
**KEY POINTS**

# Friday, May 18, 2018

## SESSION III: PASSIVE IMMUNOTHERAPY: TARGETING THE TUMOR MICROENVIRONMENT

*(Margherita Bonferroni - Cuneo, Mario Luppi - Modena)*

**08.30-09.00** LECTURE: Tumor microenvironment in MM *(Angelo Vacca - Bari)*

**09.00-09.20** SLAMF7/CS1 *(Nicola Giuliani - Parma)*

**09.20-09.40** CD38: "on target" "off tumor" effects *(Fabio Malavasi - Torino)*

**09.40-10.00** The DARPIN bispecific molecule *(Roberto Ria - Bari)*

**10.00-10.20** Role of aminoacids degrading enzymes in lympho-proliferative disease *(Francesco Di Raimondo - Catania)*

# 'Role of Bone Marrow Stromal Cells in the Growth of Human Multiple Myeloma

By Federico Caligaris-Cappio, Luciana Bergui, Maria Grazia Gregoretti, Gianluca Gaidano, Mirella Gaboli, Marina Schena, Alberta Zambonin Zallone, and Pier Carlo Marchisio

**We have verified the hypothesis that multiple myeloma (MM) may be disseminated by circulating clonogenic cells that selectively home to the bone marrow (BM) to receive the signal(s) leading to proliferation, terminal differentiation, and production of the osteoclast activating factors. Long-term cultures of stromal cells have been developed from the BM of nine patients with MM. These cells were mostly fibroblast-like elements, interspersed with a proportion of scattered macrophages and rare osteoclasts. BM stromal cells were CD54$^+$, produced high levels of interleukin-6 (IL-6) and measurable amounts of IL-1β, and were used as feeder layers for autologous peripheral blood mononuclear cells (PBMC). After 3 weeks of cocultures, monoclonal B lymphocytes and plasma cells, derived from PBMC, developed and the number of osteoclasts significantly increased. Both populations grew tightly adherent to the stromal cell layer and their expansion was matched by a sharp increase of IL-6 and by the appearance of IL-3 in the culture supernatant. These data attribute to BM stromal cells a critical role in supporting the growth of B lymphocytes, plasma cells, and osteoclasts and the in vivo dissemination of MM.**



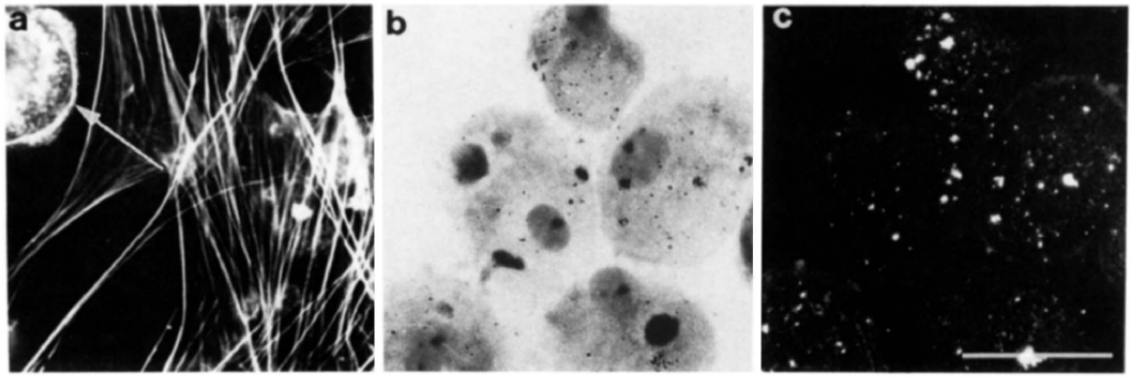



**Fig 1. Long-term cultures of MM BM aspirates are mostly formed by CD54$^+$ fibroblast-like cells, which, on staining with R-PHD, show F-actin organized in stress fibers (a, right). Fibroblast-like cells are intermingled with podosome-bearing cells (arrow in a) that represent small osteoclasts or osteoclast precursors. These cells may be bi- or polynucleated (b) and bear receptors for calcitonin, as shown by autoradiography following exposure to $^{125}$I-calcitonin (b, light field and c, dark field). Bar denotes 10 μm.**

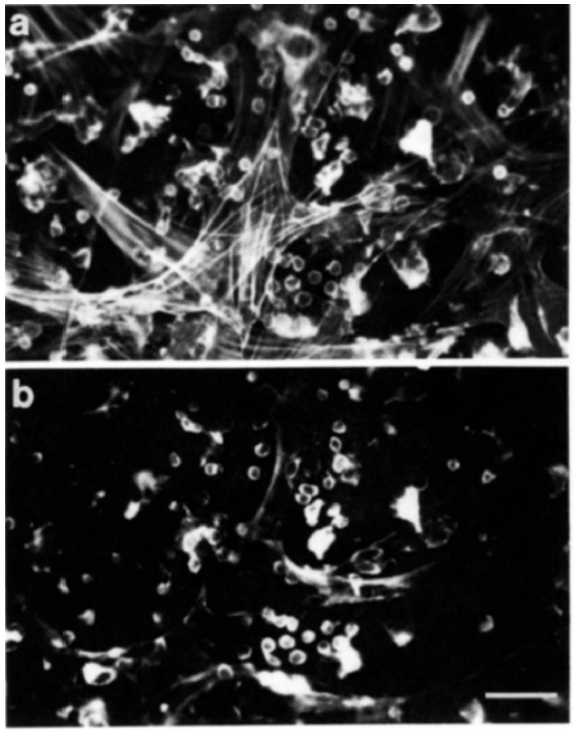



**Fig 2. Long-term cultures of MM BM stromal cells cocultured for 3 weeks with autologous PBMC show the appearance of a large number of attached lymphoid cells and plasma cells tightly adherent to the fibroblast-like layer (a). Lymphoid cells and plasma cells express the same monoclonal Ig light chain produced by the patient BM malignant plasma cells (b). The same field shows double IF staining for F-actin (a, R-PHD) and for Ig light chain (b, rabbit fluorescein-labelled anti-λ light chain). Bar denotes 20 μm.**

**Table 2. IL-1β, IL-3, and IL-6 Levels in Culture Supernatants and Patients' Sera**

| | IL-6 (ng/mL) | | | IL-1β (pg/mL) | | | IL-3 (U/mL) | | |
|---|---|---|---|---|---|---|---|---|---|
| Case* | A | B | C | A | B | C | A | B | C |
| 3 | <0.1 | 1.23 | 3.32 | 9 | 9 | 10 | U | U | 0.3 |
| 5 | <0.1 | 0.10 | 2.38 | 8 | 10 | 10 | ND | ND | ND |
| 7† | <0.1 | 3.17 | 17.80 | 11 | 9 | 12 | U | U | 0.5 |
| 8 | <0.1 | 0.78 | 3.02 | 8 | 7 | 8 | ND | ND | ND |
| 9† | <0.1 | 0.89 | 10.00 | 11 | 9 | 10 | U | U | 0.5 |

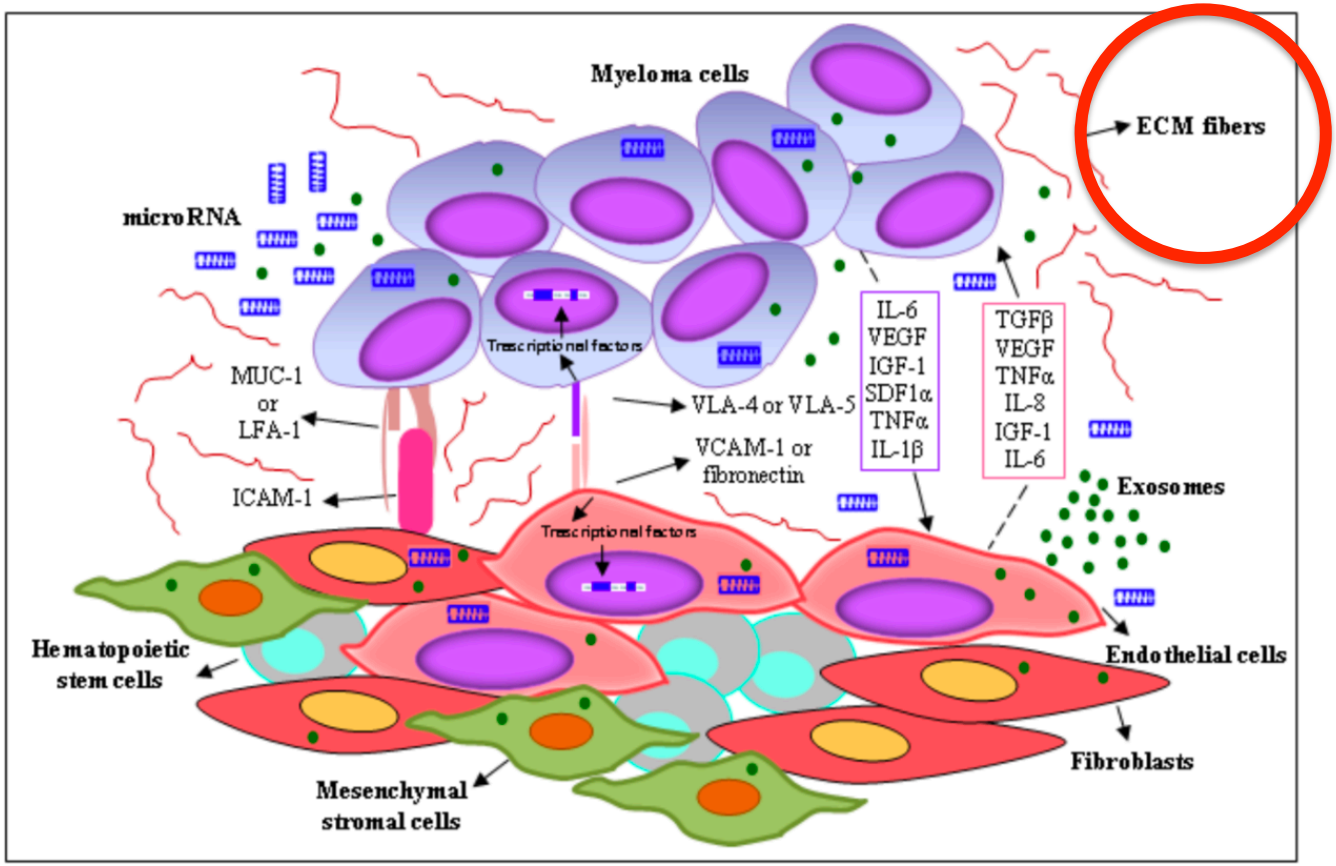
(A) Sera from the patients whose stromal cell cultures were studied for the production of ILs; (B) BM stromal cell culture supernatants (7 weeks); (C) supernatants from 3-week-old cocultures between PBMC and autologous BM stromal cells. The data are the mean of experiments performed in triplicate (IL-6 and IL-1β) or in quintuplicate (IL-3).

Transformation of a normal plasma cell into a neoplastic cell is a multistep process due to genetic and molecular events as well as to important and irreversible alterations in the bone marrow micro-environment.

The myeloma cell is immersed in the bone marrow micro-environment where it mutually interacts with stromal cells (BMSC), osteoblasts, osteoclasts, lymphocytes and endothelial cells.

*Di Marzo et al., Oncotarget 2016*

MM cells are sorrounded by a complex bone marrow microenvironment composed of ECM proteins and several cell types, including BM stromal cells (ECs, mesenchymal stromal cells, CAFs).




1. The cross-talk between MM cells and BM stromal cells is regulated by different mechanisms: cell-to-cell adhesion between MM cells and ECM components/BM stromal cells;
2. soluble factors (cytokines, chemokines, growth factors, exosomes and miRNAs released by the BM stromal cells and MM cells, with autocrine and paracrine effects).

Both mechanisms activate several signaling pathways in BM stromal cells and tumor cells, leading to MM drug resistance.

**Table 1: Adhesion molecules involved in MM drug resistance**

| PROTEINS | LIGAND | FUNCTION | REFs |
|---|---|---|---|
| Integrin $\beta_1$ | Laminin, Collagen type-VI, Fibronectin | Cell protection from cell cycle-dependent drug therapies | 44, 45 |
| Integrin $\beta_7$ | Fibronectin, E-cadherin | Cell adhesion, migration, and homing | 44, 46 |
| Integrin $\alpha_v\beta_3$ | Vitronectin, Fibronectin | Cell proliferation, protease secretion, invasion and spreading | 44, 47 |
| [1]VLA-4 ($\alpha_4\beta_1$) | Fibronectin, [2]VCAM-1 | Cell adhesion, migration, homing and invasion, angiogenesis, cytokines secretion | 48, 49, 50, 51 |
| [1]VLA-5 ($\alpha_5\beta_1$) | Fibronectin | Cell homing and migration. Its down-expression correlates with MM progression | 44, 49 |
| [2]VCAM- 1 | [1]VLA-4 | Cells migration, homing and invasion | 52 |
| [3]LFA-1 | [5]ICAM-1 | Cell adhesion, proliferation and survival, angiogenesis, tumor dissemination | 48, 50, 53 |
| [4]MUC-1 | [5]ICAM-1 | Cells adhesion, growth and survival, disease progression | 54 |
| CD44 isoforms | Hyaluronan | Cell adhesion and invasion | 44, 50 |
| CD138 (syndecan-1) | Fibronectin | Cell adhesion | 44 |

[1]VLA = Very Late Activation Antigen; [2]VCAM = Vascular Cell Adhesion Molecule; [3]LFA = Lymphocyte Function-Associated Antigen; [4]MUC = Mucin-1 antigen; [5]ICAM = Intercellular Adhesion Molecule.

Leukemia (2018) **32**, 736–743
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OPEN

ORIGINAL ARTICLE

# JAM-A as a prognostic factor and new therapeutic target in multiple myeloma

AG Solimando[1,2,3,8], A Brandl[1,2,8], K Mattenheimer[1,2], C Graf[1,2], M Ritz[1,2], A Ruckdeschel[1,2], T Stühmer[4], Z Mokhtari[1,2], M Rudelius[5], J Dotterweich[6], M Bittrich[2], V Desantis[3], R Ebert[6], P Trerotoli[7], MA Frassanito[3], A Rosenwald[5], A Vacca[3], H Einsele[2], F Jakob[6] and A Beilhack[1,2]

Cell adhesion in the multiple myeloma (MM) microenvironment has been recognized as a major mechanism of MM cell survival and the development of drug resistance. Here we addressed the hypothesis that the protein junctional adhesion molecule-A (JAM-A) may represent a novel target and a clinical biomarker in MM. We evaluated JAM-A expression in MM cell lines and in 147 MM patient bone marrow aspirates and biopsies at different disease stages. Elevated JAM-A levels in patient-derived plasma cells were correlated with poor prognosis. Moreover, circulating soluble JAM-A (sJAM-A) levels were significantly increased in MM patients as compared with controls. Notably, *in vitro* JAM-A inhibition impaired MM migration, colony formation, chemotaxis, proliferation and viability. *In vivo* treatment with an anti-JAM-A monoclonal antibody (αJAM-A moAb) impaired tumor progression in a murine xenograft MM model. These results demonstrate that therapeutic targeting of JAM-A has the potential to prevent MM progression, and lead us to propose JAM-A as a biomarker in MM, and sJAM-A as a serum-based marker for clinical stratification.

*Leukemia* (2018) **32,** 736–743; doi:10.1038/leu.2017.287

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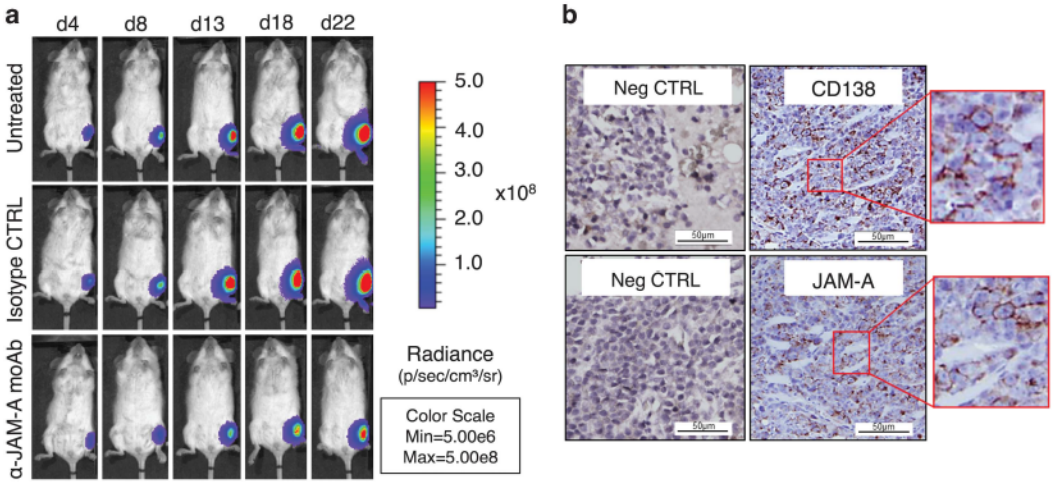
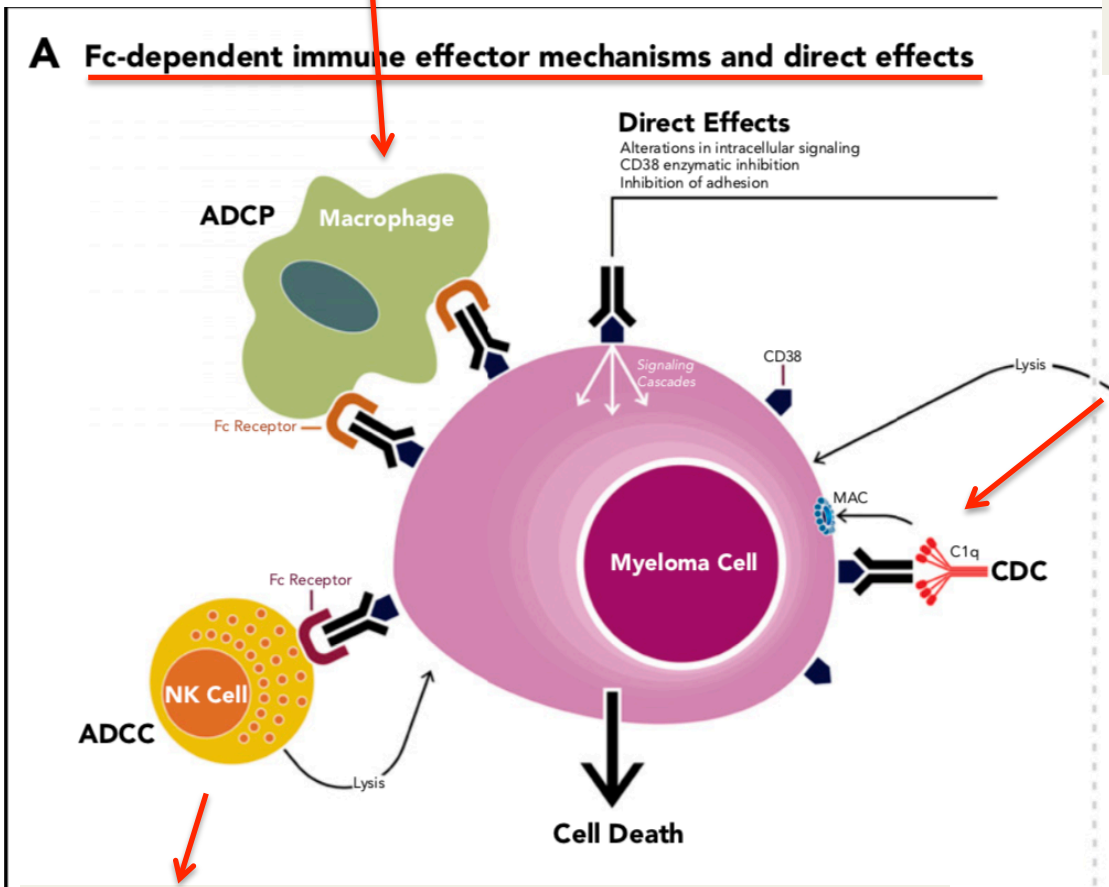


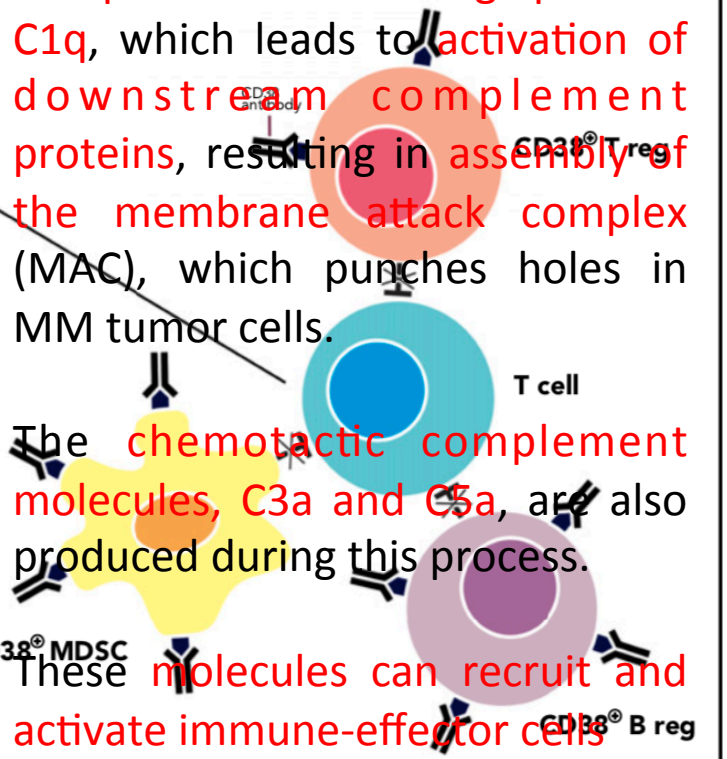
Agents capable of shifting the tumor microenvironment toward immune activation while inducing myeloma cell killing.

Phagocytosis is mediated by monocytes-macrophages-neutrophils-dendritic cells following interaction of the Fc tail of the therapeutic antibody with FcRs on these effector cells




Subsequent release of perforin and granzymes from effector cells as well as interactions with FasL and TNF–related apoptosis-inducing ligand

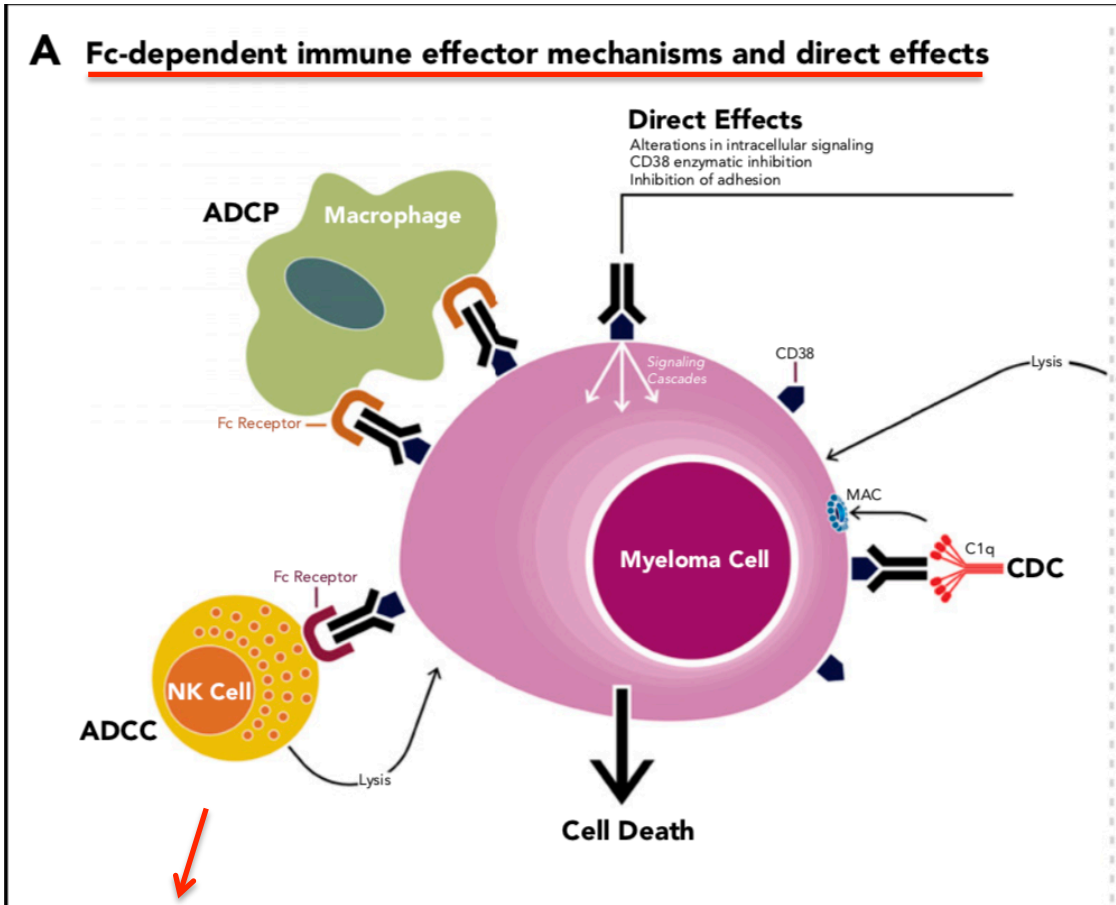
CDC is initiated following the interaction of the antibody Fc domains with the classic complement-activating protein C1q, which leads to activation of downstream complement proteins, resulting in assembly of the membrane attack complex (MAC), which punches holes in MM tumor cells.




The chemotactic complement molecules, C3a and C5a, are also produced during this process.

These molecules can recruit and activate immune-effector cells

*Van der Donk et al., Blood 2018*




CD38-targeting antibodies have pleiotropic mechanisms of action, which consists of direct effects:

1. induction of apoptosis;
2. inhibition of CD38 ectoenzyme function, which may lead to reduced adenosine levels in the BM myeloma niche.

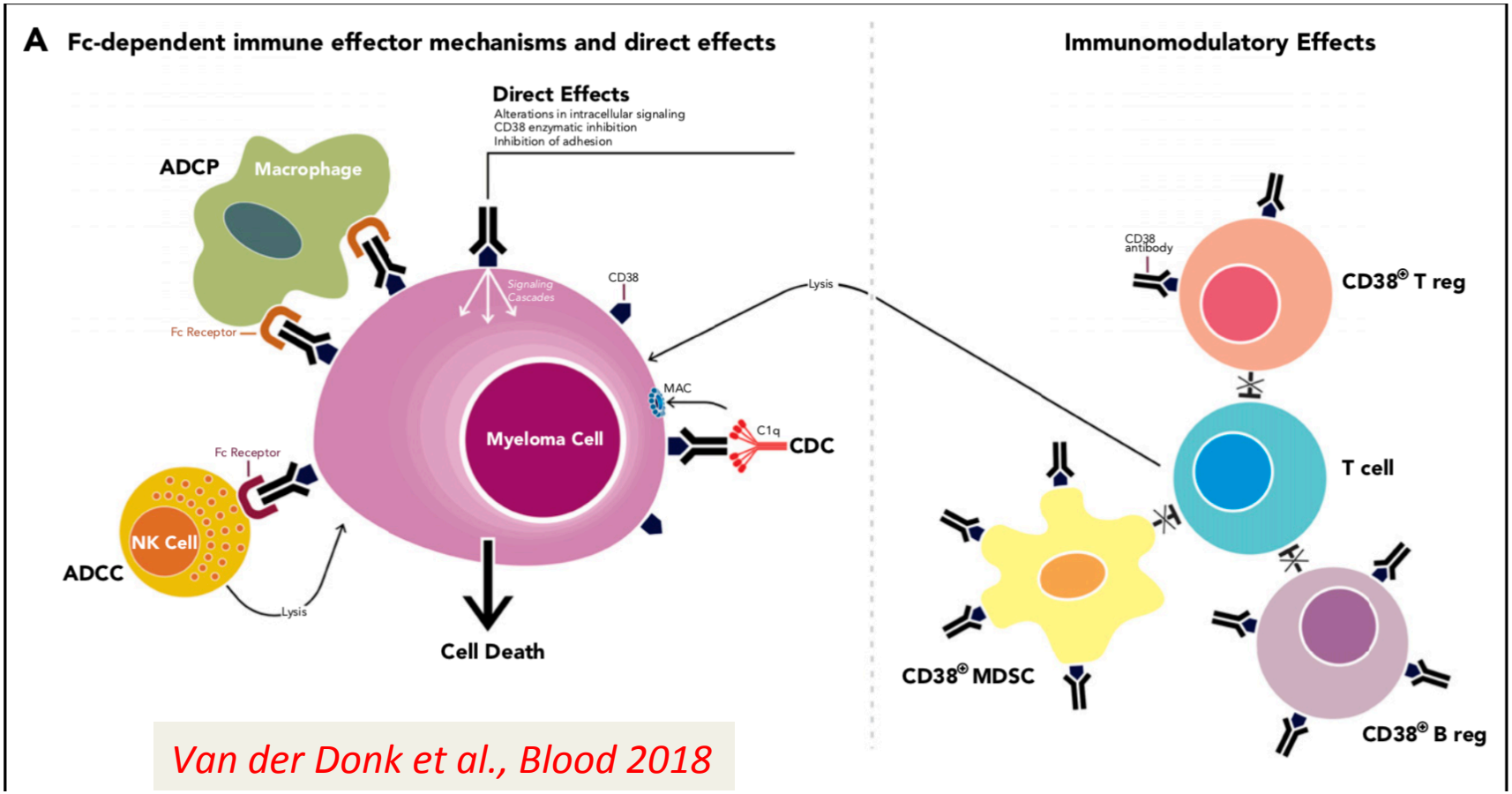
Adenosine is an immunosuppressor that helps the tumor to evade the host immune response by promoting regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), and depressing NK- and T-cell effectors.

*Van der Donk et al., Blood 2018*

CD38-targeting antibodies have pleiotropic mechanisms of action, which consists of indirect immunomodulatory effects via the eradication of :

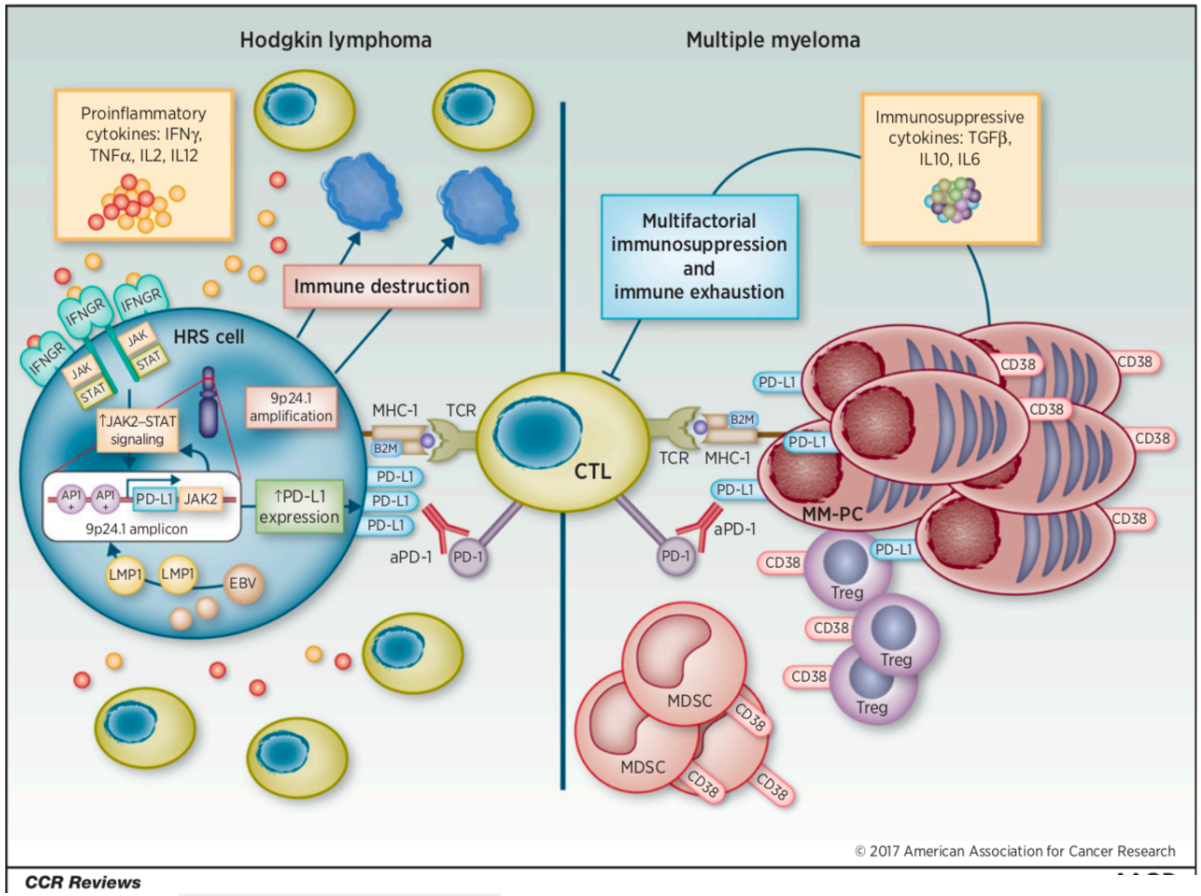
1. CD38+ Tregs,
2. CD38+ regulatory B cells (Bregs),
3. CD38+ Myeloid Derived Suppressor Cells (MDSCs) ,

which result in CD4+ and CD8+ T-cell expansion, and potentially a better host-antitumor immune response.




*Van der Donk et al., Blood 2018*

Daratumumab appears not only to deplete subpopulations of Tregs and MDSCs in the myeloma microenvironment but also to result in T-cell expansion and increased T-cell clonality suggestive of an immune mechanism of myeloma disease control.
These observations have provided rationale for investigation of daratumumab in combination with PD-1/PD-L1 blockade with or without IMiDs (NCT01592370, NCT03000452, and NCT02431208).

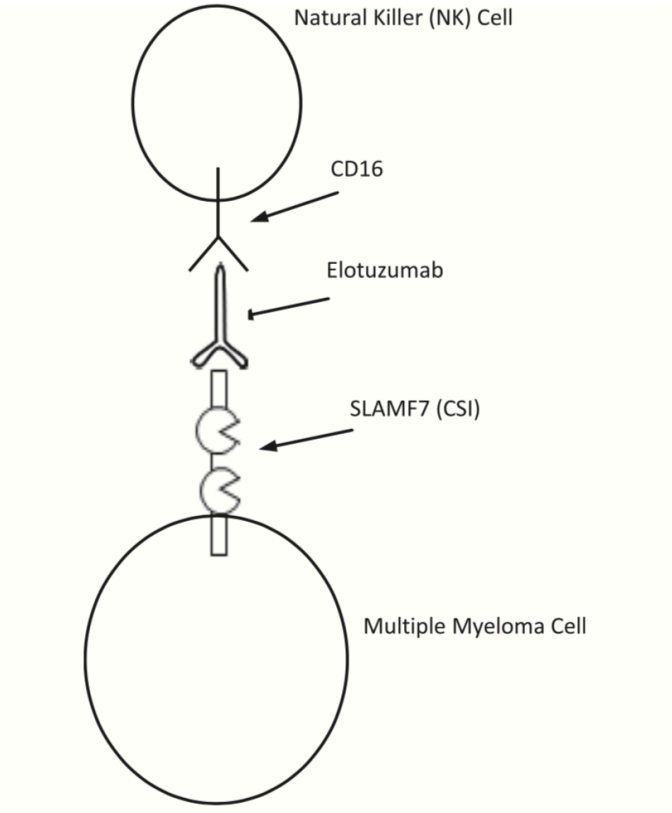



*Pianko et al.,* Clin Cancer Res; 24(5) March 1, 2018

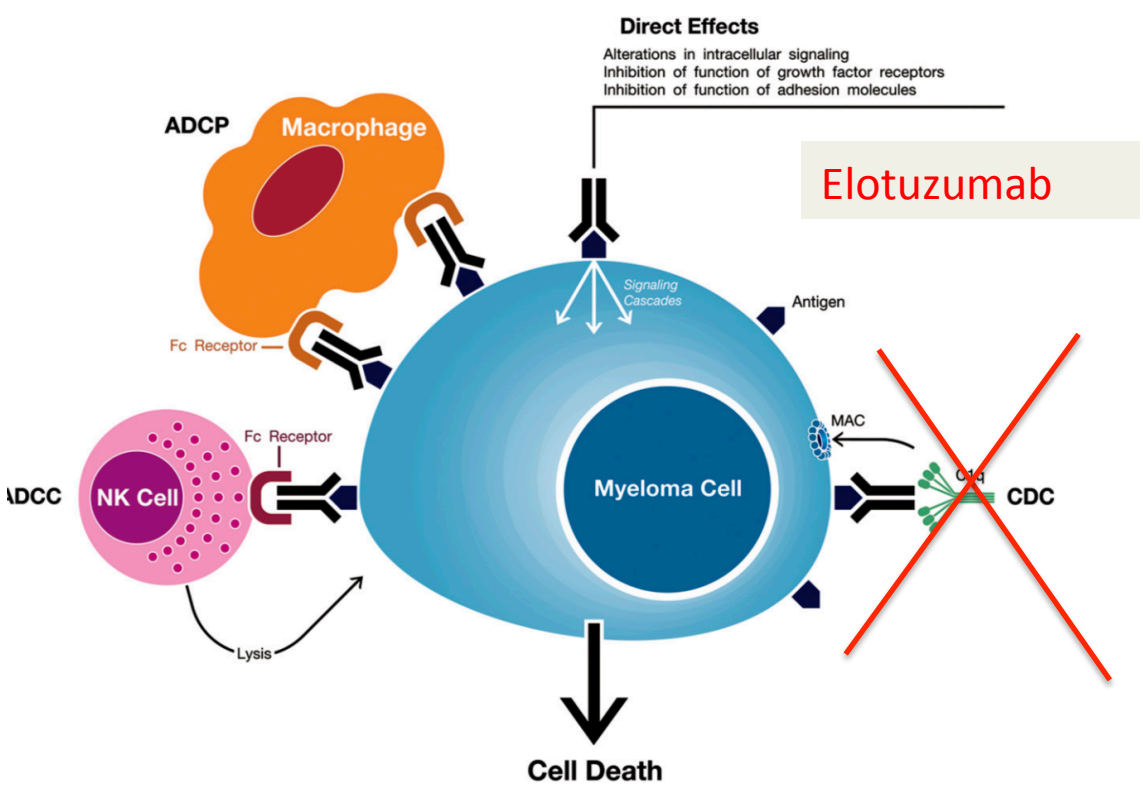
SLAMF7 is expressed on MM cells, NK cells, and a subgroup of other immune cells.

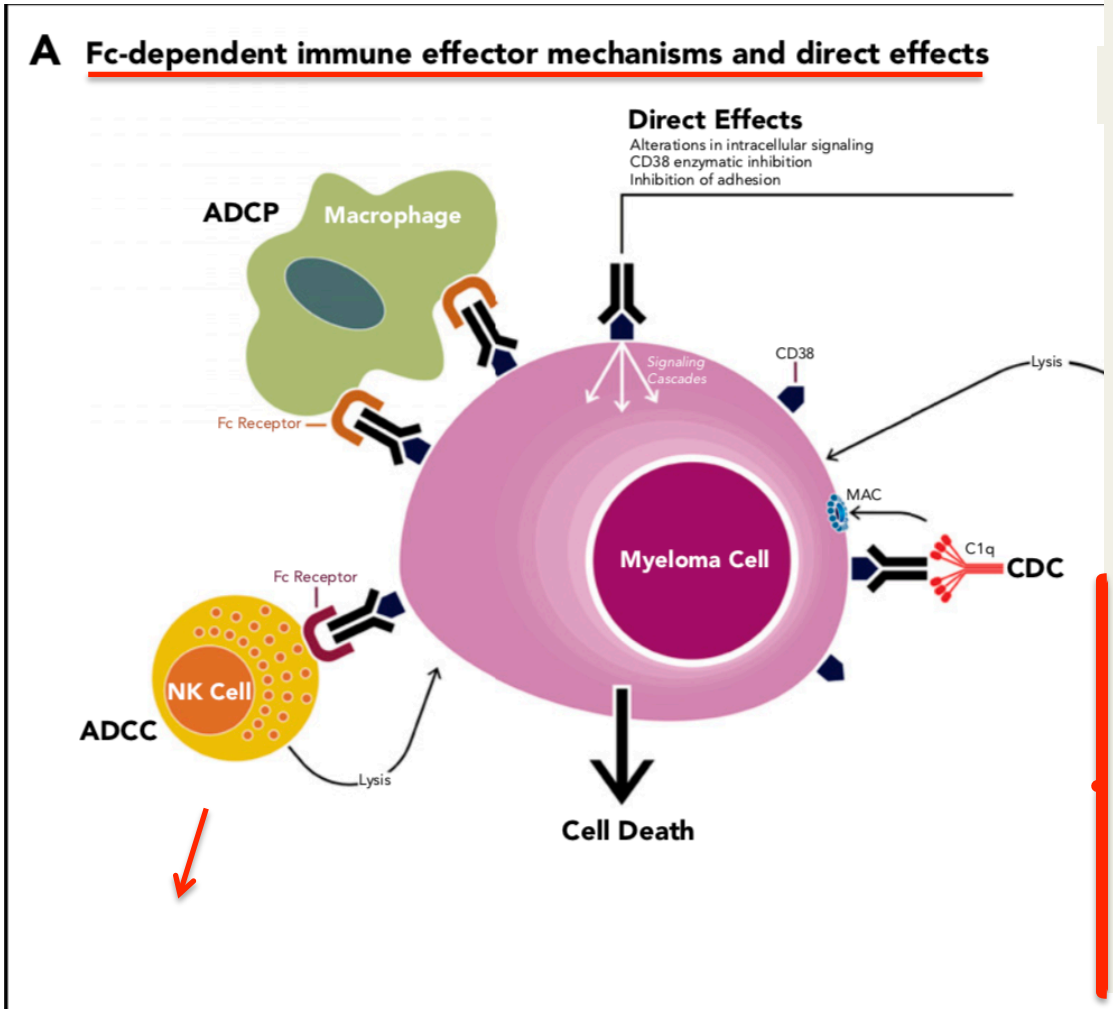
The mechanisms of action by which elotuzumab exerts its antitumor effects include ADCC and inhibition of the interactions between MM cells and stromal cells, but NOT CDC.

In addition, elotuzumab directly enhances NK cell cytotoxicity via SLAMF7 ligation.





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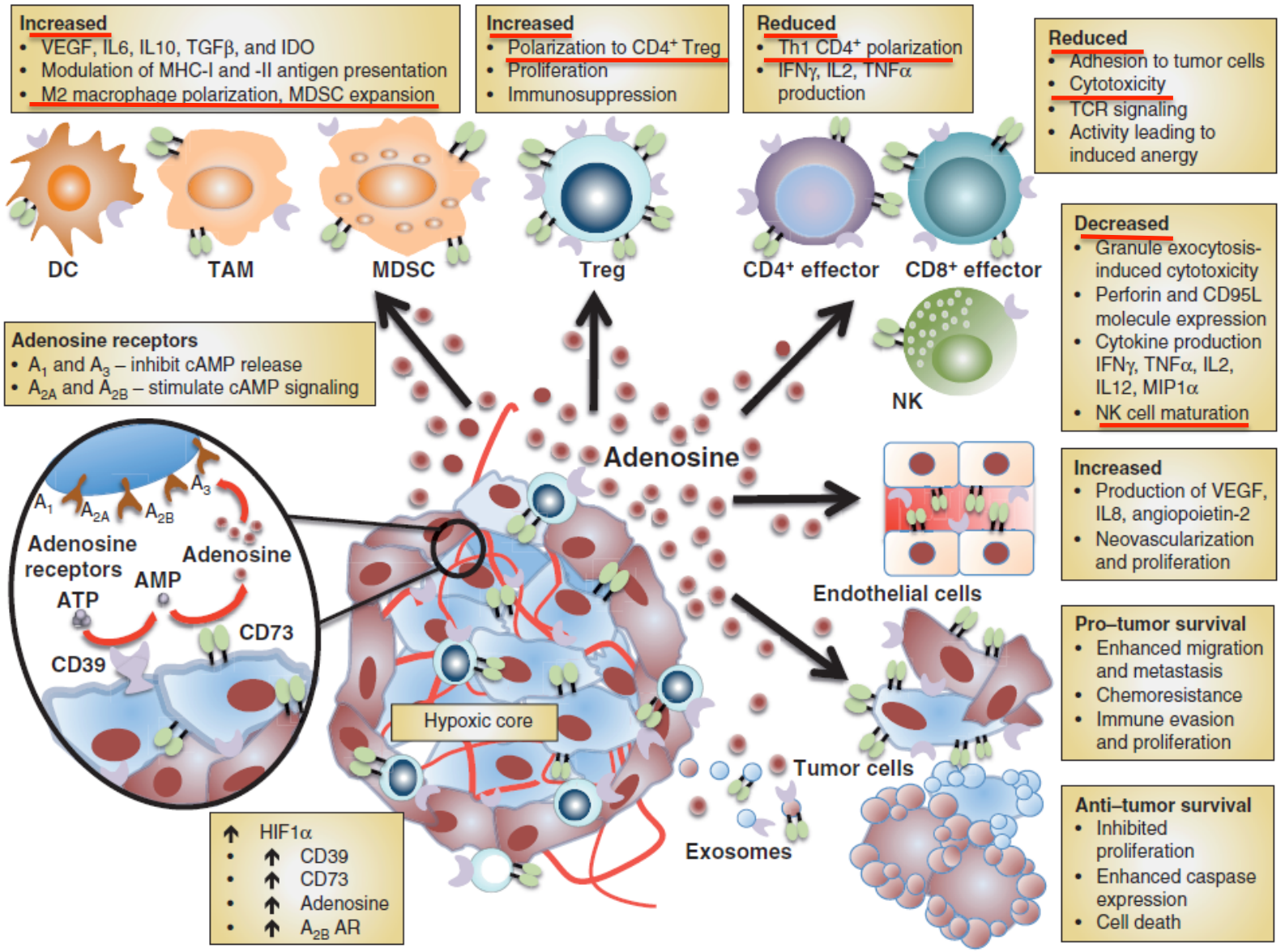
*Van der Donk et al., Blood 2018*

# Adenosine-mediated effects in the hypoxic tumor microenvironment




*Young et al., Cancer Discovery 2014*

*Ostrand-Rosenberg & Fenselau, J Immunol 2018*

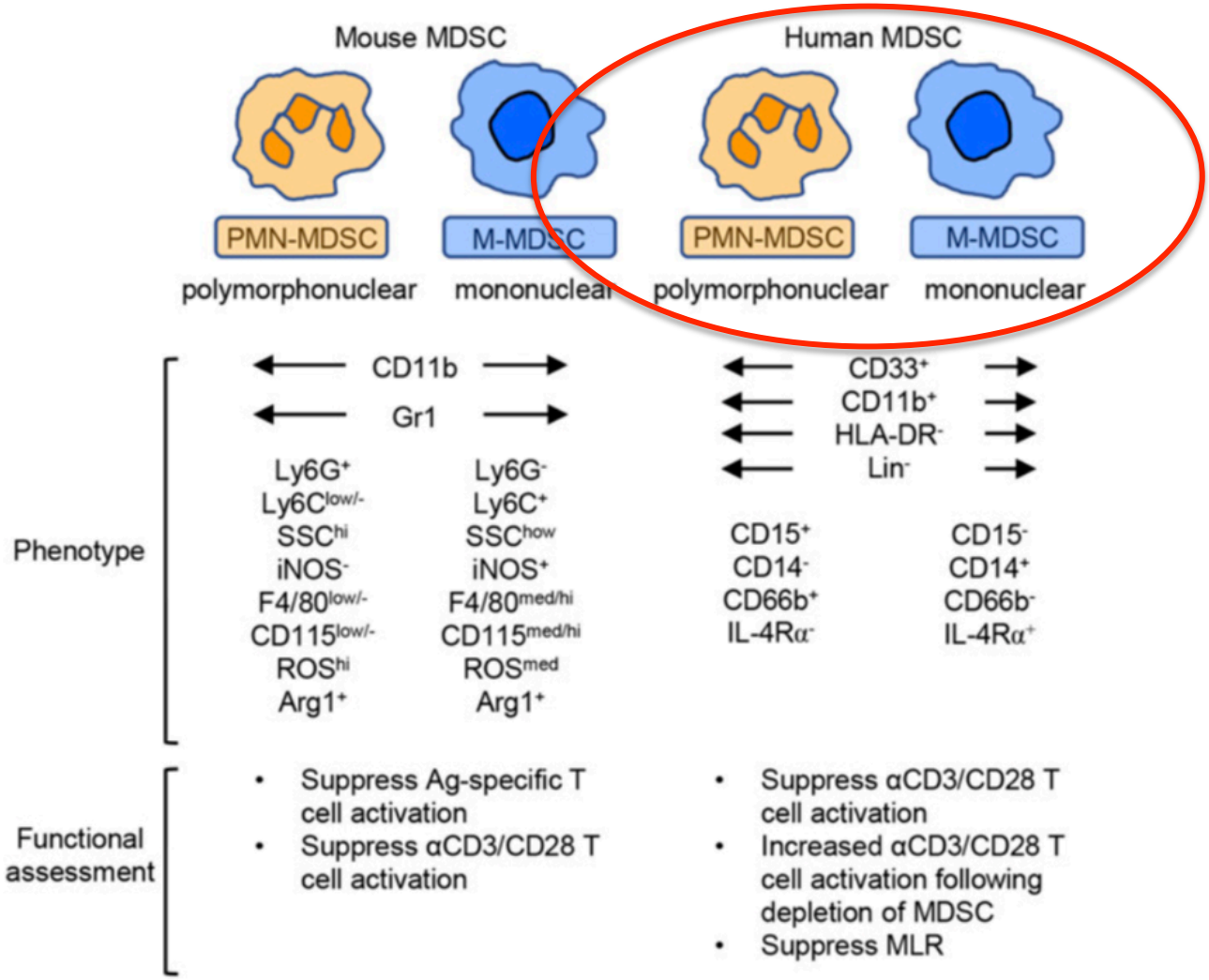
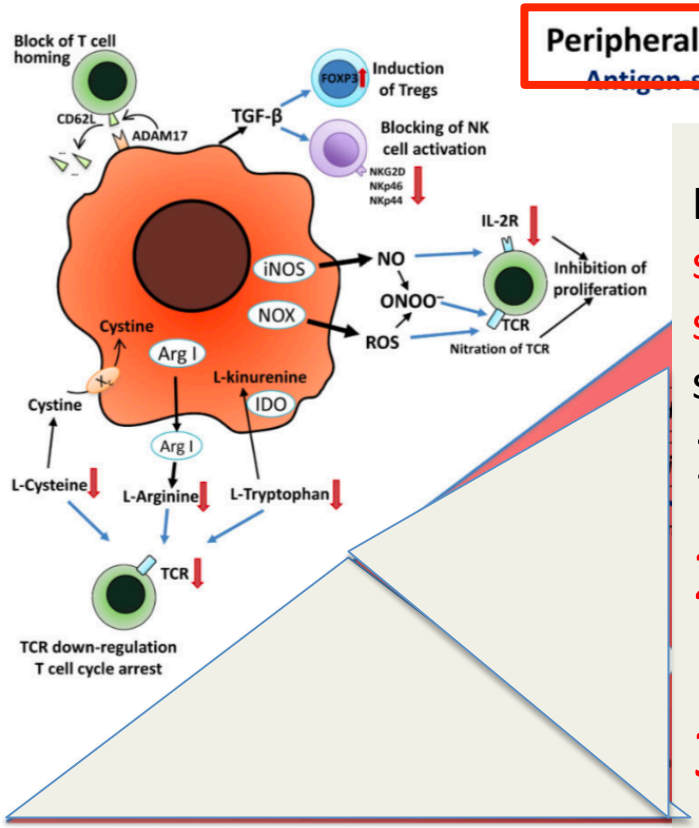



**FIGURE 1.** Phenotype and immune-suppressive functions of mouse and human M-MDSC and PMN-MDSC. $Lin^-$ indicates cells are negative for CD3, CD19, CD20, and CD56.




In peripheral lymphoid organs, immune suppression by MDSC is mainly antigen-specific, contact-dependent and utilizes several major pathways, including:

1. the production of reactive nitrogen and oxygen species (NO, ROS)
2. elimination of key nutrition factors for T cells from the microenvironment (L-arginine, L-tryptophan and L-cysteine)
3. disruption of homing of T cells (through the expression of ADAM17)
4. production of immunosuppressive cytokines (TGF-β, IL-10)
5. induction of Treg cells.

*Kumar et al.,Trend Immunol 2016*

## MDSC function in tumor site and peripheral lymphoid organs

After migration to the tumor, MDSCs are exposed to inflammatory and hypoxic tumor microenvironment.

This results in significant:

1. HIF-1α-mediated elevation of Arg1
2. upregulation of inhibitory PD-L1 on MDSC surface,
3. production of CCL4 and CCL5 chemokines attracting Tregs to the tumor.

Overall, these alterations result in more potent non-specific immunosuppressive activity of MDSCs inside the tumor.

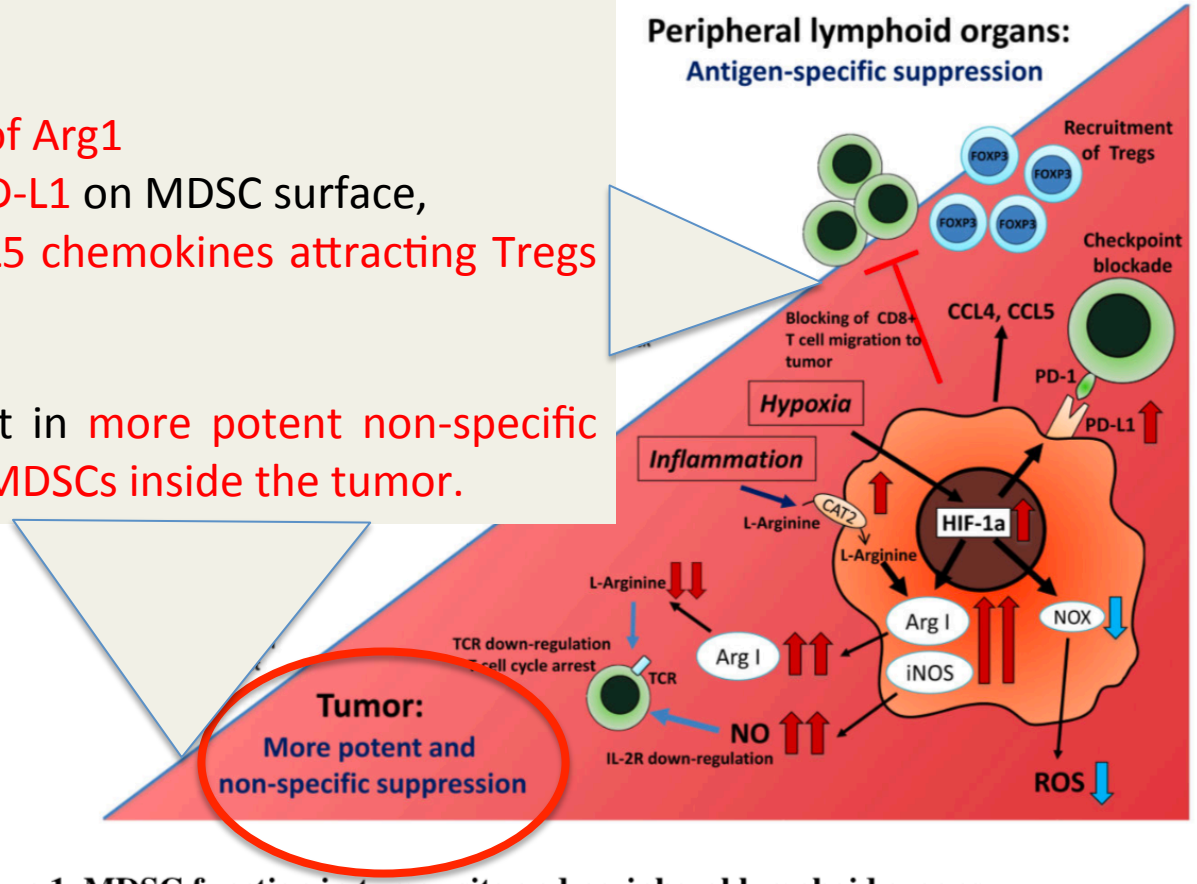



**Figure 1. MDSC function in tumor site and peripheral lymphoid organs**

*Kumar et al., Trend Immunol 2016*

A. Romano *et al*

*Br J Haematol 2014*

Hodgkin disease

**Table III.** Univariate analysis of progression-free survival.

| Factor | N patients (advanced stage) | PFS at 36 months (advanced stage) | Log-Rank (Mantel-Cox) p | Hazard Ratio (95% CI) | N patients (whole cohort) | PFS at 36 months (whole cohort) | Log-Rank (Mantel-Cox) p | Hazard Ratio (95% CI) |
|---|---|---|---|---|---|---|---|---|
| PET-2 | | | | | | | | |
| negative | 34 | 90·6% | **0·0002***** | 0·01 (0·001–0·13) | 54 | 90·0% | **<0·0001***** | **0·005 (0·0004–0·06)** |
| positive | 6 | 33·3% | | | 6 | 33·3% | | |
| $CD34^+$MDSC | | | | | | | | |
| low | 21 | 93·3% | **0·0007***** | 0·04 (0·007–0·26) | 47 | 95·7% | **<0·0001***** | **0·02 (0·004–0·11)** |
| high | 9 | 44·4% | | | 13 | 46·2% | | |
| $CD34^+$MDSC (continuous variable) | | | | | | | **<0·0001***** | **1·92 (1·41–2·61)** |

**Table IV.** Multivariate analysis of progression-free survival.

| Variable | HR | 95% CI for HR | *P*-value |
|---|---|---|---|
| Extra-nodal involvement | 3·5 | 0·53-23·63 | 0·19 |
| PET-2 positivity | 1·3 | 0·27-6·02 | 0·76 |
| WBC ($\geq 15 \times 10^9$/l) | 2·4 | 0·46-12·21 | 0·30 |
| $CD34^+$MDSC ($\geq 0{\cdot}0045 \times 10^9$/l) | **6·8** | **1·12-41·19** | **0·03** |

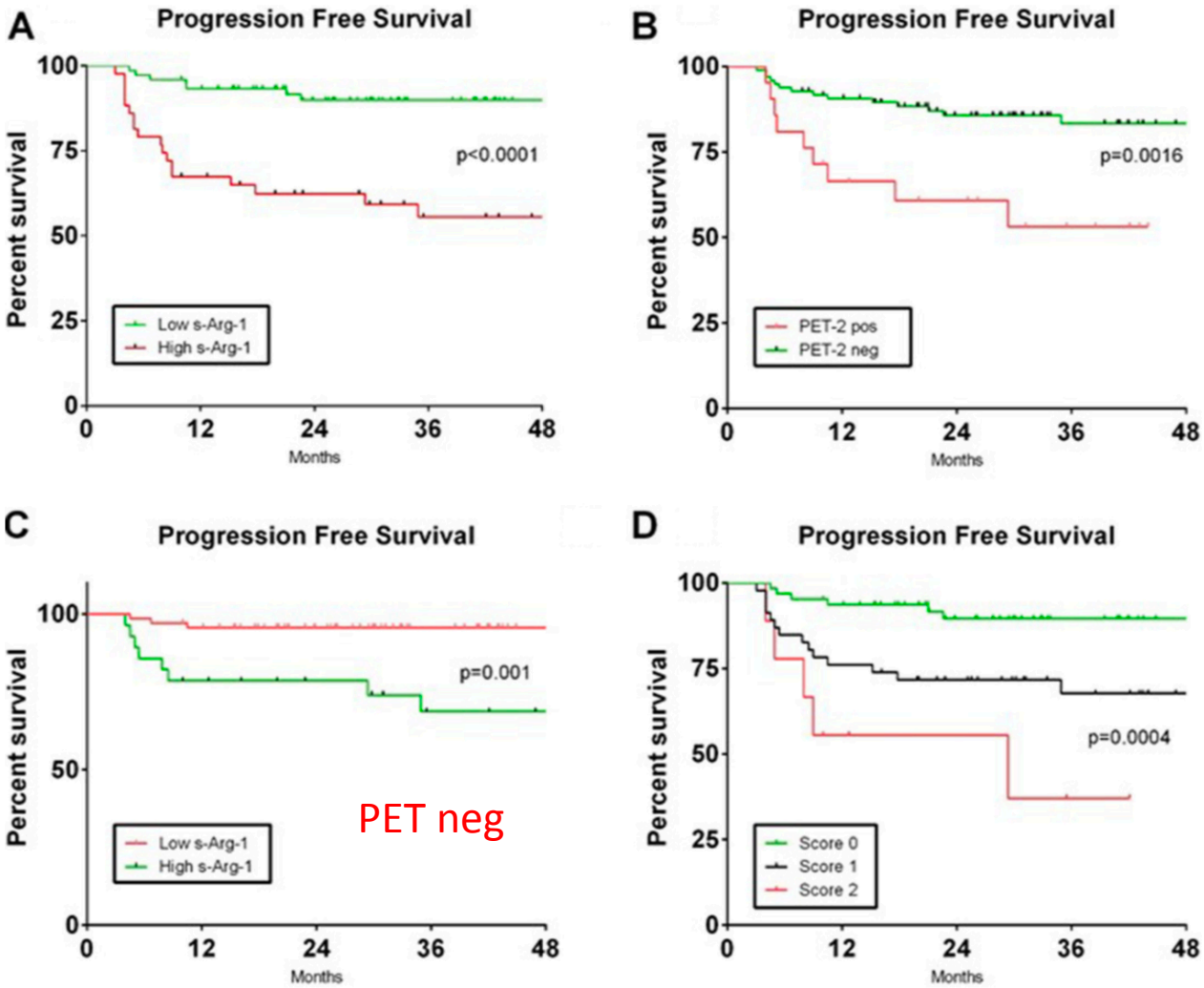



**Figure 5: Progression free survival based on combination of PET-2 status and s-Arg-1 level.** Kaplan-Meier curves of progression free survival based on s-Arg-1 at diagnosis (panel **A**) and PET-2 scan (panel **B**) in the whole cohort of 118 patients are shown. Progression free survival in the cohort of patients carrying negative PET-2 based on s-Arg-1 is shown in (panel **C**). A score was developed based on PET-2 status after two cycles of chemotherapy and s-Arg-1 level at diagnosis (Panel **D**). Patients with low s-Arg-1 and negative PET-2 scan had score 0, patients with high s-Arg-1 or positive PET-2 scan had score 1 and patients with high s-Arg-1 and positive PET-2 scan had score 2.

*Romano et al., Oncotarget 2016*

**TABLE 1. FDA-approved agents with reported effects on human MDSCs**

| Agent | FDA-approved indications | Agent classification | General mechanism of action | Effects on human MDSCs | Effects on patient outcome in approved indication | Refs. for MDSC activity |
|---|---|---|---|---|---|---|
| ATRA | APL | Differentiating agent | Binds ERK1/2 and RARs; ↑GSH synthase; ↑GSH; ↓ROS | Reduces frequency of circulating total and mo-MDSCs by differentiating cells into mature myeloid cells | ↑OS; ↑PFS; ↑RR | [12, 14, 15] |
| Vitamin D | Secondary hypoparathy- and hyperparathy-roidism | Differentiating agent | Inhibits ERK signaling and promotes apoptosis via up-regulation of BAX | Reduces frequency of circulating $CD34^{Pos}$ MDSC by differentiating cells into mature myeloid cells | Unknown; currently in clinical trials for cancer treatment | [25–27] |
| Sunitinib | RCC, GIST, and PNET | RTK inhibitor | Inhibits activity of RTKs, including PDGFR and VEGFR | Restricts MDSC development; reduces number and/or suppressive function of circulating total and PMN-MDSCs | ↑OS; ↑PFS; ↑RR (RCC and GIST) | [35, 37] |
| Bevacizumab | Colon and several other cancers | Angiogenesis inhibitor | Inhibits angiogenesis by inhibiting VEGF binding to VEGFR | Decreases circulating total and $KIT^{Pos}$ MDSC frequency | ↑PFS; ↑OS (cervical and colon cancer) | [42, 43] |
| Tadalafil | ED and BPH | PDE5 inhibitor | NO and arginase inhibition; ↓Arg1 expression; ↓NOS2 expression; ↓ iNOS expression; catalyzes the hydrolysis of cGMP [7]; ↑ intracellular [cGMP]; ↑NO2; ↑penile BP | Reduces number and/or suppressive function of bone marrow and circulating total and mo-MDSCs | Unknown; currently in clinical trials for cancer treatment | [52, 53, 56] |
| Ipilimumab | Melanoma | Check-point inhibitor | CTLA-4 antibody promotes immune activation. | Reduces frequency of circulating PMN-MDSC<br>Reduces the number of $ARG1^{+}$ myeloid cells | ↑OS; ↓ROP; ↑RR; ↑disease control rate | [71, 72] |
| Vemurafenib | Melanoma with BRAF V600E mutations | B-raf/Mek inhibitor | Inhibits mutated B-RAF<br>↓IL-6; ↓IL-10; ↓VEGF production by the tumor | Indirect action; reduces frequency of circulating mo-MDSC | ↑OS; ↓ROP; ↑RR; ↑PFS | [75] |

**TABLE 2. Active clinical trials targeting MDSCs in human cancer treatments**

| Agent(s) | Type of cancer(s) tested | Agent classification | General mechanism of action | Proposed effects of human MDSCs | Trial number |
|---|---|---|---|---|---|
| Arginine-rich nutritional supplement with surgery | Colon cancer | Differentiating agent | Increases availability of arginine | Reduces MDSC-suppressive function | NCT01885728 |
| Capecitabine + bevacizumab | Recurrent glioblastoma | Cytotoxic agent + VEGF inhibitor | Kills rapidly dividing cells and blocks angiogenesis | Capecitabine ↓MDSC number<br>Bevacizumab ↓MDSC-mediated angiogenesis and possibly MDSC recruitment to the tumor | NCT02669173 |
| Cisplatin + CKM + celecoxib + DC vaccine versus cisplatin + celecoxib + DC vaccine | Ovarian cancer | COX-2-selective inhibitor + arginase inhibitor + chemokine modulator + cytotoxic agent | Celecoxib prevents COX-2 from making PGs out of arachidonic acid, which regulates inflammation; COX-2 inhibitors also function as arginase inhibitors; CKM may alter cell signaling; cisplatin kills rapidly dividing cells. | Celecoxib ↓MDSC-suppressive function<br>Cisplatin ↓MDSC frequency | NCT02432378 |
| DC vaccine with or without gemcitabine | Sarcoma | Vaccine + nucleoside and cytotoxic agent | Gemcitabine is a nucleoside and kills rapidly dividing cells. | Gemcitabine ↓MDSC frequency | NCT01803152 |

TABLE 2. (*continued*)

| Agent(s) | Type of cancer(s) tested | Agent classification | General mechanism of action | Proposed effects of human MDSCs | Trial number |
|---|---|---|---|---|---|
| | | | is an IL-1R antagonist | | |
| PF-04136309 + FOLFIRINOX | Pancreatic adenocarcinoma | CCR2 antagonist + a cytotoxic agent | PF-04136309 inhibits the inflammatory response and metastasis in some tumors. FOLFIRINOX consists of leucovorin, fluorouracil, oxaliplatin, and irinotecan, which kill rapidly dividing cells. | PF-04136309 proposed to ↓MDSC trafficking to tumor. | NCT01413022 |
| Omaveloxolone (RTA-408) in combination with ipilimumab or nivolumab | Melanoma | NRF2 activation + immune checkpoint inhibitor | In mice, RTA-408 reduces tumor nitrotyrosine burden, inhibits the activity of MDSCs, and augments T cell anticancer activity. Ipilimumab is an anti-CTLA-4 mAb that alters T cell activation and proliferation and reduces $T_{reg}$ function; nivolumab is an anti-PD-1 inhibitor. | RTA-408 proposed to ↓MDSC-suppressive function. | NCT02259231 |
| Tadalafil | Squamous cell carcinoma | PDE5 inhibitor | NO and arginase inhibitor Tadalafil: ↓Arg1 expression; ↓NO2 expression; ↓ iNOS expression | ↓MDSC frequency and suppressive function | NCT01697800 |
| Tadalafil + anti-MUC-1 vaccine | HNSCC | PDE5 inhibitor + vaccine | NO and arginase inhibitor. Tadalafil: ↓Arg1 expression; ↓NO2 expression; ↓ iNOS expression | ↓MDSC frequency and suppressive function | NCT02544880 |

NEOPLASIA

# Bone Marrow Neovascularization, Plasma Cell Angiogenic Potential, and Matrix Metalloproteinase-2 Secretion Parallel Progression of Human Multiple Myeloma

By Angelo Vacca, Domenico Ribatti, Marco Presta, Monica Minischetti, Monica Iurlaro, Roberto Ria, Adriana Albini, Federico Bussolino, and Franco Dammacco

To assess whether the progression of plasma cell tumors is accompanied by angiogenesis and secretion of matrix-degrading enzymes, bone marrow biopsy specimens from 20 patients with monoclonal gammopathy of undetermined significance (MGUS), 18 patients with nonactive multiple myeloma (MM), and 26 patients with active MM were evaluated for their angiogenic potential and matrix-metalloproteinase (MMP) production. A fivefold increase of the factor VIII+ microvessel area was measured by a planimetric method of point counting in the bone marrow of patients with active MM as compared with nonactive MM and MGUS patients ($P < .01$). When serum-free conditioned media (CM) of plasma cells isolated from the bone marrow of each patient were tested in vivo for their angiogenic activity in the chick embryo chorioallantoic membrane (CAM) assay, the incidence of angiogenic samples was significantly higher ($P < .01$) in the active MM group (76%) compared with nonactive MM (33%) and MGUS (20%) groups. Moreover, a linear correlation ($P < .01$) was found between the extent of vascularization of the bone marrow of a given patient and the angiogenic activity exerted in the CAM assay by the plasma cells isolated from the same bone marrow. In vitro, a significantly higher fraction of the plasma cell CM samples from the active MM group stimulated human umbilical vein endothelial cell (HUVEC) proliferation (53%, $P < .01$), migration (42%, $P < .05$), and/or monocyte chemotaxis (38%, $P < .05$) when compared with nonactive MM and MGUS groups (ranging between 5% and 15% of the samples). Also, immunoassay of plasma cell extracts showed significantly higher ($P < .01$) levels of the angiogenic basic fibroblast growth factor (FGF)-2 in the active MM patients than in nonactive MM and MGUS patients (153 ± 59, 23 ± 17, and 31 ± 18 pg FGF-2/100 μg of protein, respectively). Accordingly, neutralizing anti–FGF-2 antibody caused a significant inhibition (ranging from 54% to 68%) of the biological activity exerted on cultured endothelial cells and in the CAM assay by plasma cell CM samples from active MM patients. Finally, in situ hybridization of bone marrow plasma cells and gelatin-zymography of their CM showed that active MM patients express significantly higher ($P < .01$) levels of MMP-2 mRNA and protein when compared with nonactive MM and MGUS patients, whereas MMP-9 expression was similar in all groups. Taken together, these findings indicate that the progression of plasma cell tumors is accompanied by an increase of bone marrow neovascularization. This is paralleled by an increased angiogenic and invasive potential of bone marrow plasma cells, which is dependent, at least in part, by FGF-2 and MMP-2 production. Induction of angiogenesis and secretion of MMPs by plasma cells in active disease may play a role in their medullary and extramedullary dissemination, raising the hypothesis that angiostatic/anti-MMP agents may be used for therapy of MM.



1. Increased neovascularization of the bone marrow stroma in patients affected by MM.

2. The grade of this neovascularization seems to increase during the evolution from MGUS to MM

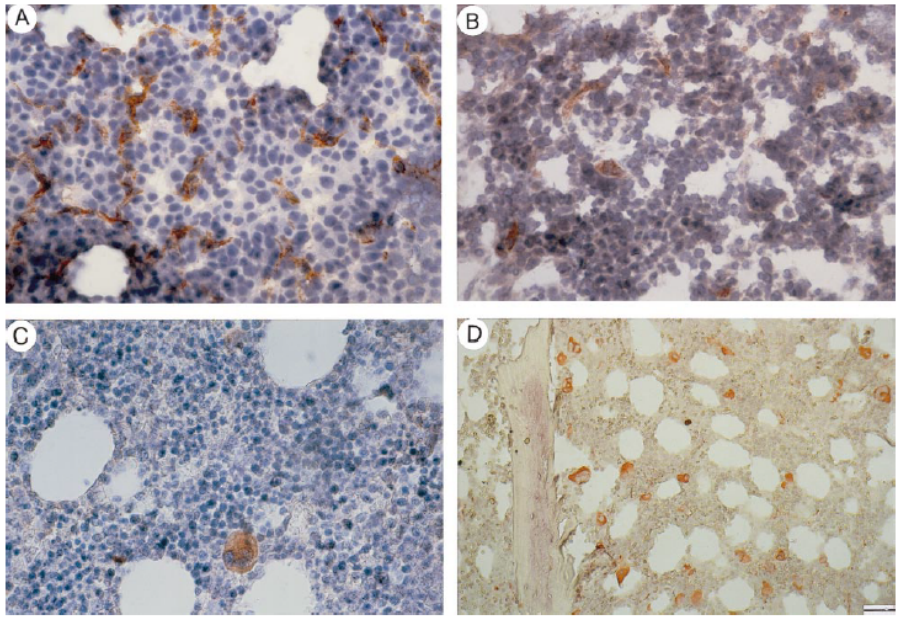



Fig 1. Staining with factor VIII of bone marrow from patients with (A) MM at relapse, (B) MM at plateau, (C) MGUS, and (D) a control subject

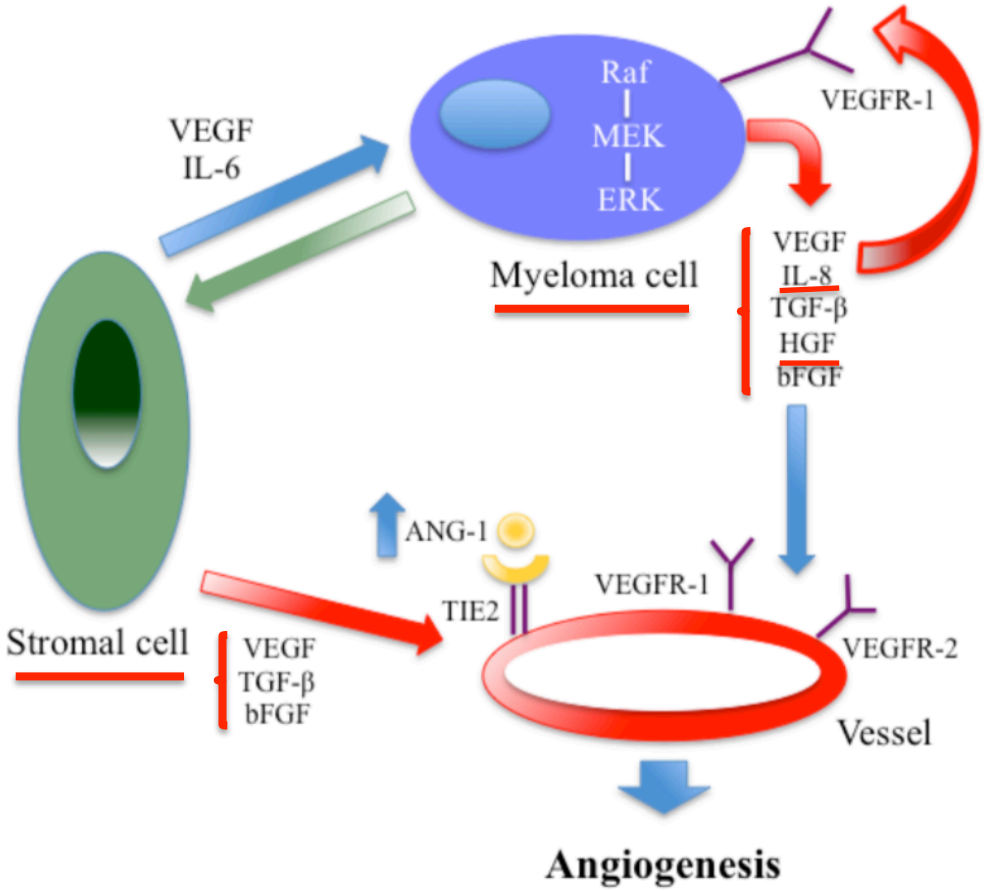



**Figure 4: Autocrine and paracrine VEGF-mediated pathways in multiple myeloma: both are important for tumor angiogenesis and growth.** A close relationship between VEGF and IL-6 has been found in the paracrine pathways.



This process is triggered by neoangiogenetic factors, which are produced from the BMSC, [such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (b-FGF), and TGF-β, and from the myeloma cells themselves (such as VEGF, IL-8, hepatocyte growth factor (HGF) and TGF-β).

Designed ankyrin repeat proteins (DARPin®) are among the promising non-immunoglobulin binding proteins.
DARPin® molecules are high-affinity binding proteins with high target specificity and high biophysical stability.

MP0250 is a multi-domain DARPin® drug candidate with binding specificities for VEGF-A (the main isoform of VEGF), HGF and human serum albumin and it is the first multi-functional DARPin® protein in clinical trials.

www.impactjournals.com/oncotarget/ Oncotarget, 2018, Vol. 9, (No. 17), pp: 13366-13381

Research Paper

**Targeting angiogenesis in multiple myeloma by the VEGF and HGF blocking DARPin® protein MP0250: a preclinical study**

Luigia Rao[1,*], Kim De Veirman[2,*], Donato Giannico[1], Ilaria Saltarella[1], Vanessa Desantis[1], Maria Antonia Frassanito[1], Antonio Giovanni Solimando[1], Domenico Ribatti[3], Marcella Prete[1], Andreas Harstrick[4], Ulrike Fiedler[4], Hendrik De Raeve[5], Vito Racanelli[1], Karin Vanderkerken[2,*] and Angelo Vacca[1,*]

1. **MP0250 inhibits *in vitro* MMEC functions involved in angiogenesis**
2. **MP0250 is more effective than single VEGF- and HGF-neutralizing mAbs**
3. **MP0250 abrogates *in vivo* angiogenesis**
4. **MP0250 impacts angiogenesis in syngeneic 5T33MM model**
5. **MP0250 improves bortezomib activity in MM**

# BM mesenchymal stromal cell–derived exosomes facilitate multiple myeloma progression

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1. MM **BMMSCs release exosomes** that are **transferred to MM cells**, thereby resulting in **modulation of tumor growth** in vivo.
2. **Exosomal *microRNA* (*miR*) content differed between MM and normal BM-MSCs**, with a **lower content of the tumor suppressor *miR-15a***.
3. **MM BM-MSC–derived exosomes had higher levels of oncogenic proteins, cytokines, and adhesion molecules** compared with exosomes from the cells of origin.
4. Importantly, whereas MM BM-MSC–derived exosomes promoted MM tumor growth, **normal BM-MSC exosomes inhibited the growth of MM cells.**

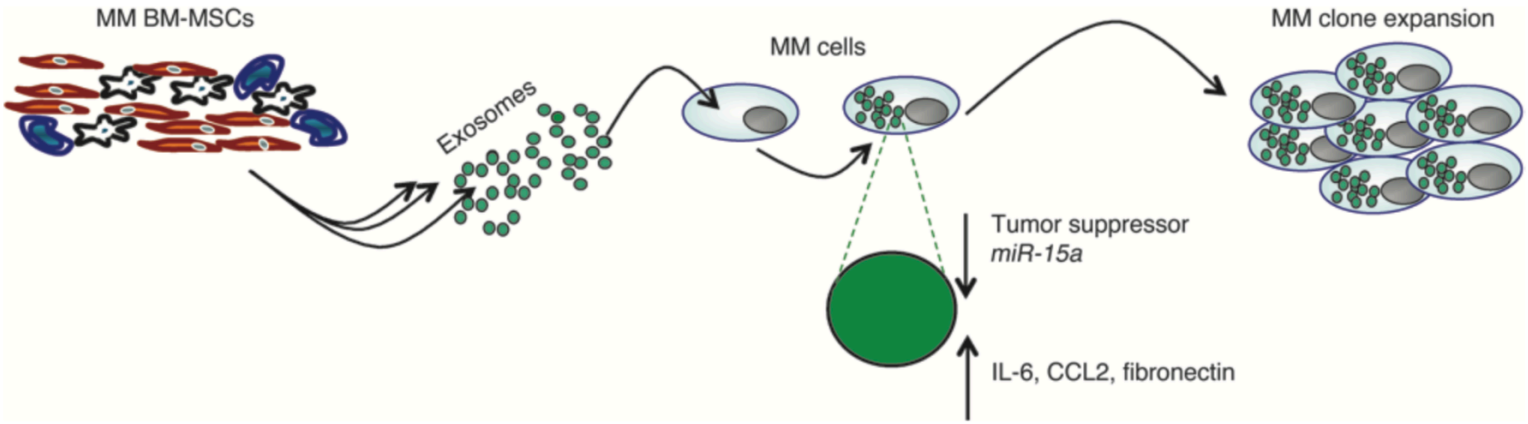



**Figure 8**
BM-MSC–derived exosomes support MM clone expansion.

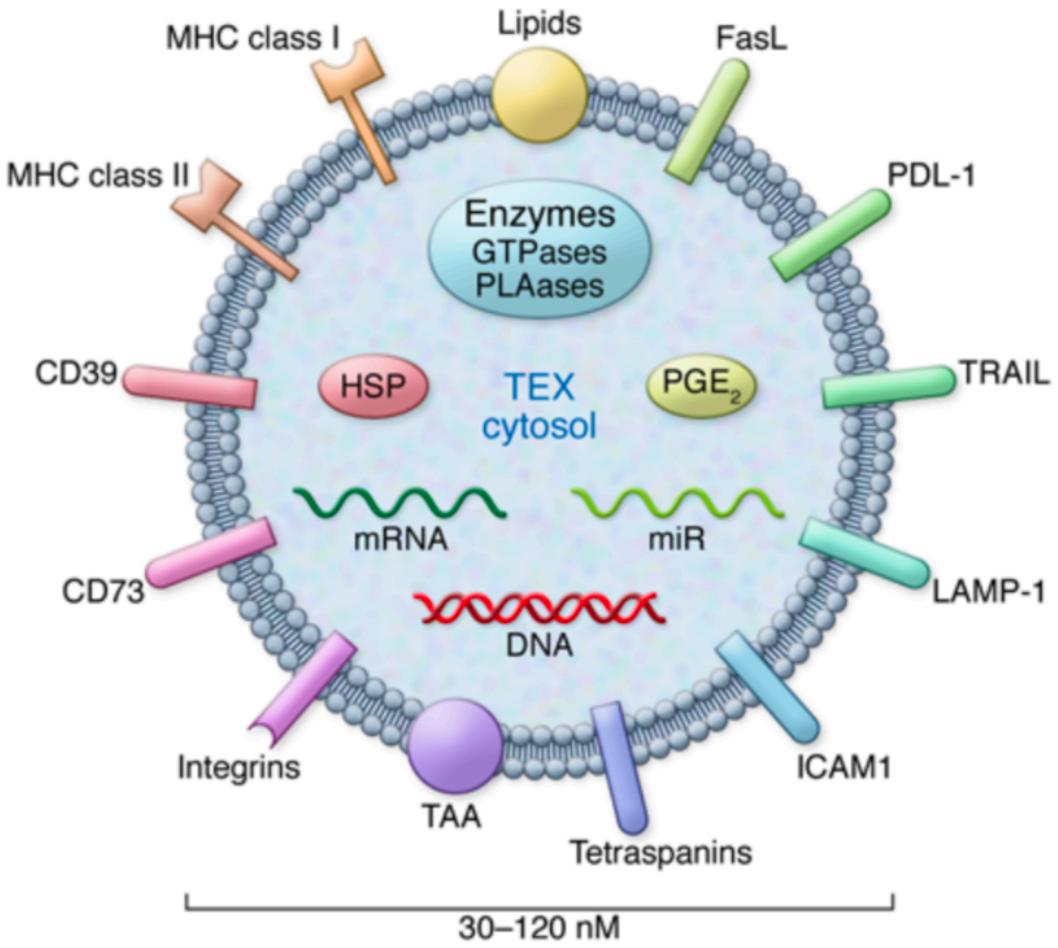



**Figure 2. Components of the TEX cargo.** TEX may contain immunoinhibitory ligands and immunostimulatory molecules. The intravesicular content includes nucleic acids and various cytosolic components from the cytosol of a parent cell. Reproduced with permission from *Biochemical Society Transactions* (50).

TEX produced and released by tumor cells, induce:

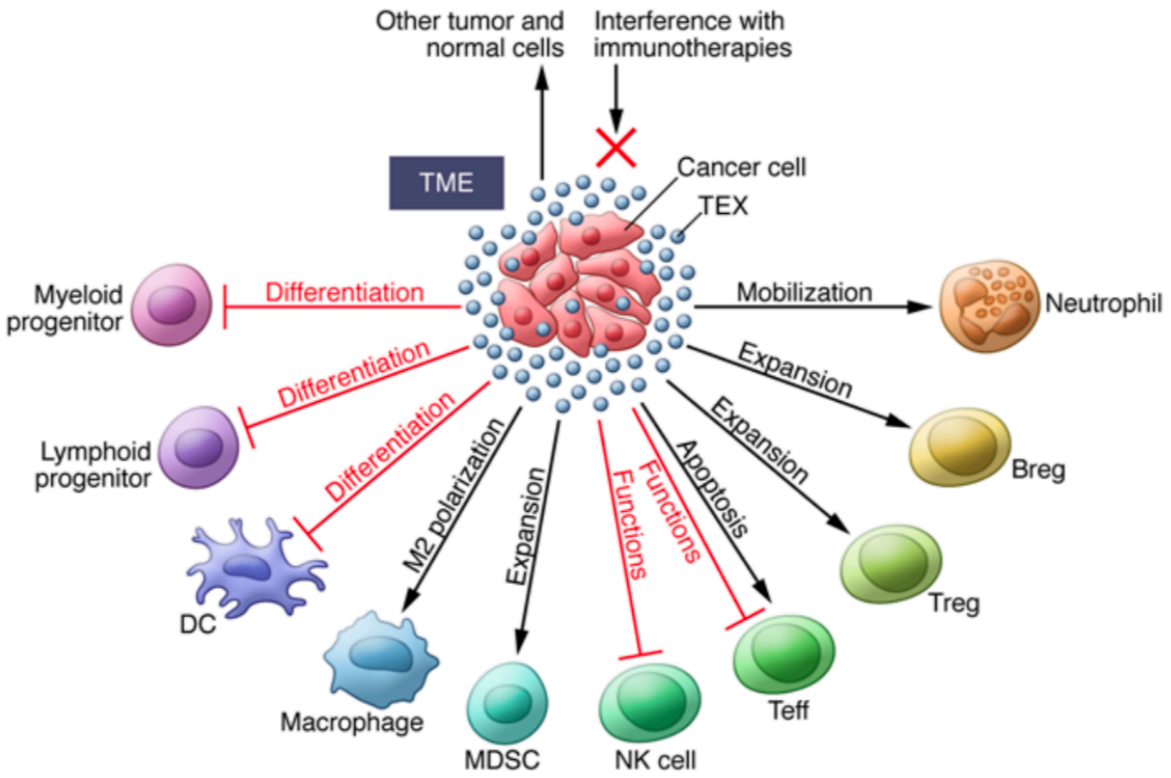
(a) inhibition of functions necessary for antitumor responses;

(b) apoptosis of activated Teffs;

(c) expansion and upregulation of suppressive activity in Tregs, MDSCs, and regulatory B cells (Bregs);

(d) interference with cellular differentiation;

- TEX carrying TAAs can interfere with immuno-therapies.
- TEX may also mediate autocrine effects and influence the functions of normal cells present in the TME.



3D dynamic culture of reconstructed human multiple myeloma microenvironments in bioreactor may represent a useful platform for drug testing and for studying tumor-stroma molecular interactions.

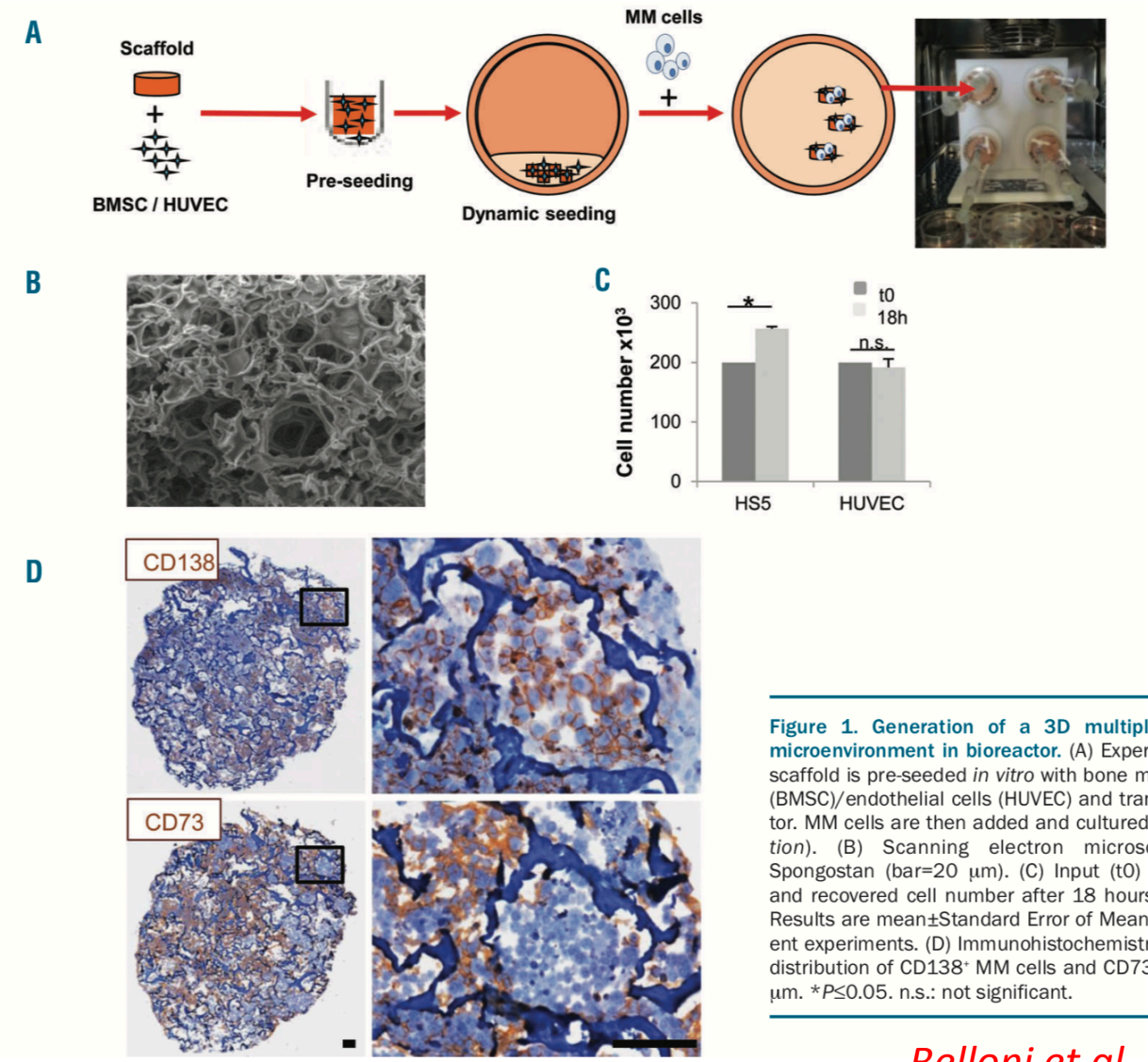



**Figure 1. Generation of a 3D multiple myeloma (MM) microenvironment in bioreactor.** (A) Experimental procedure: scaffold is pre-seeded *in vitro* with bone marrow stromal cells (BMSC)/endothelial cells (HUVEC) and transferred to bioreactor. MM cells are then added and cultured (see *Methods section*). (B) Scanning electron microscopy analysis of Spongostan (bar=20 μm). (C) Input (t0) (200x10³/scaffold) and recovered cell number after 18 hours (h) of 3D culture. Results are mean±Standard Error of Mean of three independent experiments. (D) Immunohistochemistry showing uniform distribution of CD138⁺ MM cells and CD73⁺ stroma. Bar=100 μm. *$P \leq 0.05$. n.s.: not significant.

*Belloni et al., Haematologica 2018*

# KEY POINTS

1. Increasing knowledge on BM microenvironment in MM:

- Relevance of BM stroma (cell-cell adhesion)
- Relevance of BM stroma (soluble factors):
  Growth factors
  MDSC-Arginasi
  Exosomes
- New immunotherapies take advantage of and generate such knowledge (daratumumab-elotuzumab-darpins)

2. New laboratory technical advances (3D)

3. Need to translate knowledge and therapies to smoldering MM and MGUS.