Monoclonal antibodies, tumor targeting and immunotherapeutical strategies with SLAM-F7 and CD38 (Bologna 2019)

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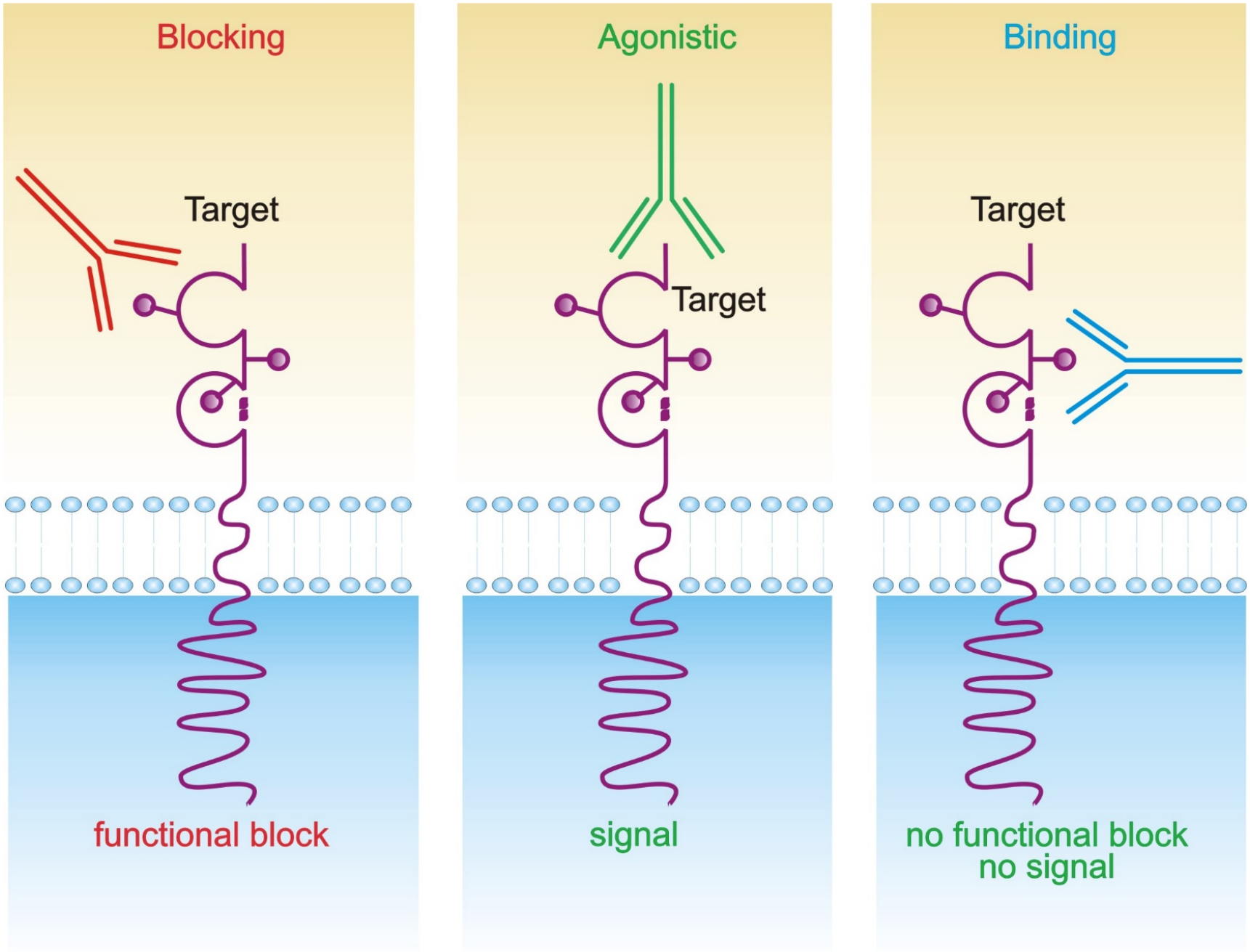
Conflicts of Interest.

- Jenssen (USA): Advisory Board, research grants
- Celgene: reasearch grants
- Sanofi: Advisory Board
- Tusk Immunotherapeutics: Scientific Board
- Centrose: Scientific Board

Events occurring after target binding by monoclonal antibodies

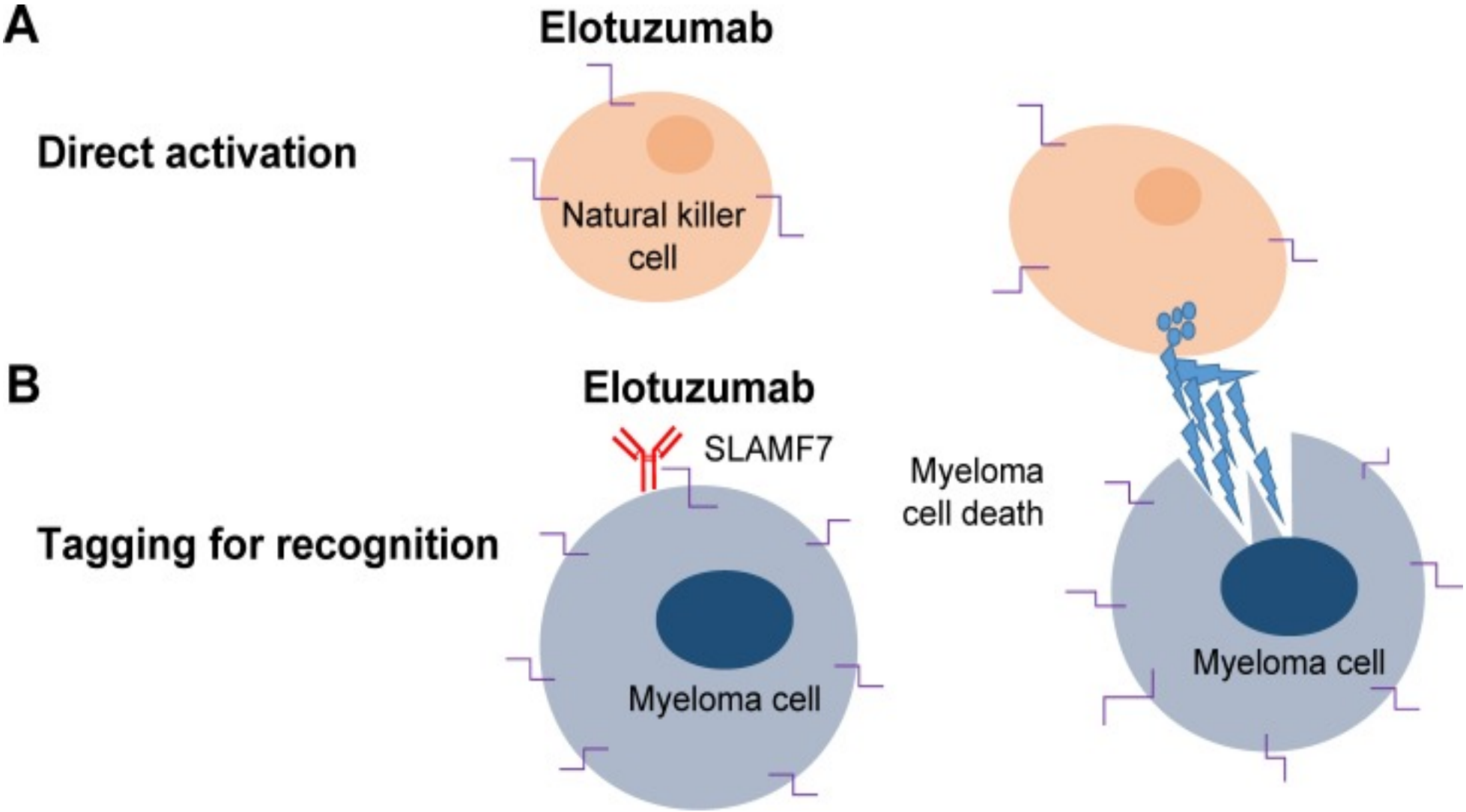


Main human FcRs and specific tissue distribution and function

	CD16A	CD32A	CD32B	CD64
Signals	positive signals	positive signals	negative signals	positive signals
Tissue Distribution	NK cell, Monocyte, DC, Macrophage	Monocyte, DC, Platelets, Macrophage, Neutrophil	B cell, Plasma cell, Monocyte, DC, Macrophage, Neutrophil, Mast cell	Monocyte, DC, Macrophage

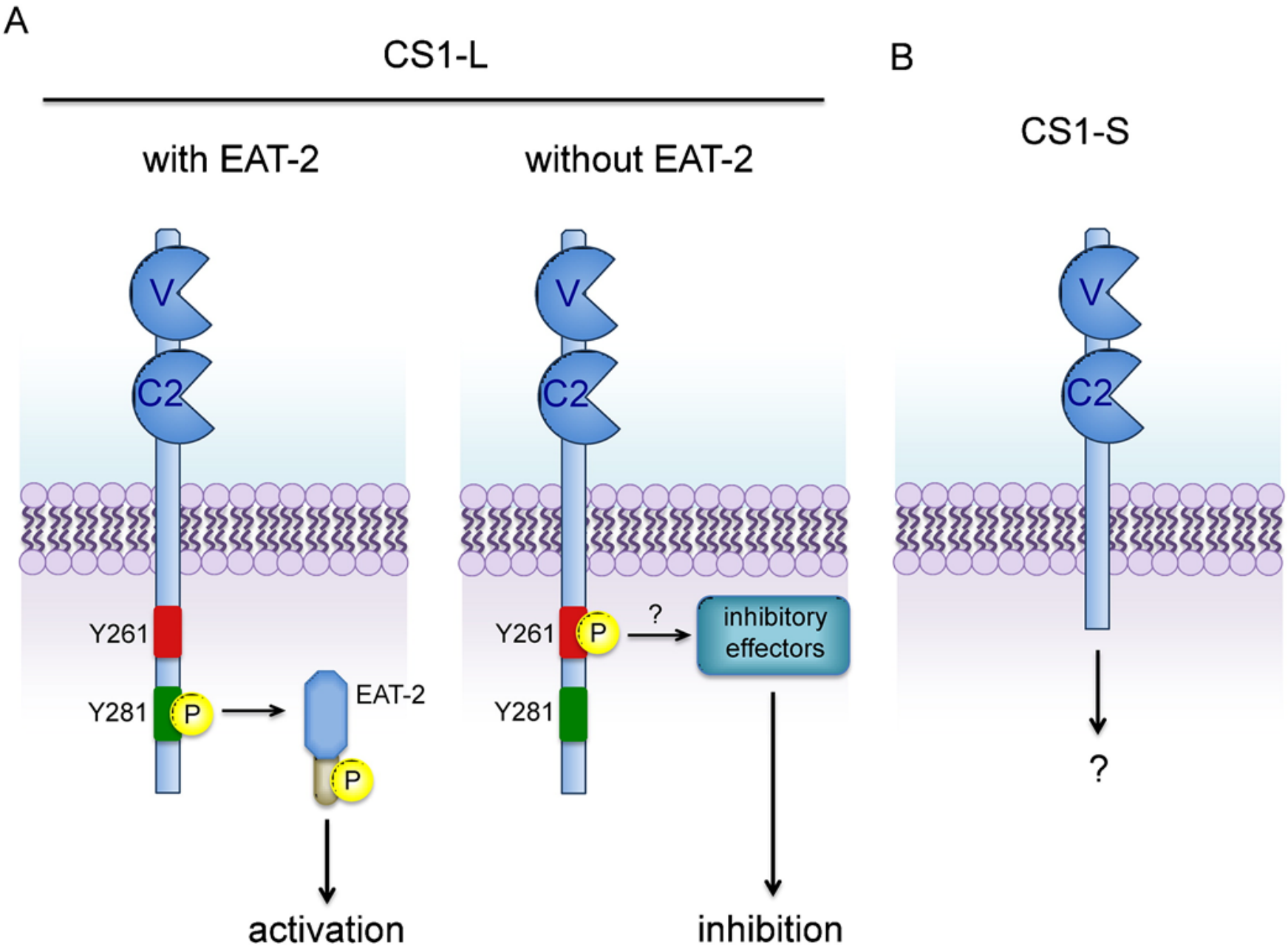
SLAMF7

Elotuzumab: proposed mechanisms of action in multiple myeloma



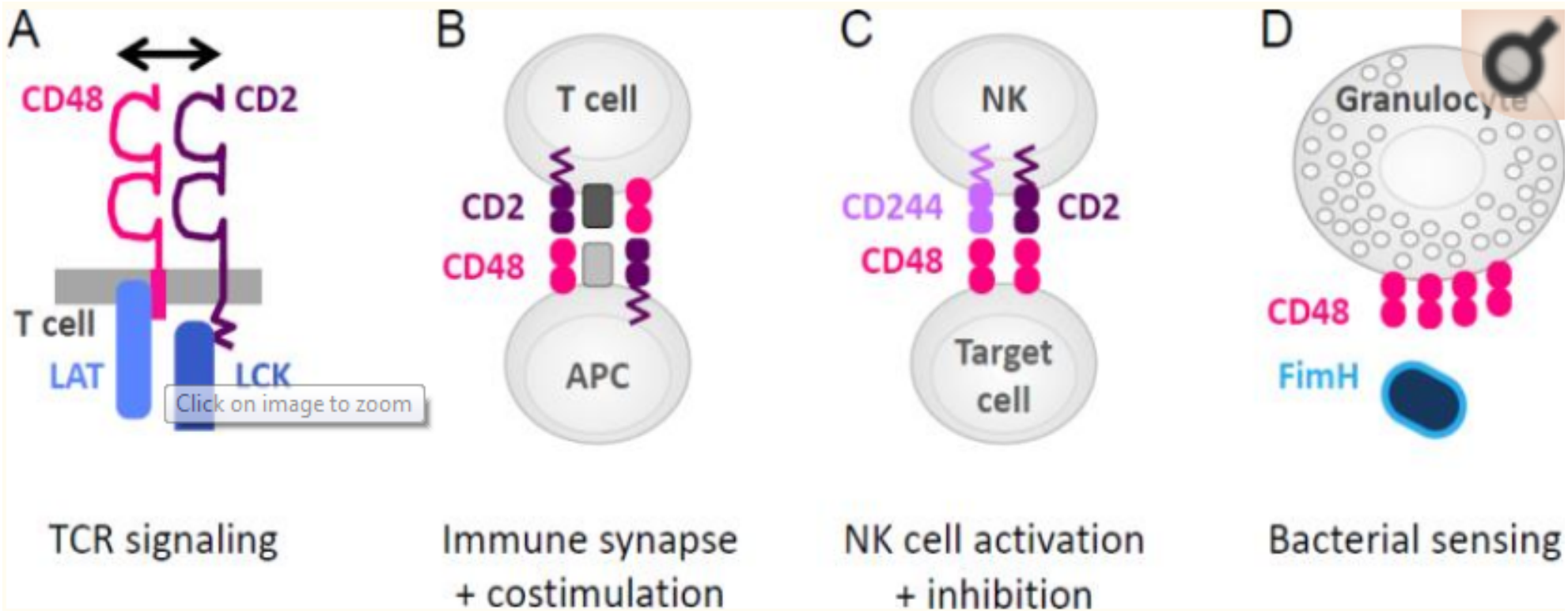
R. Friend, …….. and S. Z. Usmani. Drug Design, Development and Therapy, 11: 893-900, 2017

Two CS1 (SLAMF7) isoforms differentially regulate immune cell functions



Guo et al. Mol Cell Biol. 2015 Jan;35(1):41-51

Mechanism of action of CD48 (SLAMF2) along with SLAM members



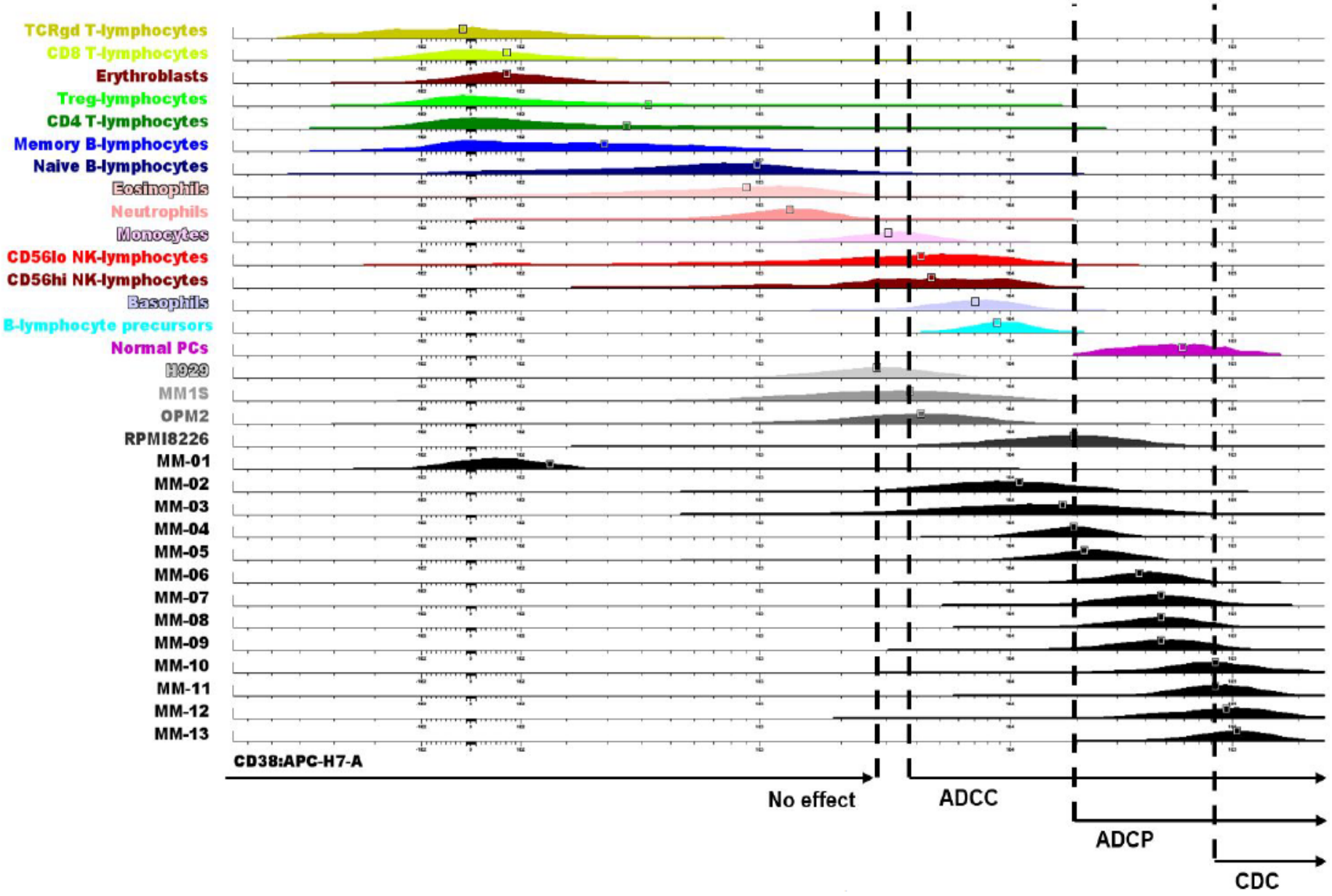
McArdel SL, Therorst C, Sharpe A, *Clin Immunol*, 2016.

CD48 (SLAMF2) IS OVEREXPRESSED IN THE MAJORITY OF MM AND MAY REPRESENT AN ALTERNATIVE TARGET WHEN SLAMF7 IS WEAKLY EXPRESSED.

ASHOUR R ET AL. INT. J. HEMATOL. 2019

Isatuximab

MoA triggered by Isatuximab in function of the CD38 levels expressed by normal and MM cells



L. Moreno,…….and B. Paiva. *Clin. Cancer Res.* (2019)

L. Moreno, ……., J. F. San Miguel and B. Paiva.
The Mechanism of Action of the anti-CD38 monoclonal antibody Isatuximab in Multiple Myeloma.
Clin Cancer Res. 2019 May 15;25(10): 3176-3187.

Result #1

Isa induces internalization of CD38, but not its significant release from MM cells

L. Moreno, ……., J. F. San Miguel and B. Paiva.
The Mechanism of Action of the anti-CD38 monoclonal antibody Isatuximab in Multiple Myeloma.
Clin Cancer Res. 2019 May 15;25(10): 3176-3187.

Results # 2

a) Isa induces direct apotosis on MM with high CD38 levels.

b) Isa induces sensitization of MM with high CD38 to bortezomib + dexamethasone

c) No apoptosis by direct transmembrane signaling in MM cells with CD38 levels similar to those of patients.

d) No significant gene deregulation on RPMI8226 upon antibody ligation

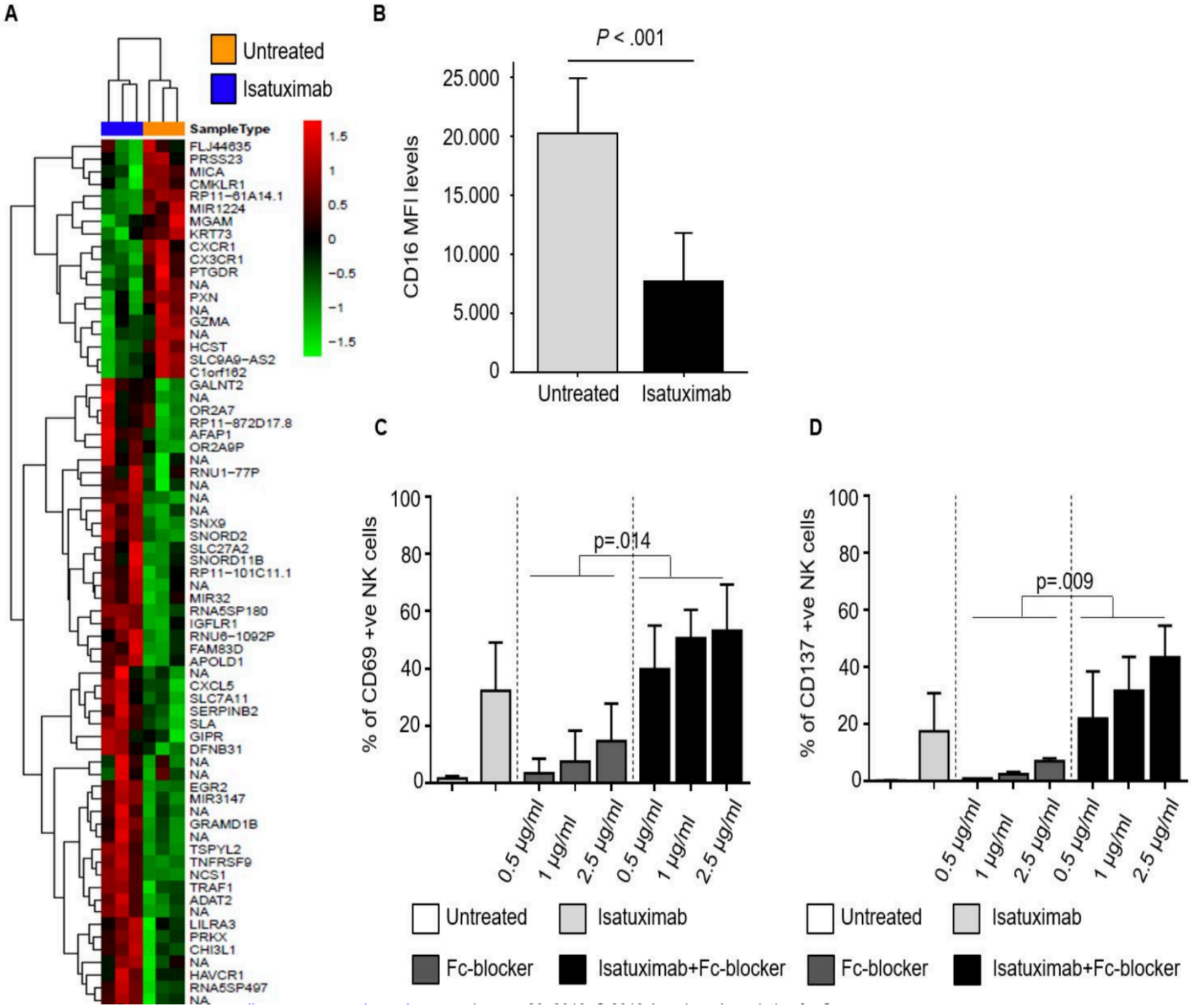


Fig. 4. (A) Heat-map of genes differentially expressed in NK cells exposed to RPMI8228 with Isa. (B) CD16 after Isa in BM NK cells from MM patients. Percentage of CD69 (C) and CD137 (D) cells in NK cells exposed to RPMI8226 and Isa, in the presence of a Fc blocker

Activation and depletion of NK cells after Isa treatment

a) ADCC mediated by Isa leads to killing of high and low CD38 MM cells. At the same time, Isa depletes high CD38 NK cells.

b) Transcriptome of NK was evaluated after co-culture with RPMI8226 with (and without) Isa.

c)TNFRSF9 (4-1BB, CD137) was up-modulated, also at the protein level.

d) CD16 was down-regulated during the cross-talk.

e) CD69 and CD137 are increased when NK cells are treated with Fc blockers.

f) Isa activates NK cells by Fc-binding and CD38 trans-membrane signaling

Effecs of Isatuximab on $CD38^{+}$ subsets of T regs in MM patients

1) CD38 expression is higher on Tregs than Tcons in MM patients
2) CD38 surface levels and % of $CD38^{high}$ are increased by lenalidomide (len) and pomalidomide (pom)
3) Isatuximab induces <Treg
   >Tcons (enhanced by lenalidomide and pomalidomide)
4) Isatuximab induces <Foxp3 in Tregs
   <IL10
   restores Tcons proliferation and function
5) Isatuximab treatment >lysis of MM cells by CD38+ T and NK cells
6) Co-cultures of MM with Tcons → induction of Tregs

   The induced Tregs (iTregs) express CD38, CD25, Foxp3 higher than natural Tregs
7) Isatuximab decreases iTregs induced by Mm and BM stromal cells

   This effect is induced by inhibition of cell-cell contacts and release of GFβ/IL10
8) CD38 levels correlate with differential inhibition by Isatuximab of Tregs from MM

Conclusion: Isatuximab preferentially blocks immunosuppressive Tregs and restore immune effector functions.

Feng X. et al., …. and Anderson K. C. Clin Cancer Res, 2017

Adaptive anti-tumor immunity in response to antibody treatment: *in vivo* vaccination

Hypothesis: the fitness of the immune system at baseline would be predictive for clinical response to Isatuximab.

Results: MM patients were screened for endogeneous antibody responses against a total of 27 myeloma-associated tumor antigens.

<u>Antibody response</u>

2 patients: no pre-existing antibody response and no response during treatment. → Likely, a compromised immune system already at baseline.

2 patients: pre-existing antibody responses against several antigens. During Isatuximab treatment, new and increasing autologous IgG antibody responses against additional MM-associated antigens.

Target antigens identified were MAGEC2 and NY-GESO-1 (an IgG response). The latter molecule was temporary target of IgM (new response).

<u>Cell response:</u>

No T cell response in the 2 non-responders after Isatuximab therapy.

$CD8^+$ T cells were identified against NY-GESO-1 and CD38 after Isatuximab therapy.

$CD4^+$ and $CD8^+$ T cell response was observed against CD38 in the second responder patient.

Clinical effects

The 2 patients with humoral and cell responses to MM-associated antigens showed significant responses to Isatuximab treatment. Patients showed complete remission (CR) and disappearance of the serological markers. The effects were durable.

The 2 non-responders showed progression of disease, after 2-12 months of disease stabilization.

Conclusions

1) Generation of anti-CD38 and anti-MM-associated antigens antibodies during Isatuximab treatment.
2) MM with anti-MM immune response appear to develop additional anti-tumor responses during Isatuximab treatment.
3) Tumor-specific immune fitness might be associated with positive clinical response.

Caveat: the results were obtained from a small clinical sample. However, these are indications supporting the view that Isatuximab treatment favours an adaptive anti-tumor immunity (*in vivo* vaccination)

Atanackovic D., et al., *Leukemia*, 2019.

Isatuximab-induced killing of MM cells is dependent on the expression of CD38 and of complement inhibitory receptors: response predictive markers?

ADCC and ADCP: MM cells should express >100,000 molecules/cell.

CDC: 250,000 molecules/cell are insufficient for implementing CDC. Indeed, MM lines with >250,000 molecules/cell may be resistant to Isa-mediated CDC. Induced over-expression of CD38 is not sufficient to re-sensitize MM cells.

The authors investigated the role of inhibitors of the complement cascade (CD46, CD55 and CD59 receptors) to explain these observation.

CD46

CD55 50,000 mol/cell is a threshold to suppress Isa-mediated CDC

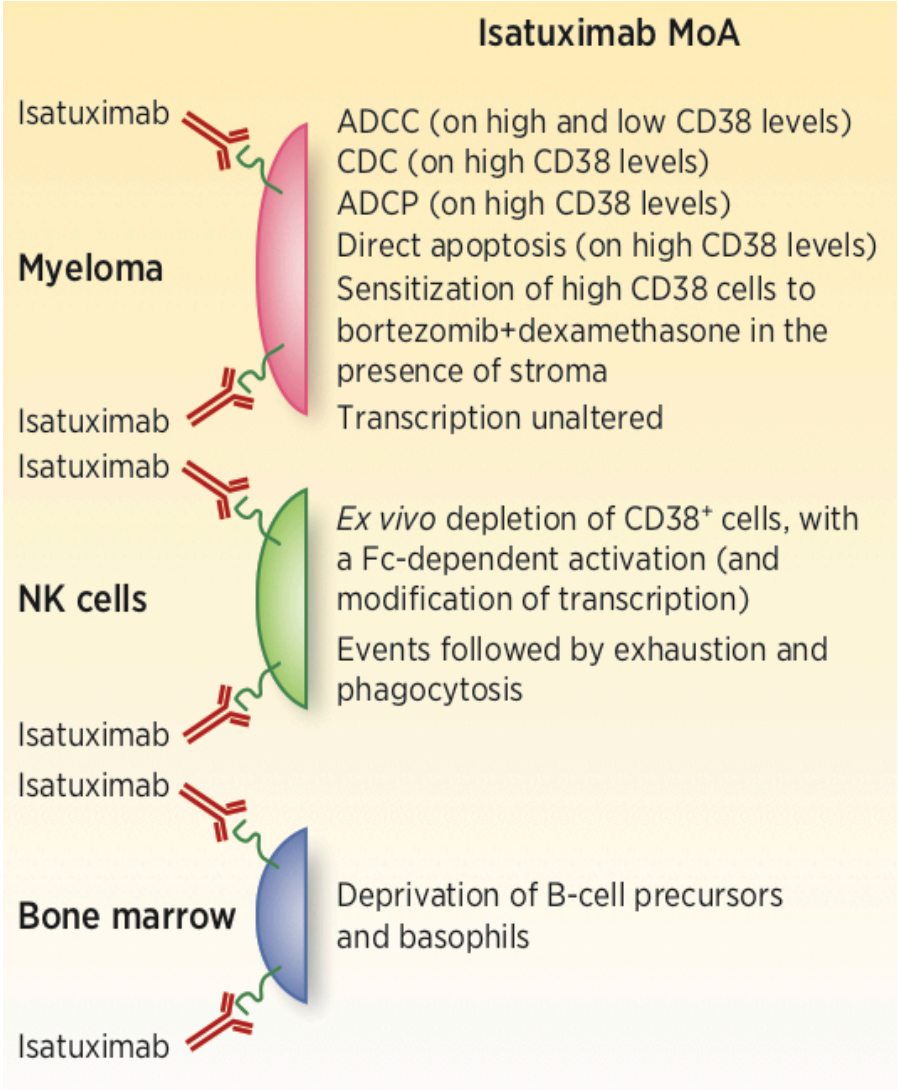
CD59

High levels of at least one of each is sufficient for inducing resistance to Isa-mediated CDC, even with high CD38.

Conclusion: High levels of CD38 along with low levels of CD59 (and *bona fide* of the other inhibitors) are important for Isa-mediated CDC.

Z. Song ……..and B. Paiva, M. Chiron and F. Adrian, Cancer Research, 2019

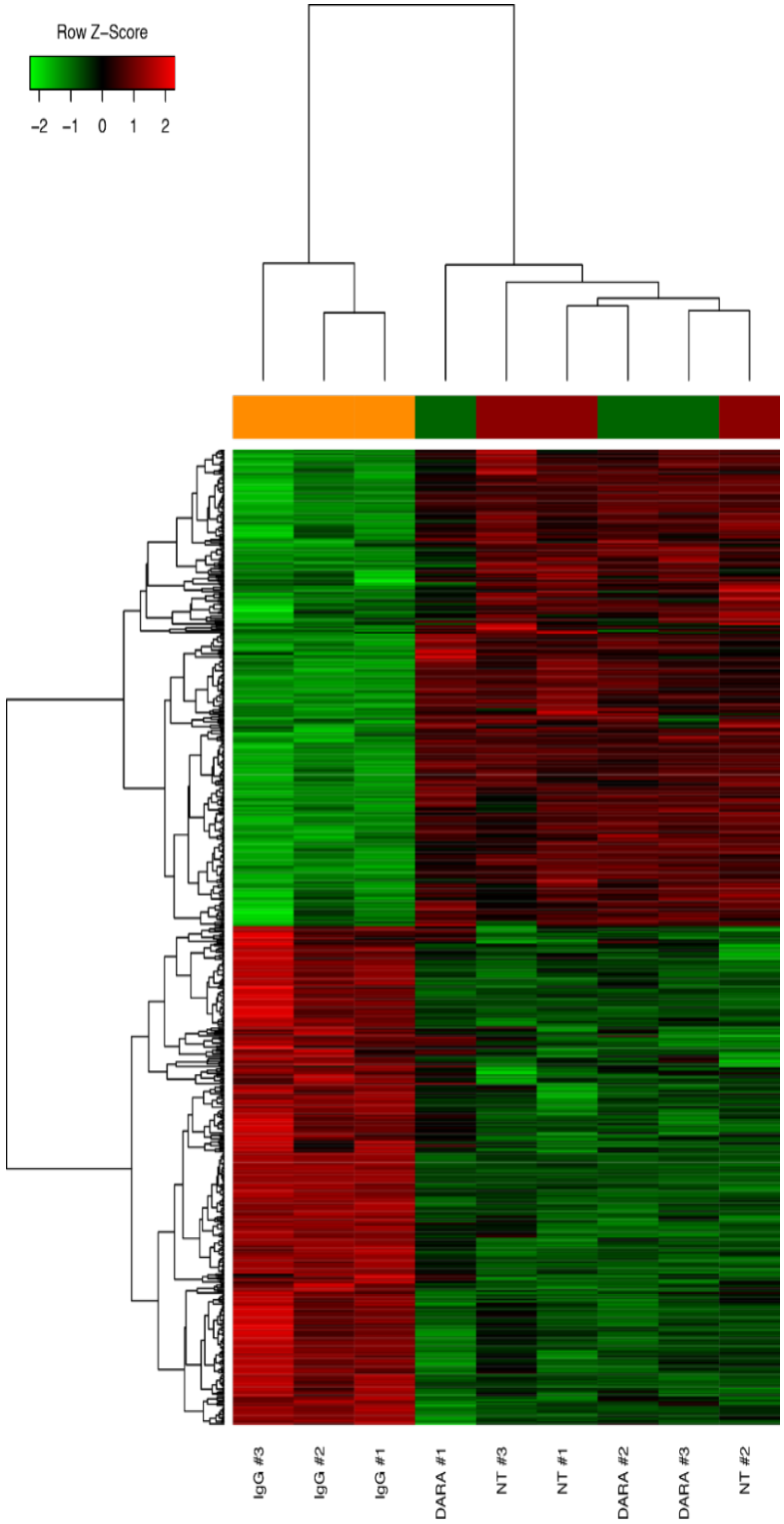
Summary of the effects implemented by Isatuximab in an *in vitro* setting.



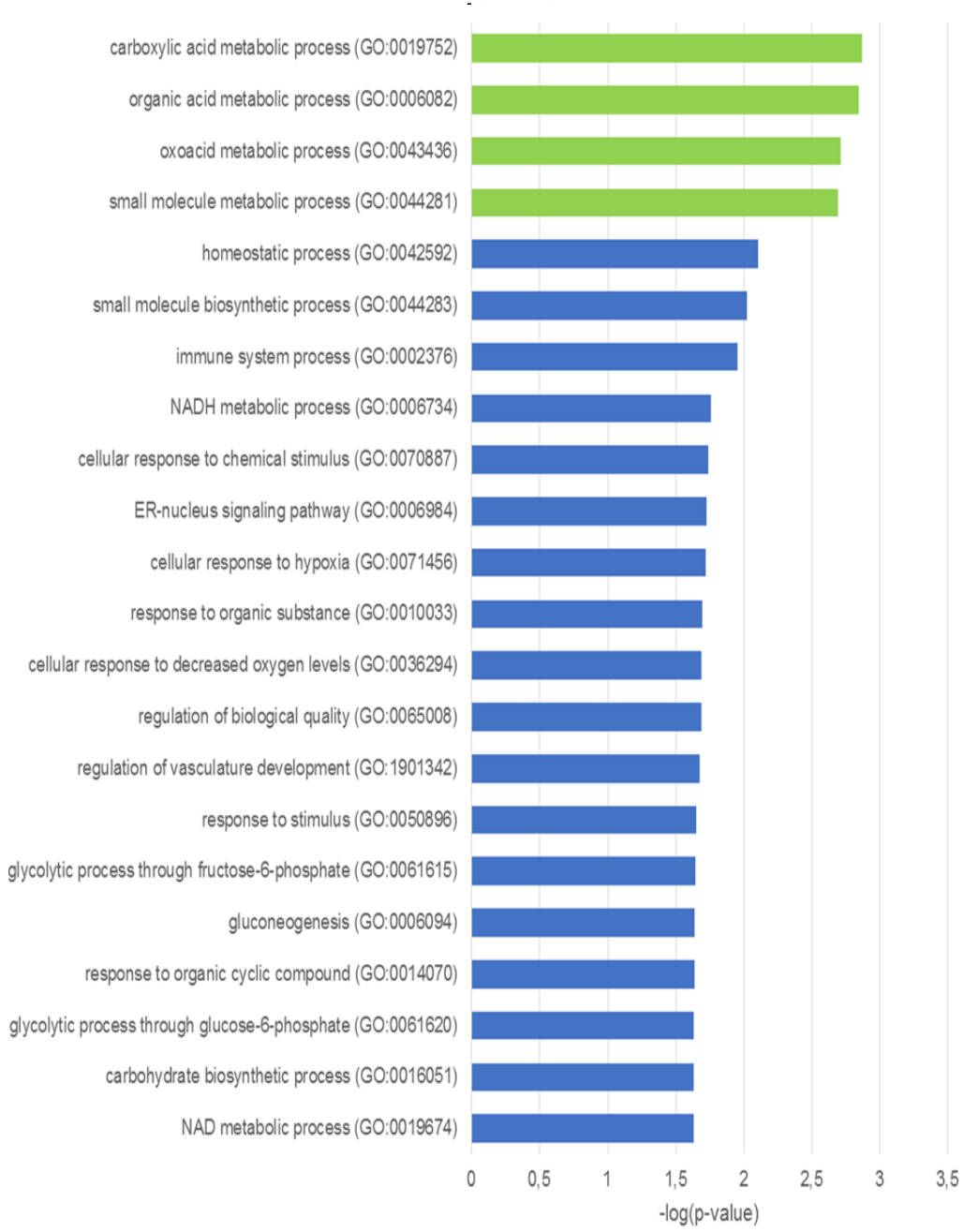
F. Malavasi & A. C. Faini, *Clinical Cancer Research*, 2019, modified

CD38

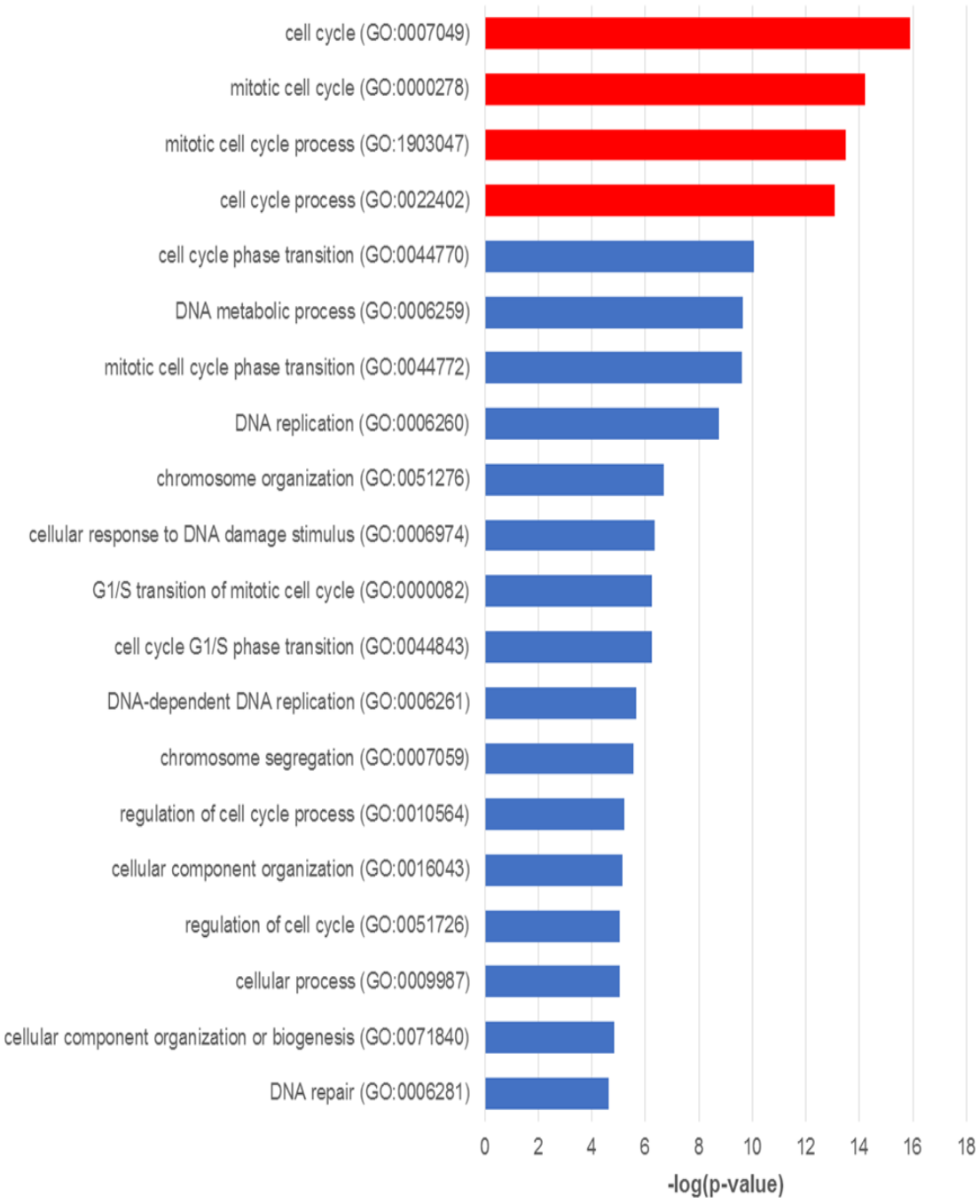
Heatmap showing up- and down-regulated genes in human myeloma upon Dara treatment vs irrelevant IgG ligation



Up-regulated genes in human myeloma upon Dara

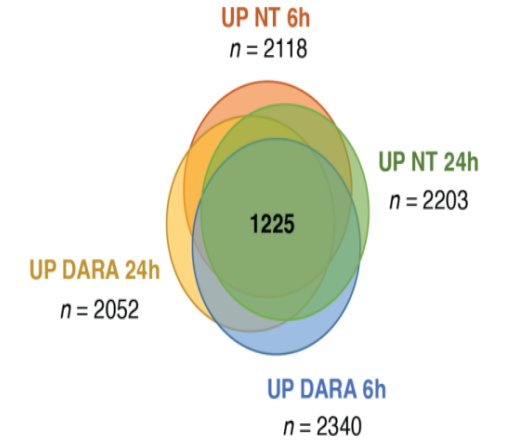


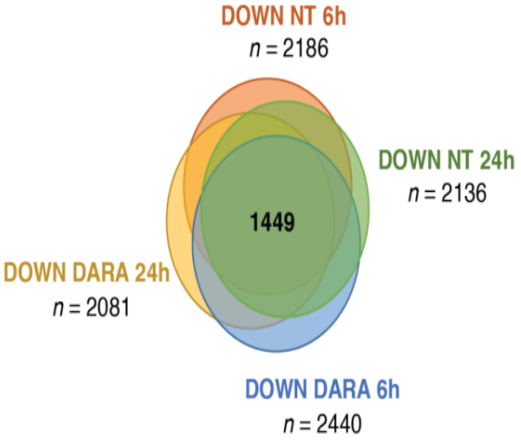
Down-regulated genes in human myeloma upon Dara ligation

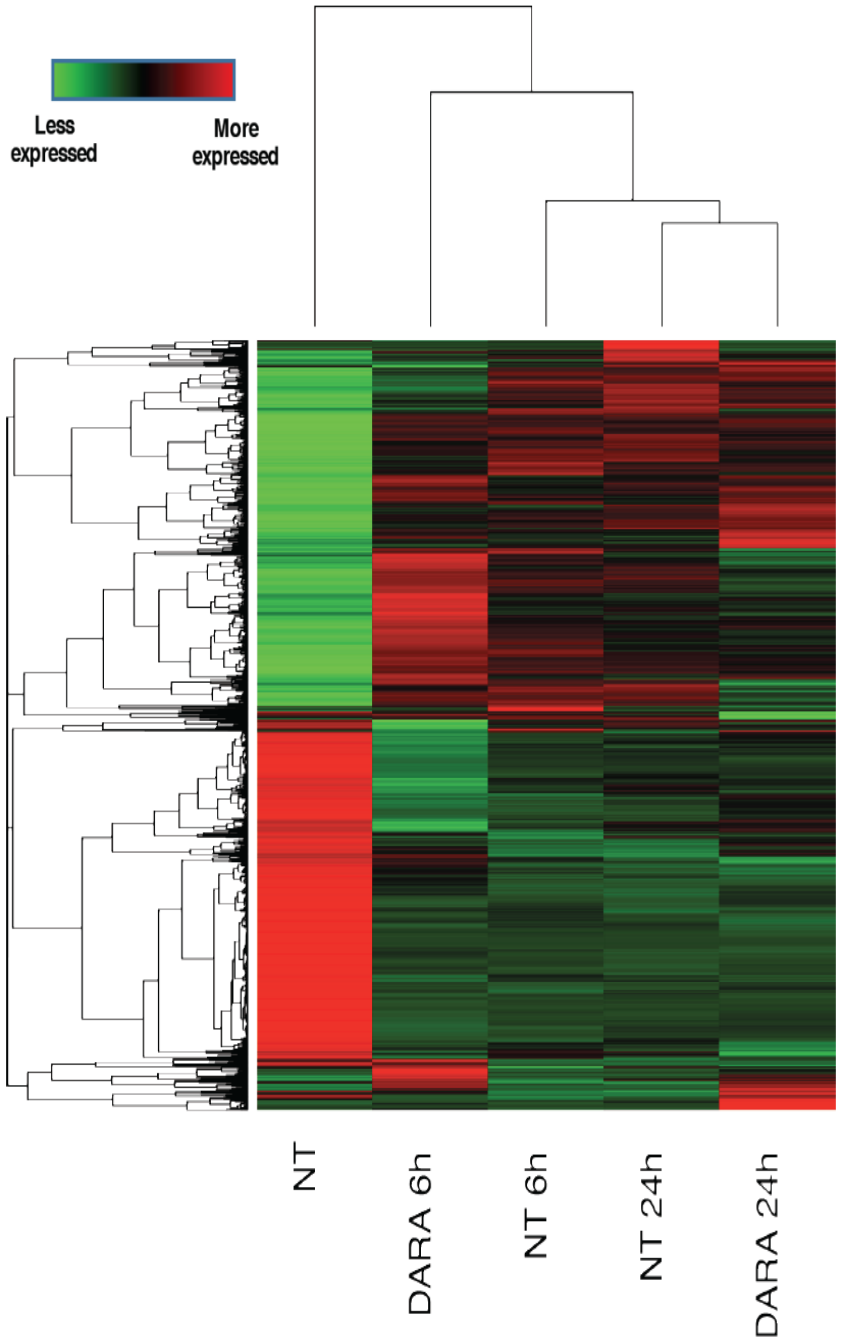


Whither MV from multiple myeloma:

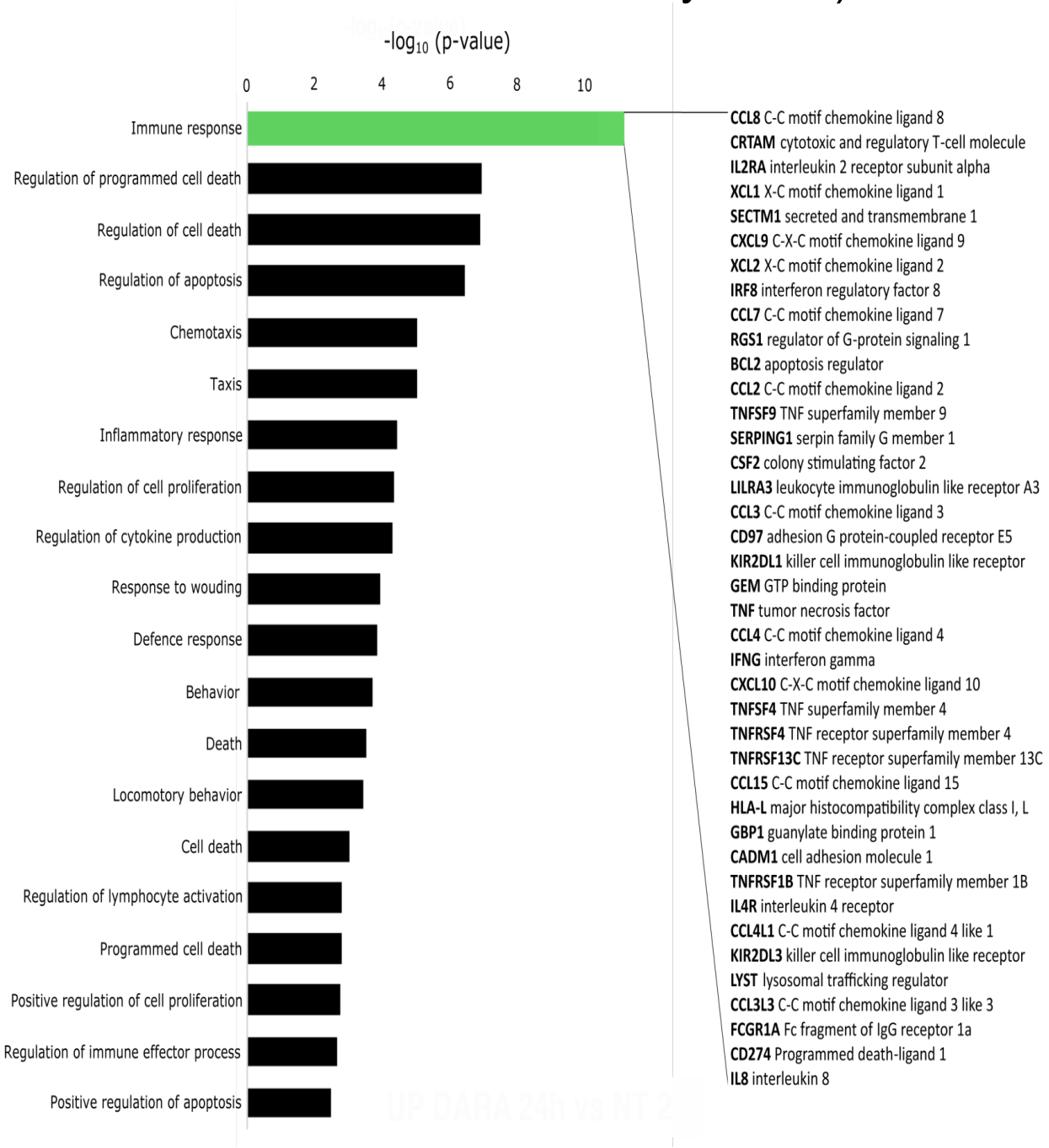
Molecular effects observed on NK cells ($CD16^+/CD56^+$)







Comparative analysis of up modulated genes (RNA polyA) after exposure of NK cells to MV-DARA (control: MV from untreated myeloma)



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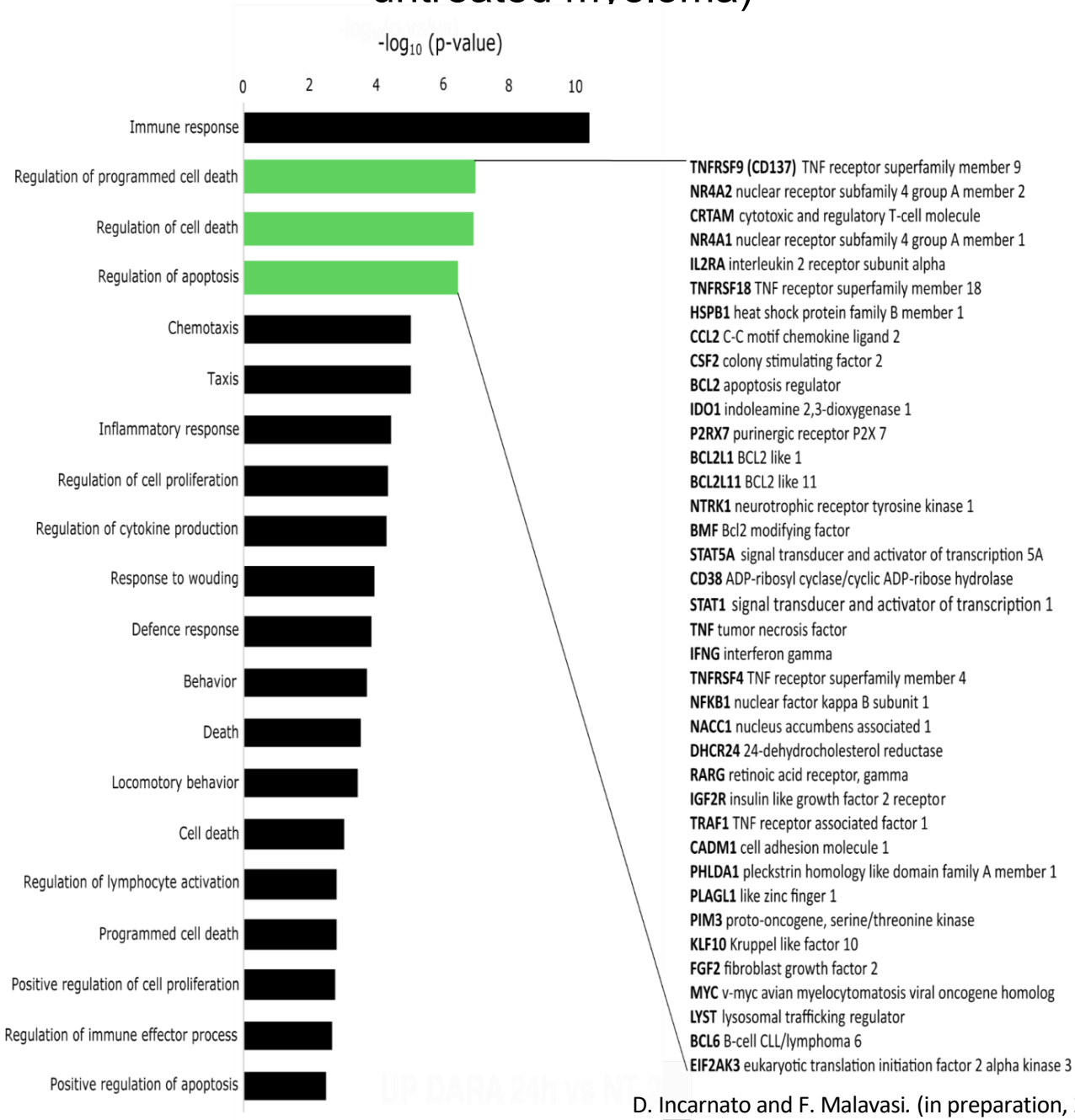
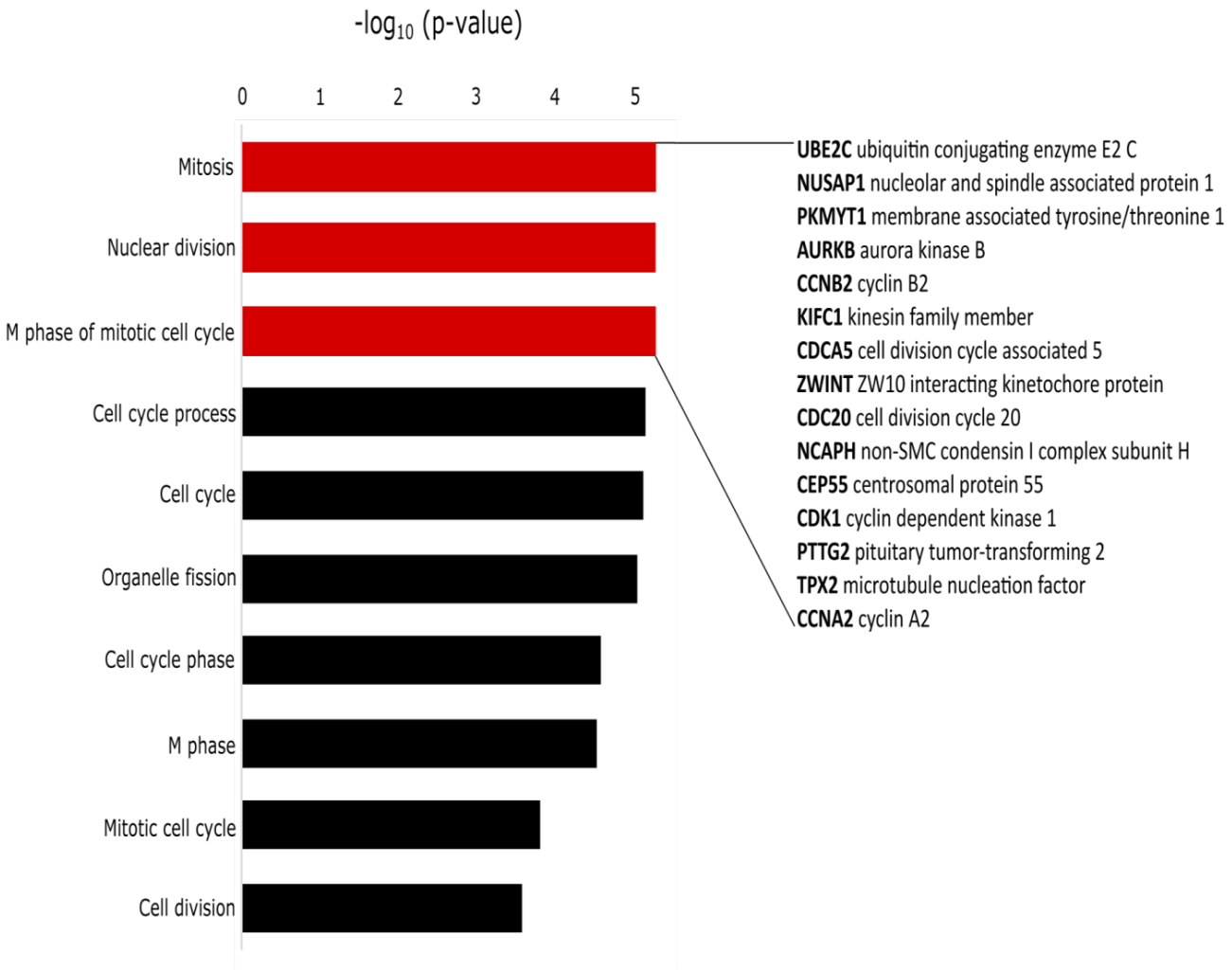


D. Incarnato and F. Malavasi. (in preparation, 2019)

Comparative analysis of down modulated genes (RNA polyA) after exposure of NK cells to MV-DARA (control: MV from untreated myeloma)



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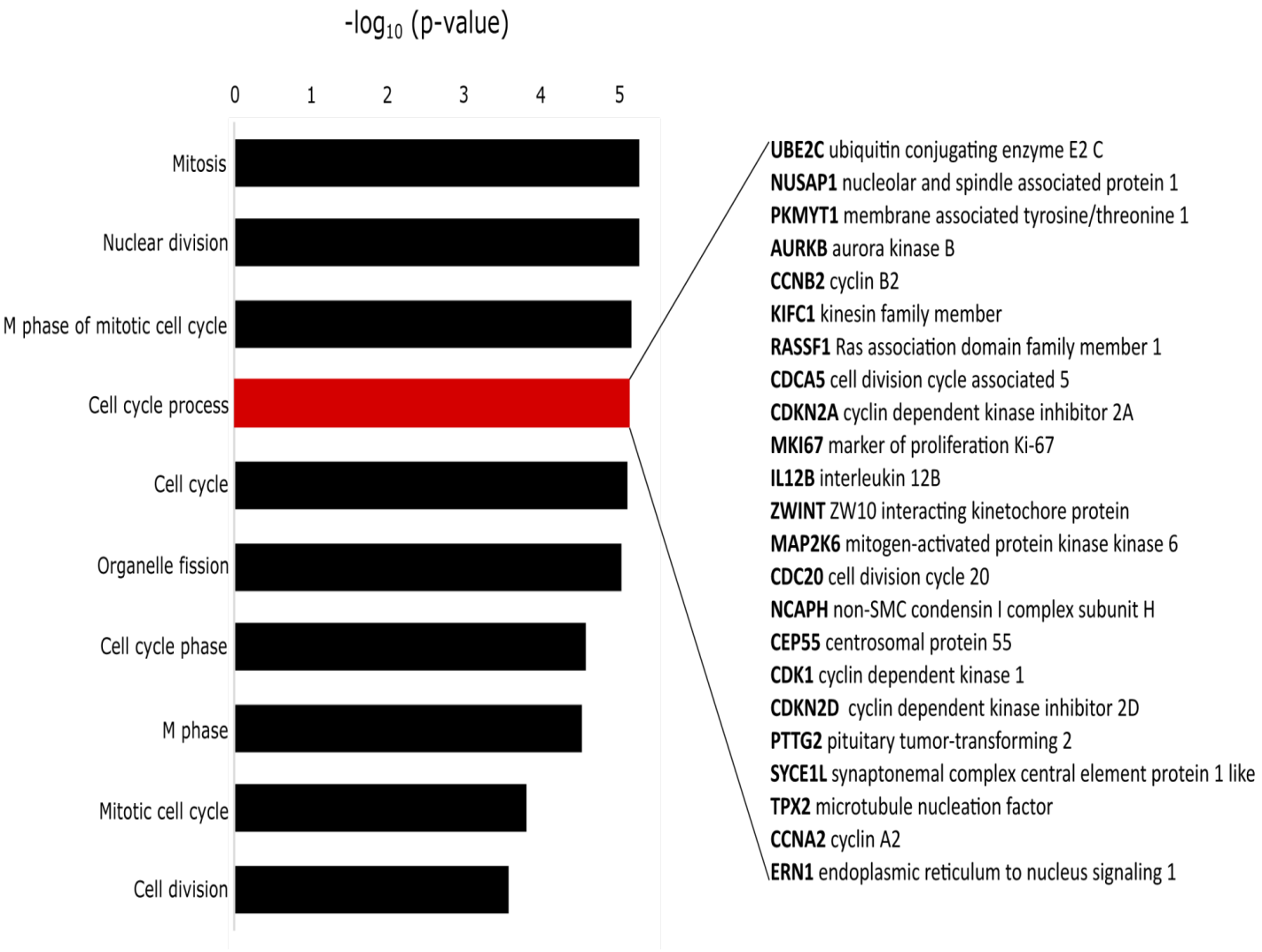
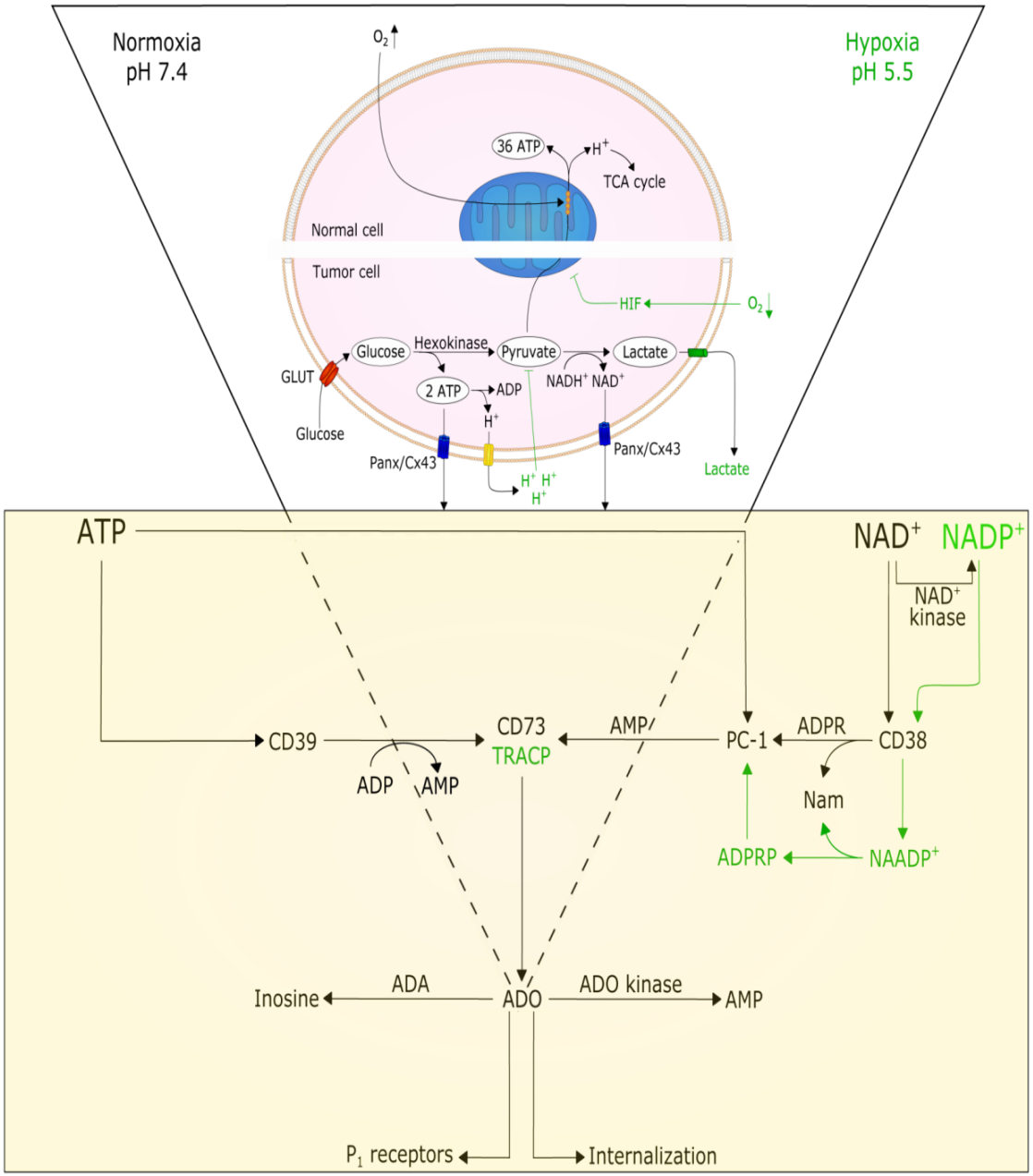
Comparative analysis of down modulated genes (RNA polyA) after exposure of NK cells to MV-DARA (control: MV from untreated myeloma)

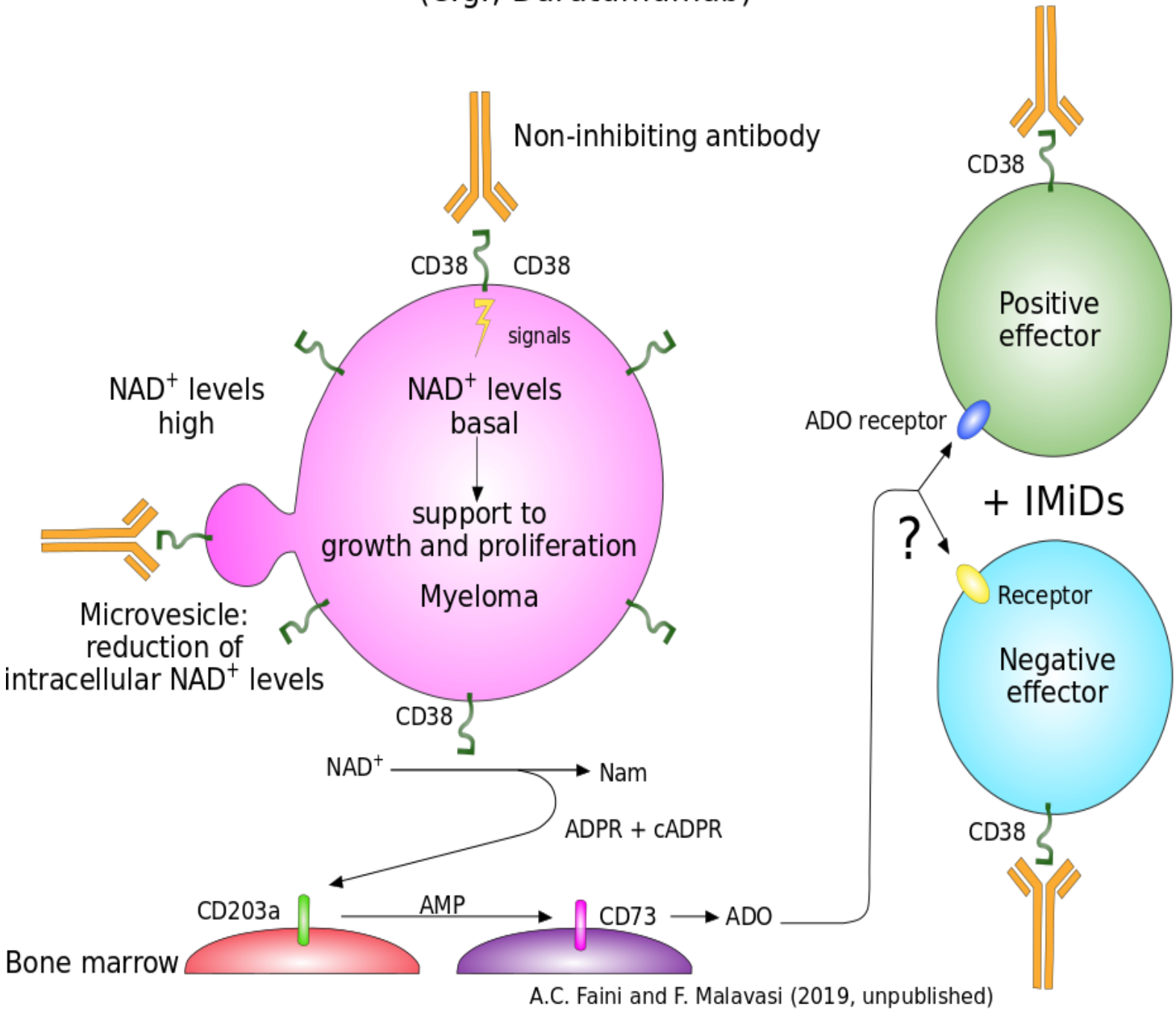


D. Incarnato and F. Malavasi (in preparation, 2019)

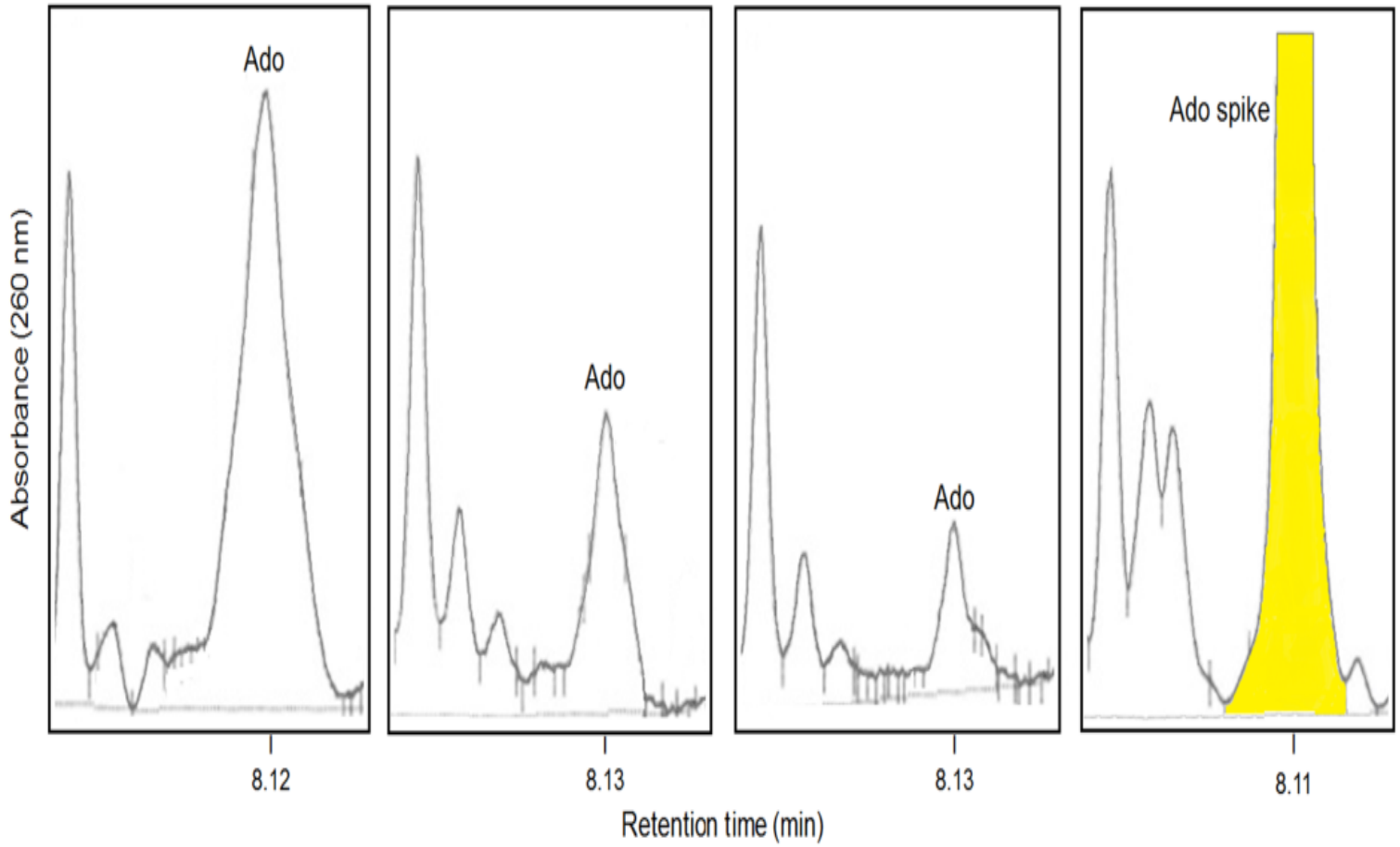
Interplay among ectoenzymes, their substrates and products in the BM niche



A. Chillemi, ……. A.L. Horenstein and F. Malavasi (Frontiers Immunol., 8: 307, 2017)


A Hypothesis: NON-INHIBITING THERAPEUTIC ANTIBODIES
(e.g., Daratumumab)
Non-inhibiting antibody
CD38
CD38
signals
NAD+ levels high
NAD+ levels basal
support to growth and proliferation
Myeloma
Microvesicle: reduction of intracellular NAD+ levels
CD38
NAD+
Nam
ADPR + cADPR
CD203a
AMP
CD73
ADO
Bone marrow
CD38
Positive effector
ADO receptor
+ IMiDs
?
Receptor
Negative effector
CD38
A.C. Faini and F. Malavasi (2019, unpublished)

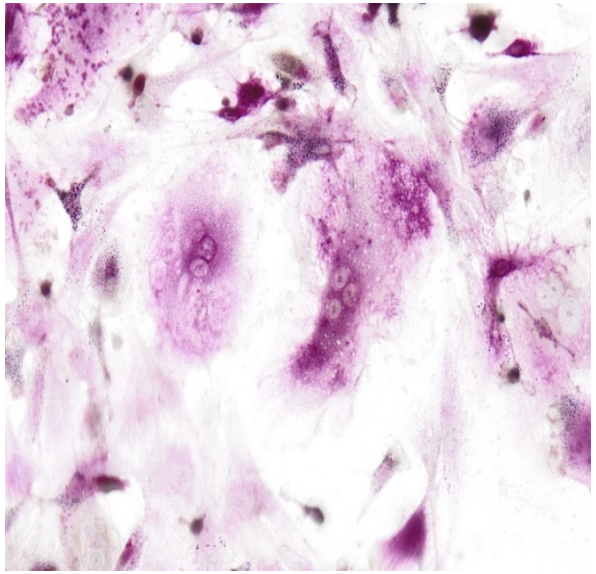
Proof-of-principle: BM plasma from MM patients contains ADO



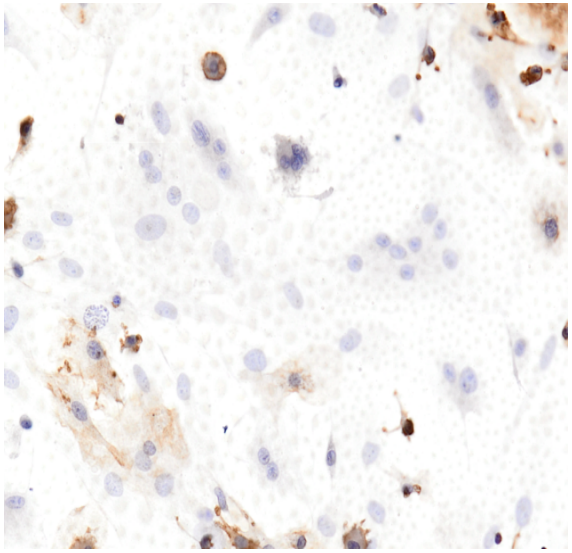
Horenstein A.L. et al. *Molecular Medicine* (2016)

Human Osteoclasts express ADO receptors

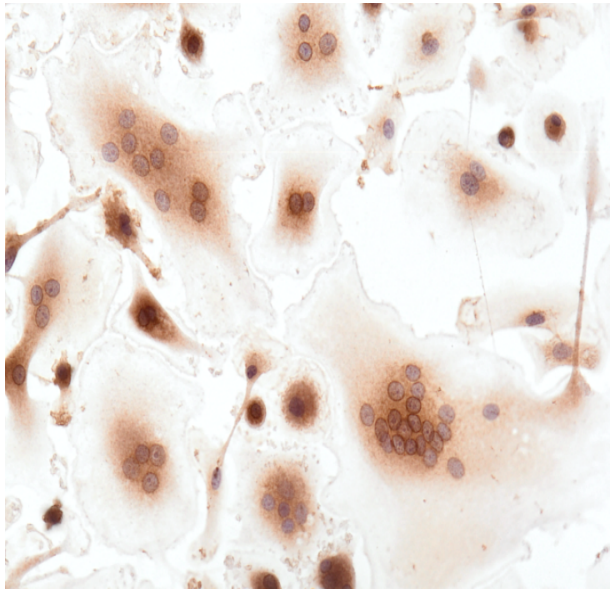
TRAP



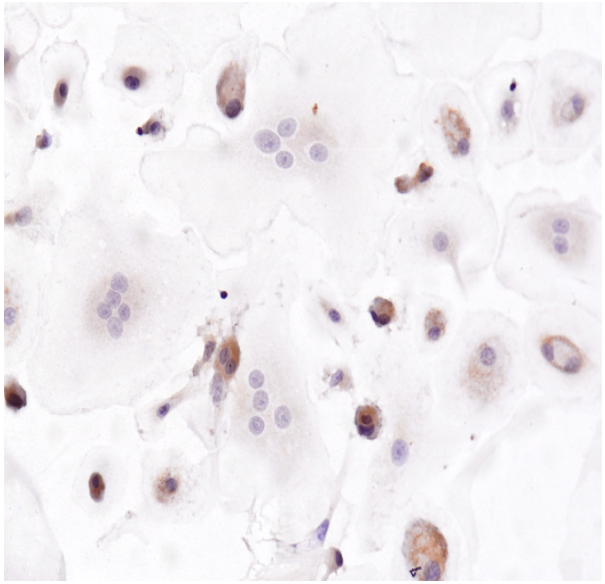
ADORA1



ADORA2a

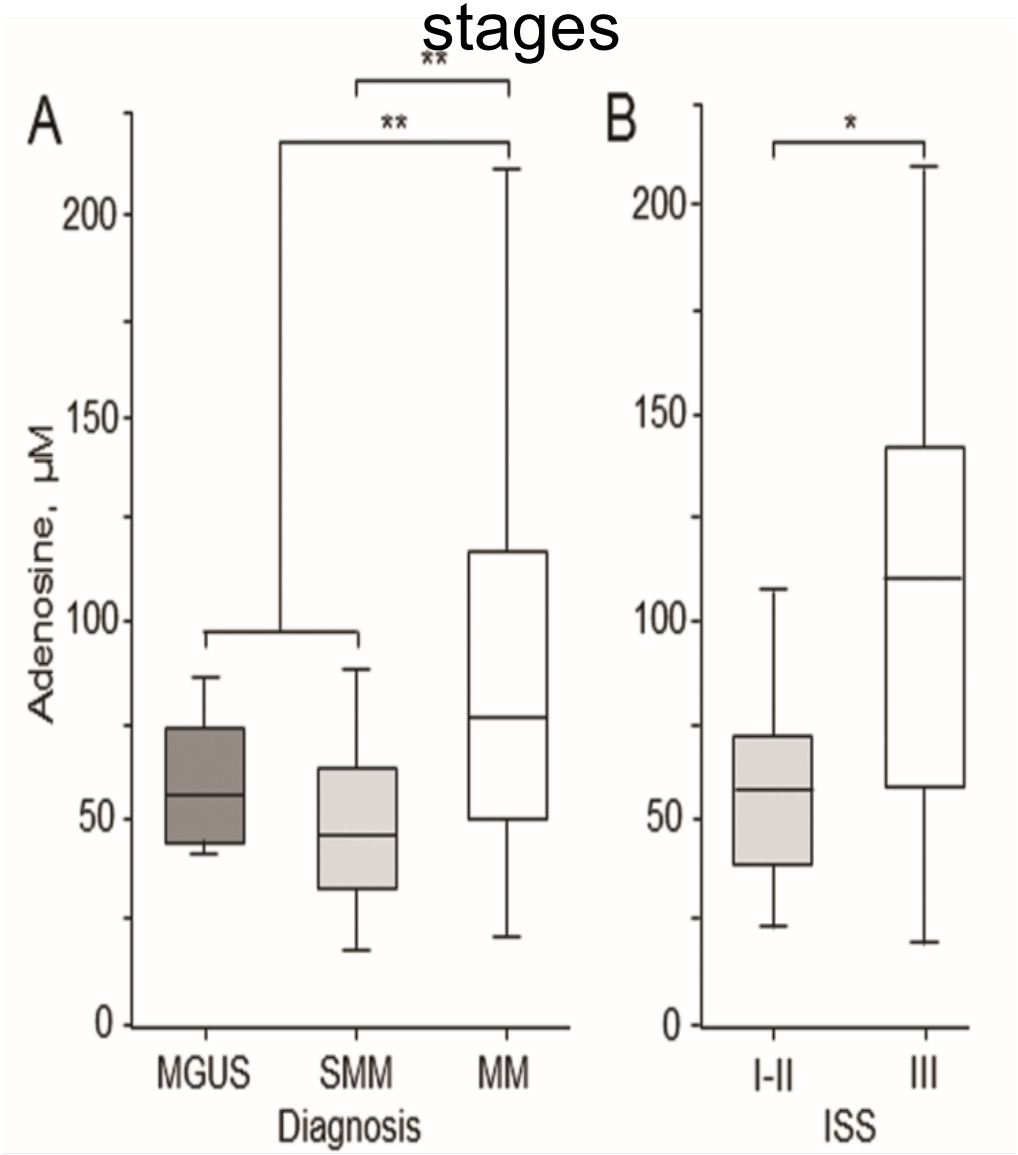


ADORA2b



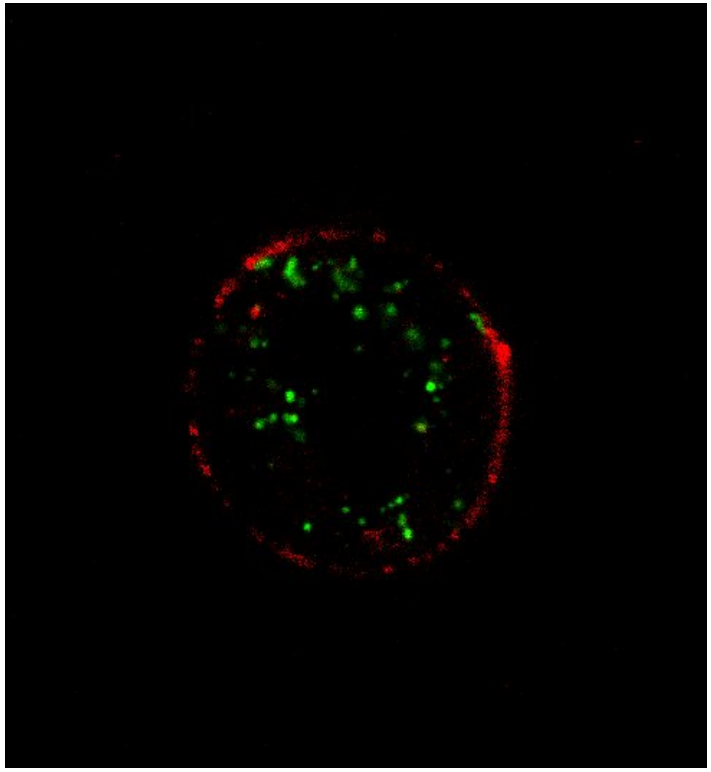
V. Quarona (unpublished)

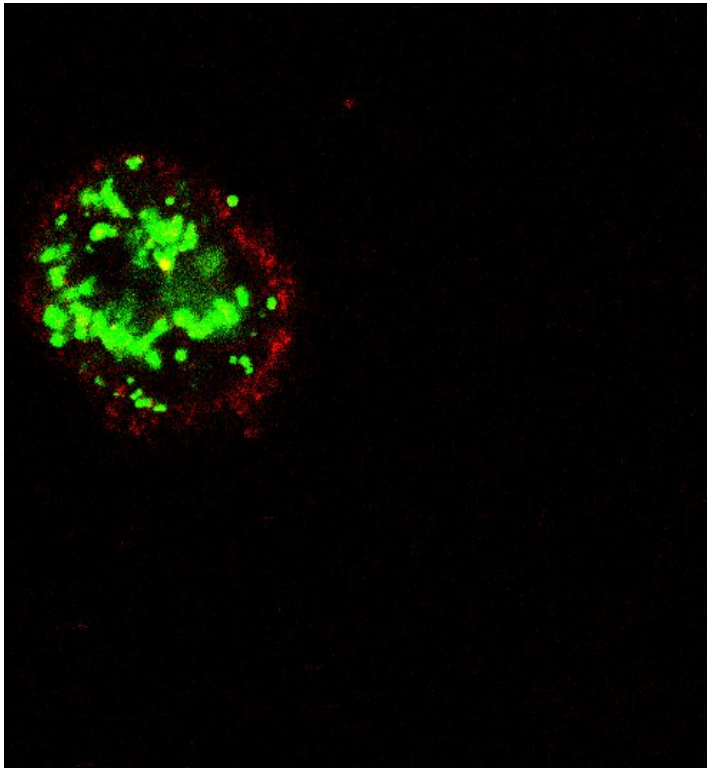
BM plasma ADO levels in different MM stages



Horenstein A.L. et al. *Molecular Medicine* (2016)

Internalization into dendritic cells of MVs from myeloma membranes

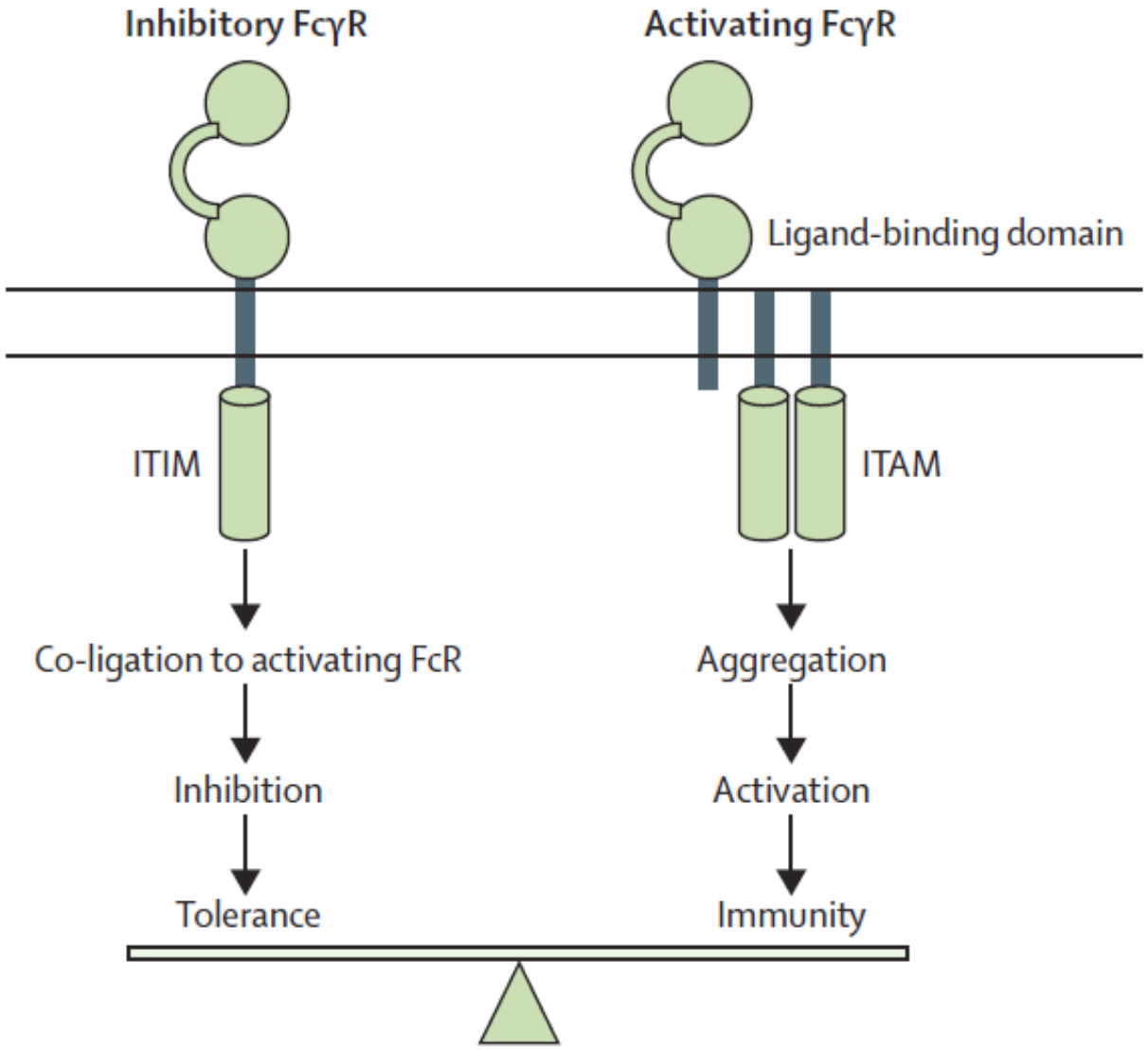




RED=CD80-Alexa546
GREEN=DiO-labeled MVs

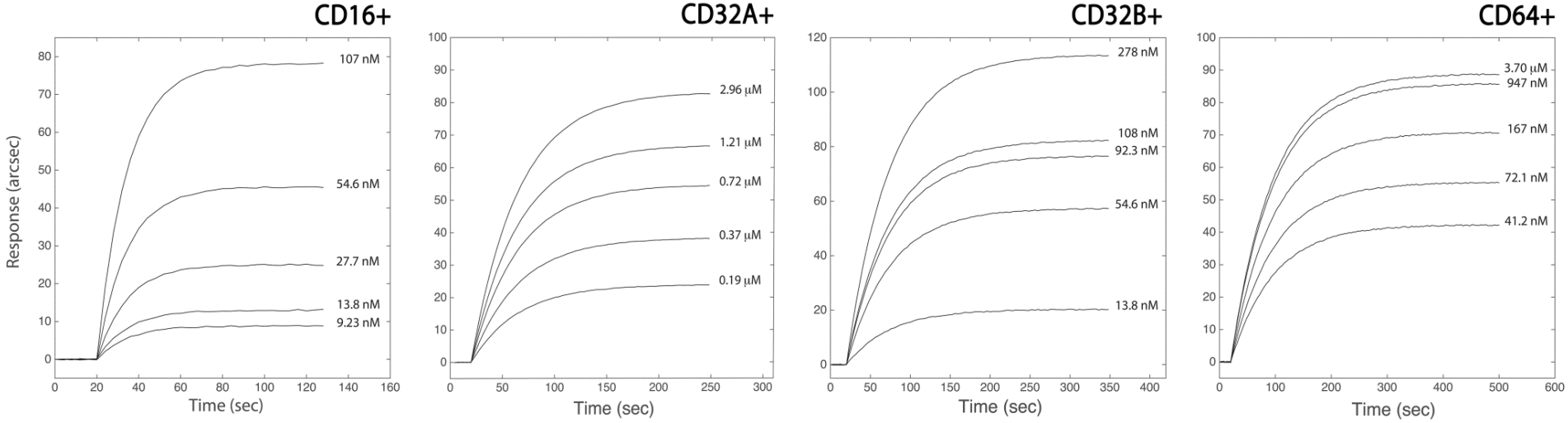
Faini A.C. and Malavasi f., 2019, in preparation

FcReceptors: friends or foes?



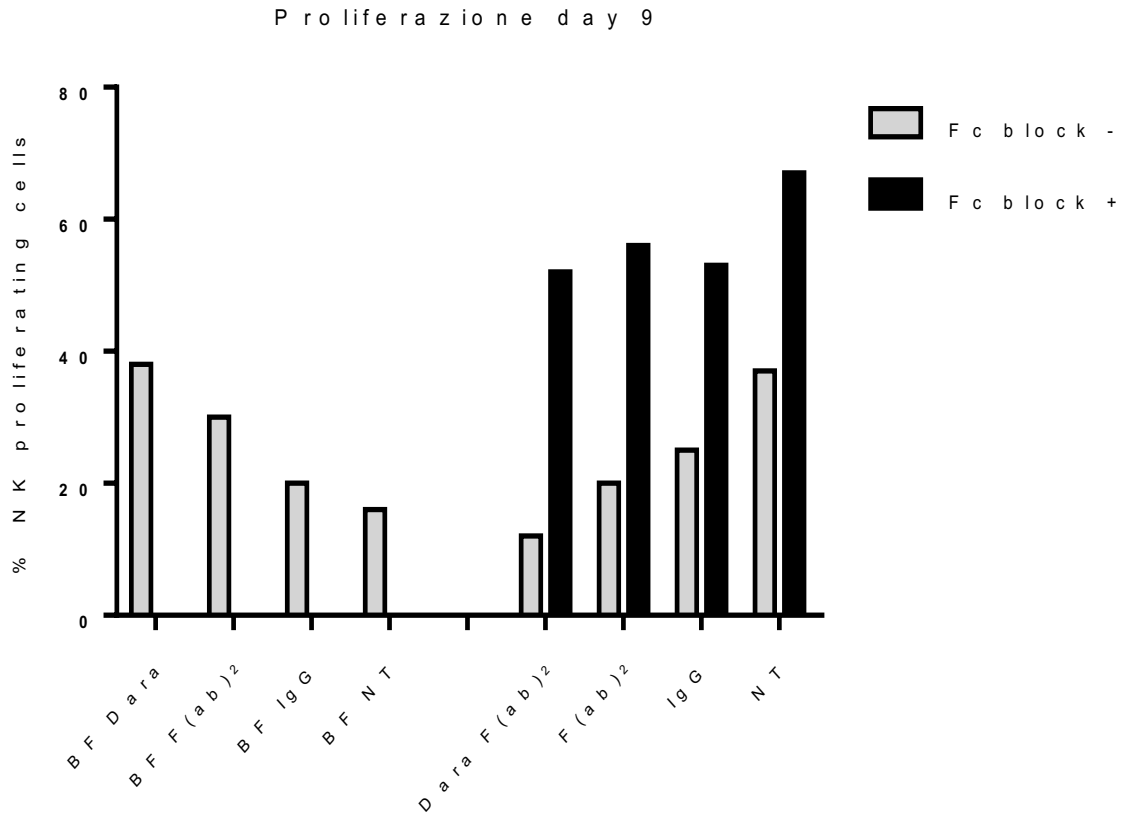
Kinetic and equilibrium parametrics of the Dara FcR recognition process

(SPR biosensor, Iasys *Plus* – Affinity sensors, Cambrudge, UK)

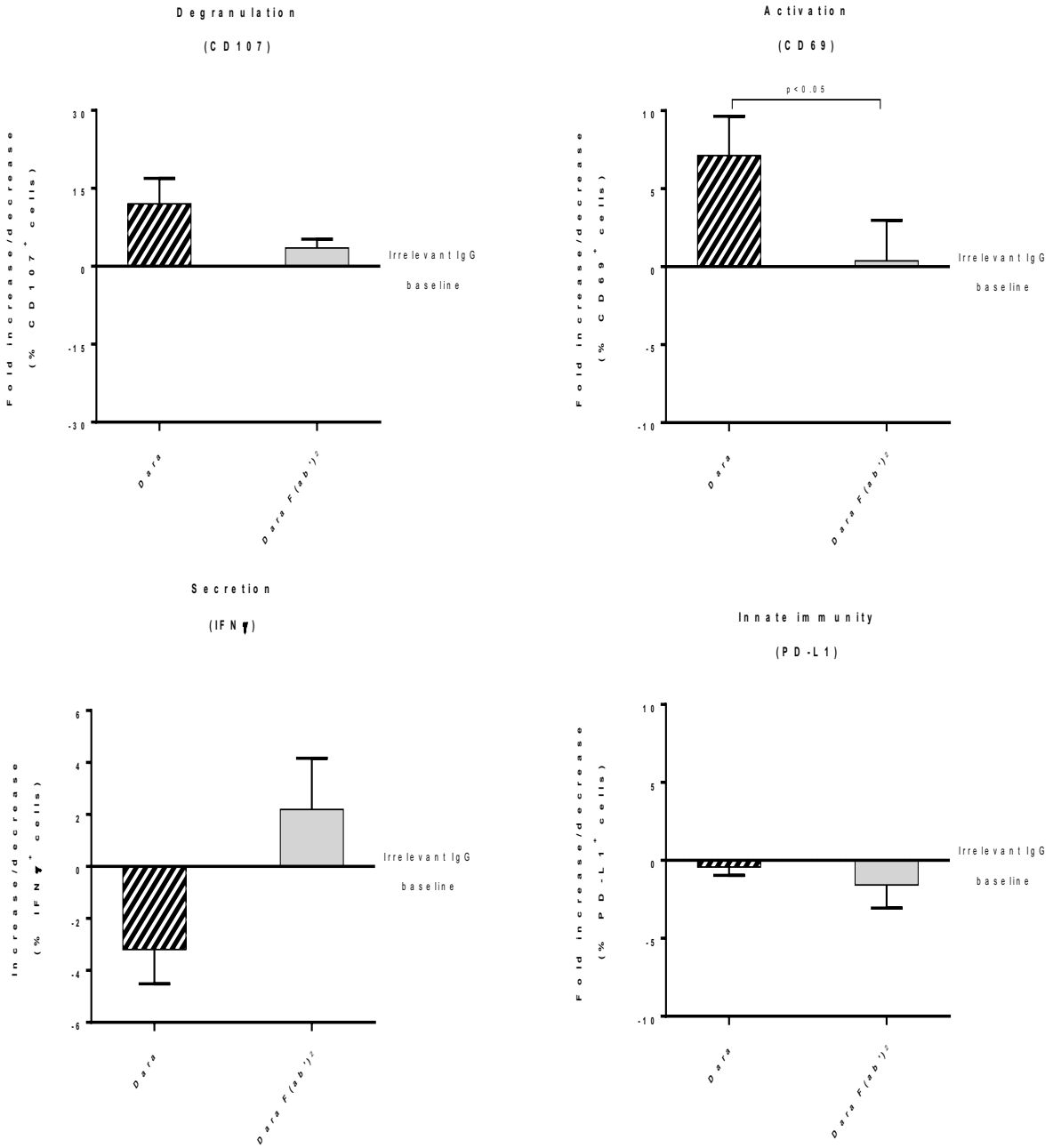


Courtesy of M. Cuccioloni, unpublished.

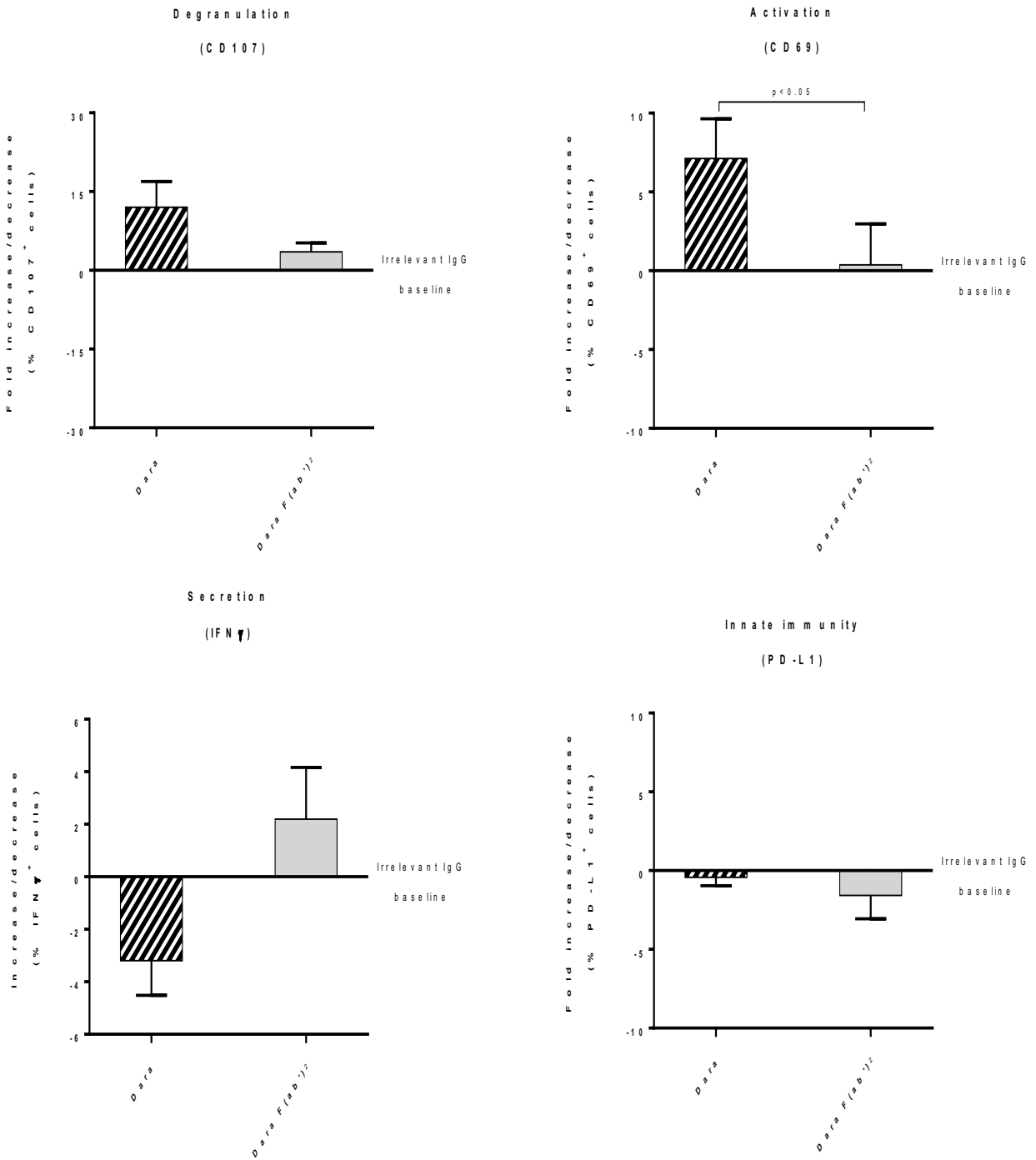
Insoluble vs soluble Dara-mediated effects on NK proliferation



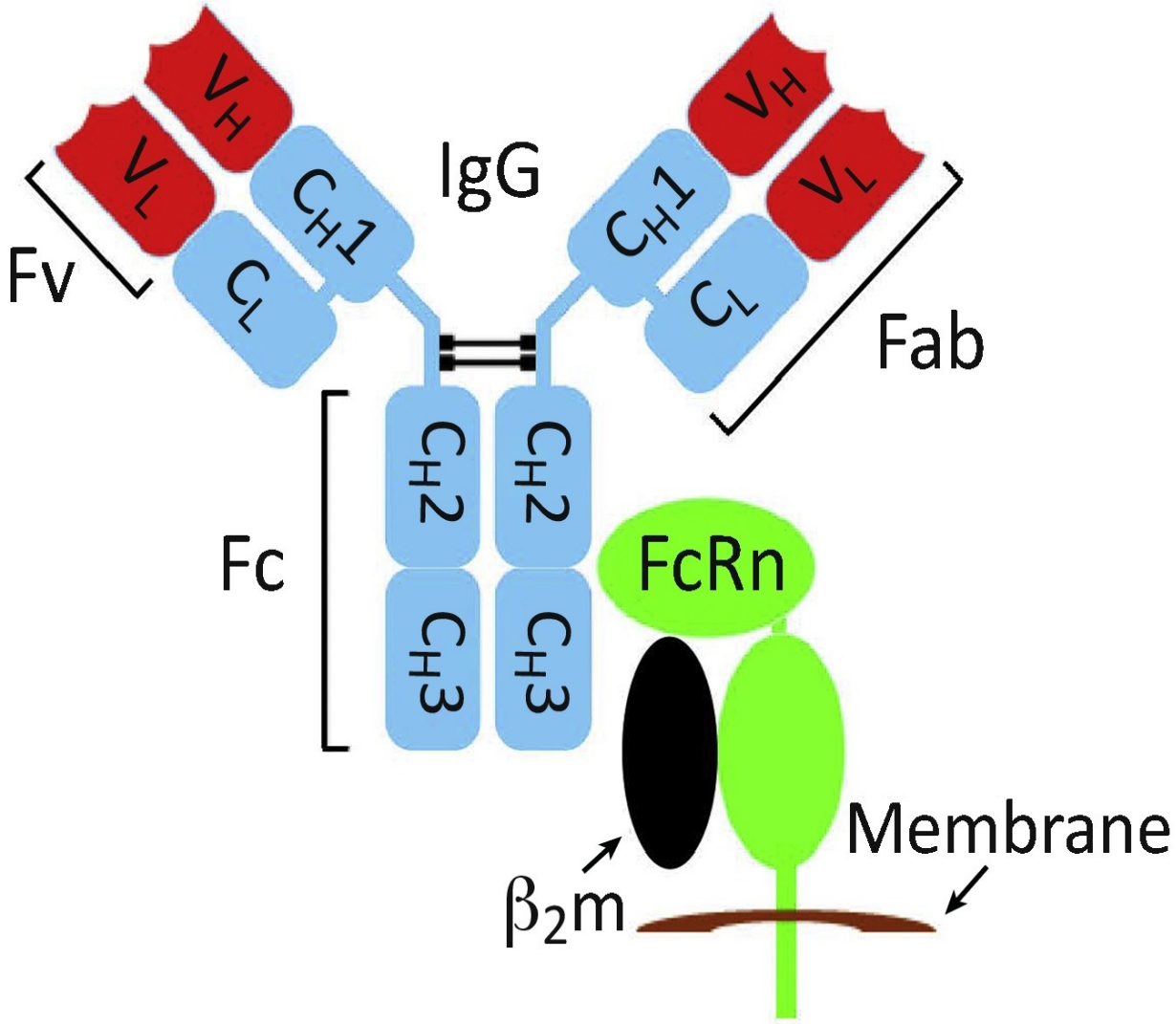
SOLUBLE DARATUMUMAB



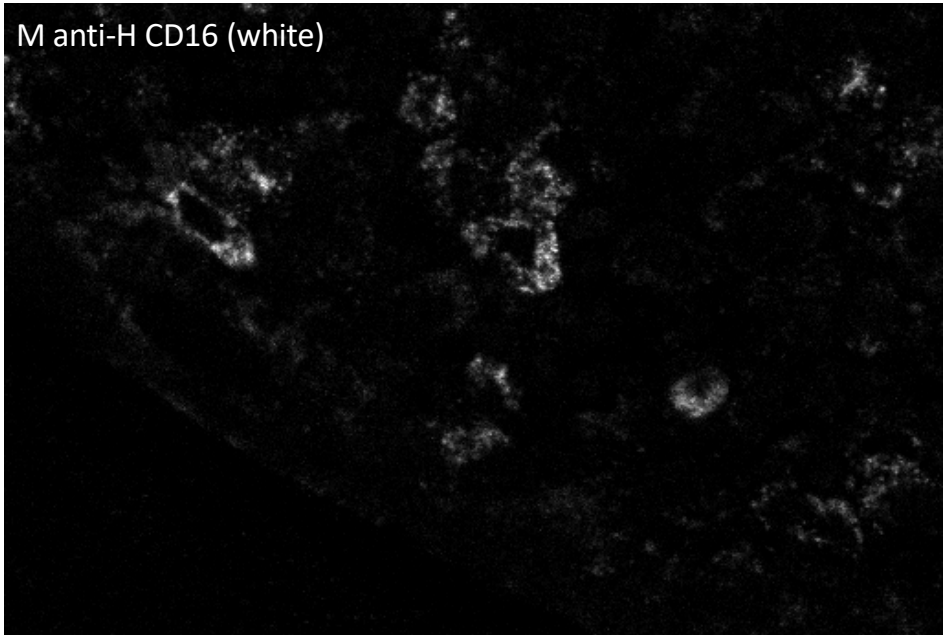
SOLUBLE DARATUMUMAB

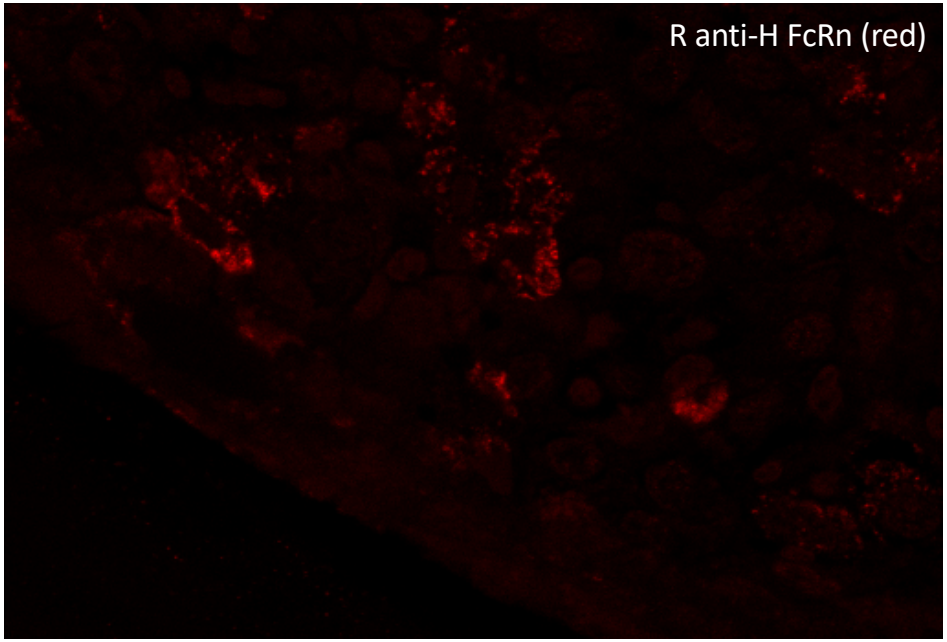


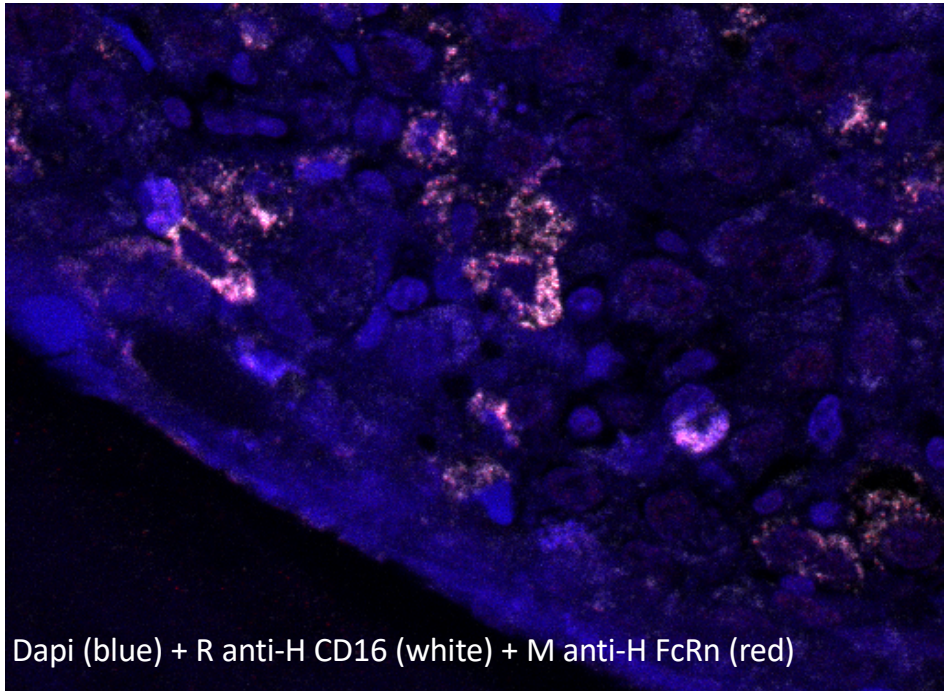
FcRn

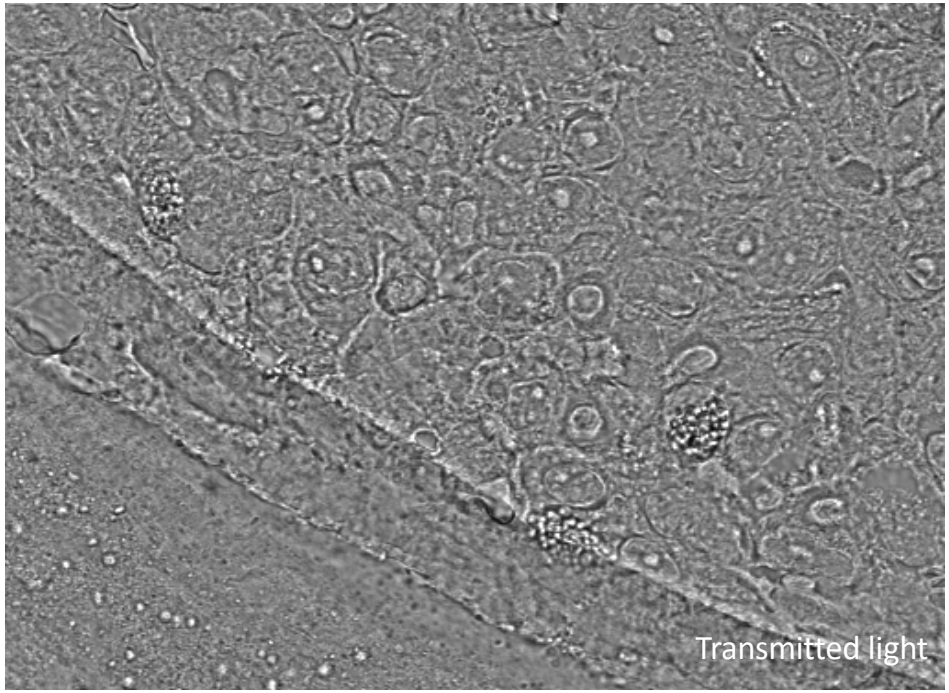

IgG
Fv
Fab
Fc
V_H
V_L
C_H1
C_L
C_H2
C_H3
FcRn
β_2m
Membrane

Osteomedullary biopsy from MM patient: confocal analysis of $CD16^{+}/FcRn^{+}$cells



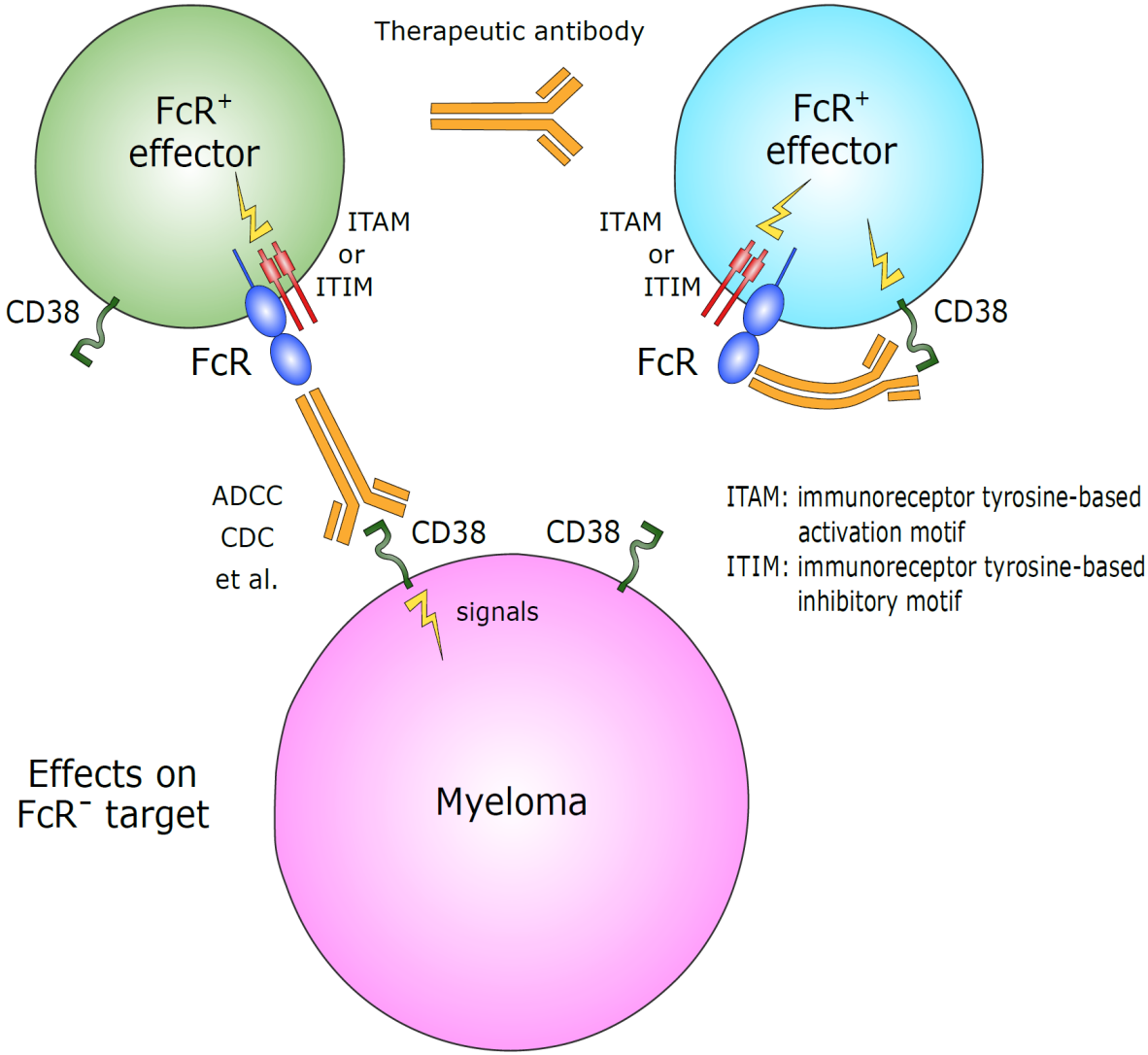






V. Quarona (2018)

Distinct effects of therapeutic antibodies on tumor target and on efffectors: a hypothesis

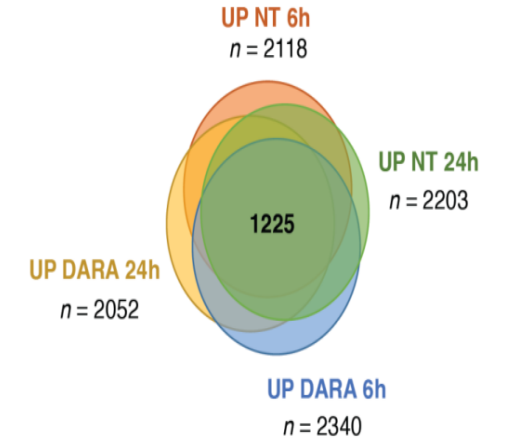


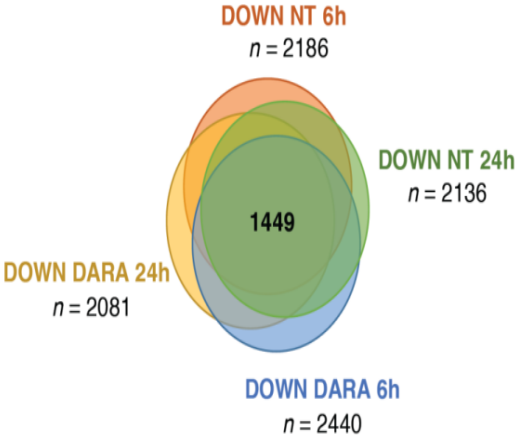
A. C. Faini and F. Malavasi. Clinical Cancer Research, 2019

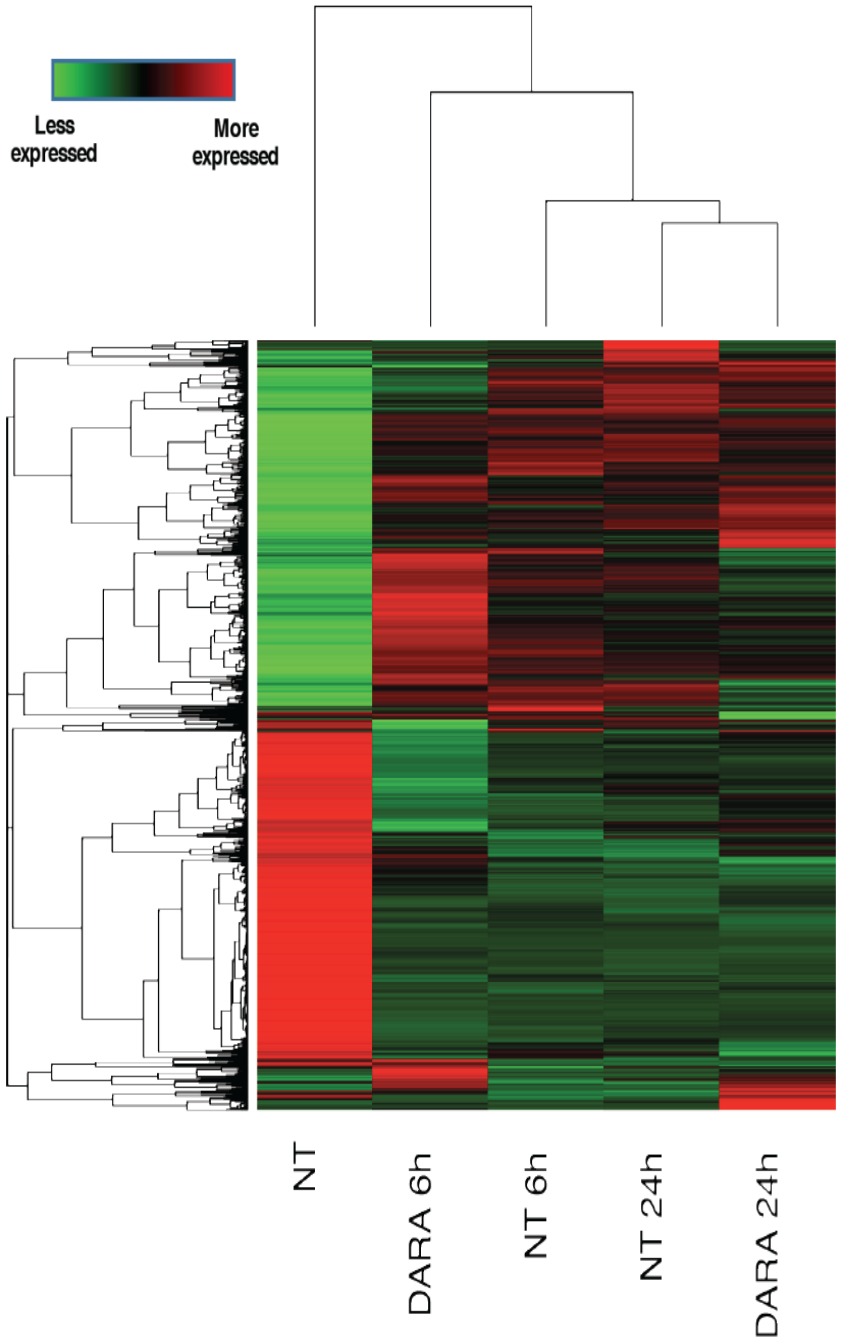
- THE END

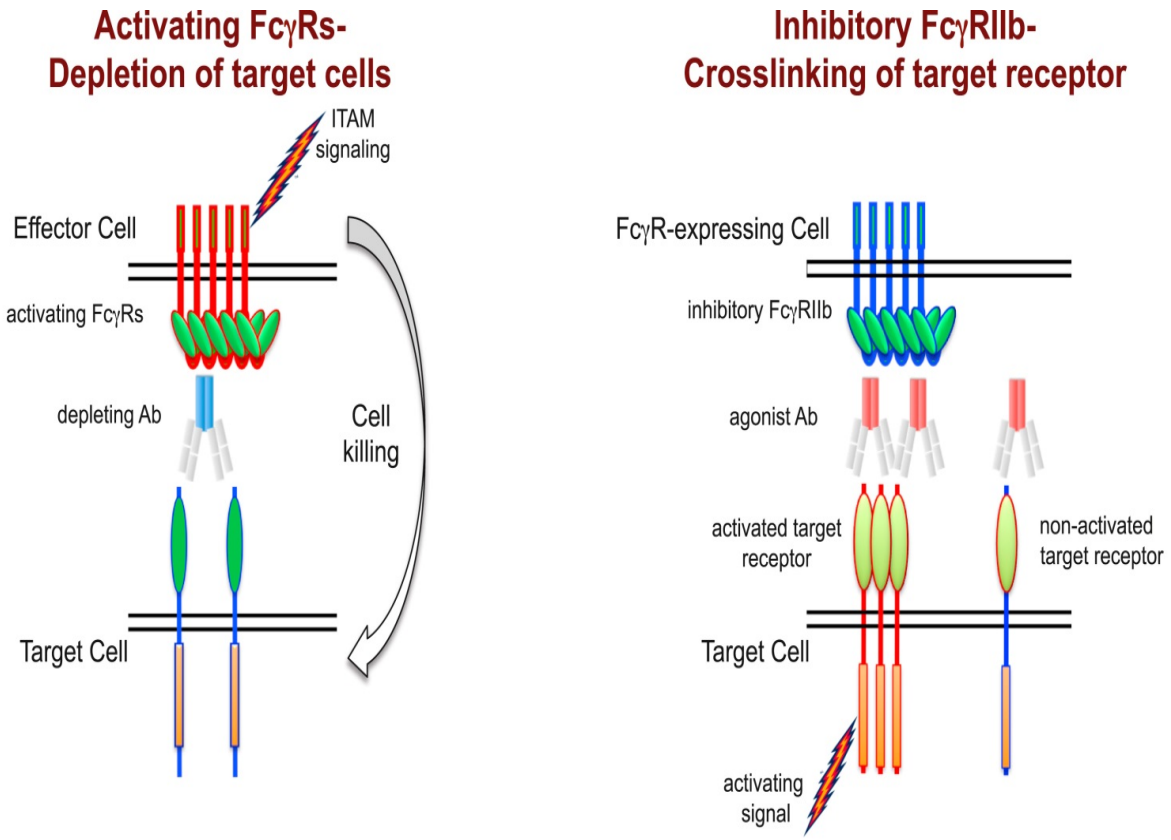
Whither MV from multiple myeloma:

Molecular effects observed on NK cells ($CD16^+/CD56^+$)









Examples:

α-PD-L1 → Depletion of myeloid cells in the TEM

α-CD73 → ? → Depletion of tumor cells; Activation of neutrophils in the TEM

α-CTLA4, α-OX40, α-GITR → Depletion of regulatory T cell in the TEM

α-CD40 → Activation of DCs

α-DR5 → Induced apoptosis

α-MARCO → Reprograming of TAMs

Dahan R and Ravetch J. V. . *Cancer Cell* 2016 30, 369-371

Immune modulation induced by Daratumumab on relapsed/ refractory MM patients (RRMM).

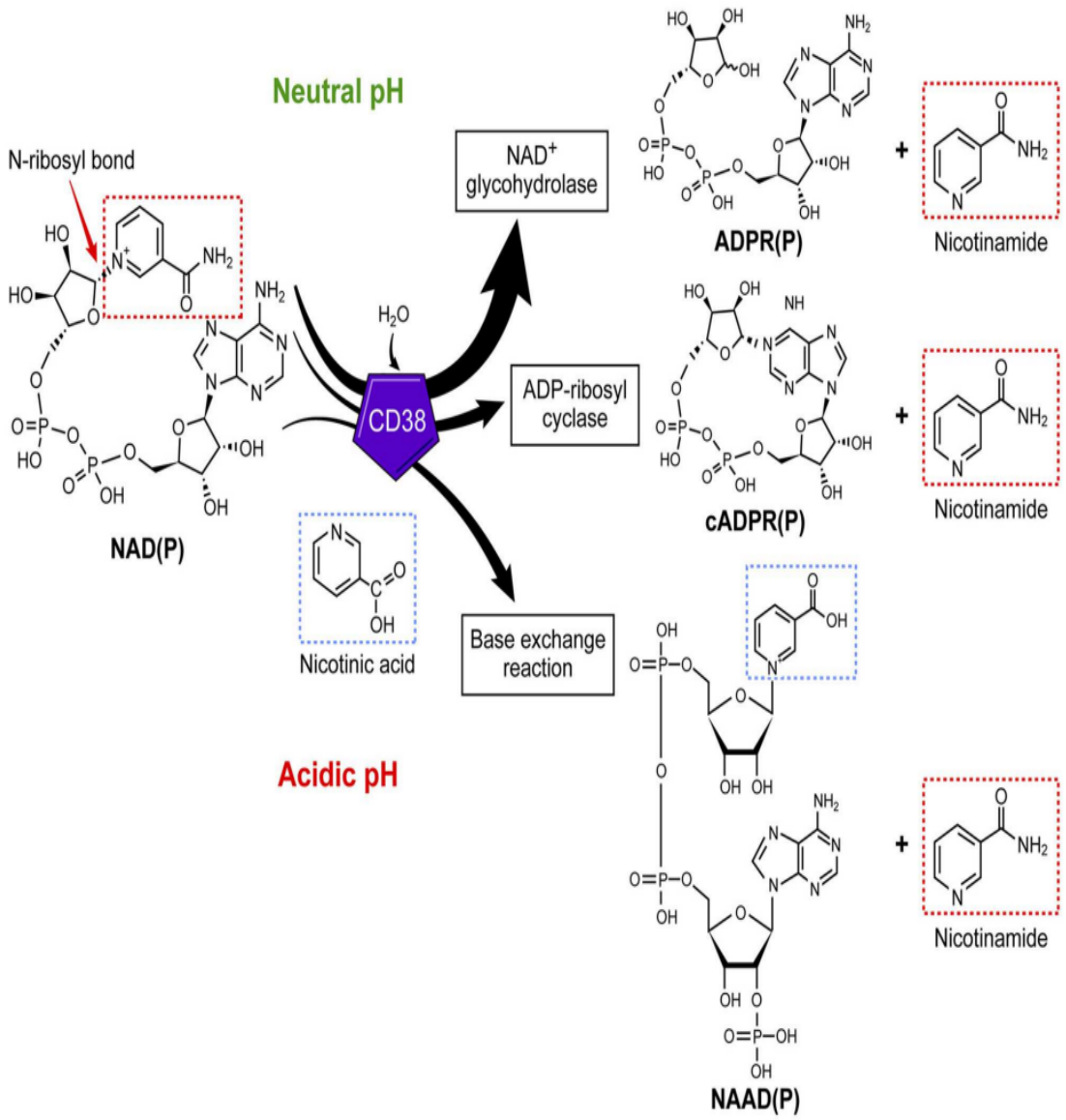
Strategy: analysis of whole peripheral blood
preparations and BM samples (baseline *versus* on treatment) from monotherapy SIRIUS and GEN501 studies**.**
Analysis: by high-parameter mass cytometry.
Results:
General: Expansion of cytotoxic T lymphocyte populations and reduction of immune-suppressive cells.
Details:
1) surface CD38 was decreased on immune cells on the whole blood from SIRIUS study.
2) NK cells were significantly depleted.
3) Residual NK cells: i) >surface CD69 and CD127, ii) <CD45RA. iii) trends for increase of CD25, CD27 and CD137. iv) Granzyme B was decreased.
3) At the same time, the immune suppressive populations are depleted, while CD38 basophiles were reduced.

After 2 months of Dara therapy:
4) whole blood samples shifted to $CD8^+$ prevalence, with high granzyme B positivity.

Conclusion: An increased cytotoxicity to MM cells, mainly obtained via $CD8^+$ T lymphocytes rich in cytolytic granzyme B, represents an adaptative response expected to sustain the depth of the response.

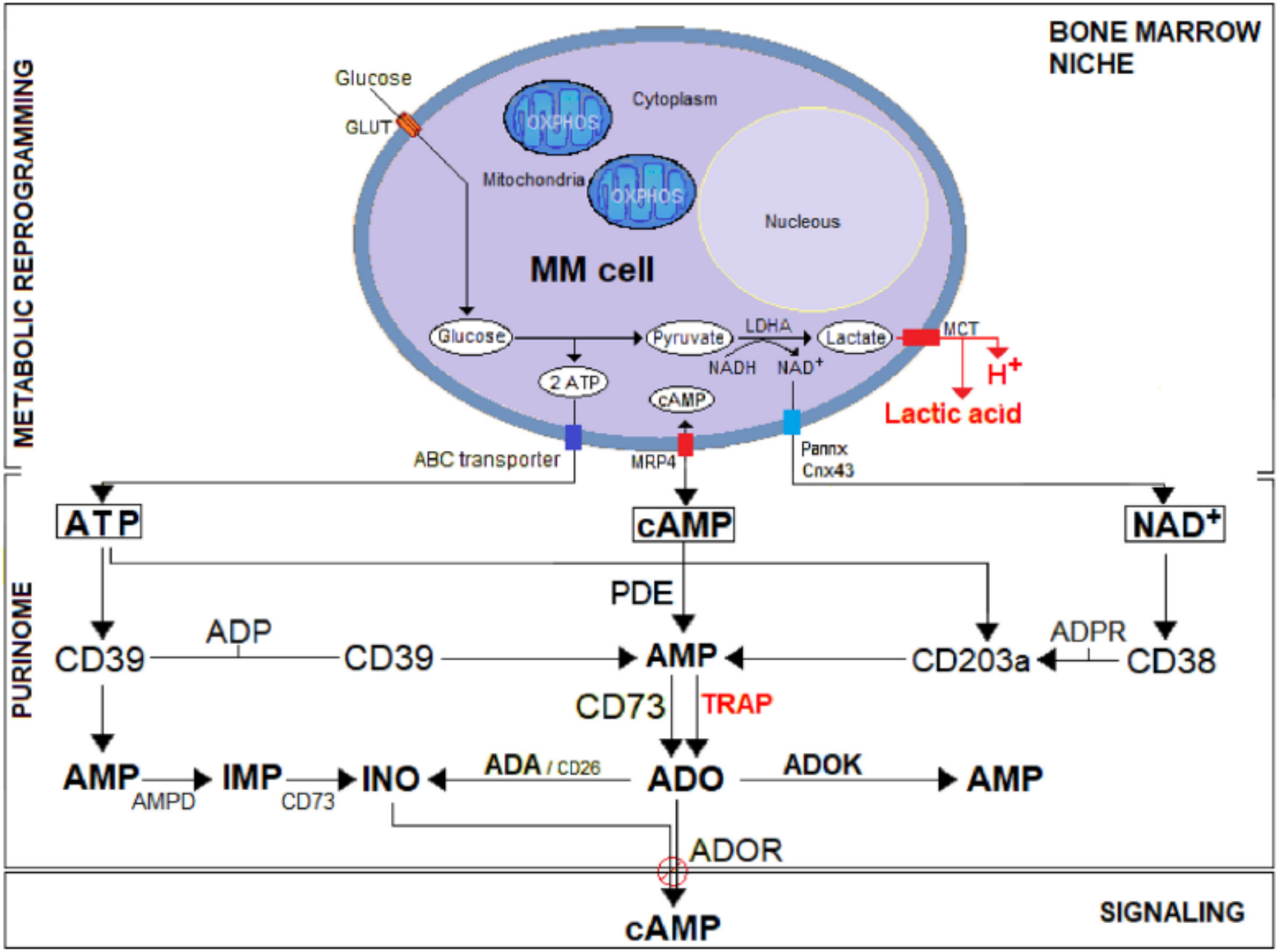
Adams HC 3°, ……van de Donk NWCJ, Sasser AK and Casneuf T. Cytometry Am March 2019

CD38 in NAD$^+$ metabolism



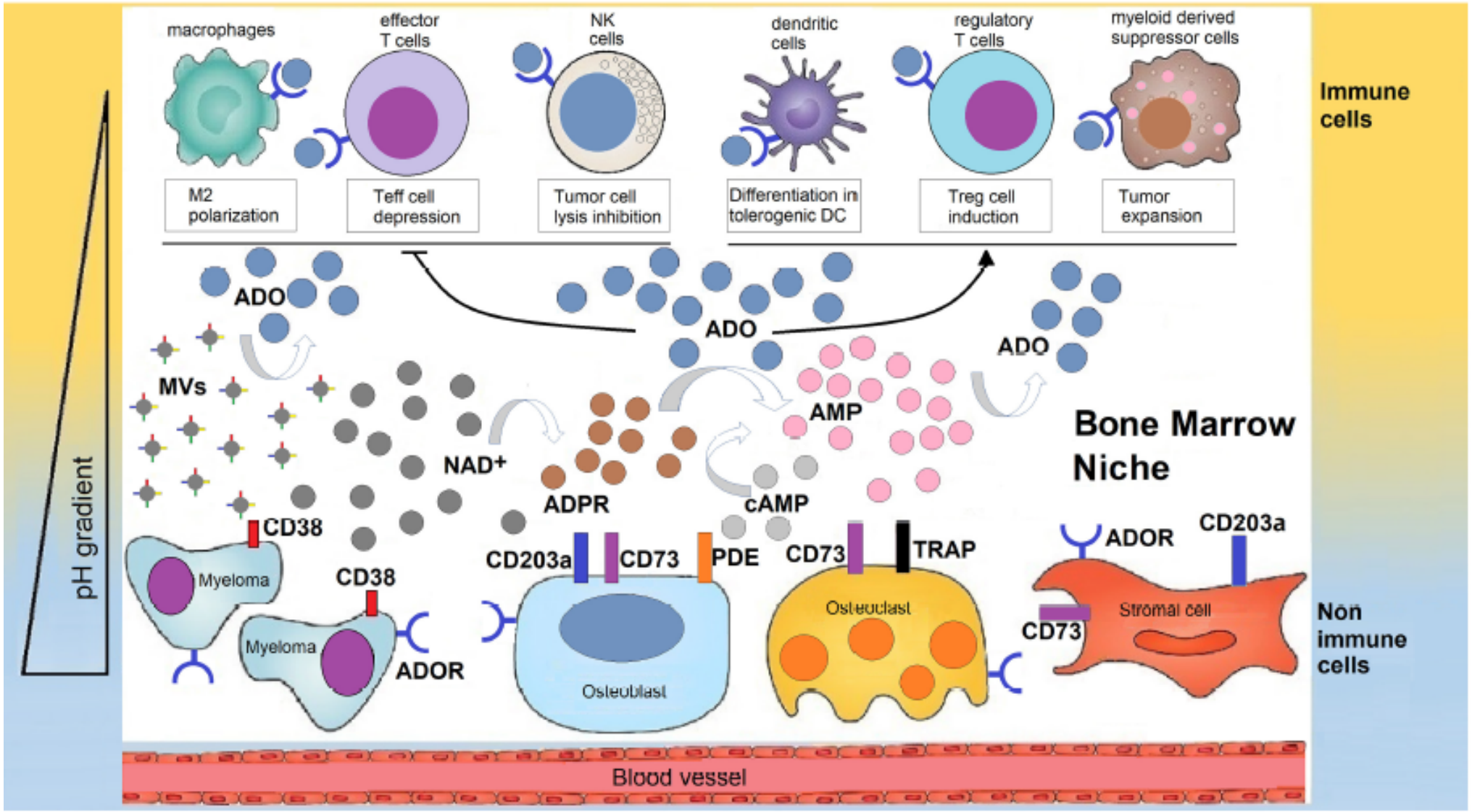
Hogan KA, Chini CCS and Chini EN, Front Immunol, 10: 1187, 2019

Purinome in the MM environment during metabolic reprogramming of the bone marrow niche.



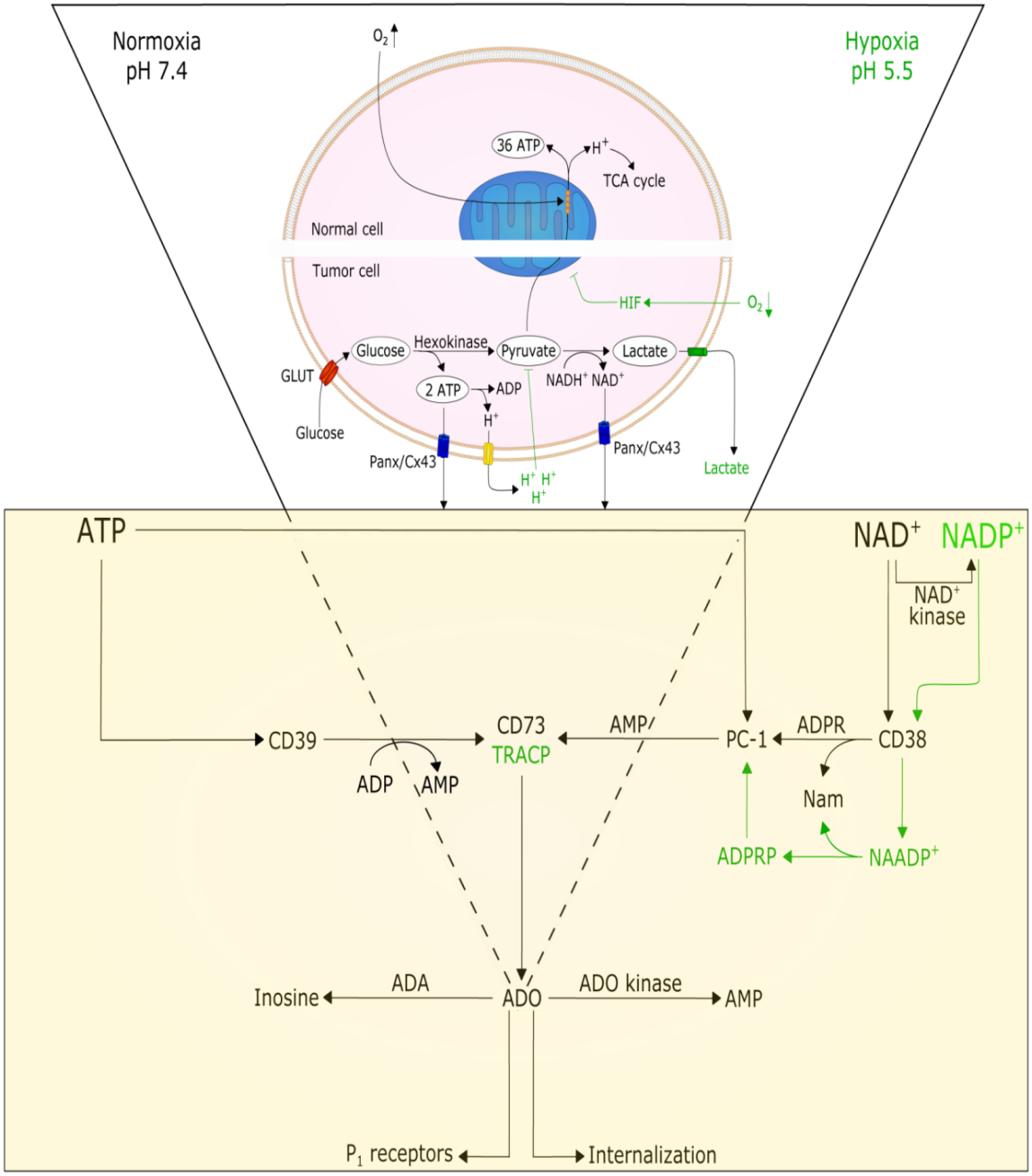
A.L. Horenstein,…… and F. Malavasi. *Frontiers Immunol.* (10:

Human bone marrow sheltering malignant plasma cells, bone and immune cells supports the production of adenosine
with generation of a tolerant niche



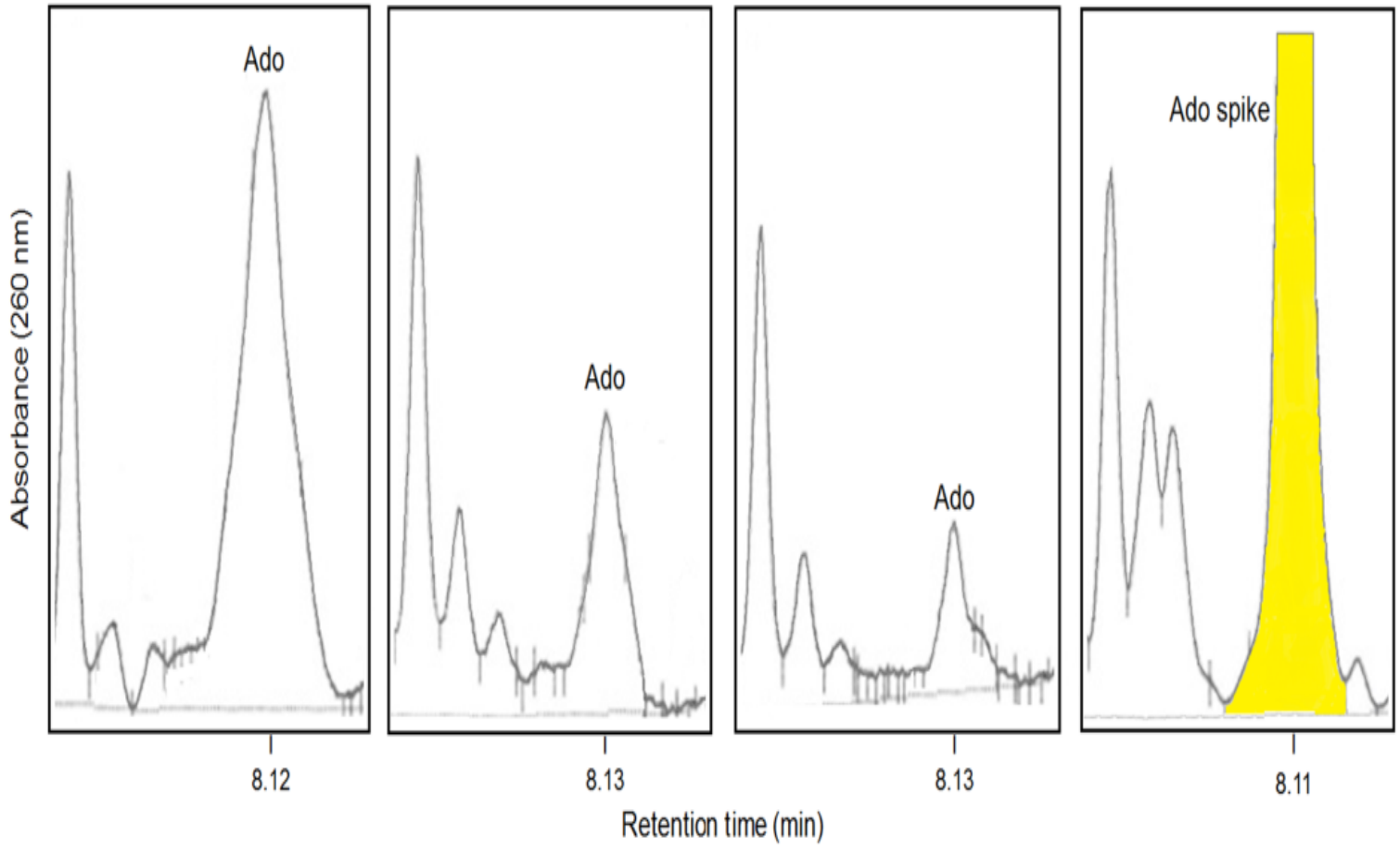
A.L. Horenstein……and F. Malavasi (*Frontiers Immunol.* 10: 760, 20

Interplay among ectoenzymes, their substrates and products in the BM niche

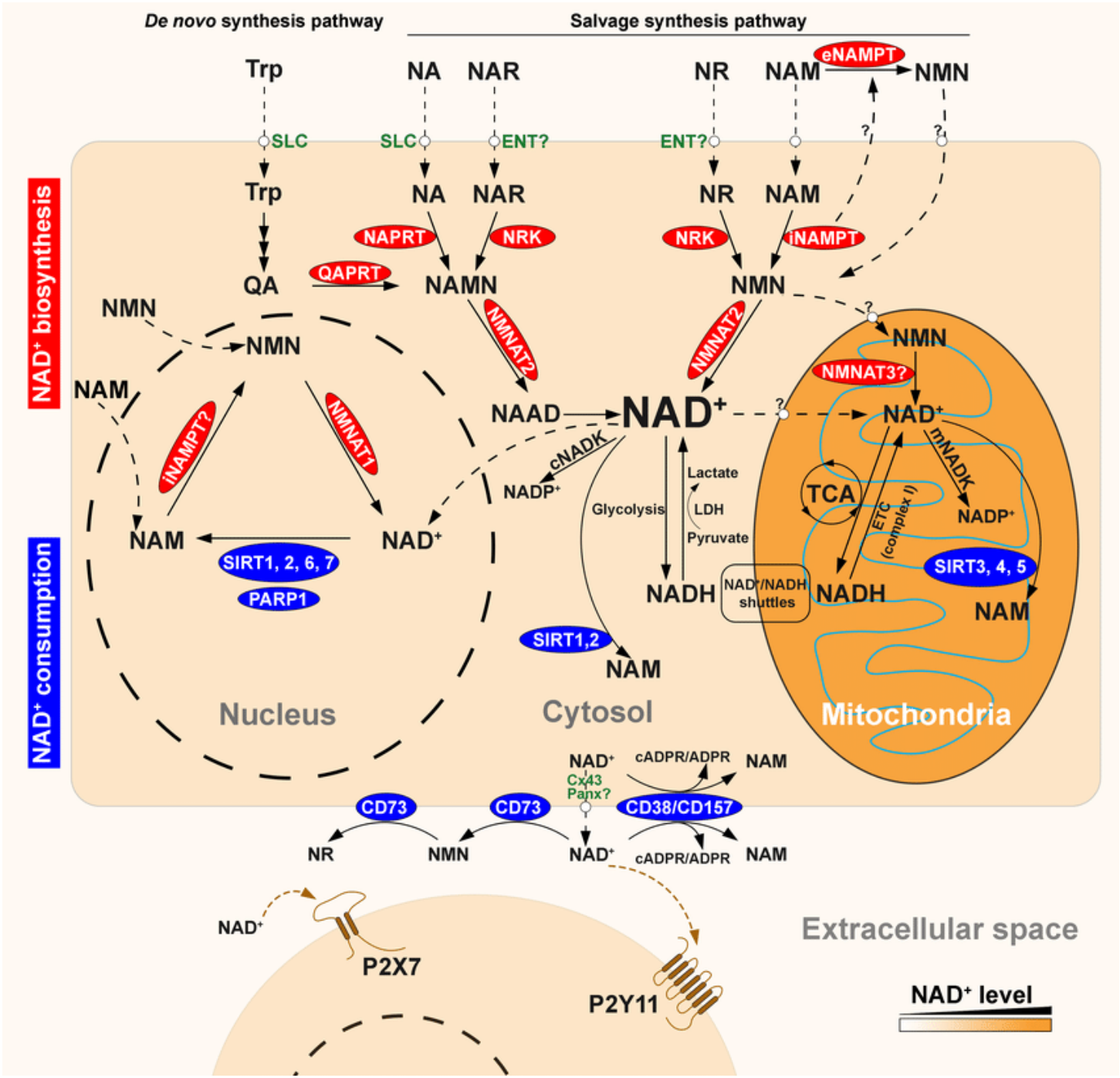


A. Chillemi, ……. A.L. Horenstein and F. Malavasi (Frontiers Immunol., 8: 307, 2017)

Proof-of-principle: BM plasma from MM patients contains ADO



Horenstein A.L. et al. *Molecular Medicine* (2016)



Y. Zhu et al., Phrmacology and Therapeutics, 2019