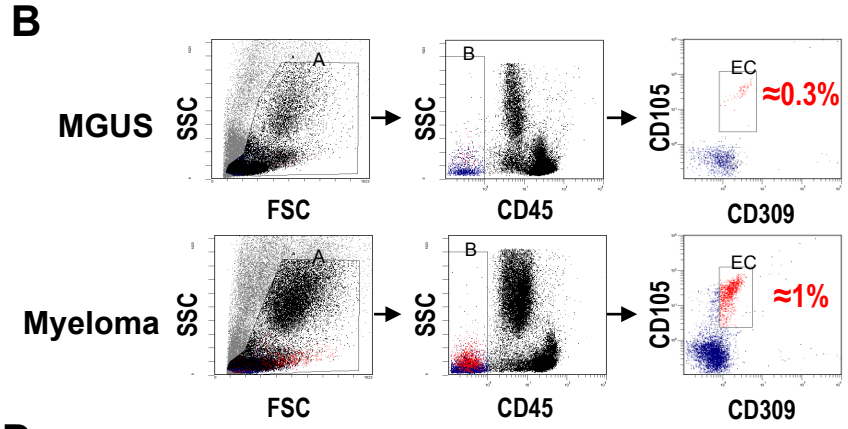


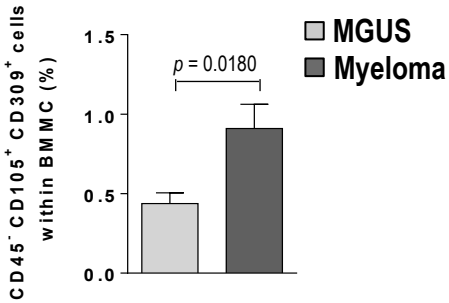
Interactions between endothelial cells and T cells in myeloma microenvironment

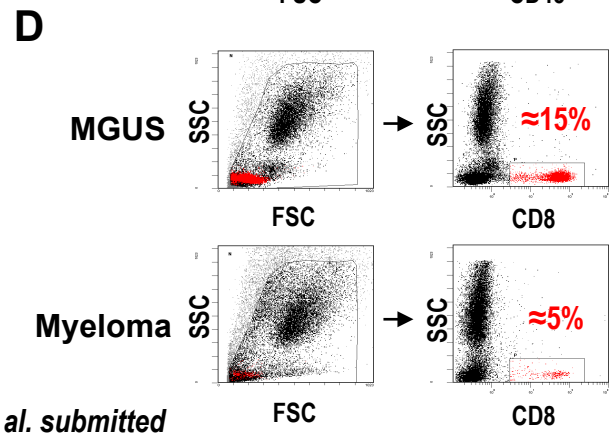
Prof. V. Racanelli, Drs. A. Solimando, P. Leone, G. Di Lernia, my lab

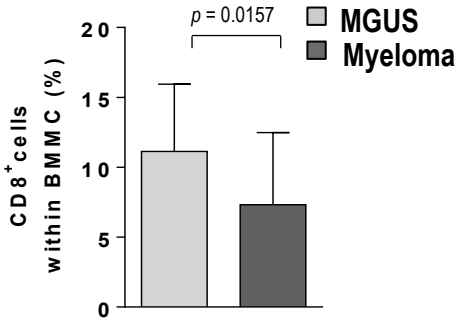






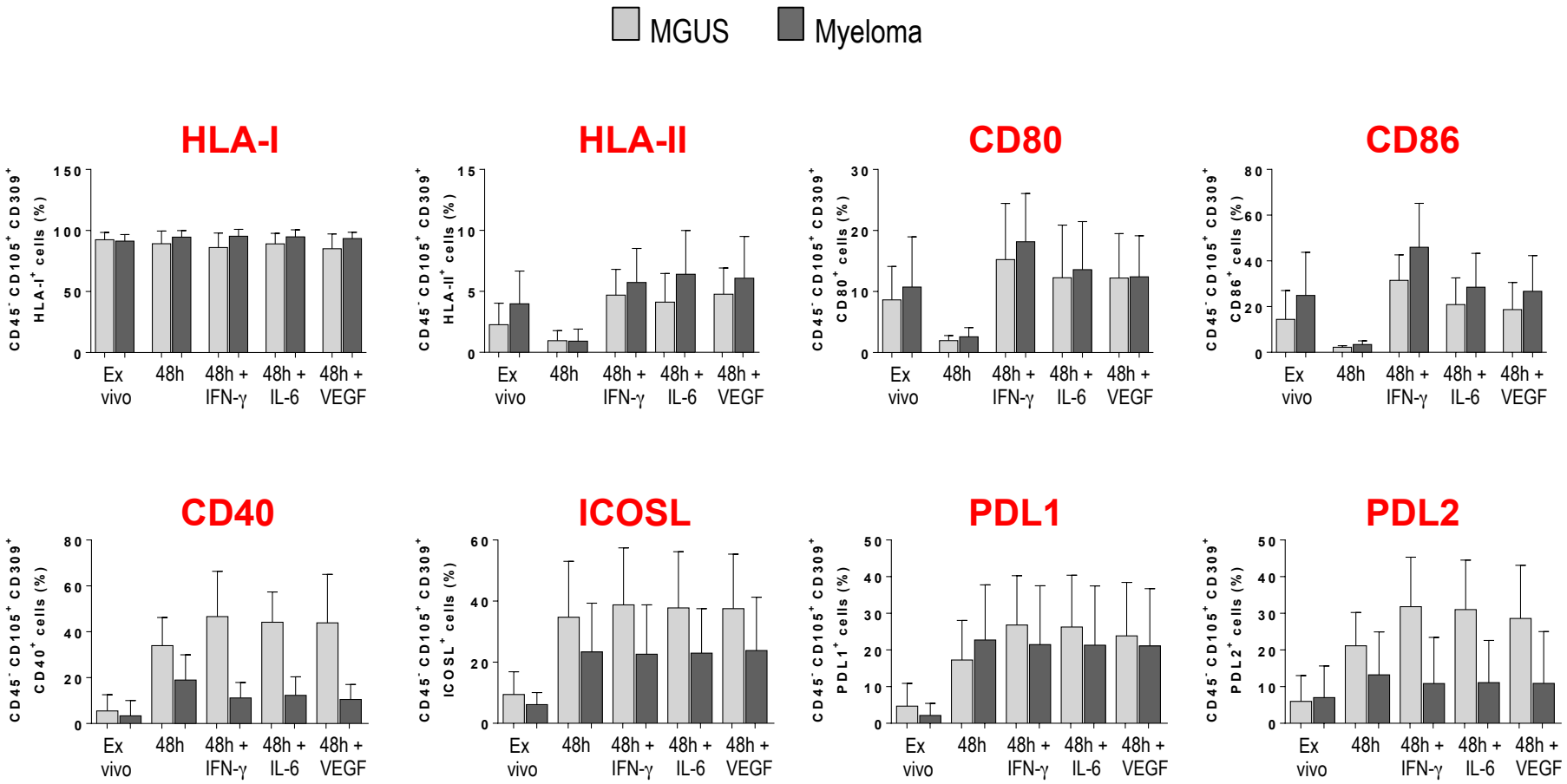




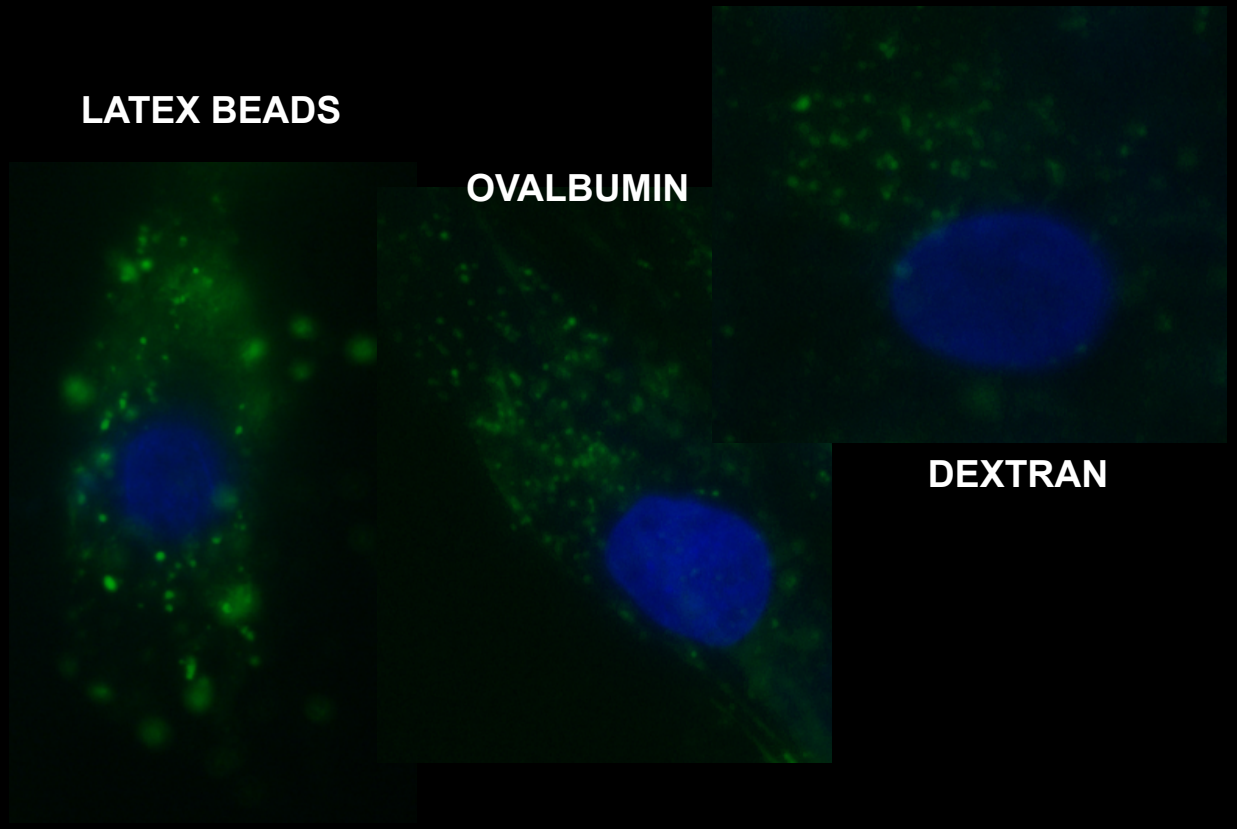


Racanelli et al. submitted

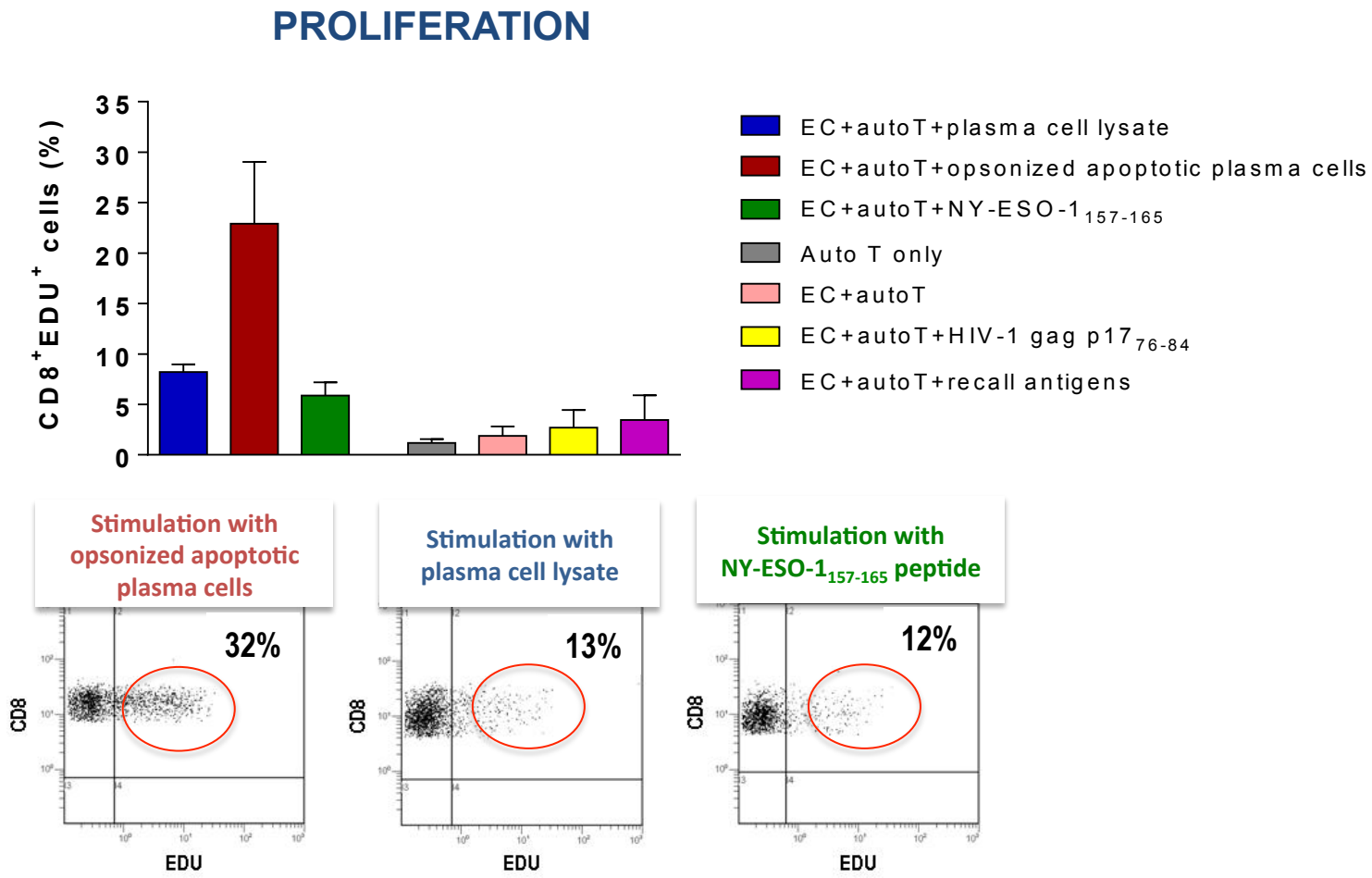
Phenotype of bone marrow endothelial cells in active myeloma



Antigen uptake by myeloma endothelial cells



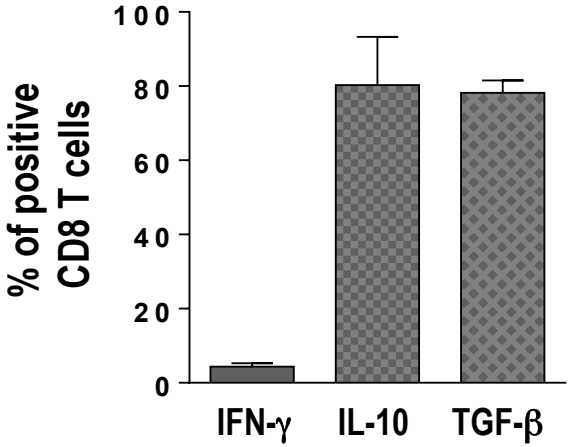
Capacity of bone marrow endothelial cells to stimulate autologous $CD8^+$ T cells (from bone marrow) (1)

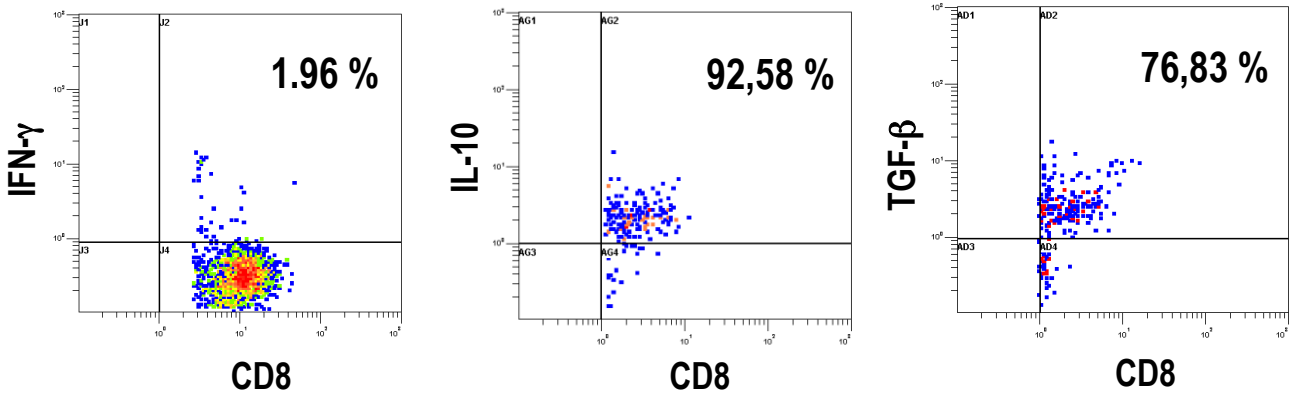


Capacity of bone marrow endothelial cells to stimulate autologous $CD8^+$ T cells (from bone marrow) (2)

Autologous CD8 T cells +
opsonized apoptotic plasma cells + endothelial cells

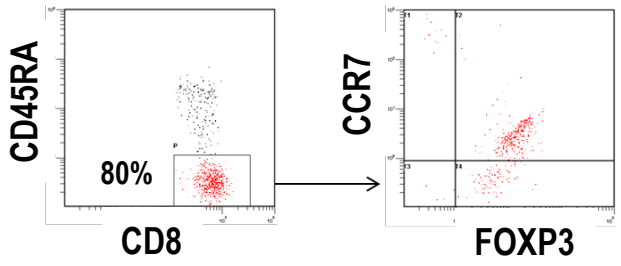
CYTOKINE PRODUCTION



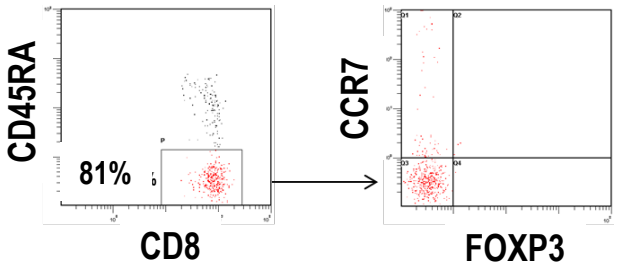


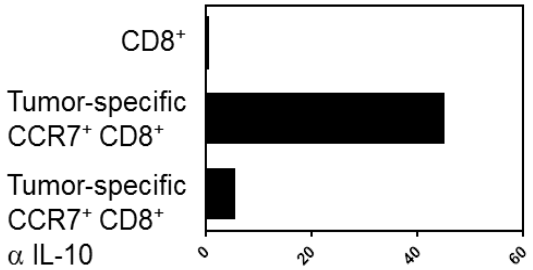
Antigen-specific suppressor capacity of endothelial cell-reactive $CCR7^{+}CD8^{+}$ T cells (4 experiments)

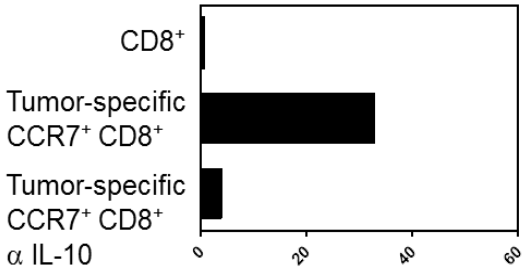
Stimulated with endothelial cells

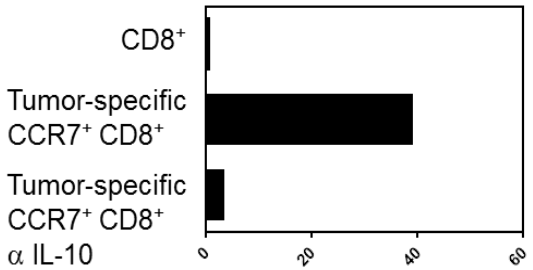


Stimulated with dendritic cells

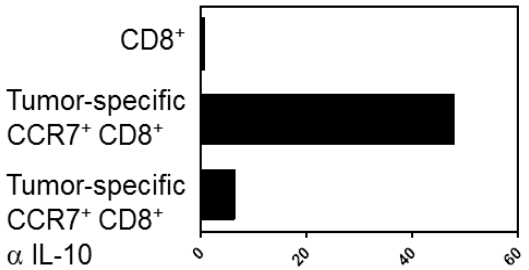








% inhibition tumor-specific $CCR7^{-}CD8^{+}$ T cell proliferation



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

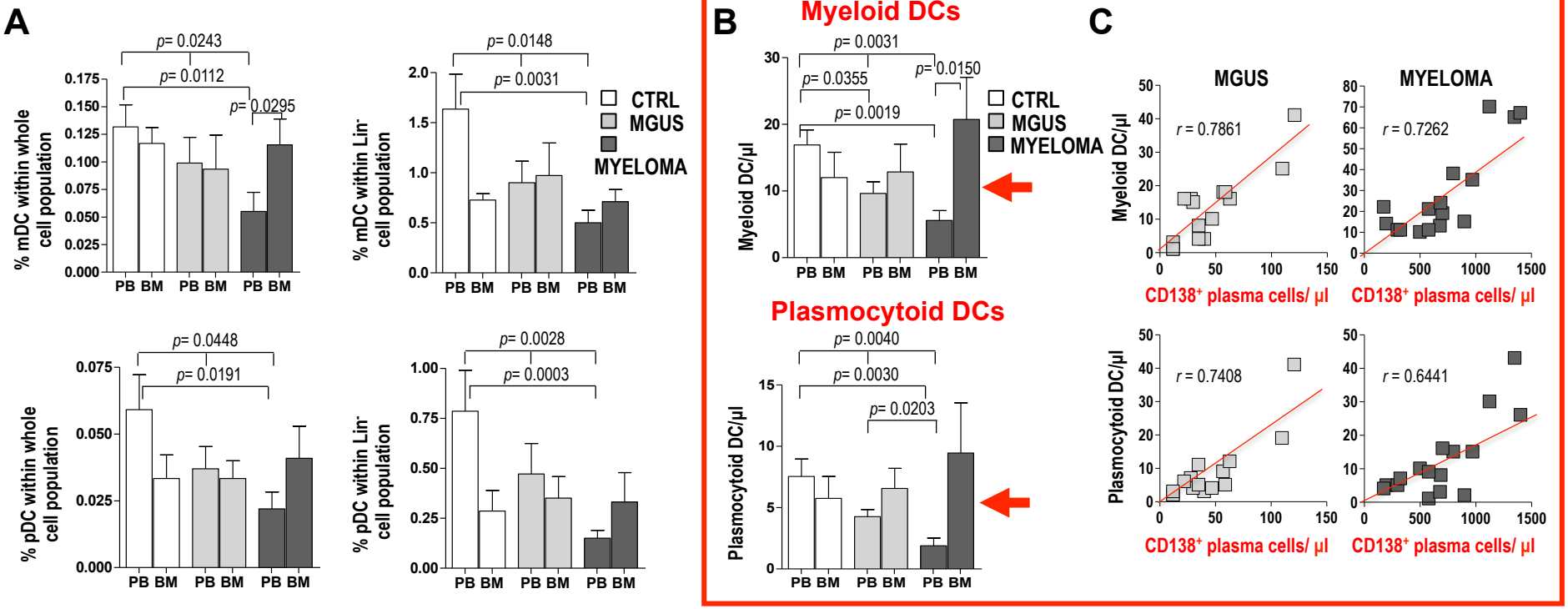
CONCLUSIONS

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

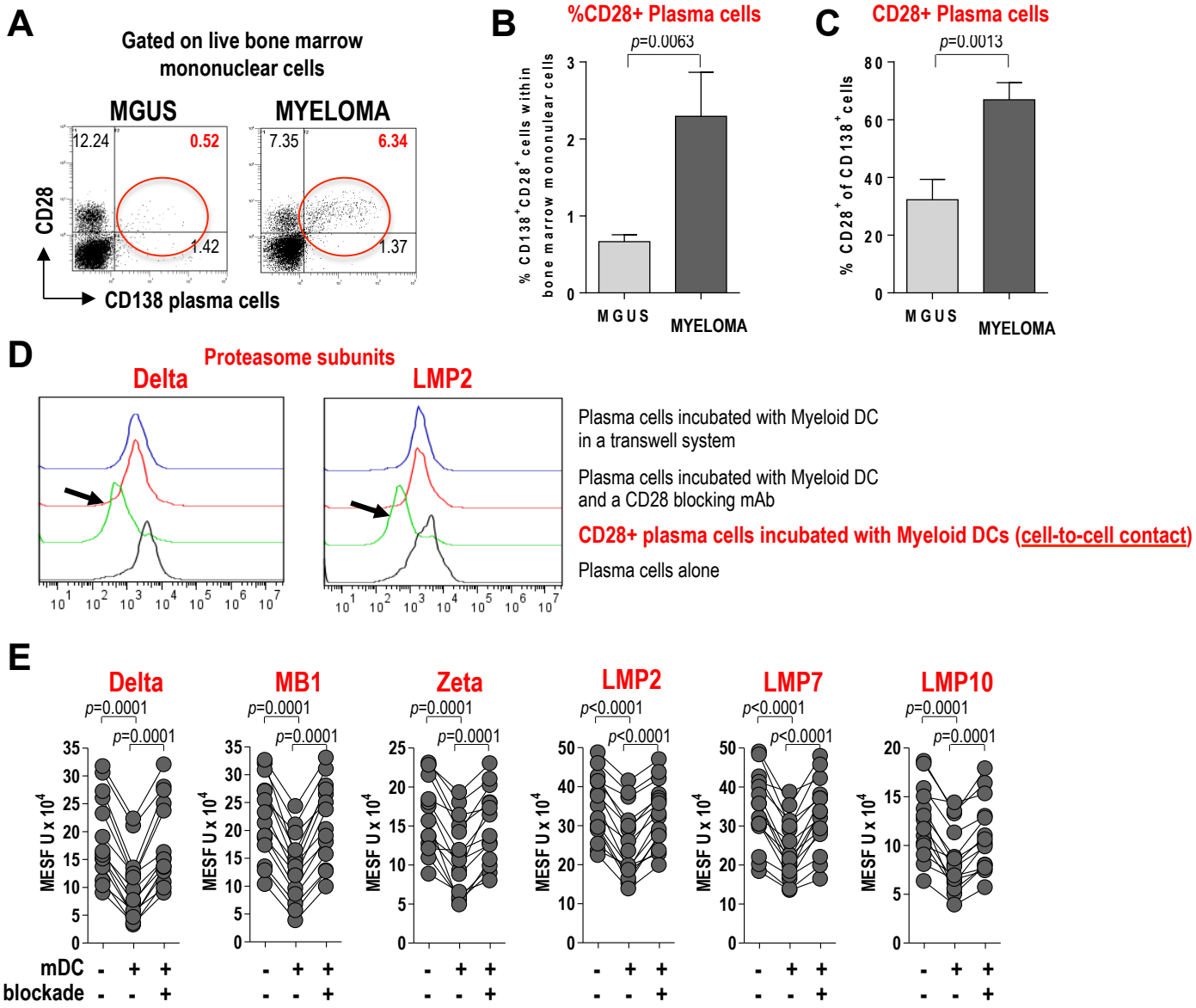
Frequency of DCs in whole blood and marrow samples

Dr. P. Leone, my lab

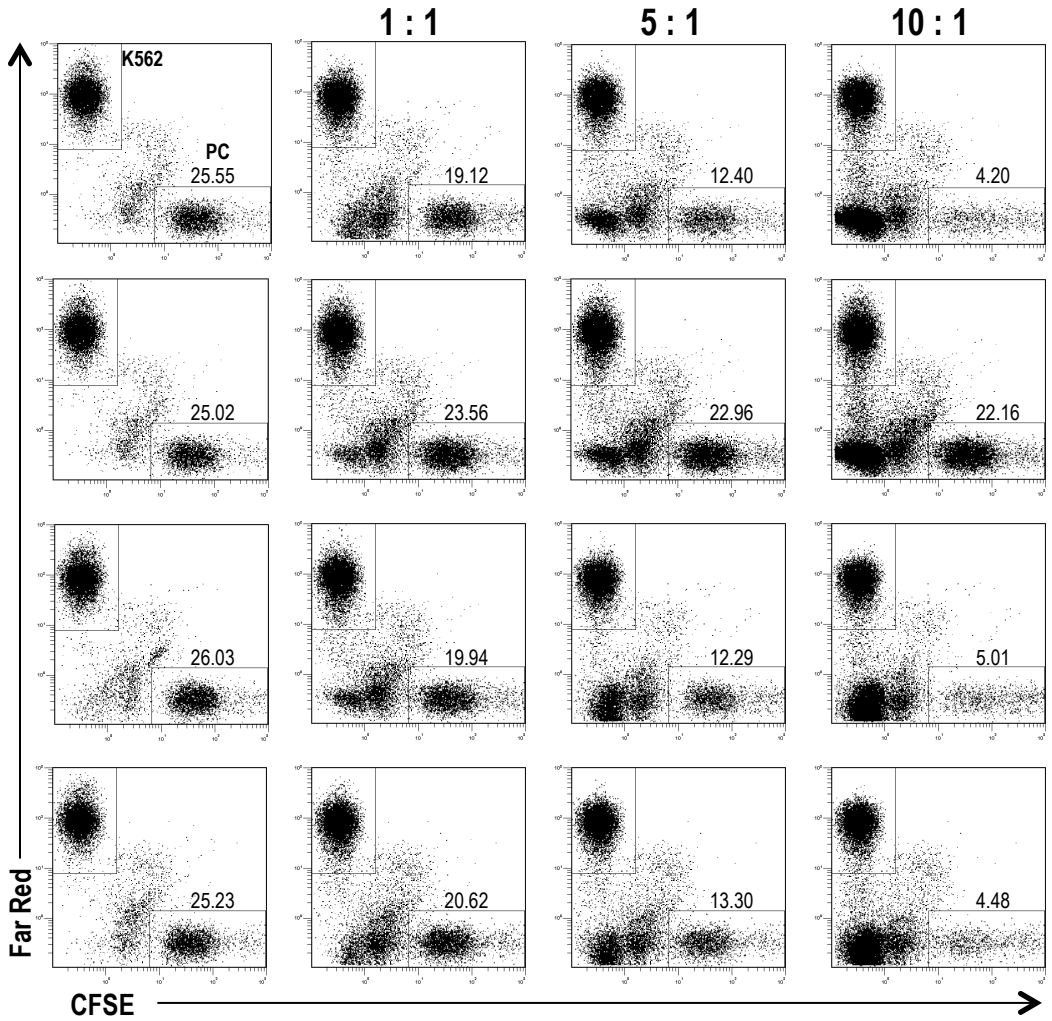


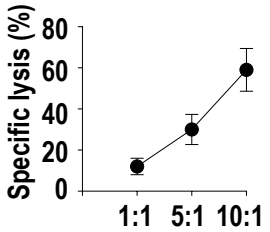
Leone et al, Blood 2015

CD28+ plasma cells and their T cell evasion

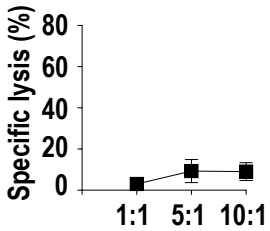


Effect of DCs on tumor plasma cell susceptibility to CD8^{+} T cell–mediated killing

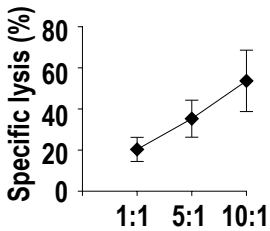




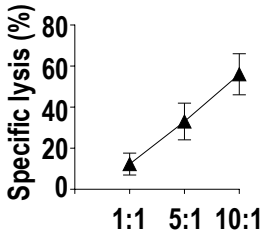
No Plasma cells pre-incubation



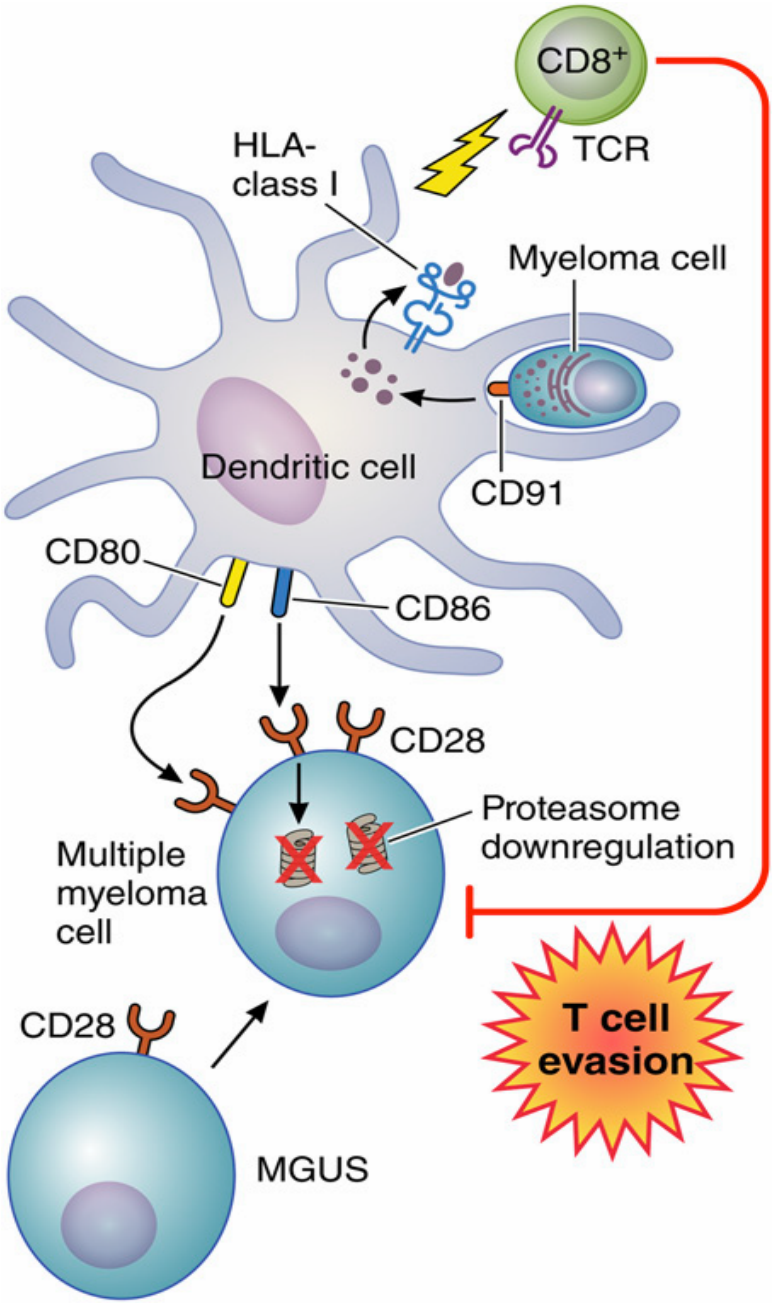
CD28+ plasma cells pre-incubation with Myeloid DCs (cell-to-cell contact)



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



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



Comment on Leone et al, page 1443

Myeloma escape from immunity: an “inside” job

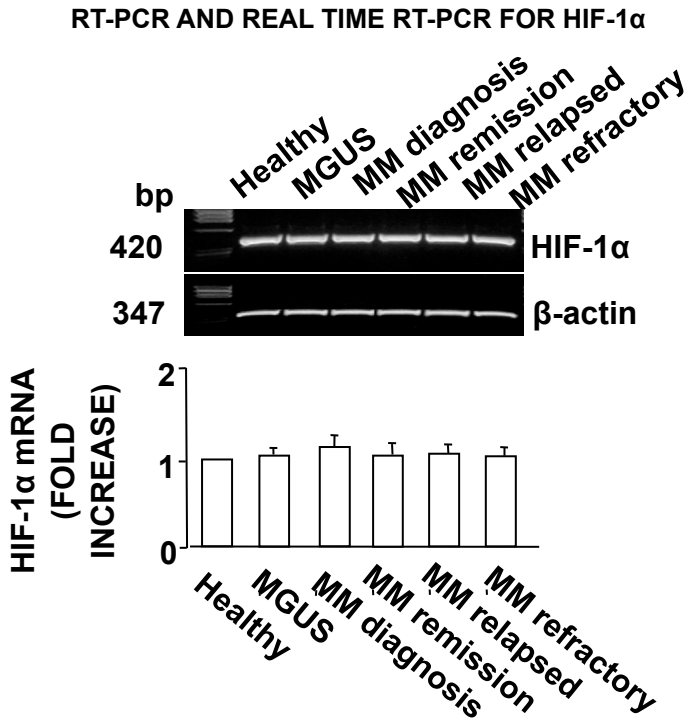
Aaron P. Rapoport UNIVERSITY OF MARYLAND SCHOOL OF MEDICINE

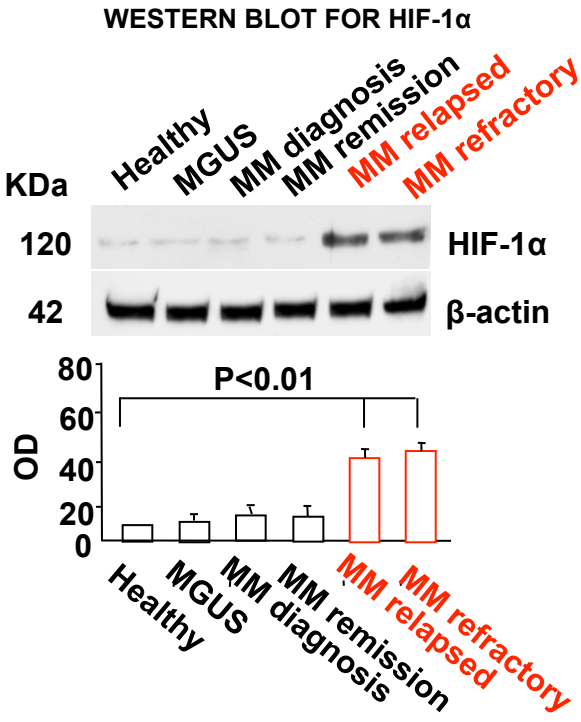
In this issue of Blood, Leone et al describe a novel mechanism mediated by bone marrow dendritic cells (DCs) that impairs T-cell recognition and killing of myeloma cells.

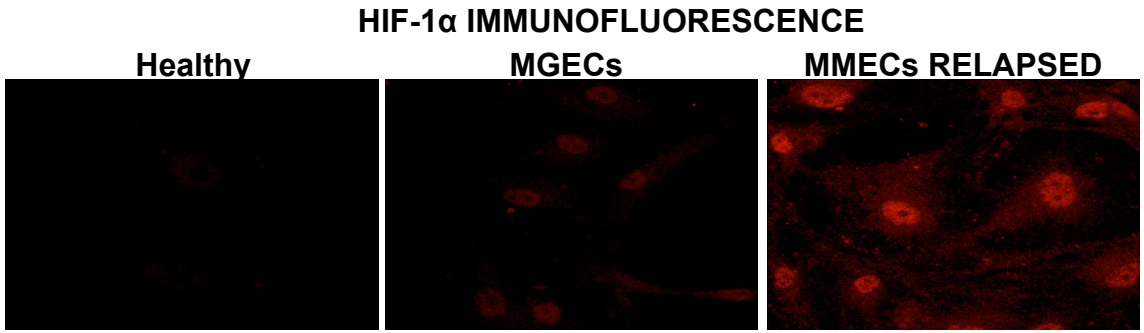
DCs protect tumor plasma cells from CD8+ T cell killing

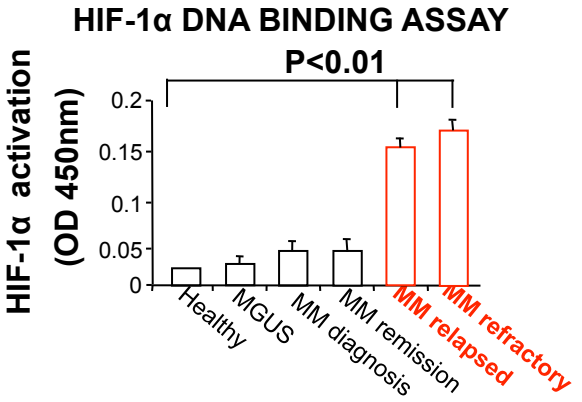
Leone et al., Blood. 2015 Sep 17; 126(12):1443-51. Epub 2015 Jul 16.

Normoxic expression and activation of HIF-1α in myeloma endothelial cells correlates with progression steps



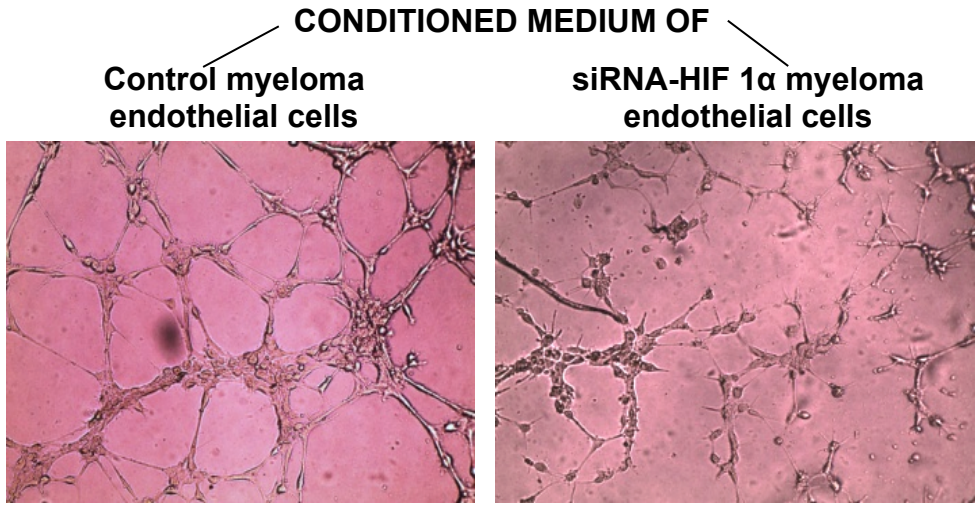


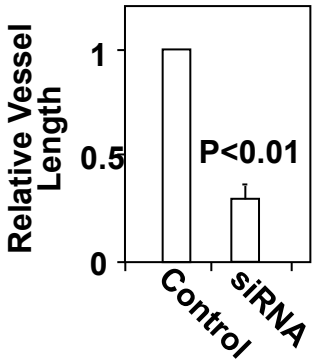


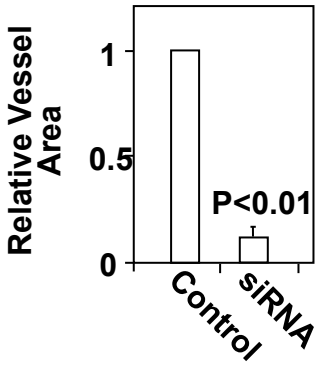


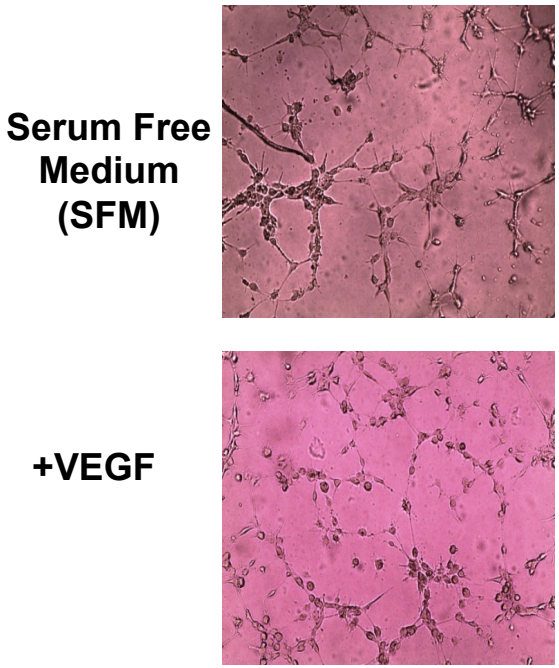
Ria et al. Clin. Cancer Res., 2014

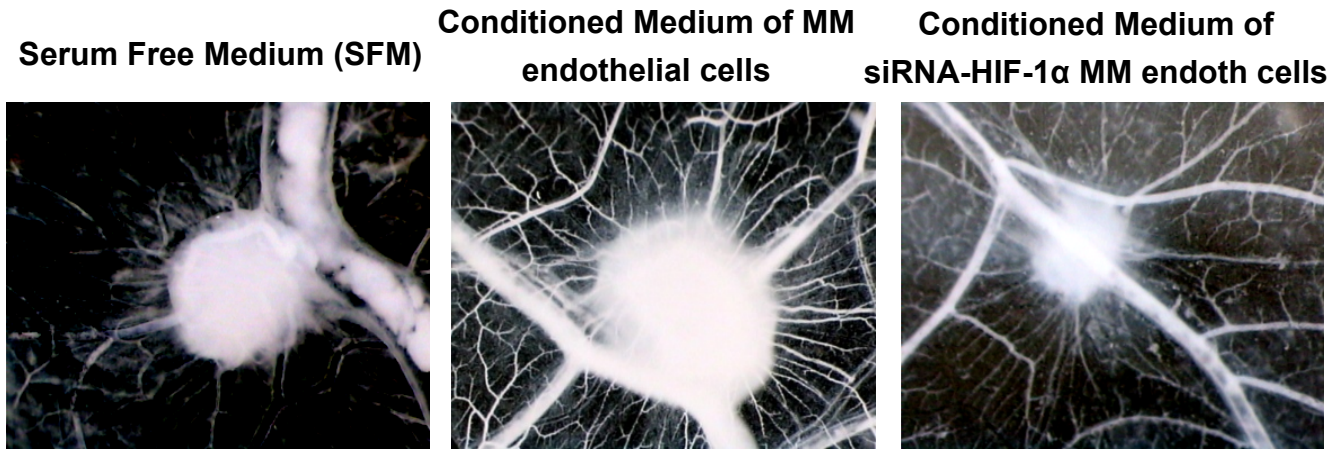
HIF-1α knockdown impairs angiogenic myeloma endothelial cells in relapsed and resistant patients both *in vitro* and *in vivo*



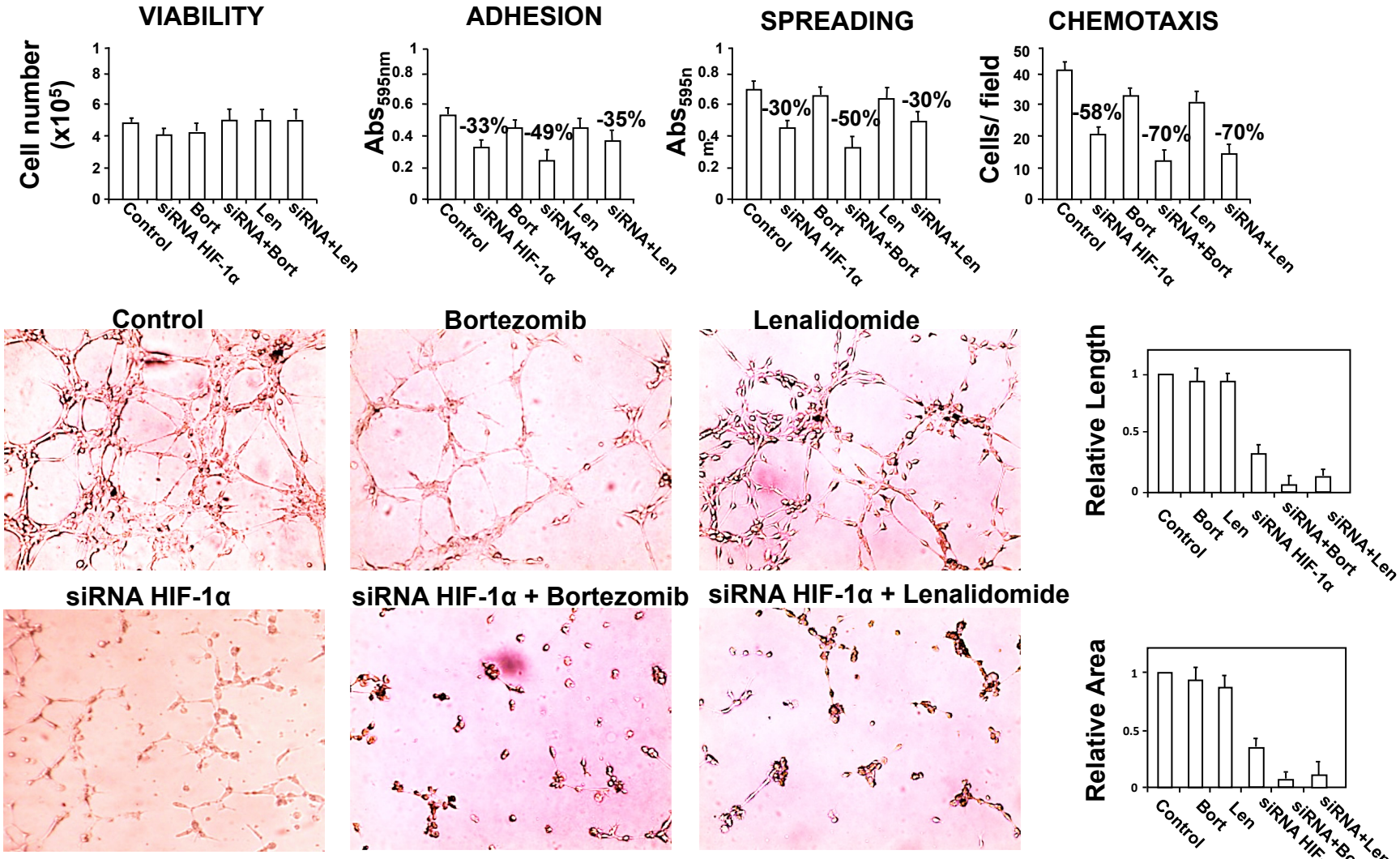








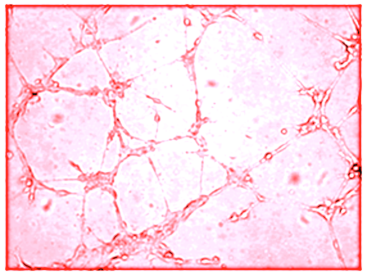
Restoring sensitivity to bortezomib and lenalidomide by HIF-1α knockdown



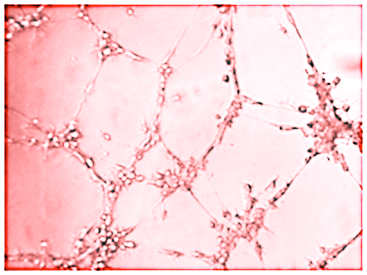
Ria et al., Clin. Cancer Res. 2014

Restoring sensitivity to antiangiogenic drugs by HIF-1α inhibition

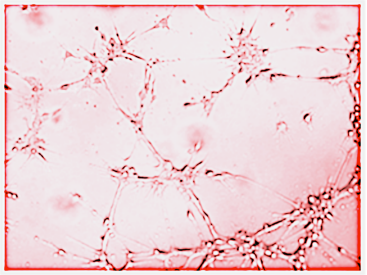
Control



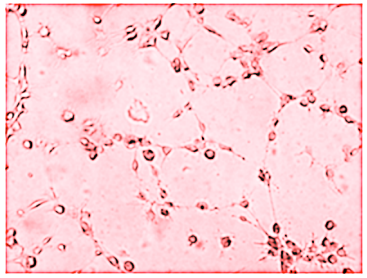
+ Lenalidomide



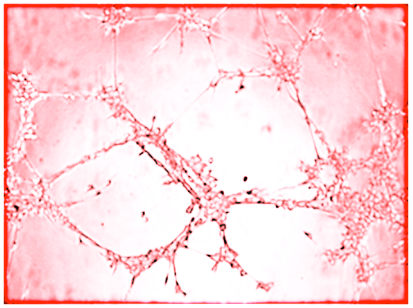
+ Thalidomide



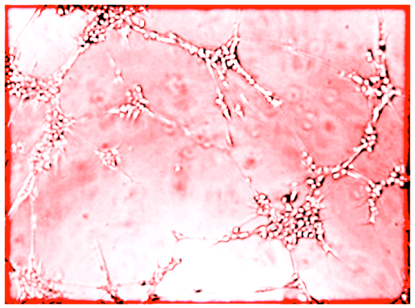
+ Bortezomib



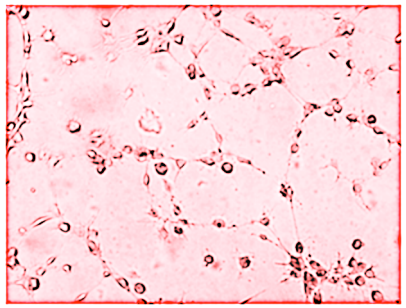
Panobinostat 20nM



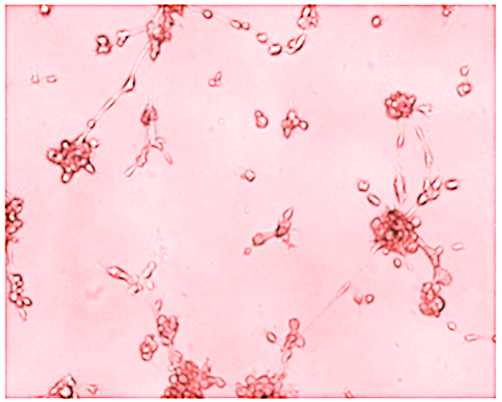
Panobinostat 50nM



Panobinostat 100nM



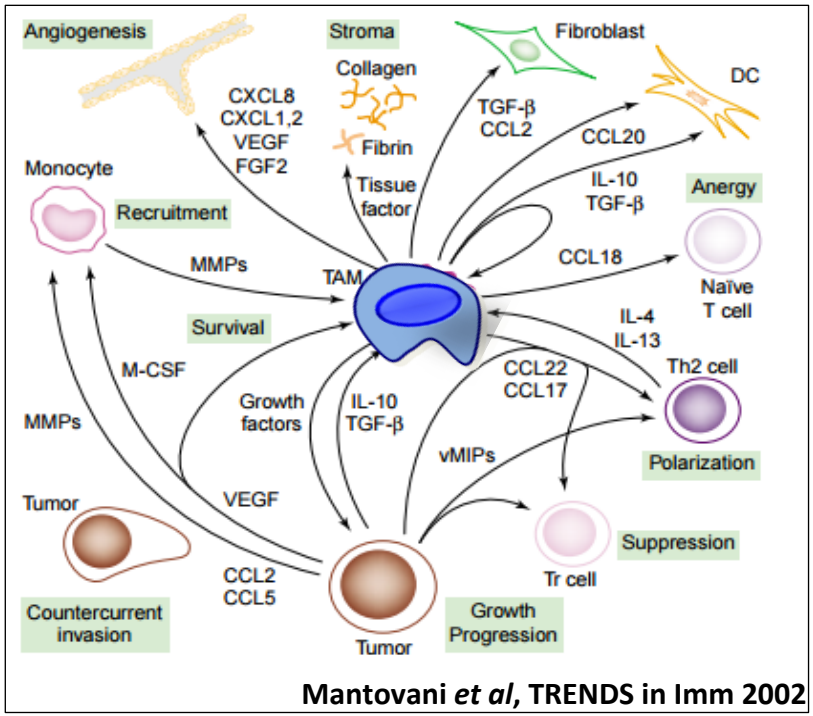
Panobinostat + Bortezomib



PATIENT AT 3rd RELAPSE

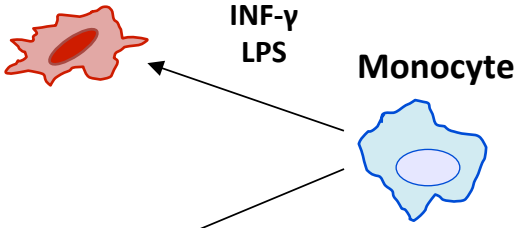
Panobinostat is a Histone Deacetylase Inhibitor (HDACi)

Macrophage polarization in myeloma microenvironment



Mantovani *et al*, TRENDS in Imm 2002

M1 or classic activation



TAM/M2 or alternative activation

POLARIZED M2 CELLS REGULATE TUMOR GROWTH AND PROGRESSION, ADAPTIVE IMMUNITY, STROMA FORMATION AND ANGIOGENESIS

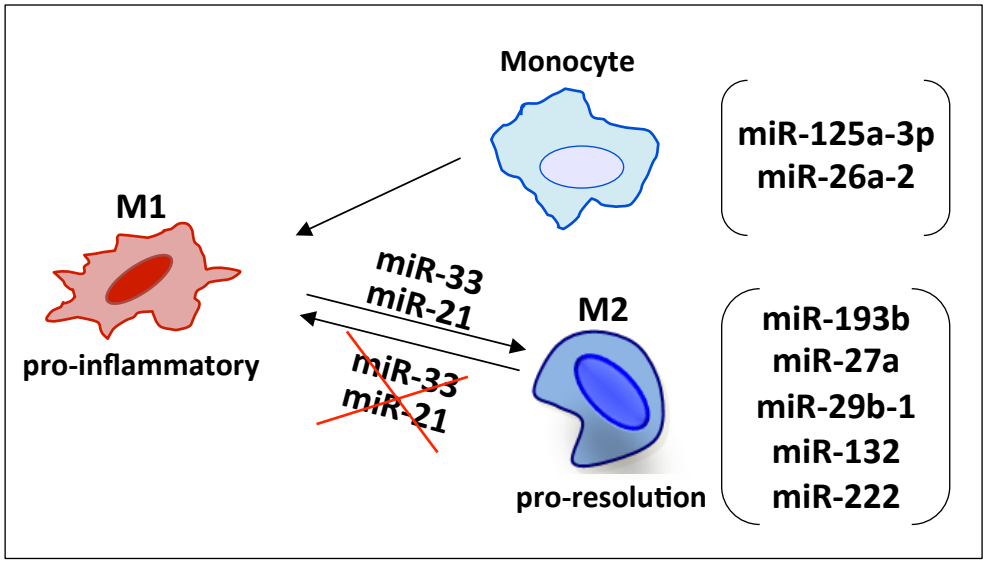
Di Marzo et al., Oncotarget 2016

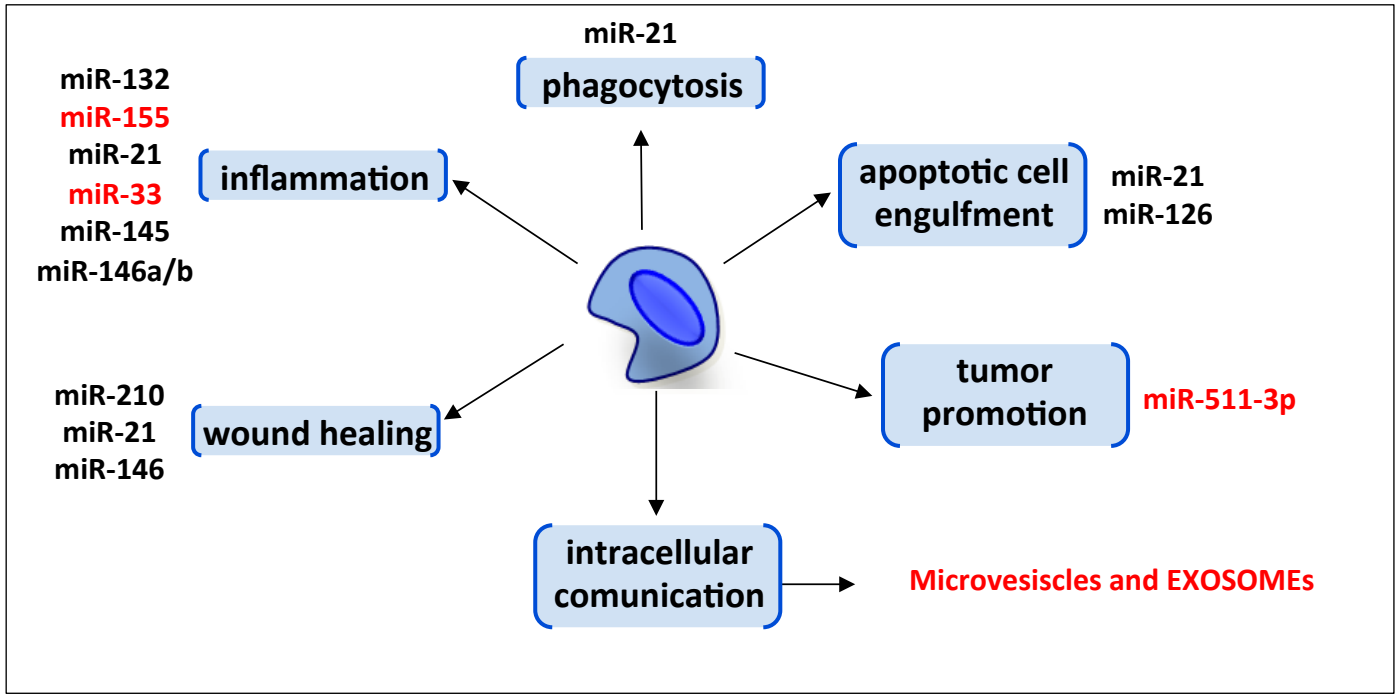
Subtypes	M1	M2
Expression marker	CD-80 CD-86 iNOS TLR-2 TLR-4	CD-163 CD-14 CD-23 MR Scavenger ReceptorA/B
Cytokines	IL-1 IL-6 IL-12 TNF-α	IL-1ra IL-10 IL-13 TGF-β
Chemokines	CCL-10 CCL-11 CCL-5 CCL-8 CCL-9 CCL-2 CCL-3 CCL-4	CCL-17 CCL-22 CCL-24 CCL-16 CCR-2 CCL-18

New players in macrophage polarization and function

miRNA in...

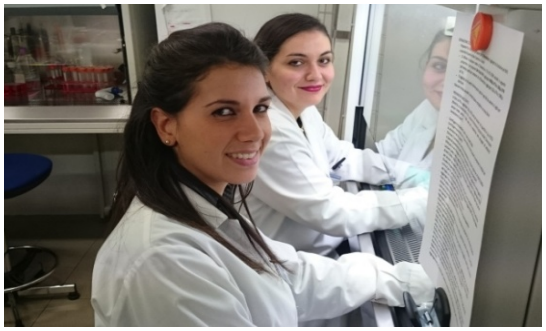
... macrophage polarization and...

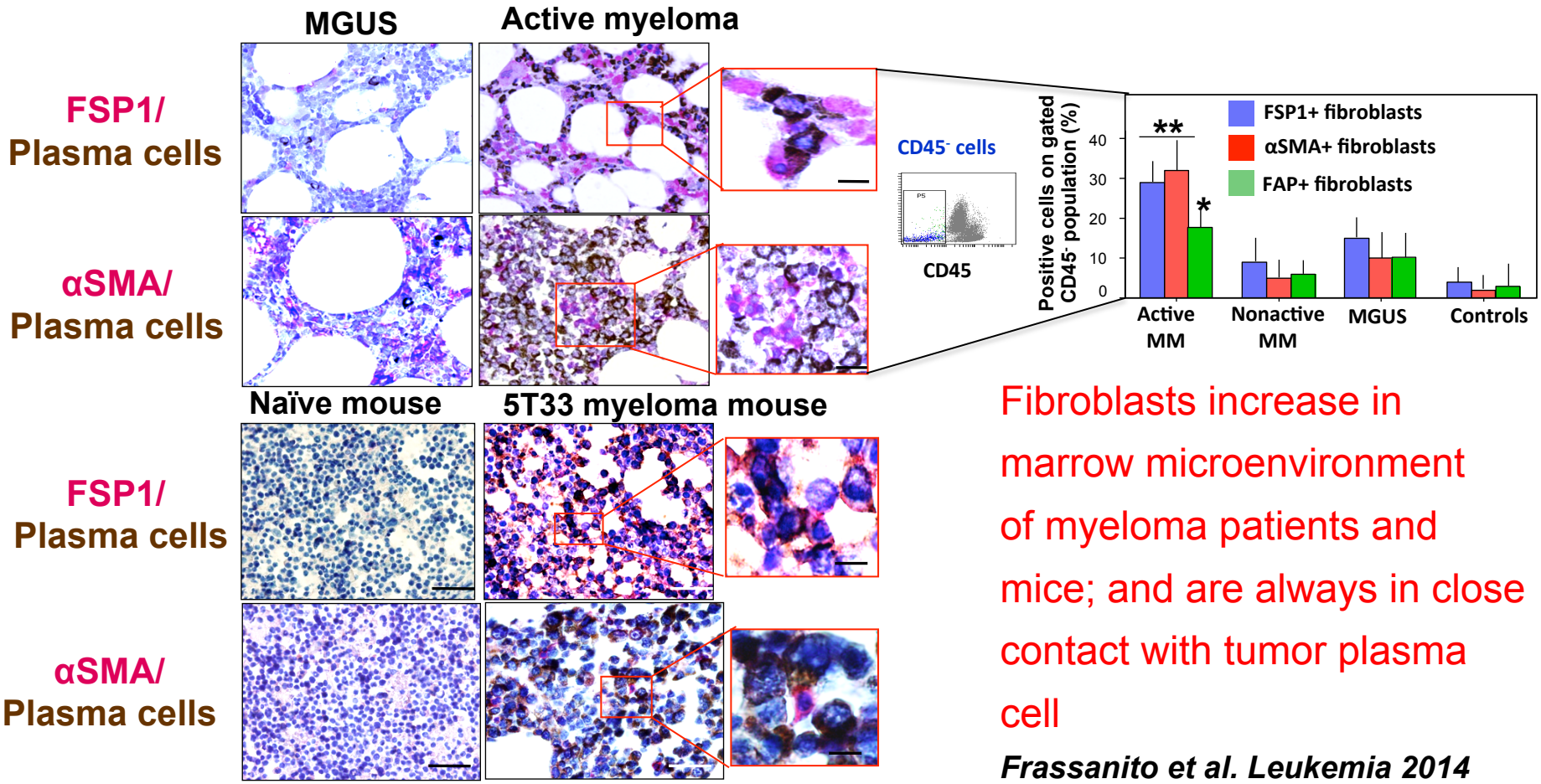




... function

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



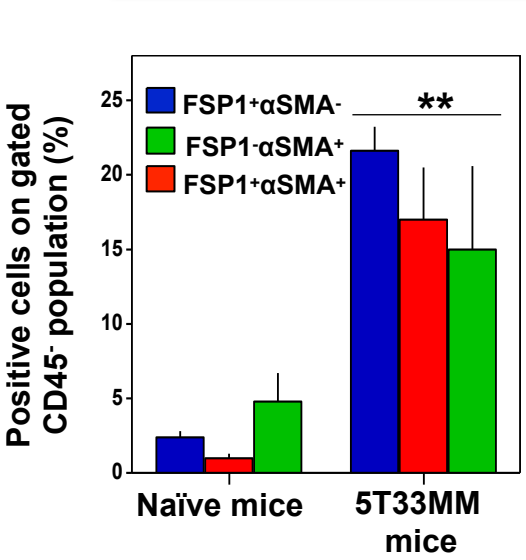


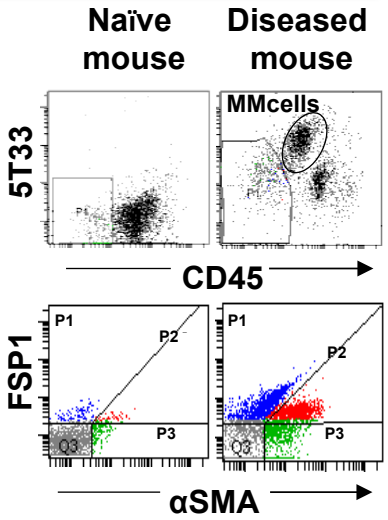
Fibroblasts increase in marrow microenvironment of myeloma patients and mice; and are always in close contact with tumor plasma cell

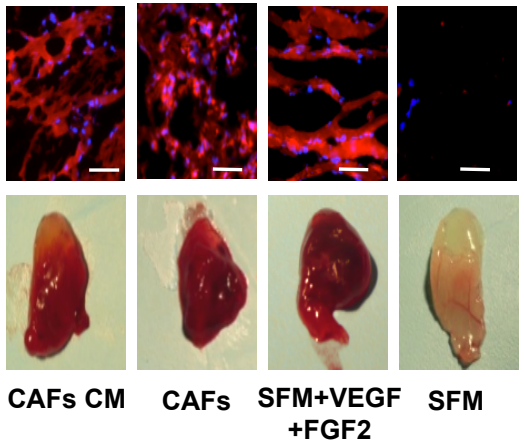
Frassanito et al. Leukemia 2014

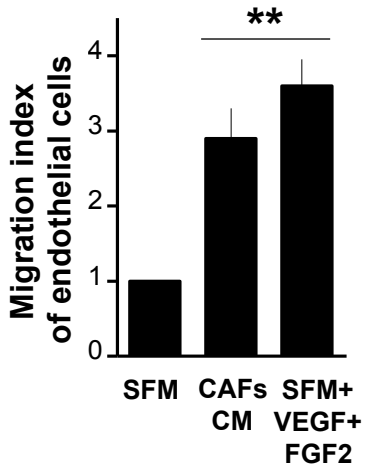
Fibroblasts induce myeloma initiation and progression

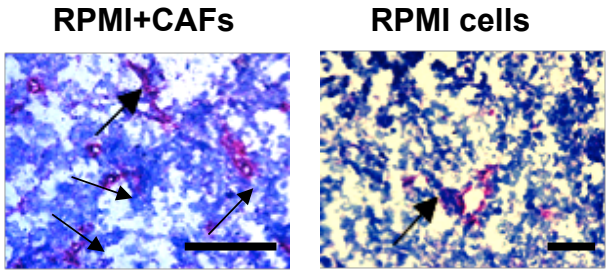
5T33MM mouse



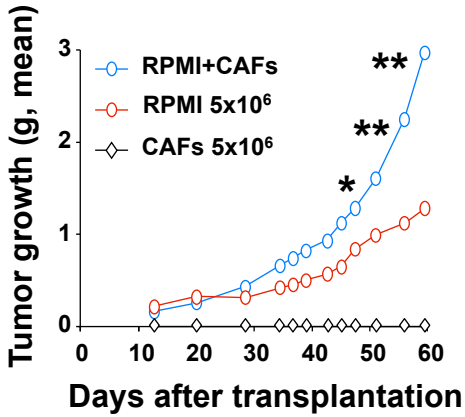


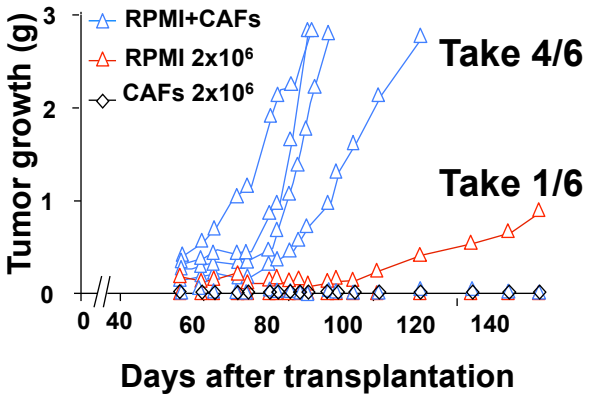


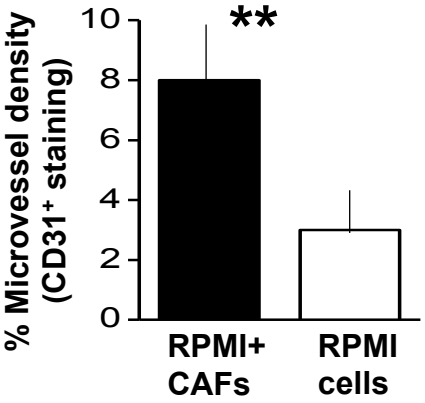




RPMI8226 cells are human myeloma plasma cells

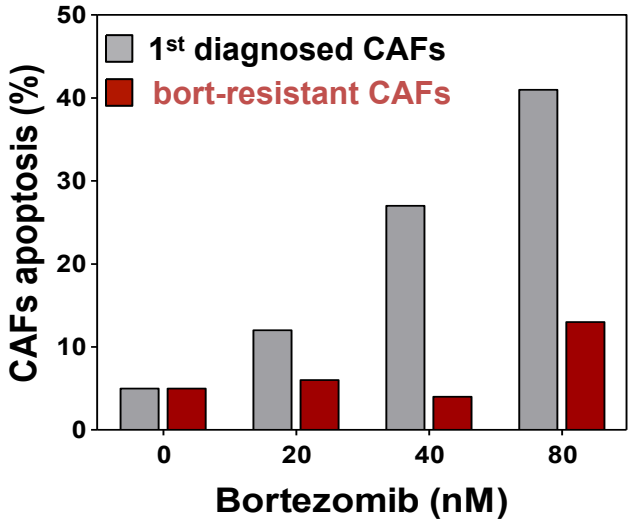






.....and bortezomib-resistance

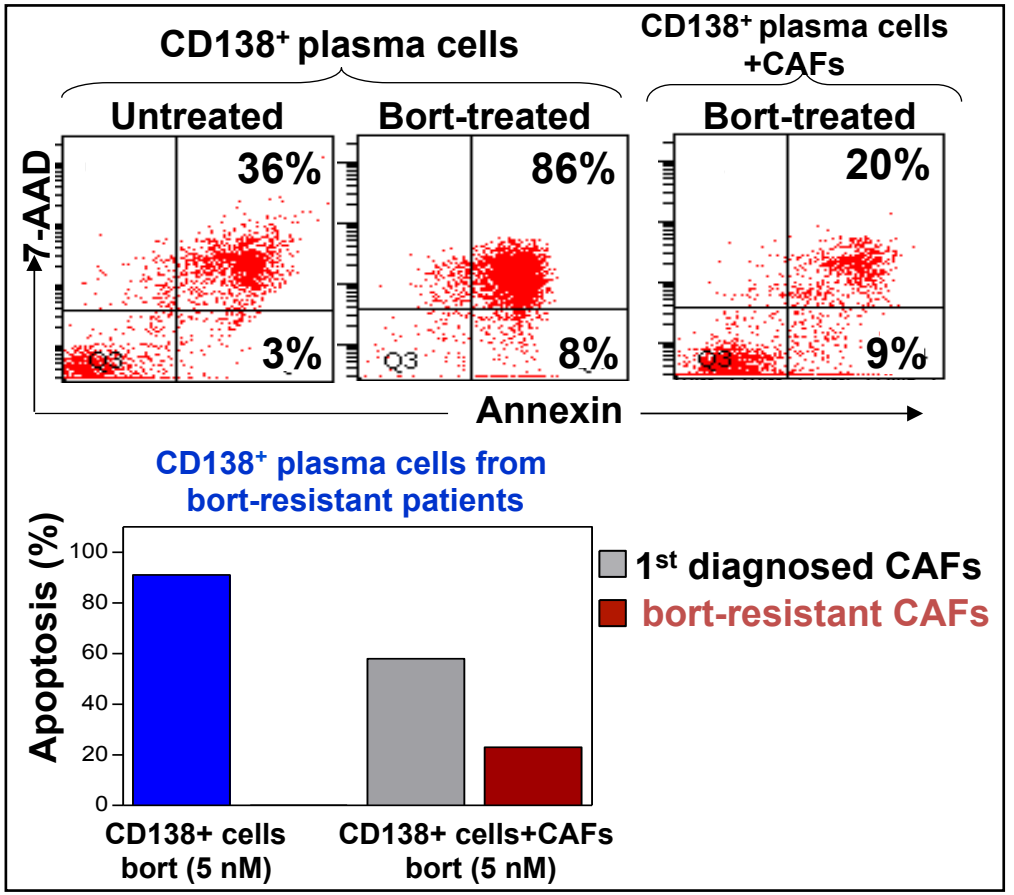
bort-induced CAFs apoptosis

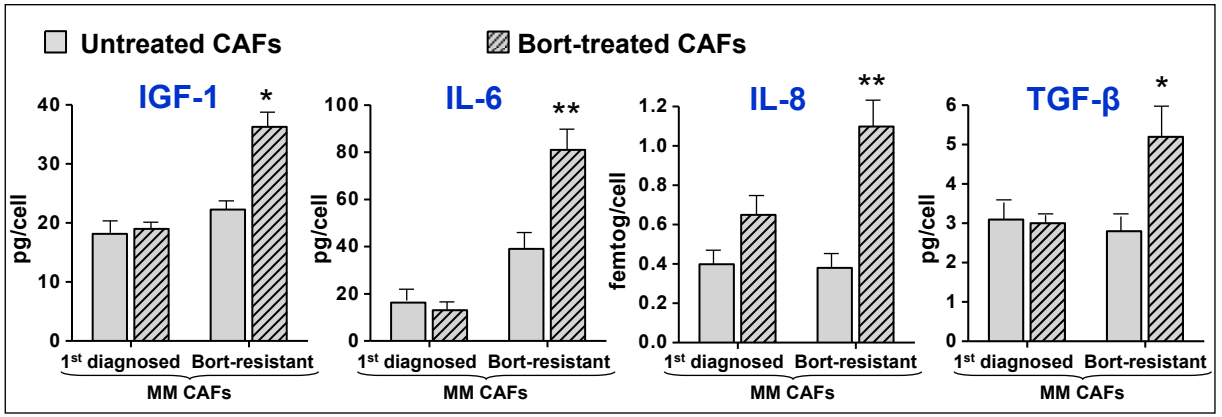


- CAFs from bort-resistant patients <u>are resistant</u> to bort *in vitro*
- CD138+ plasma cells from bort-resistant patients <u>are sensitive</u> to bort *in vitro*
- CAFs from bort-resistant patients <u>prevent</u> bort-induced apoptosis of CD138+ plasma cells by releasing soluble growth factors for plasma cells

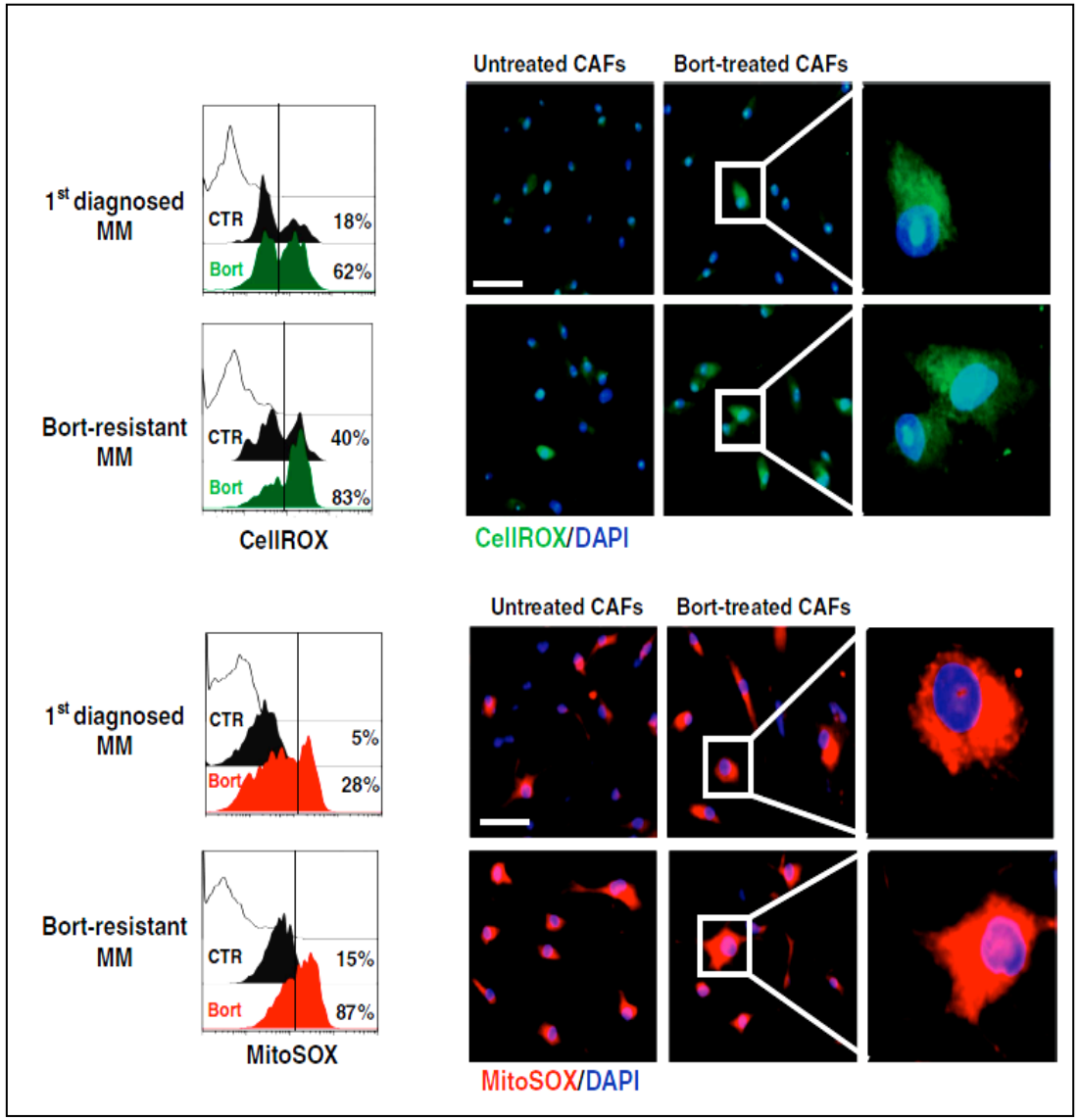
Frassanito et al. Leukemia 2016

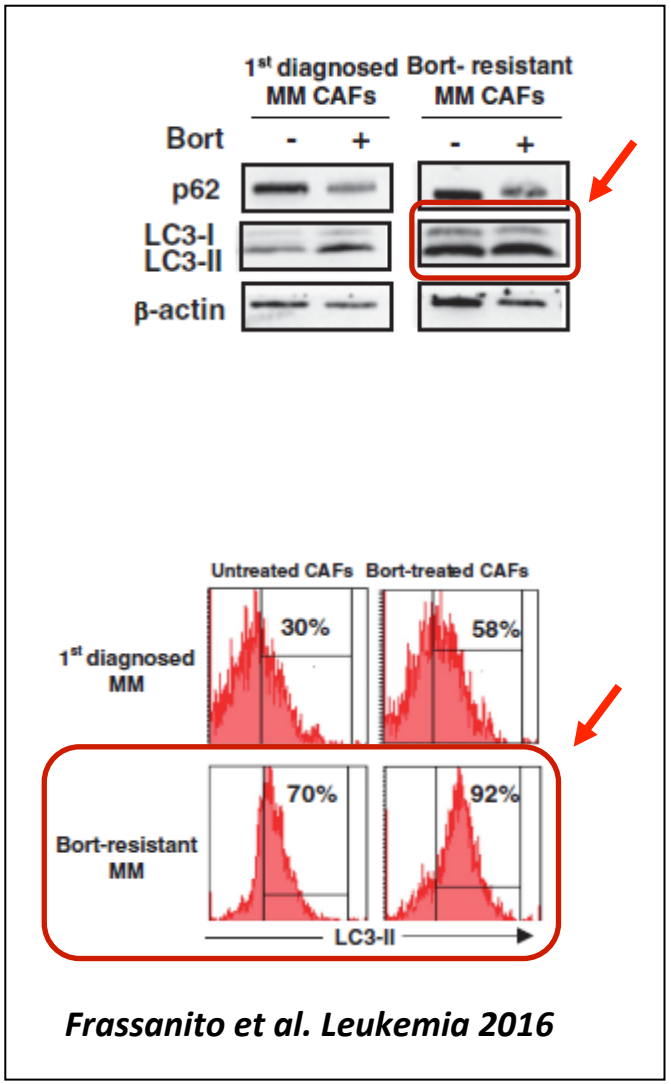
bort-induced apoptosis of myeloma plasma cells





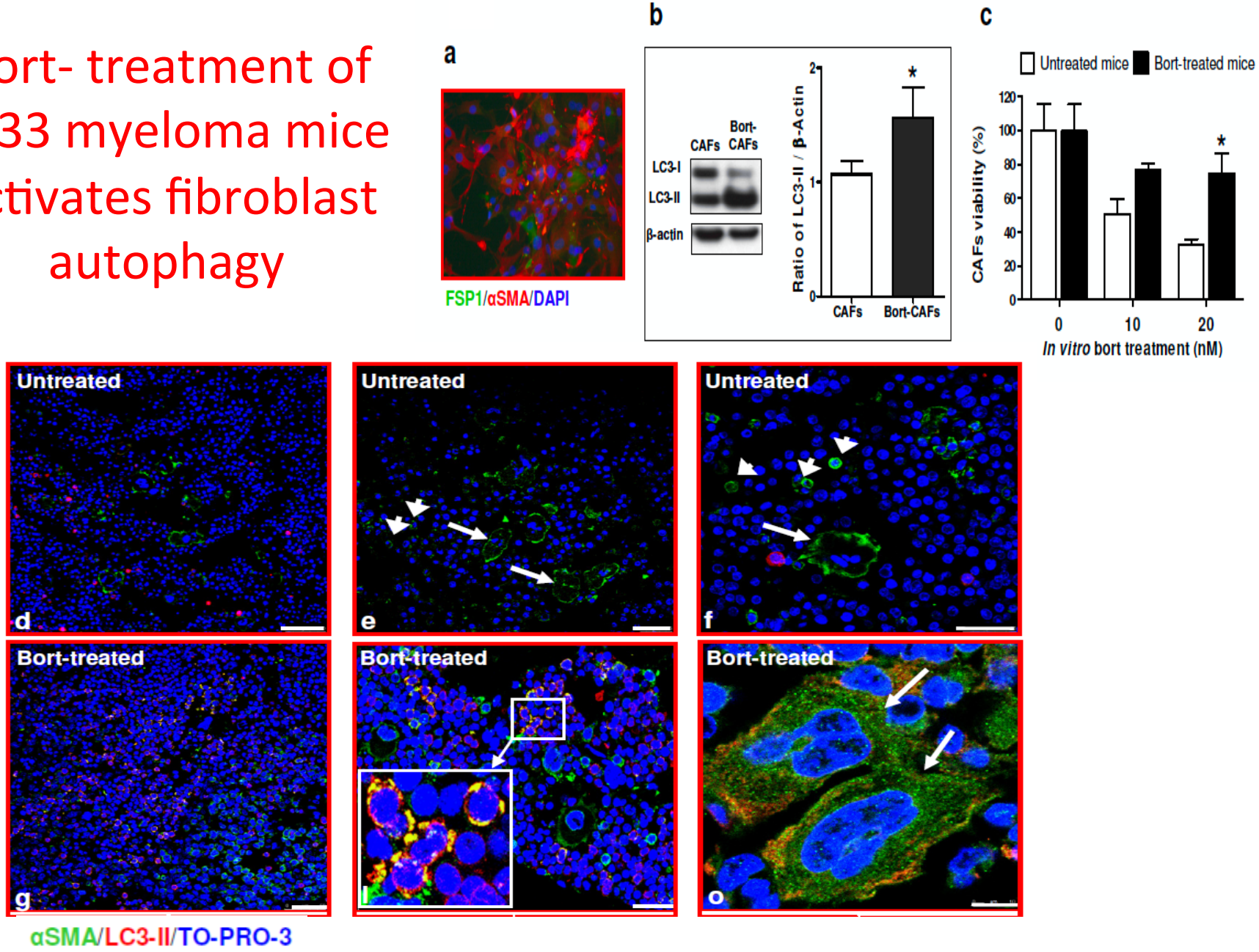
Bortezomib induces cellular stress and activates pro-survival autophagy in bort-resistant CAFs





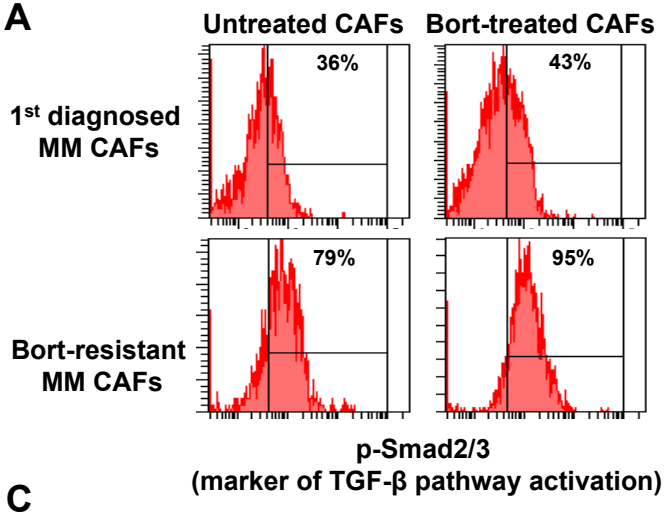
Frassanito et al. Leukemia 2016

Bort- treatment of 5T33 myeloma mice activates fibroblast autophagy

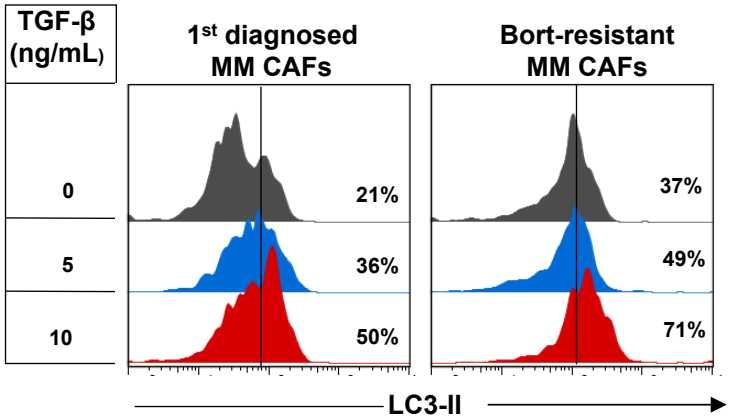


Role of TGFβ in bortezomib resistance of myeloma CAFs

A

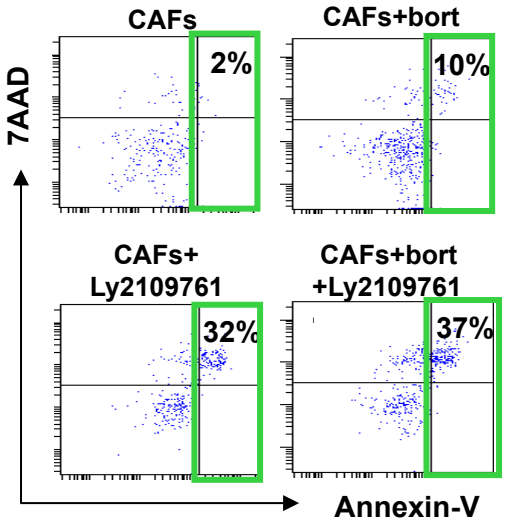


B



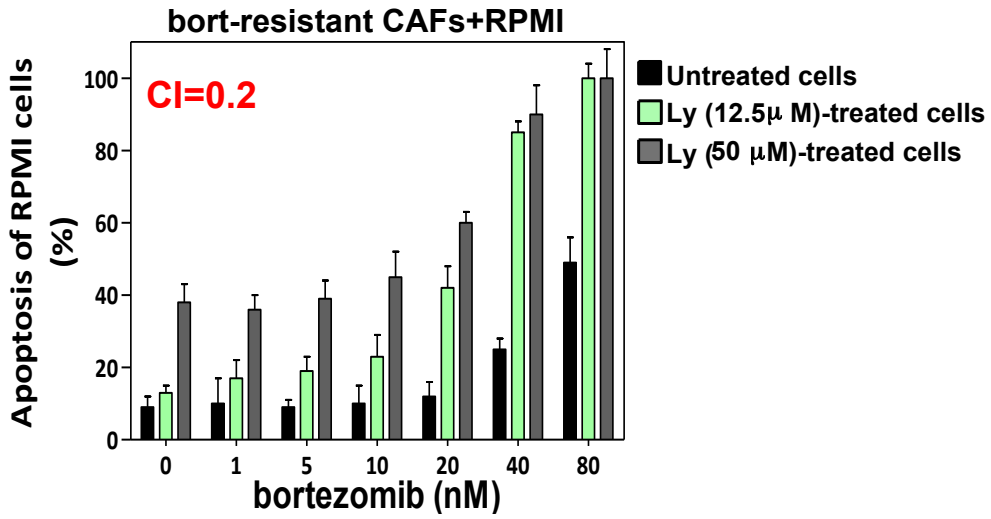
C

Treatment of cocultures CAFs + myeloma plasma cells with TGF-βR inhibitor LY2109761 and bortezomib induces CAFs apoptosis…



D

...and restores the bort-induced apoptosis of myeloma plasma cells



TGF-βR inhibitors may be envisaged as a therapeutic strategy in bortezomib-resistant MM patients

CONCLUSIONS

Bone marrow fibroblasts of patients with myeloma resistant to bortezomib form a protective niche for plasma cells and confer them resistance to bortezomib

BY INHIBITING TGF-β PATHWAY OF THESE RESISTANT FIBROBLASTS THE SENSITIVITY TO BORTEZOMIB OF PLASMA CELLS CAN BE RESTORED

miRNAs and Exosomes: new players in tumor microenvironment

www.impactjournals.com/oncotarget/ Oncotarget, Vol. 7, No. 37

Review

Microenvironment drug resistance in multiple myeloma: emerging new players

Lucia Di Marzo[1,*], Vanessa Desantis[1,*], Antonio Giovanni Solimando[1], Simona Ruggieri[2], Tiziana Annese[2], Beatrice Nico[2], Ruggiero Fumarulo[3], Angelo Vacca[1] and Maria Antonia Frassanito[3]

[1] Department of Biomedical Sciences and Human Oncology, Internal Medicine Section, University of Bari Medical School, Bari, Italy

[2] Department of Basic Medical Sciences, Neurosciences and Sensory Organs, University of Bari Medical School, Bari, Italy

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[*] These authors have contributed equally to this work

Correspondence to: Angelo Vacca, *email:* angelo.vacca@uniba.it

Keywords: cancer-associated fibroblasts, drug resistance, exosomes, microRNAs, multiple myeloma

Received: May 05, 2016 *Accepted:* July 11, 2016 *Published:* July 26, 2016

www.impactjournals.com/oncotarget/ Oncotarget, Advance Publications 2016

Cytoskeleton-centric protein transportation by exosomes transforms tumor-favorable macrophages

Zhipeng Chen[1,*], Lijuan Yang[1,*], Yizhi Cui[1], Yanlong Zhou[1], Xingfeng Yin[1], Jiahui Guo[1], Gong Zhang[1], Tong Wang[1], Qing-Yu He[1]

[1] Key Laboratory of Functional Protein Research of Guangdong Higher Education Institutes, Institute of Life and Health Engineering, College of Life Science and Technology, Jinan University, Guangzhou 510632, China

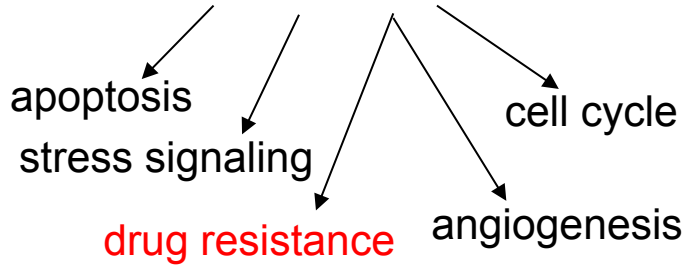
[*] These authors have contributed equally to this work

Correspondence to: Qing-Yu He, *email:* tqyhe@jnu.edu.cn
Tong Wang, *email:* tongwang@jnu.edu.cn

Keywords: exosomes, tumor-associated macrophages, proteome, transportation, cytoskeleton-centric

Received: June 16, 2016 *Accepted:* August 21, 2016 *Published:* September 1, 2016

miRNAs in tumor microenvironment



Citation: Oncogenesis (2016) **5**, e250; doi:10.1038/oncsis.2016.52

www.nature.com/oncsis

ORIGINAL ARTICLE

Modification of tumor cell exosome content by transfection with wt-p53 and microRNA-125b expressing plasmid DNA and its effect on macrophage polarization

M Trivedi[1,3,4], M Talekar[1,4], P Shah[1], Q Ouyang[1] and M Amiji[1,2]

MiR-125b Is Critical for Fibroblast-to-Myofibroblast Transition and Cardiac Fibrosis

Varun Nagpal, PhD; Rahul Rai, MBBS; Aaron T. Place, PhD; Sheila B. Murphy, MS; Suresh K. Verma, PhD; Asish K. Ghosh, PhD; Douglas E. Vaughan, MD

Published in final edited form as:

Cancer Discov. 2012 December ; 2(12): 1100–1108. doi:10.1158/2159-8290.CD-12-0206.

MicroRNAs Reprogram Normal Fibroblasts into Cancer Associated Fibroblasts in Ovarian Cancer

Anirban K. Mitra[1], Marion Zillhardt[1], Youjia Hua[2], Payal Tiwari[1], Andrea E. Murmann[2], Marcus E. Peter[2,+], and Ernst Lengyel[1,+]

ORIGINAL MANUSCRIPT

miR-27 is associated with chemoresistance in esophageal cancer through transformation of normal fibroblasts to cancer-associated fibroblasts

Koji Tanaka, Hiroshi Miyata*, Keijiro Sugimura, Shuichi Fukuda, Takashi Kanen
Kotaro Yamashita, Yasuhiro Miyazaki, Tsuyoshi Takahashi, Yukinori Kurokawa
Makoto Yamasaki, Hisashi Wada, Kiyokazu Nakajima, Shuji Takiguchi,
Masaki Mori and Yuichiro Doki

Oncogene (2013) **32**, 414 - 421

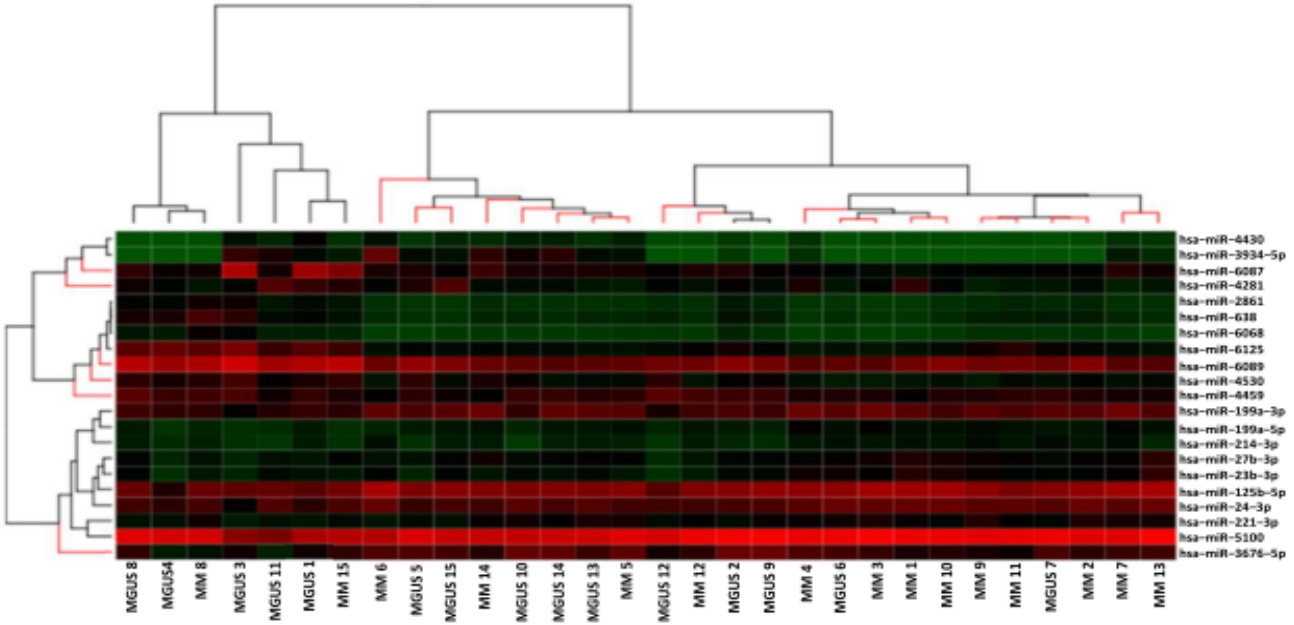
www.nature.com/onc

ORIGINAL ARTICLE

microRNA-125b inhibits tube formation of blood vessels through translational suppression of VE-cadherin

F Muramatsu[1,3], H Kidoya[1,3], H Naito[1], S Sakimoto[1] and N Takakura[1,2]

Differential miRNAs expression profile in CAFs of myeloma *vs.* MGUS



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

qRT-PCR

The top miRNAs UP REGULATED are:

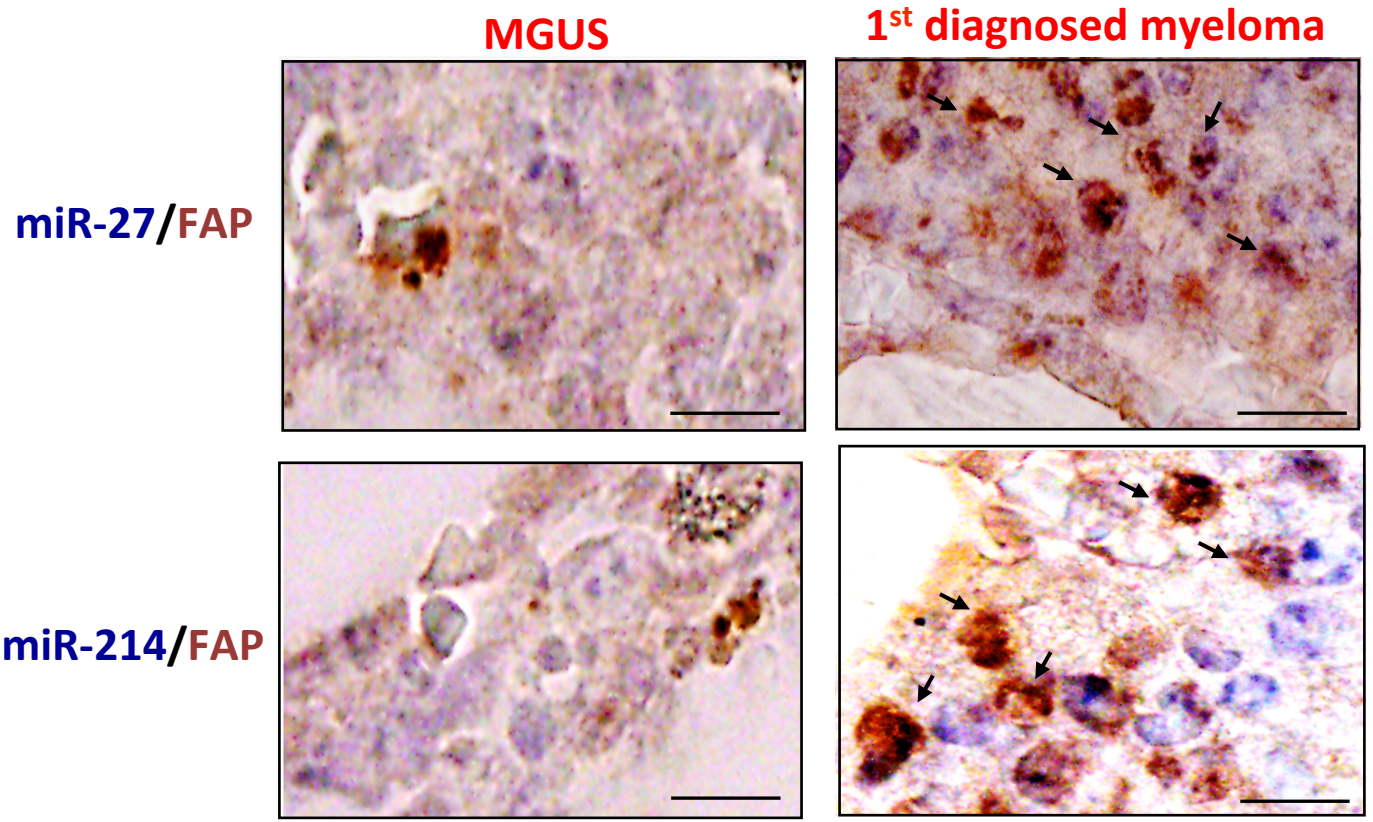
- hsa-miR-23b-3p – fold change: 0.351
- **hsa-miR-27b-3p – fold change: 0.366**
- hsa-miR-125b-5p - fold change: 0.431
- **hsa-miR-214-3p – fold change: 0.342**
- hsa-miR-199a-5p – fold change: 0.33

The top miRNAs DOWN REGULATED are:

- hsa-miR-3960 – fold change: - 0.277
- hsa-miR-6087 – fold change: - 0.401
- hsa-miR-6068 – fold change: - 0.287
- hsa-miR-2861 – fold change: - 0.312
- hsa-miR-642a-3p – fold change: - 0.285

Frassanito et al., submitted

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

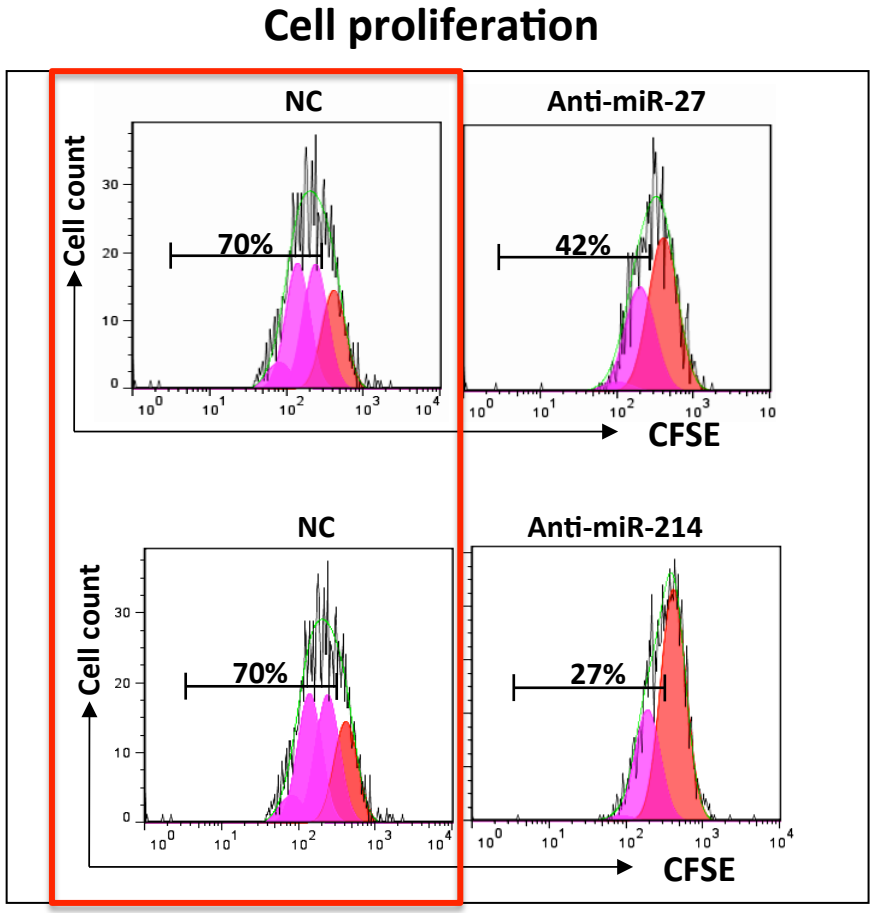


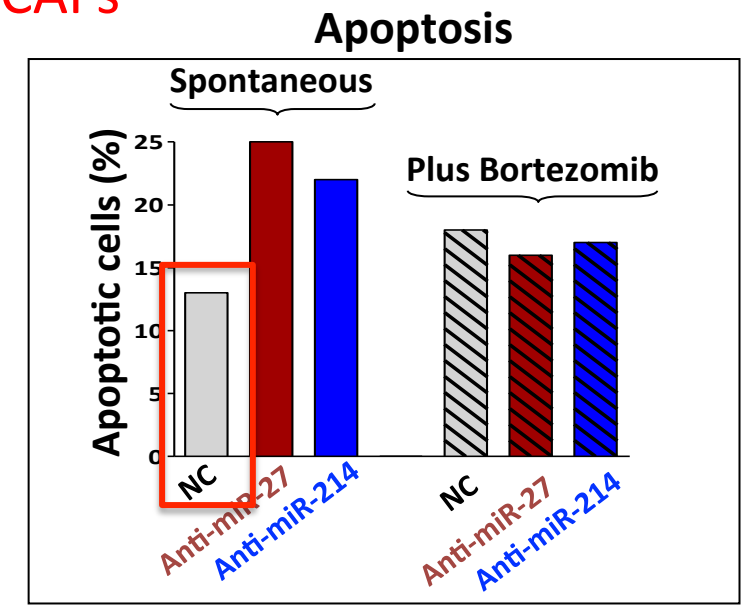
In situ hybridization

CAFs co-expression of FAP (brown) and miR (blue) gives dark-brown dots

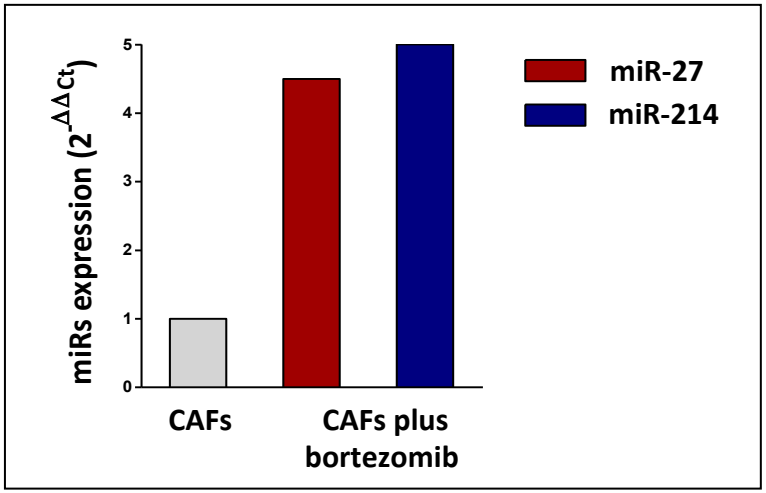
Frassanito et al. submitted

miR-27 and miR-214 induce proliferation and prevent apoptosis in myeloma CAFs



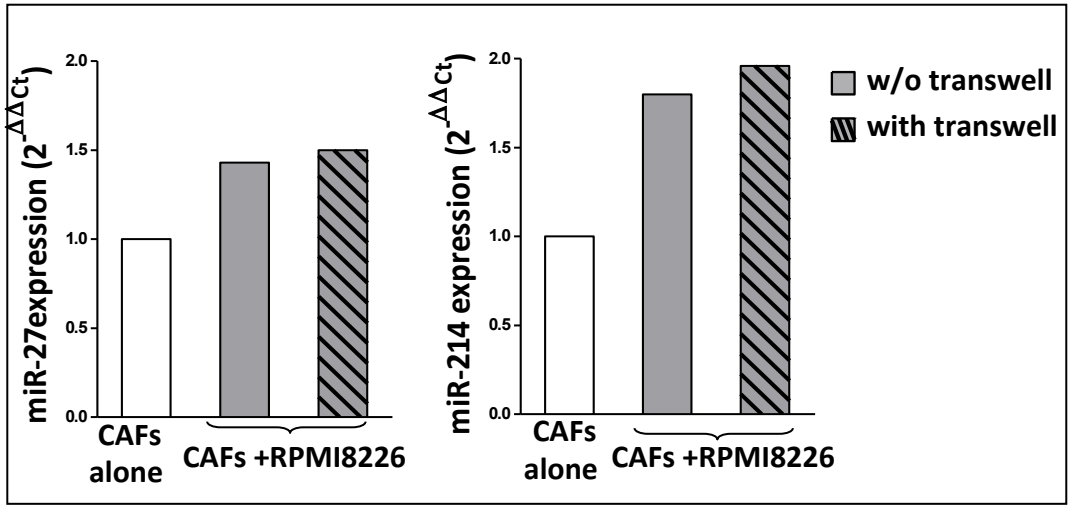


Bortezomib induces miR-27 and miR-214 expression

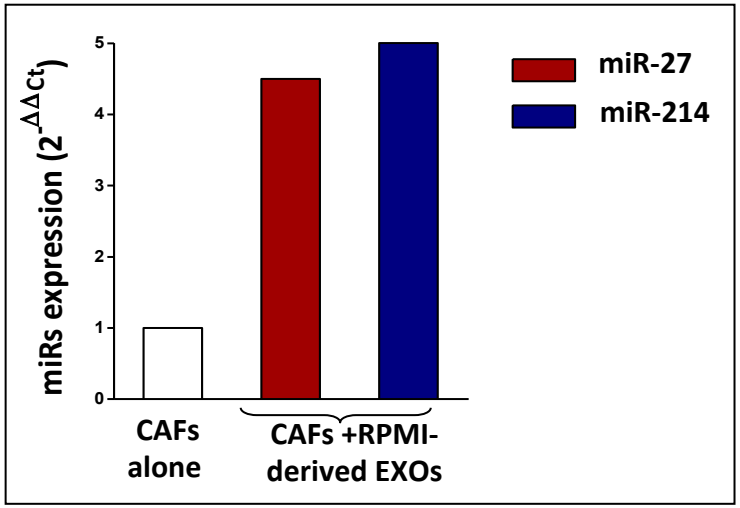


Myeloma cells and their exosomes (EXOs) induce miR-27 and miR-214 expression in MGUS CAFs and cell activation

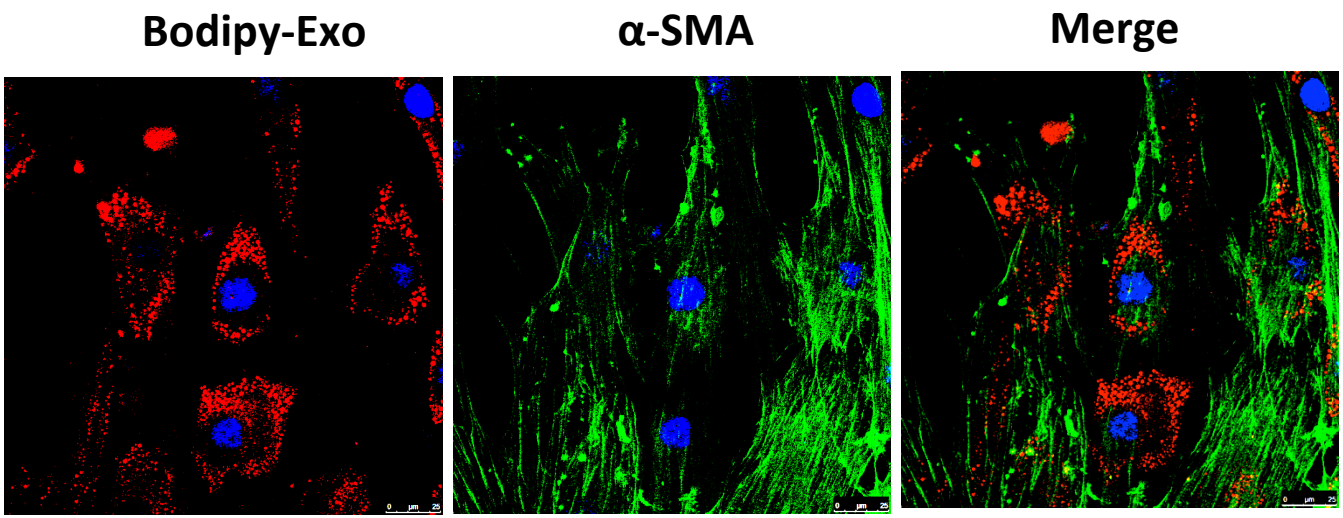
MGUS-CAFs + RPMI8226 cocultures



MGUS-CAFs + RPMI8226-derived EXOs

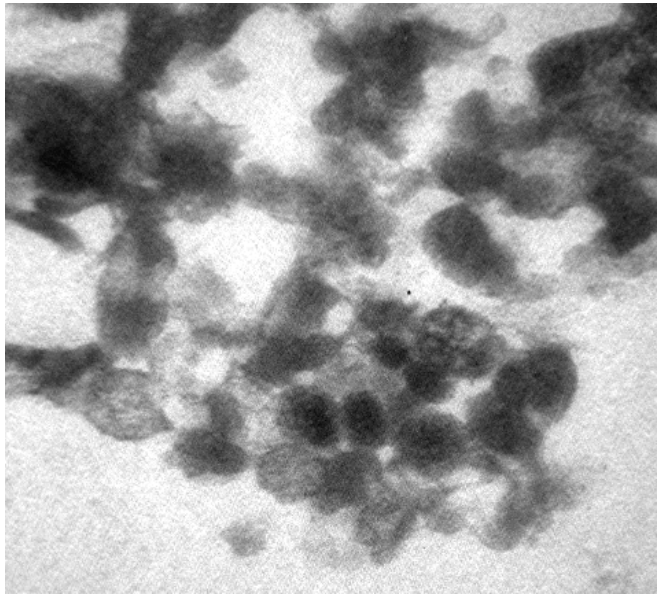


Uptake of myeloma-derived EXOs by MGUS-CAFs

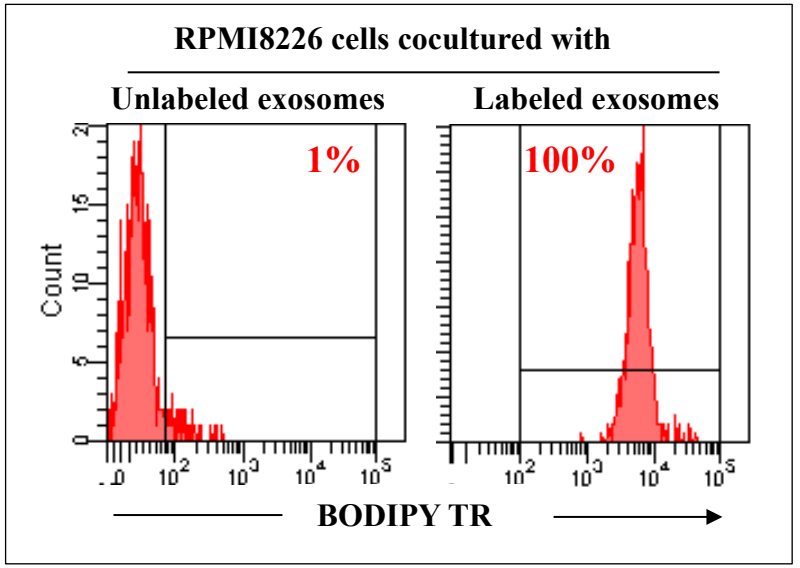


CAFs-derived exosomes and their uptake by myeloma cells

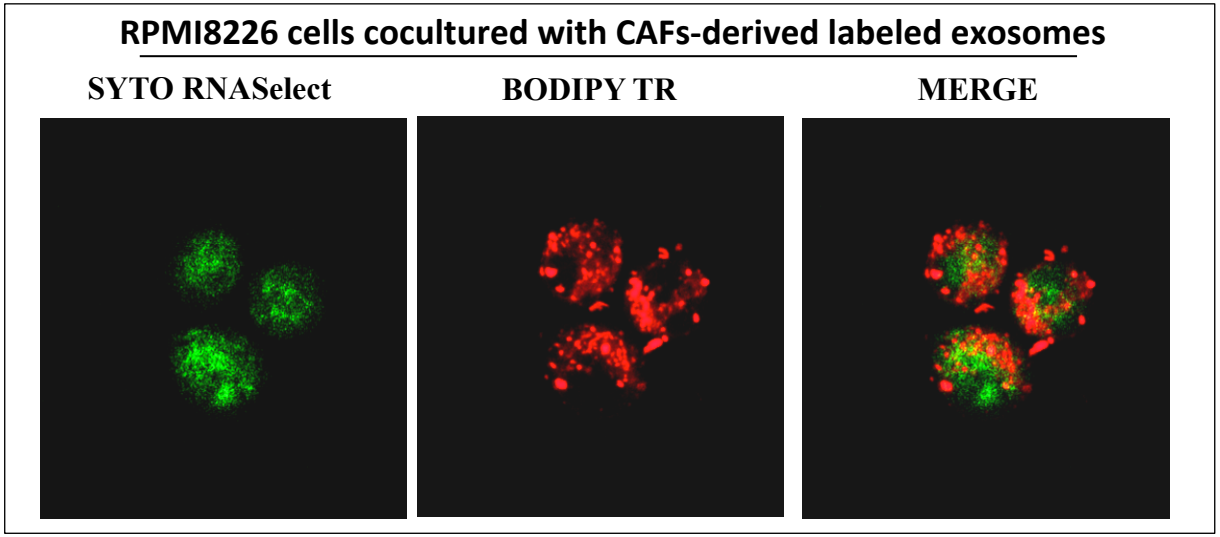
TRANSMISSION ELECTRON MICROSCOPY IMAGE



FLOW CYTOMETRY ANALYSIS OF EXOSOMES UPTAKE BY RPMI8226 PLASMA CELLS



DUAL IMMUNOFLUORESCENCE CONFOCAL LASER SCANNING MICROSCOPY IMAGES OF CAFS-DERIVED EXOSOMES UPTAKE BY RPMI8226 PLASMA CELLS

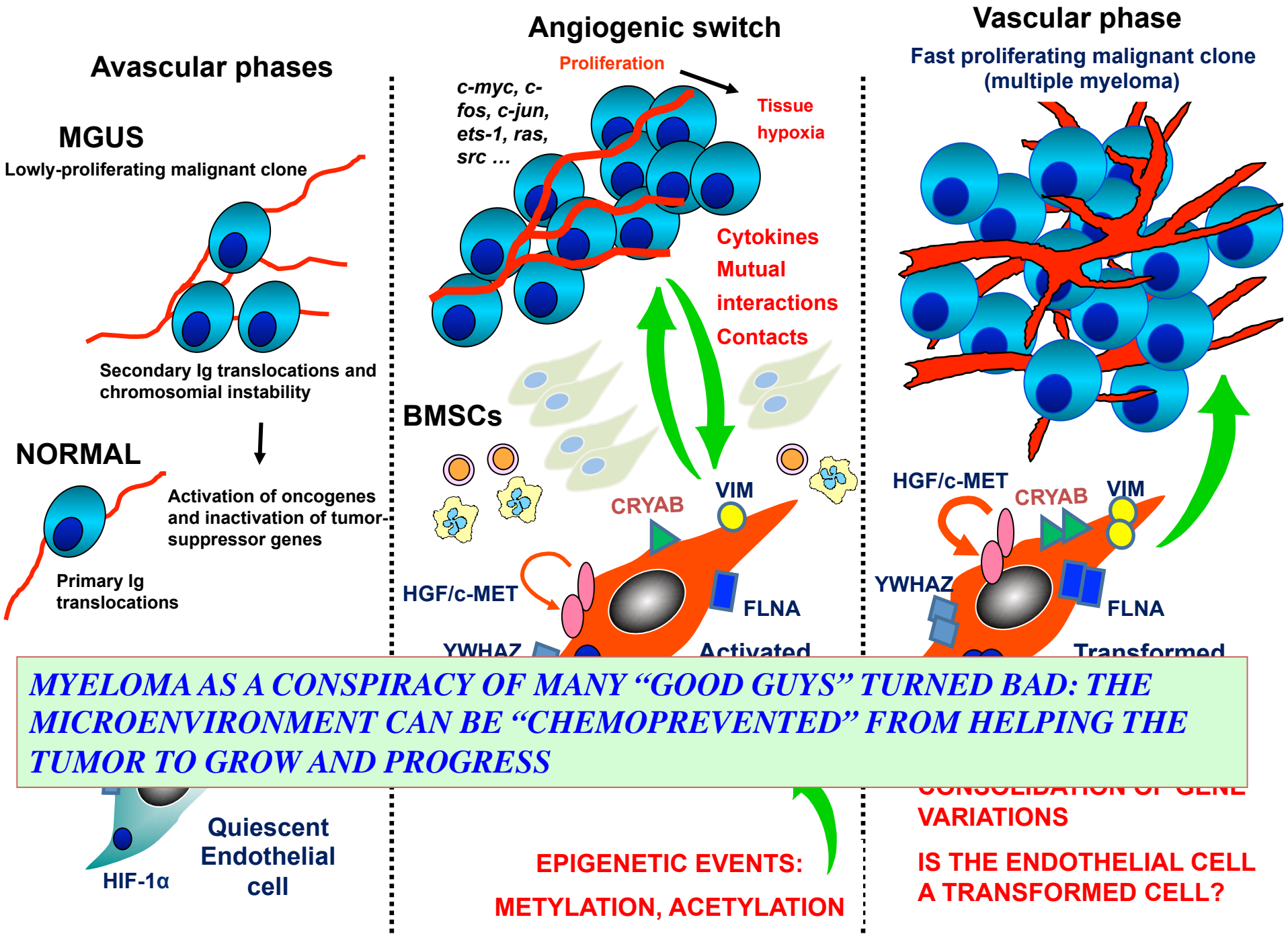


CONCLUSIONS

- **Myeloma CAFs phenotype and functional activity is regulated by a specific miRNAs profile, including miR-27 and miR-214, induced by myeloma cells, through exosomes release, and bortezomib;**

- **In turn, CAFs release exosomes swallowed by myeloma cells (and others BMSCs ?) activating several functional cell pathways**

Exosome-mediated cross-talk between myeloma cells and CAFs results in a protective niche favouring tumor progression and drug resistance.

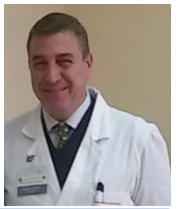

Avascular phases
MGUS
Lowly-proliferating malignant clone
Secondary Ig translocations and chromosomial instability
NORMAL
Activation of oncogenes and inactivation of tumor-suppressor genes
Primary Ig translocations
Angiogenic switch
Proliferation
c-myc, c-fos, c-jun, ets-1, ras, src …
Tissue hypoxia
Cytokines
Mutual interactions
Contacts
BMSCs
CRYAB
VIM
HGF/c-MET
FLNA
YWHAZ
Activated
Vascular phase
Fast proliferating malignant clone (multiple myeloma)
HGF/c-MET
CRYAB
VIM
YWHAZ
FLNA
Transformed
MYELOMA AS A CONSPIRACY OF MANY "GOOD GUYS" TURNED BAD: THE MICROENVIRONMENT CAN BE "CHEMOPREVENTED" FROM HELPING THE TUMOR TO GROW AND PROGRESS
HIF-1α
Quiescent Endothelial cell
EPIGENETIC EVENTS:
METYLATION, ACETYLATION
VARIATIONS
IS THE ENDOTHELIAL CELL A TRANSFORMED CELL?

Policlinico di Bari

Dipartimento di Scienze Biomediche e Oncologia Umana
U.O.C. Medicina Interna "G. Baccelli"
Direttore Prof. Angelo Vacca

Phase II/ III clinical trials in Multiple Myeloma

Prof. Roberto Ria, Drs. Assunta Melaccio,
Rossella Acquaviva, my lab/ clinic



Year	Study	Enrolled patients
2013	Denosumab- AMG162: Treatment of Bone Disease in Subjects with Newly Diagnosed Multiple Myeloma	5
	CA204006: Len/Dex +/-Elotuzumab in Previously Untreated MM	2
	CC-4047-MM-010: Pomalidomide with low dose dexamethasone in refractory or relapsed and refractory MM	16
2014	MMY2084: prolonged therapy with bortezomib in relapsed and refractory MM	3
2015	IST-CAR-601: Carfilzomib, Cyclophosphamide and Dexamethasone in Newly MM	2
2016	MMY3010: Early Access Treatment for Daratumumab	3
	CC-4047-MM015: post authorisation registry of patients treated with pomalidomide for relapsed and refractory MM	6
2018	MP0250-CP201: MP0250 + Bortezomib/Dexamethasone in patients with relapsed and refractory MM	2018 - ongoing

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

Acknowledgments

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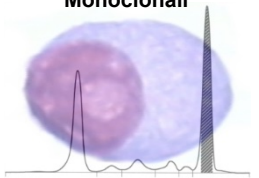
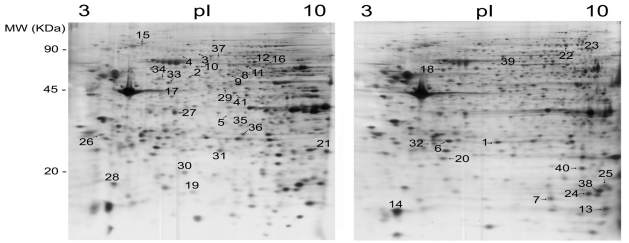


Proteomic analysis of CAFs from bort-resistant and 1st diagnosed patients

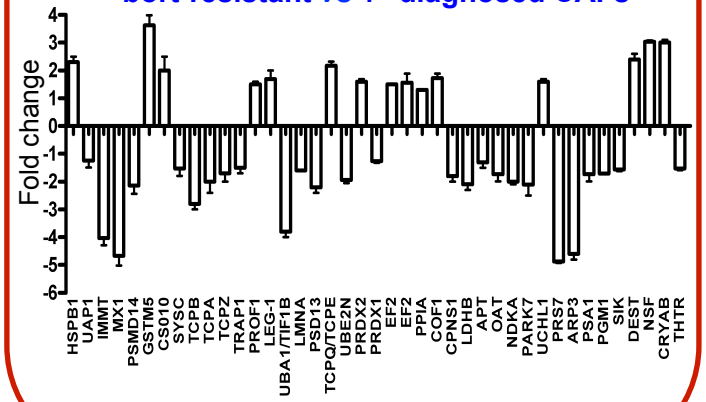
Bort-resistant *vs* 1st diagnosed CAFs

CAFs proteins from:

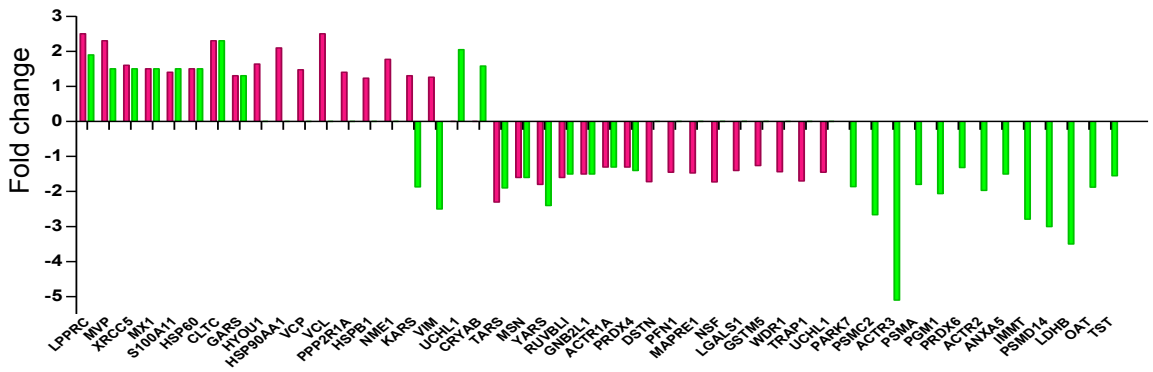
1st diagnosed patient | **bort-resistant patient**



bort-resistant *vs* 1st diagnosed CAFs

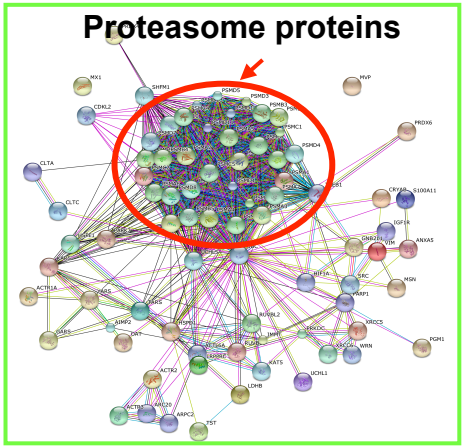


bort-treated CAFS from bort-resistant (■) and 1st diagnosed (■) patients

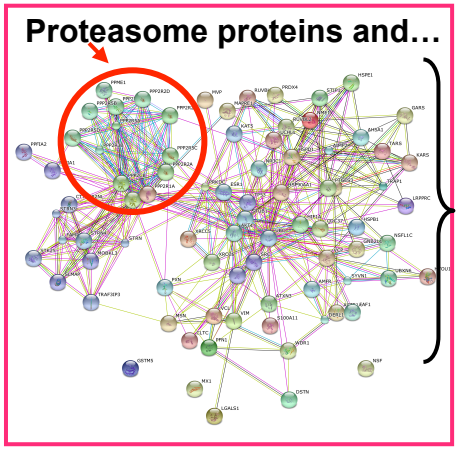


Protein-protein interactions network by STRING database

1st diagnosis CAFs



bort-resistant CAFs



…other proteins involved in oxidative stress and survival autophagy

CAFs from bort-resistant and 1st diagnosed patients show a different proteomic profile which is differentially modulated by bortezomib