CD38 e SLAMF7: caratteristiche e utilizzi in pratica clinica

Nicola Giuliani

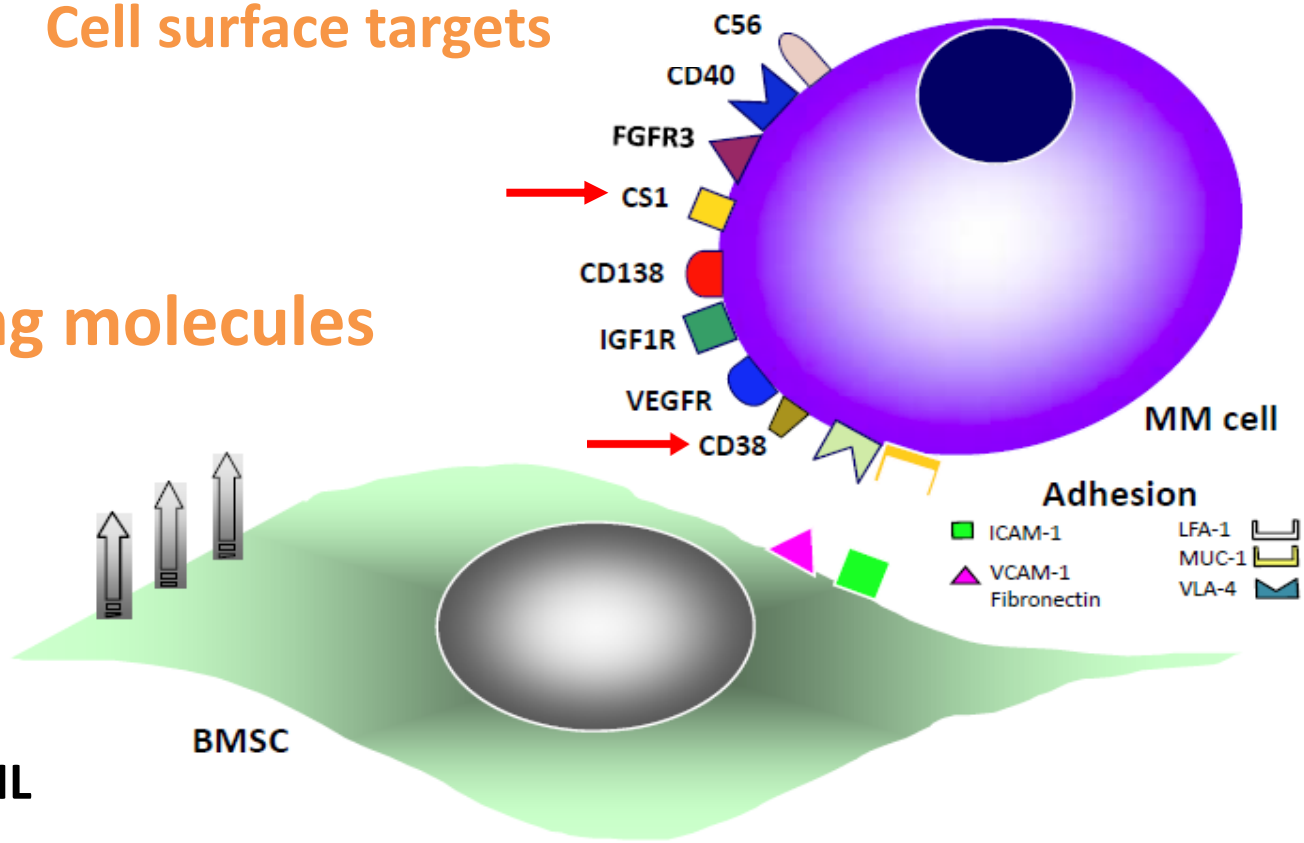
U.O. di Ematologia e CTMO e Università di Parma

Targets for monoclonal antibody therapy in multiple myeloma (MM)

Cell surface targets

Signaling molecules

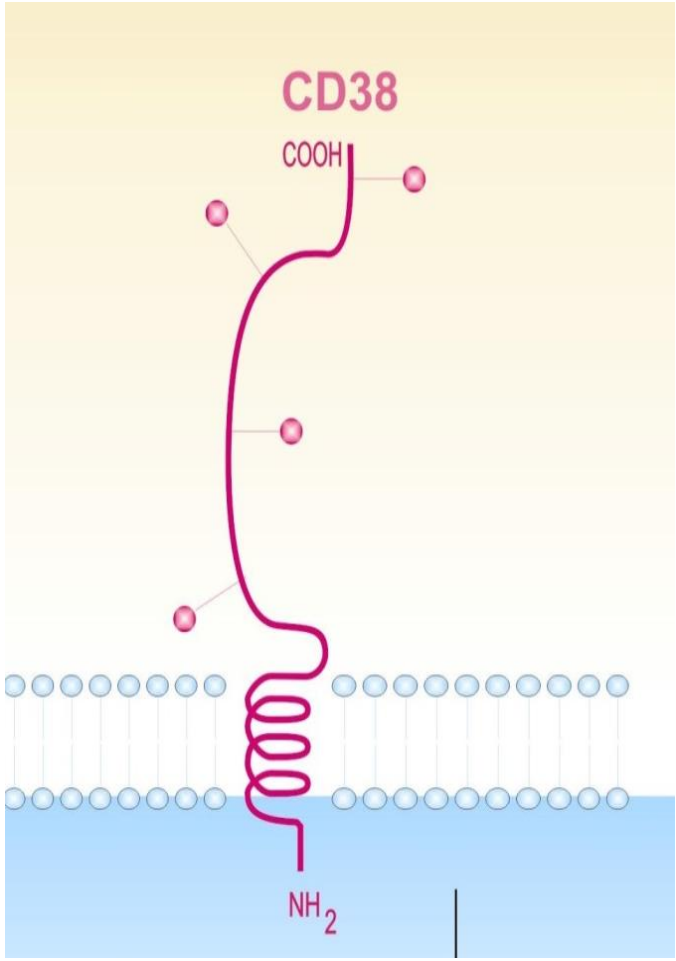
IL-6
RANKL
DKK1
VEGF
IGF-1
SDF-1α
BAFF, APRIL



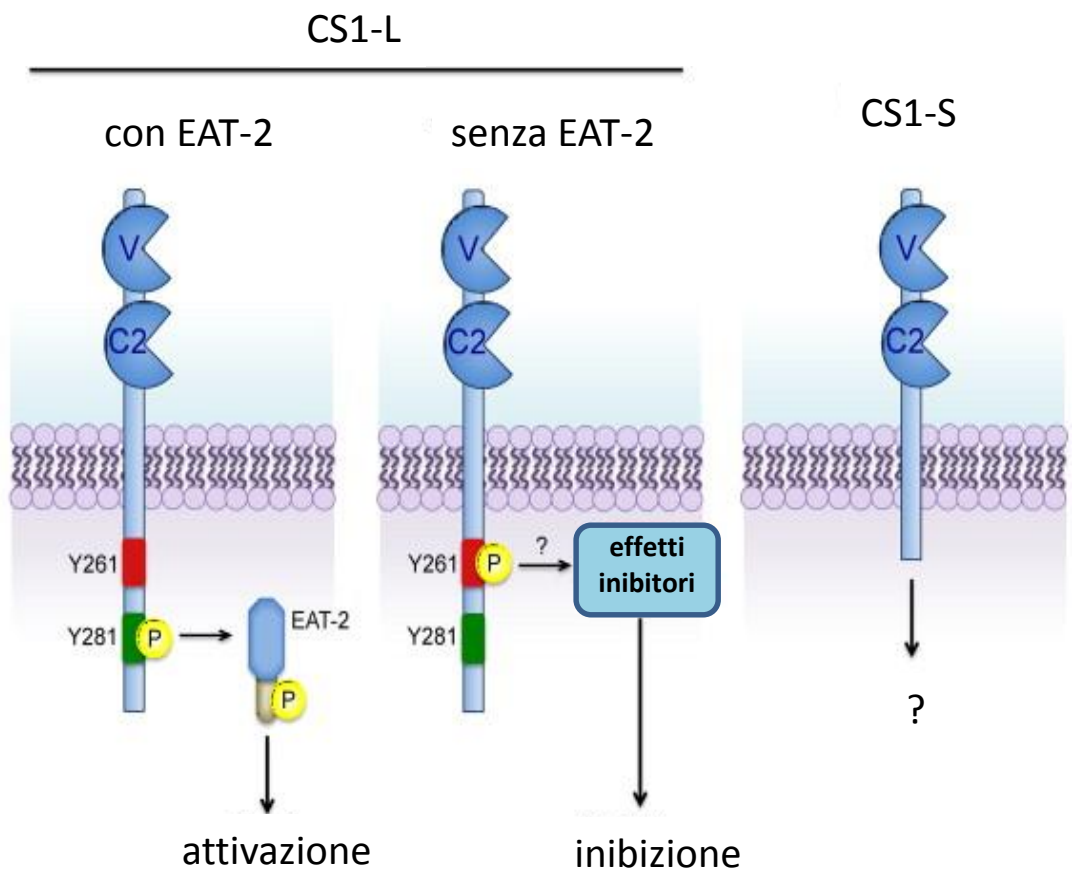
Adapted from: Anderson K et al. *J Clin Oncol,* 2012

CD38 and SLAMF7: two surface targets

CD38



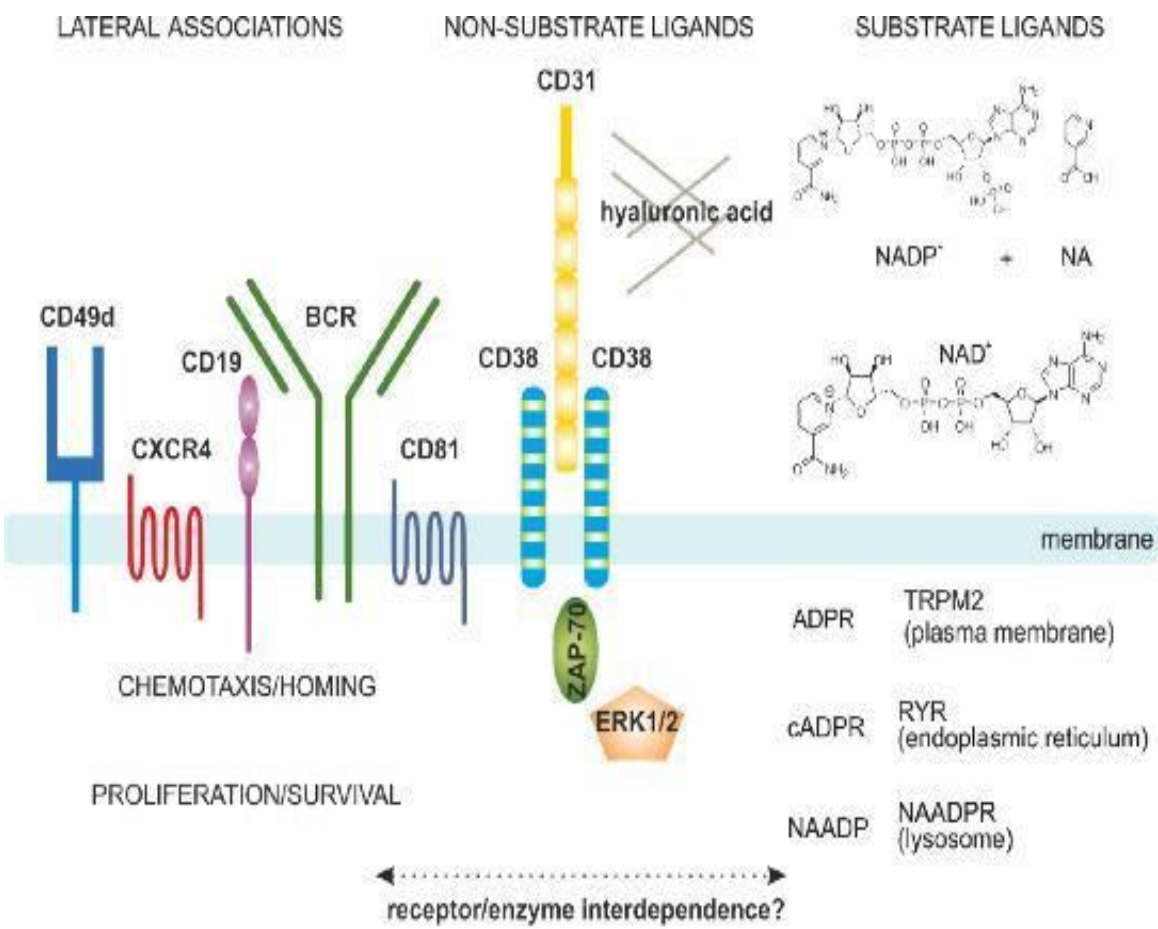
CS1 (SLAMF7)



SLAMF7: Signalling lymphocytic activation molecule F(amily)7

Malavasi F et al. *Physiol Rev*, 2008.
Vellette A et al. *Crit Rev Oncol/Hem*, 2013.

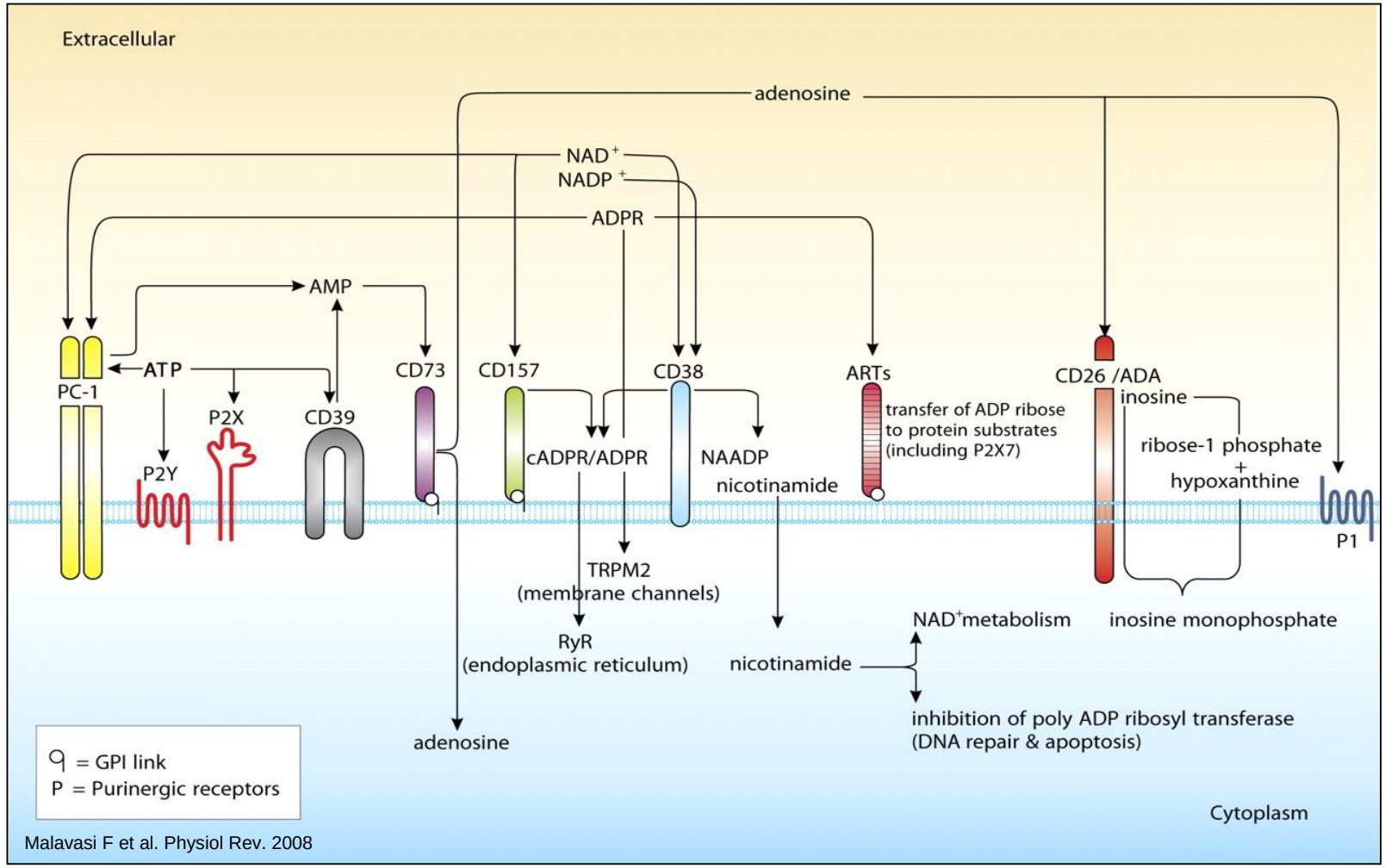
CD38 is a Cell-surface Receptor and Ectoenzyme



- **As a receptor**
  - Regulates signaling, homing, adhesion and migration in close contact with BCR complex and CXCR4.
  - Engagement with CD31 or hyaluronic acid activate ZAP-70, ERK1/2 and NFKB pathways and regulates activation and proliferation of the cell.
- **As an ectoenzyme**
  - CD38 interacts with NAD+ and NADP+, which are converted to cADPR, ADPR, and NAADP, all intracellular $Ca2^{+}$ mobilizing agents.

Malavasi F et al. *Blood*, 2011.

Biological aspects behind antibody based targeting CD38



Malavasi F et al. Physiol Rev. 2008

Courtesy Malavasi F, ASH 2016 Abs n. SCI-36

CD38 Expression in MM Cells and Other Lymphoid Tissues

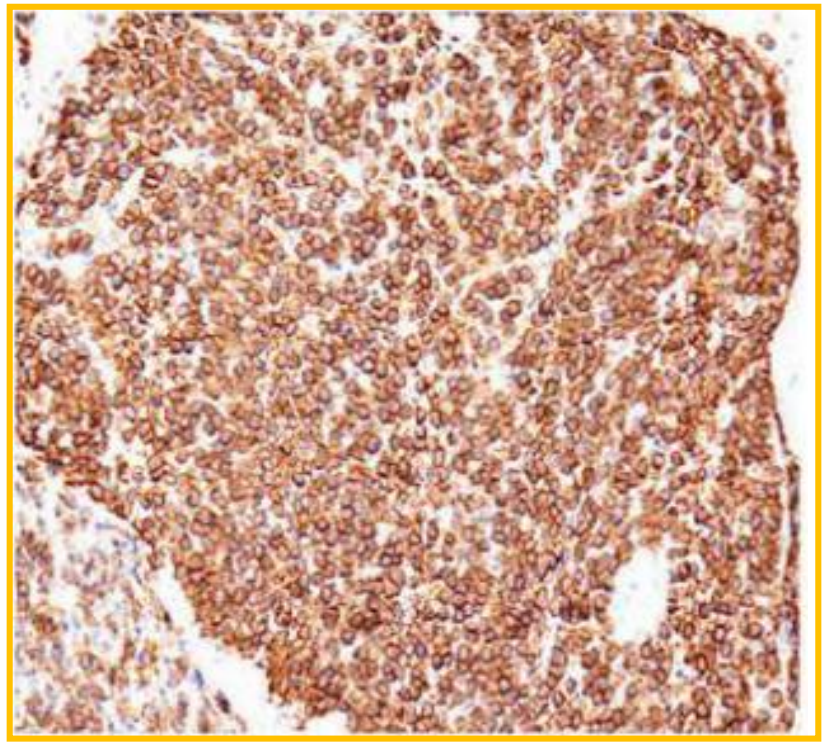
Lymphoid tissue	Cell population
Blood	T cells (precursors, activated) B cells (precursors, activated) Myeloid cells (monocytes, macrophages, dendritic cells) NK cells Erythrocytes Platelets
Cord blood	T and B lymphocytes, monocytes
Bone marrow	Precursors Plasma cells
Thymus	Cortical thymocytes
Lymph nodes	Germinal center B cells

- **Highly and uniformly expressed on myeloma cells**[1,2,3]
- **Relatively low expression on normal lymphoid and myeloid cells** and in some tissues of non-hematopoietic origin[4]
- **CD38 is not expressed on hematopoietic pluripotent cells**, which are crucial for the recovery of the long-term bone marrow

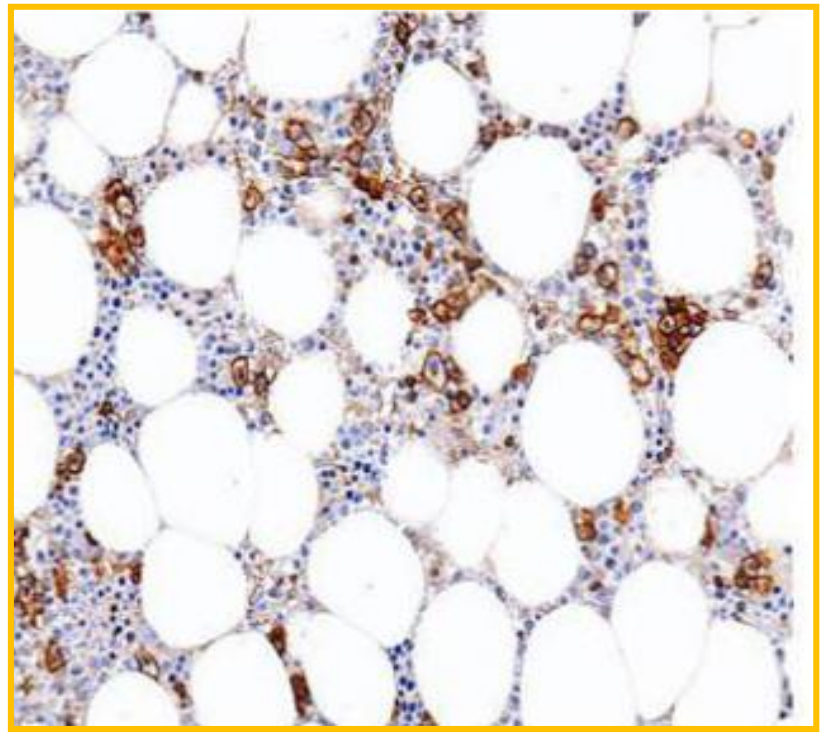
Malavasi F et al. *Physiol Rev*, 2008.
Lin P et al. *Am J Clin Pathol*, 2004.
Santonocito AM et al. *Leuk Res*, 2004.
Deaglio S et al. *Leuk Res*, 2001.

CD38 expression in bone biopsies of MM patients

In high density MM cells

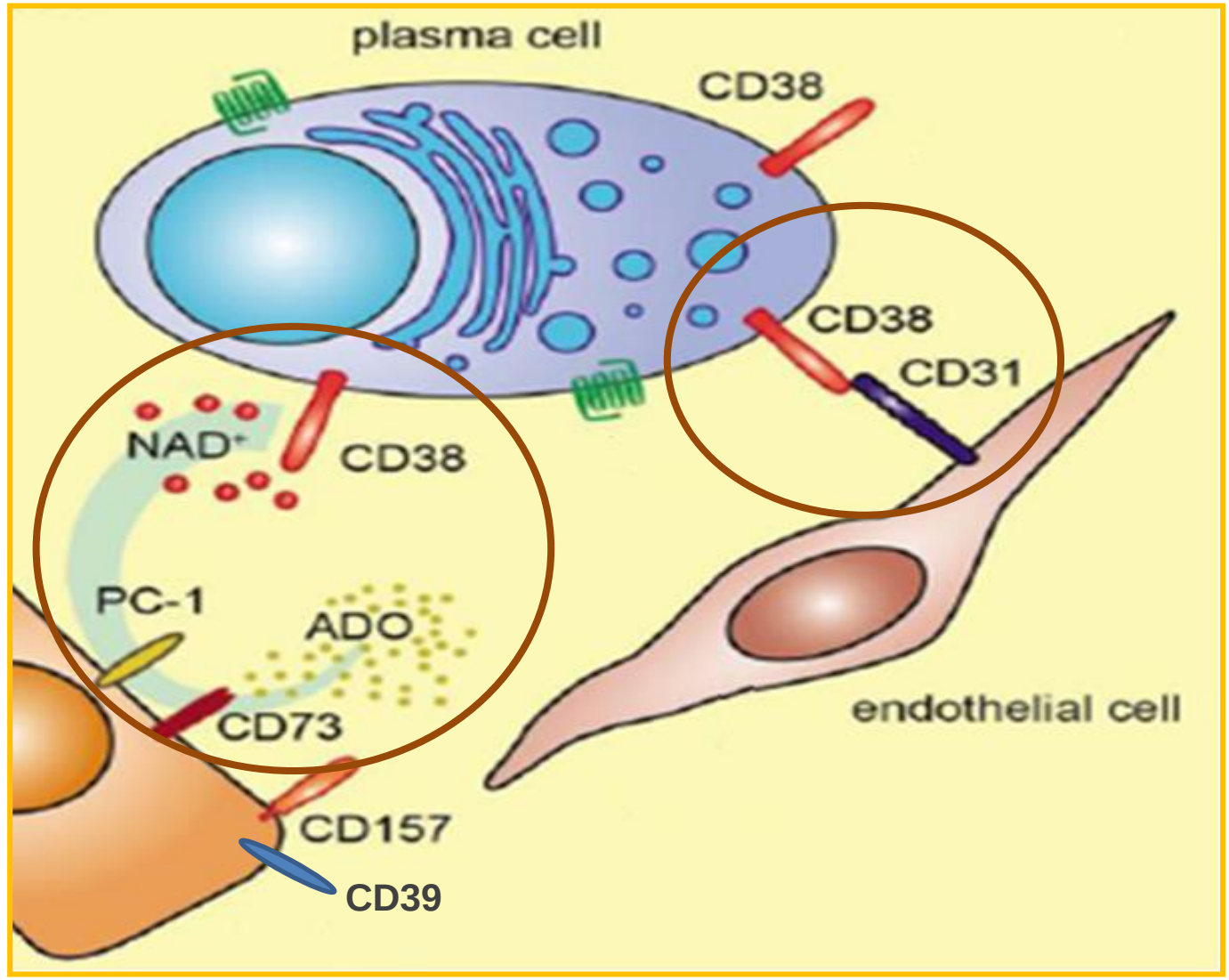


In low density MM cells



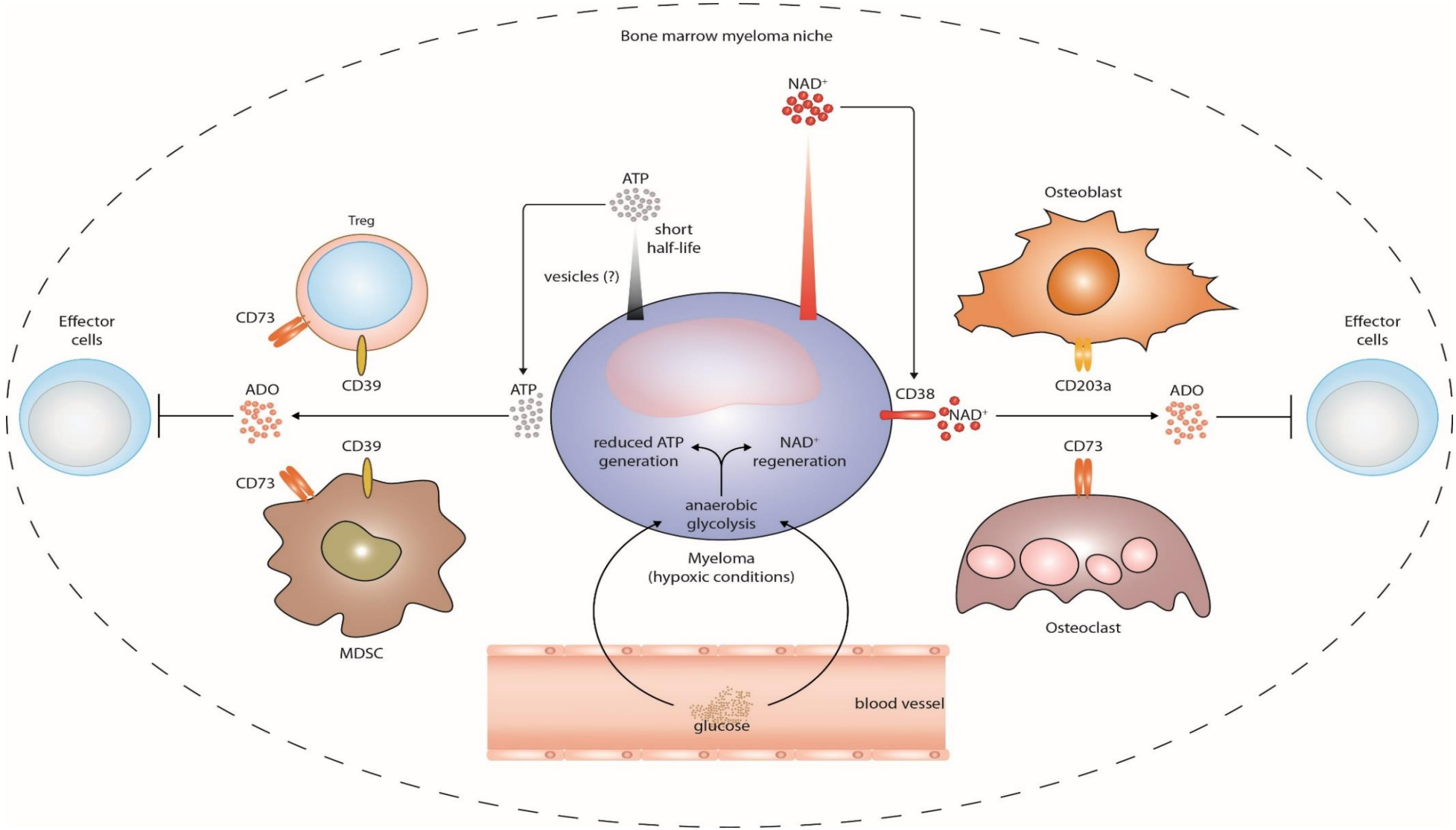
Quarona V et al. *Ann N Y Acad Sci*, 2015.

CD38 in MM microenvironment



Modified from: Chillemi A et al. *Frontiers in Bioscence*, 2014.

Metabolic balance between ATP and NAD+ in the BM niche



Quarona V et al. *Ann N Y Acad Sci*, 2015.

Rationale for targeting CD38

Functions:

1) Receptor-mediated adhesion and signaling functions
2) Enzymatic activities

   Contributes to intracellular calcium mobilization

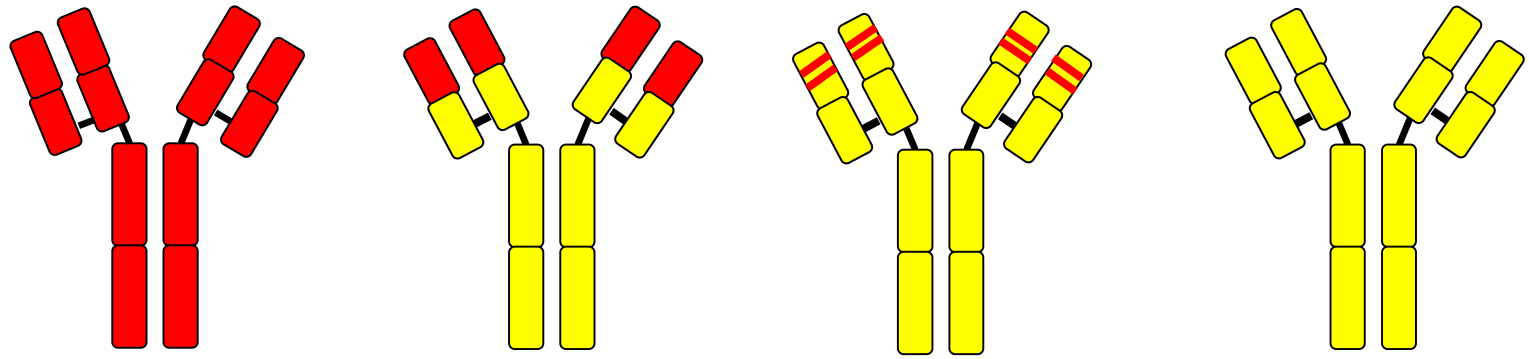
   Involved in production of adenosine: important for induction of local immunological tolerance → implicated in local survival strategy of the neoplastic plasma cell in the bone marrow milieu

Expression levels:

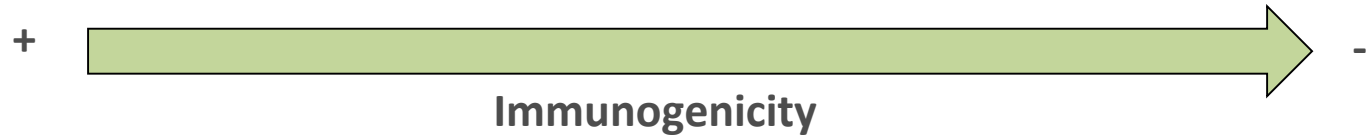
1) Low level of expression of CD38 on lymphoid and myeloid cells under normal conditions
2) High level of CD38 expression on malignant cells in MM

De Weers M et al. *J Immunol,* 2011; Chillemi A et al. *Mol Med,* 2013; Quarona V et al. *Ann N Y Acad Sci,* 2015.

Humanization of antibodies to overcome immunogenicity



Mouse	Chimeric	Humanized	Human
'momab' = fully murine (Tositumomab)	**'ximab'** = chimeric mouse or rat Ig variable regions; human constant regions (Rituximab)	**'zumab'** = humanized chimeric mAb with only complementarity determining regions being mouse origin (Bevacizumab)	**'umab'** = fully human (**Daratumumab**)



Imai K & Takaoka A *Nature Reviews Cancer,* 2006.

Anti-CD38 monoclonal antibodies

Chimeric:

Isatuximab (SAR650984)

Fully human:

Daratumumab (DARA)

MOR202 (MOR)

De Weers M et al. *J Immunol,* 2011.
https://download.ama-assn.org/resources/doc/usan/x-pub/isatuximab.pdf
http://www.morphosys.com/pipeline/proprietary-product-portfolio/mor202

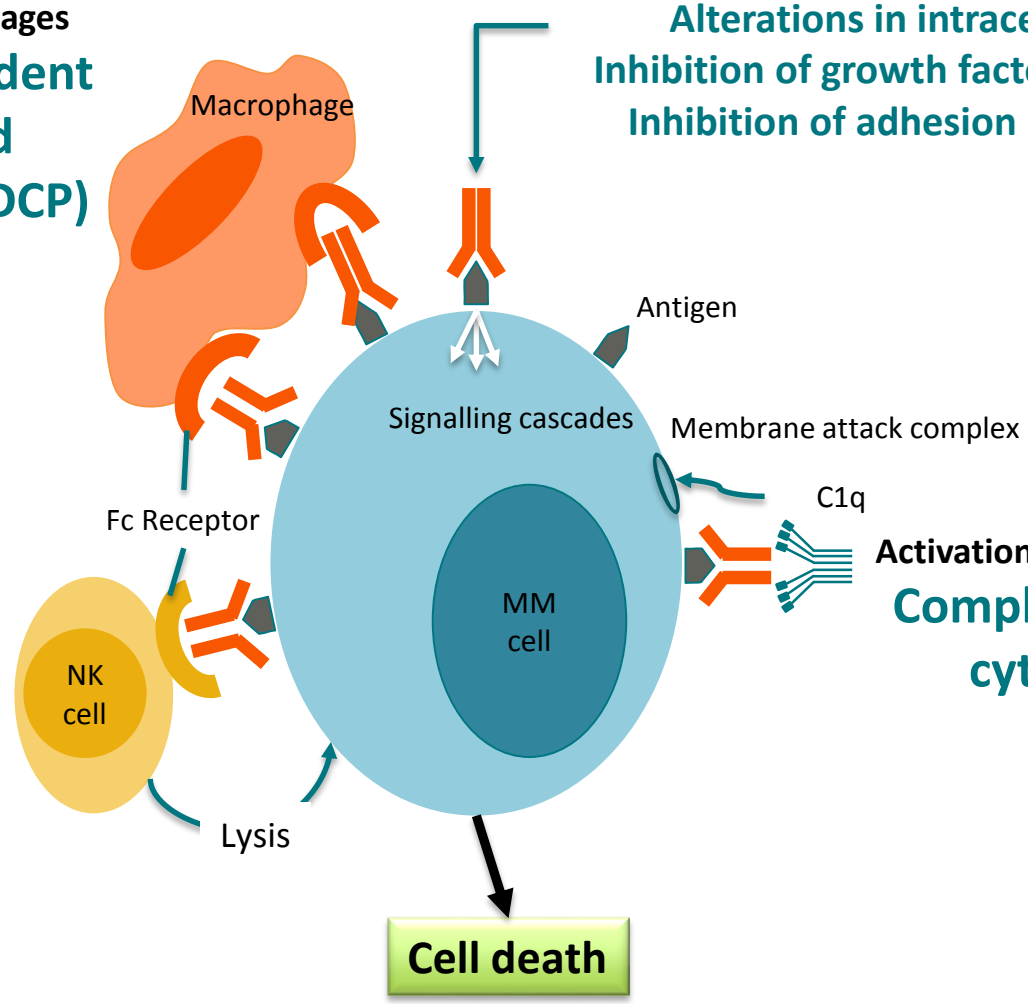
Monoclonal antibodies act through different mechanisms

Direct effects

Alterations in intracellular signalling
Inhibition of growth factor receptor function
Inhibition of adhesion molecule function

Activation of macrophages

Antibody-dependent cell-mediated phagocytosis (ADCP)



Activation of the complement system

Complement-dependent cytotoxicity (CDC)

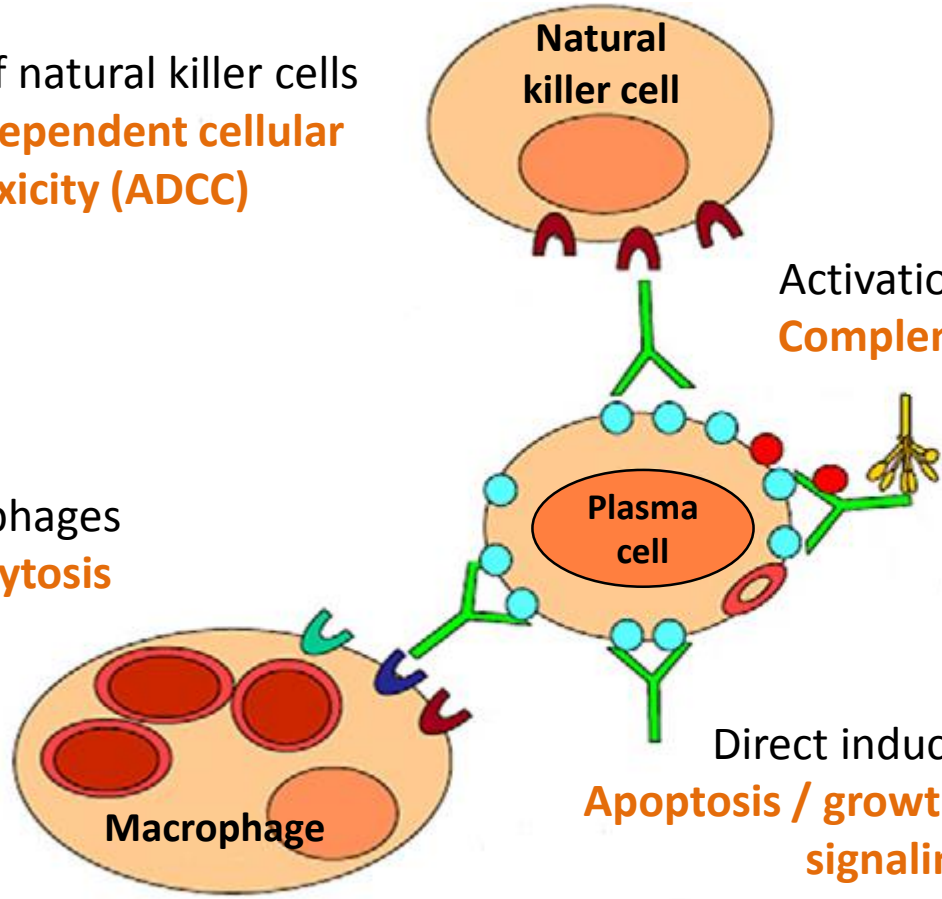
Activation of natural killer (NK) cells

Antibody-dependent cellular cytotoxicity (ADCC)

van de Donk NW et al. *Blood,* 2016.

Daratumumab (DARA): mechanisms of action

Activation of natural killer cells
Antibody-dependent cellular cytotoxicity (ADCC)



Activation of the complement system
Complement-dependent cytotoxicity (CDC)

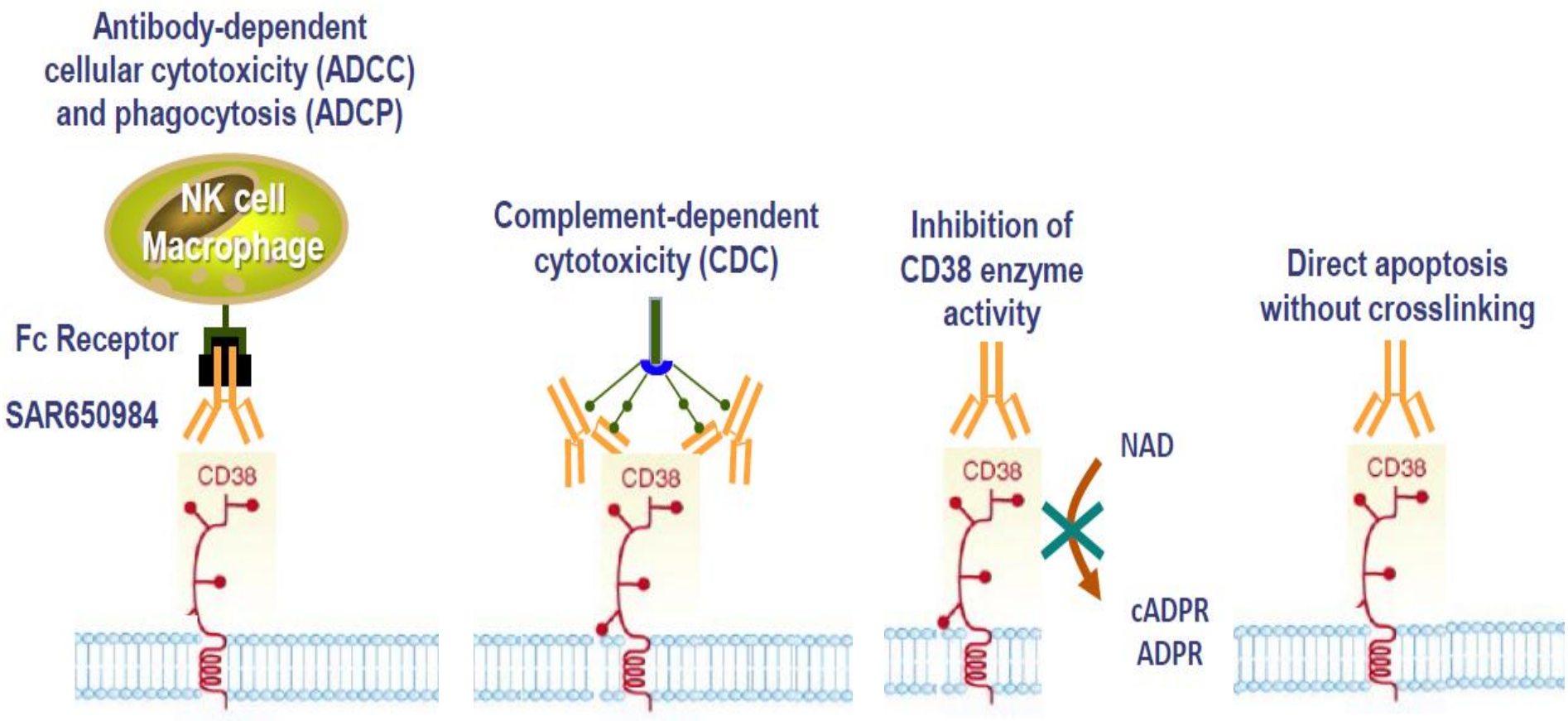
Activation of macrophages
Induction of phagocytosis

(Antibody-dependent cell-mediated phagocytosis = ADCP)

Direct induction of apoptosis
Apoptosis / growth arrest via targeting of signaling pathways

Adapted from: Golay J & Introna M *Arch Biochem Biophys*, 2012 ;Tai YT & Anderson KC *Bone Marrow Res,* 2011.

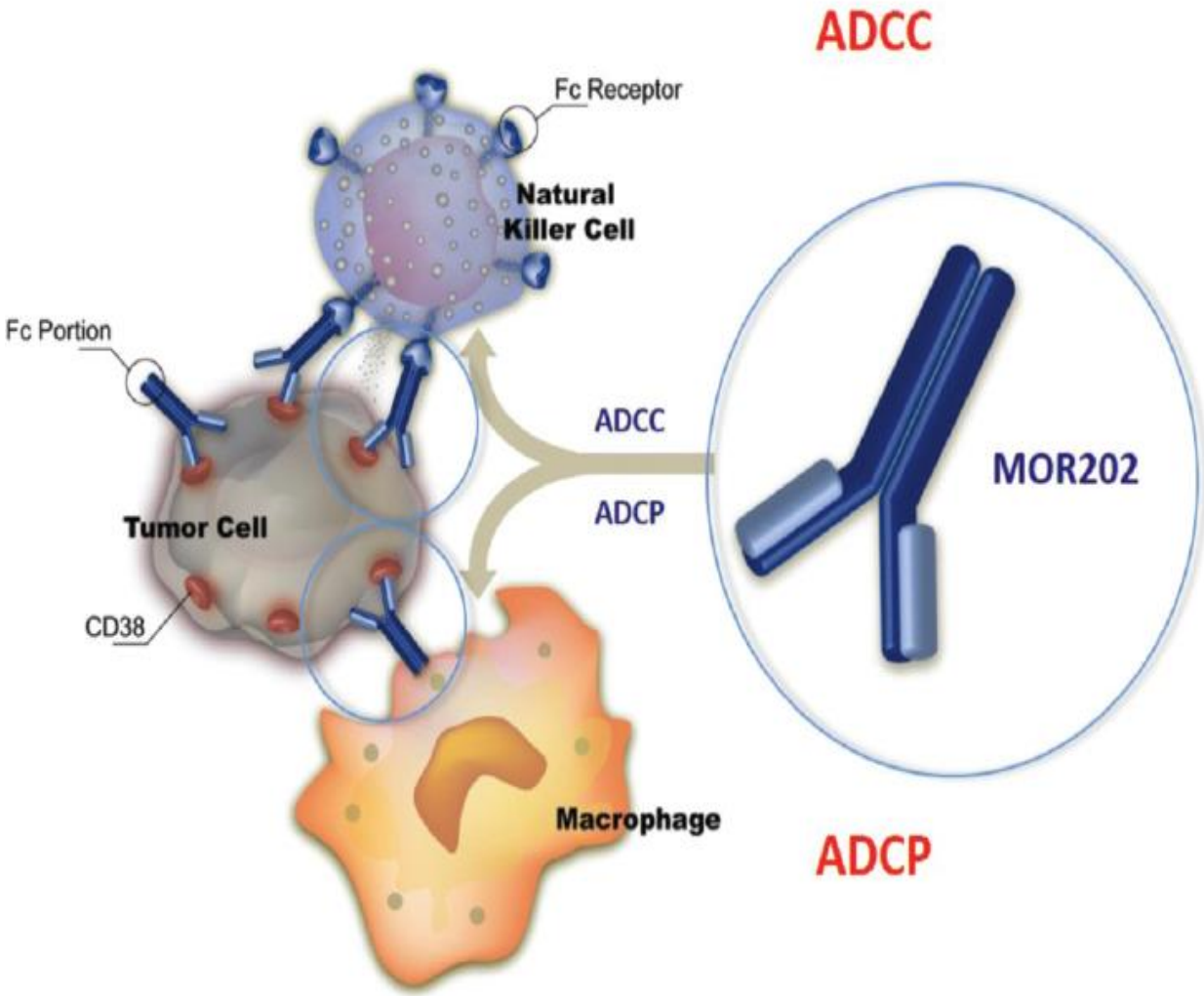
Isatuximab (SAR650984, anti-CD38) mechanisms of action



Canonical and lysosome-dependent cell death*

Deckert J et al. *Clin Cancer Res*, 2014.
Martin TG et al. ASH 2014 (Abstract 83); oral presentation.
*Jiang H et al. *Leuk,* 2016.

MOR202 (CD38) mAb: main mechanisms of action



antibody-dependent cellular cytotoxicity (ADCC)

phagocytosis (ADCP)

Raab MS et al. ASCO 2015 (Abstract 8574), poster presentation.

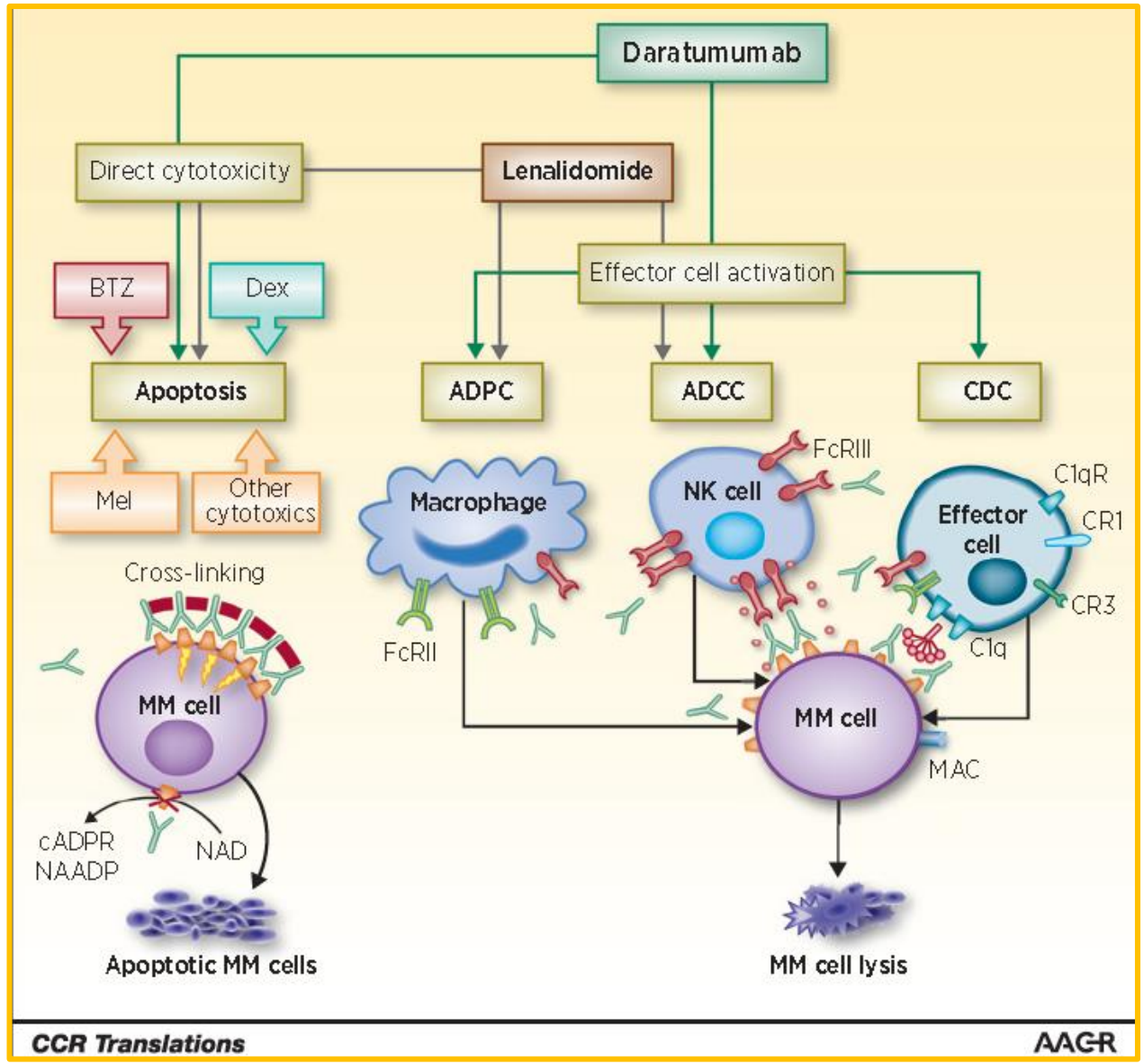
Summary of mechanisms of action of anti-CD38 mAbs

	DARA	SAR	MOR	TAK
Origin	Human	Humanized	Human	Humanized
Development phase	Phase III	Phase I/II	Phase I/IIa	Preclinical
Binding	+++	+++	++	+++
CDC (max lysis)	+++	+	+	++
Phagocytosis	+++	nd*	++	+++
ADCC (max lysis)	++	++	++	++
PCD direct	–	++	–	–
PCD crosslinking	+++	+++	+++	+++
Modulation ectoenzyme function	+	+++	–	+

nd, not determined; PCD, programmed cell death.

van Bueren L et al. Poster presented at: 56th American Society of Hematology (ASH) 2014; San Francisco, CA, USA.

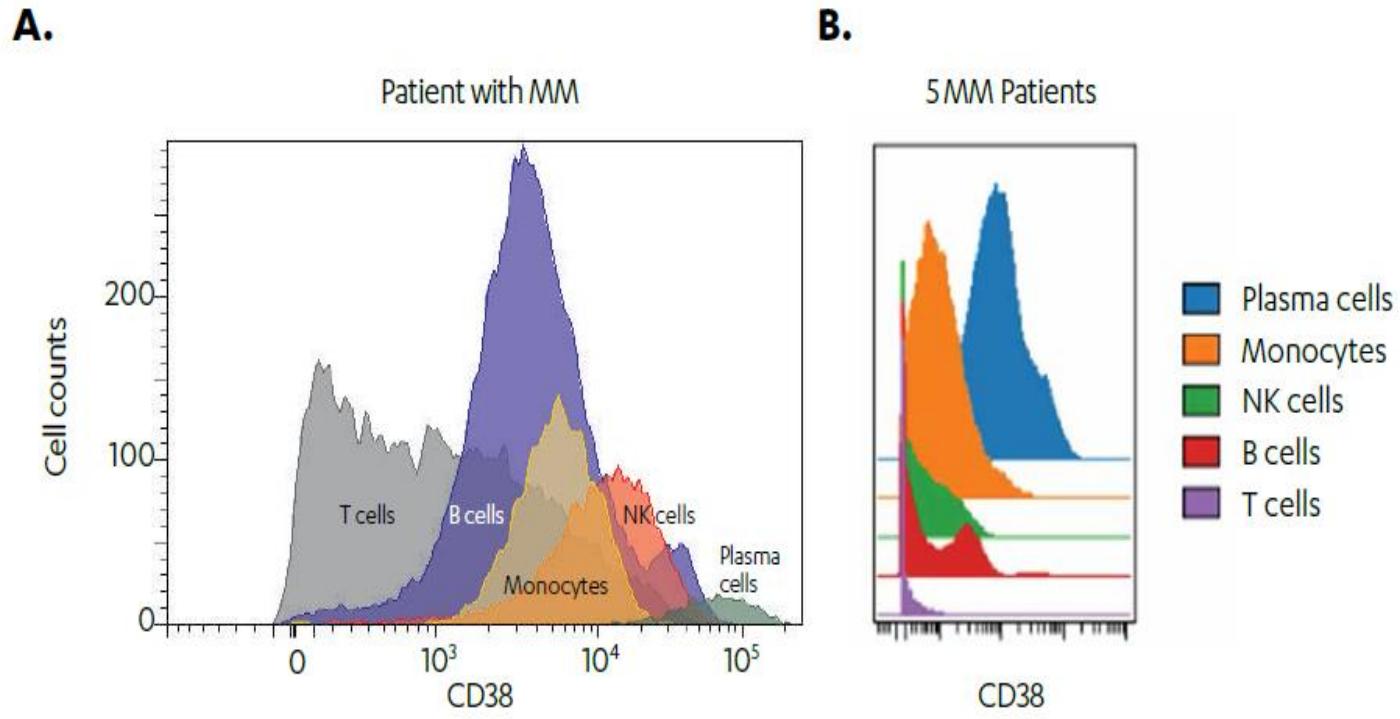
DARA in combination with other drugs: mechanisms



Laubach JP et al. *Clin Cancer Res,* 2015.

Hierarchy of CD38 expression across immune subtypes, as assessed via flow cytometry[1] (A) and CyTOF® (B)

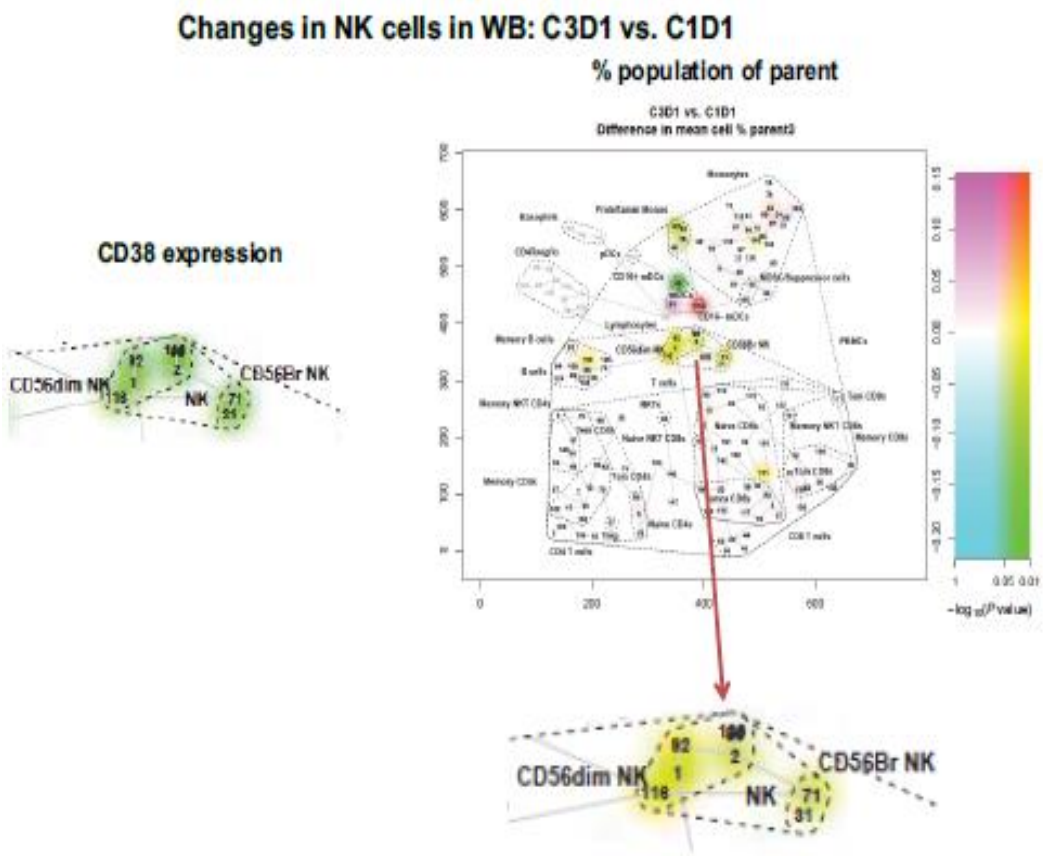
- Previous flow cytometry observations from MM BM of SIRIUS and GEN501 were confirmed, including comparable CD38-marker intensity in natural killer (NK), monocyte, and B- and T-cell compartments

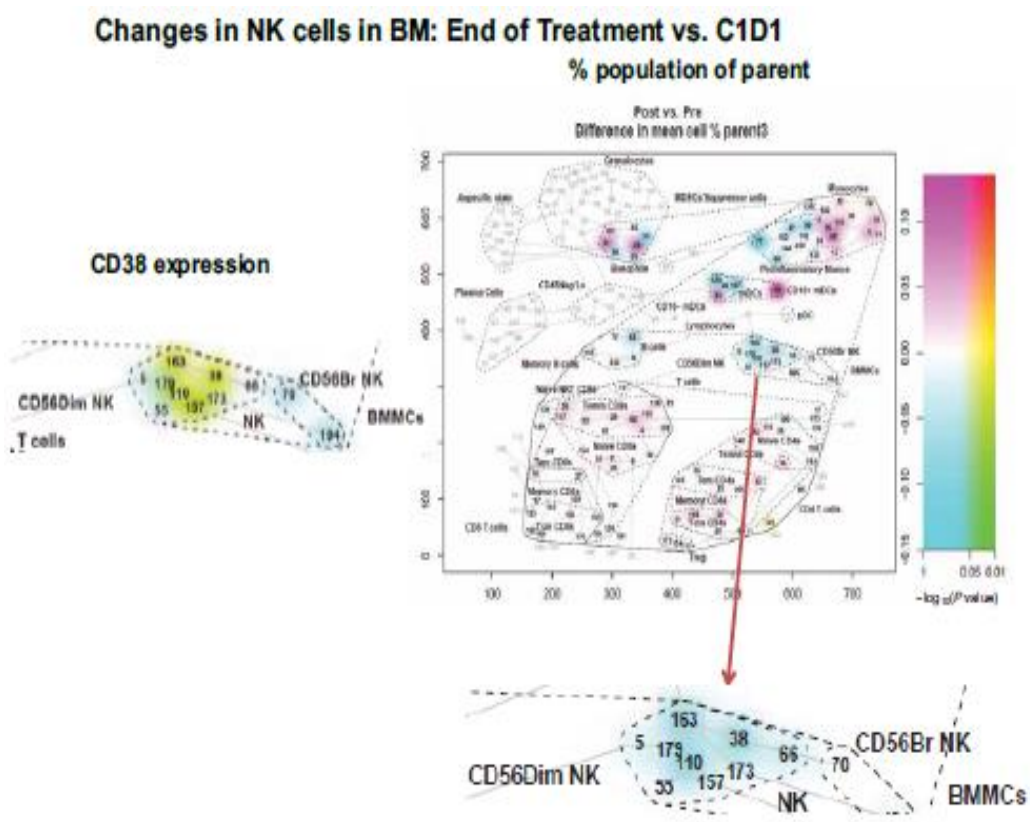


1. Krejcik J et al. *Blood*, 2016.

NK cell depletion observed from WB in SIRIUS (A) and BM in GEN501 (B)

- Along with reduced CD38 expression, the NK-cell population was depleted from WB in SIRIUS and BM in GEN501

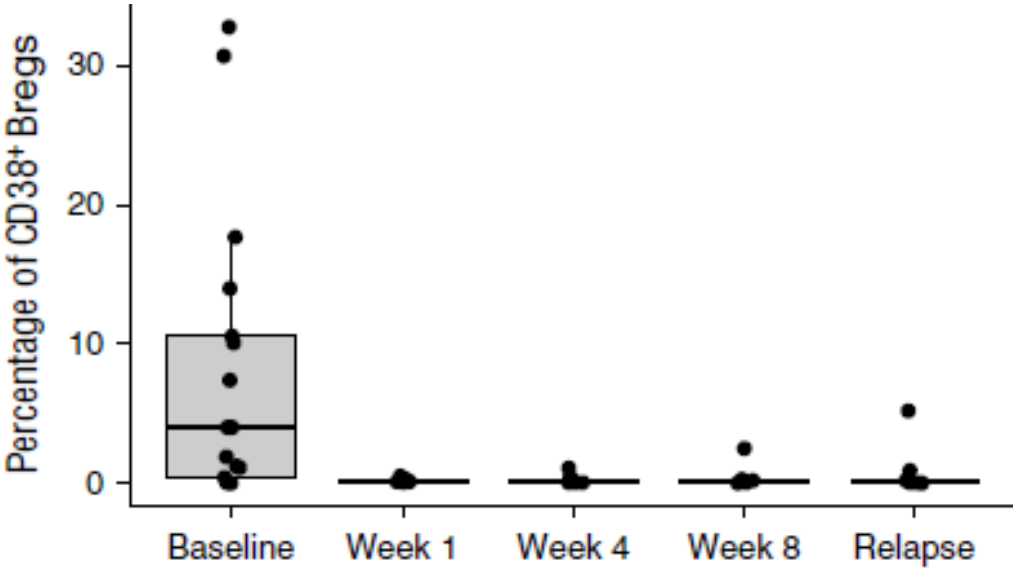




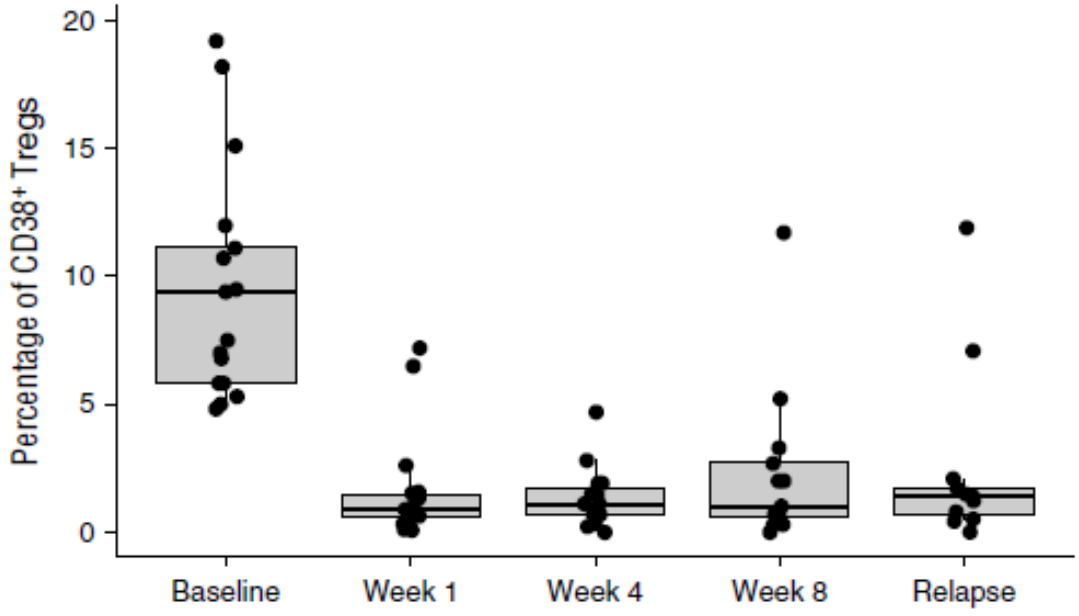
Adams H et al. ASH 2016; San Diego, CA .

Effect of DARA treatment on CD38+ Bregs and Tregs

Breg

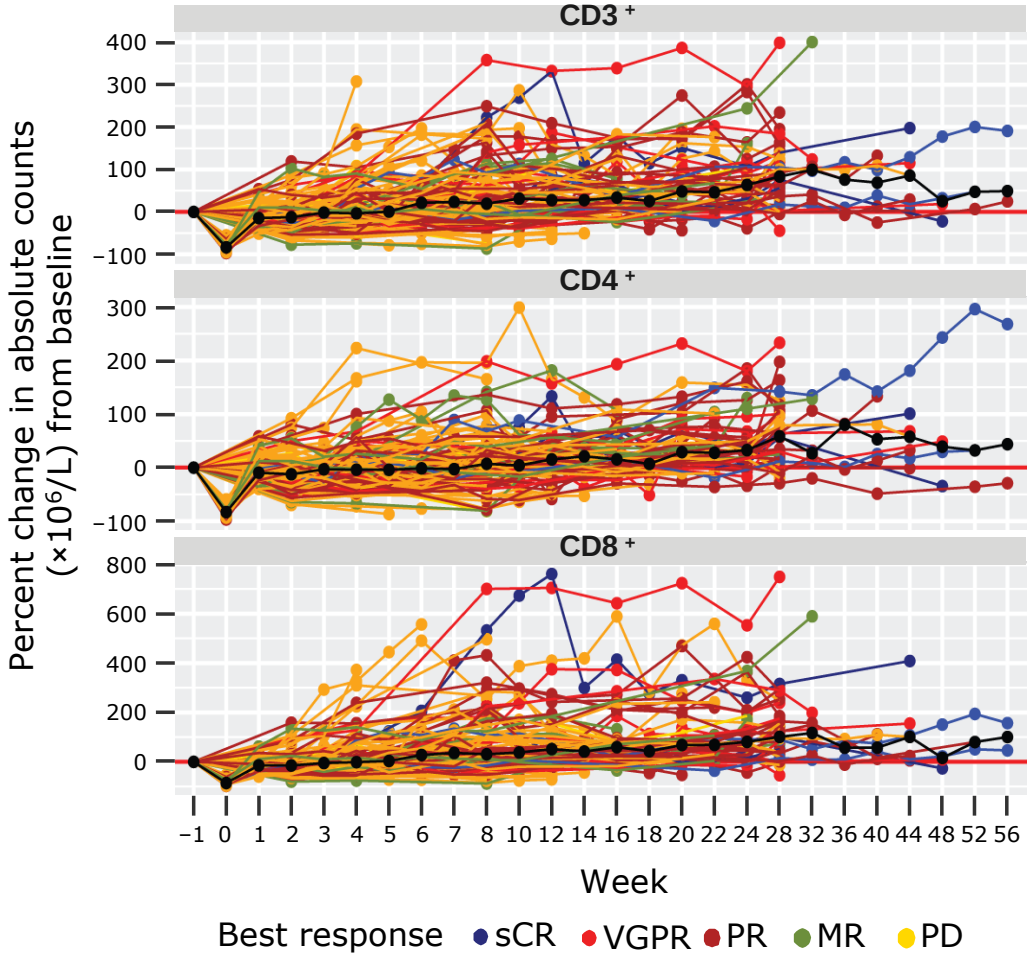


Treg



Krejcik J et al. *Blood*, 2016.

CD3+, CD4+, and CD8+ T-cell counts increase in peripheral blood with DARA treatment



- In peripheral blood (PB; n = 58), significant mean increases in $CD3^+$ (44%), $CD4^+$ (32%), and $CD8^+$ (62%) T-cell counts per 100 days were seen with DARA treatment

Krejcik J et al. ASH, 2015; Orlando, FL. Abstract 3037.

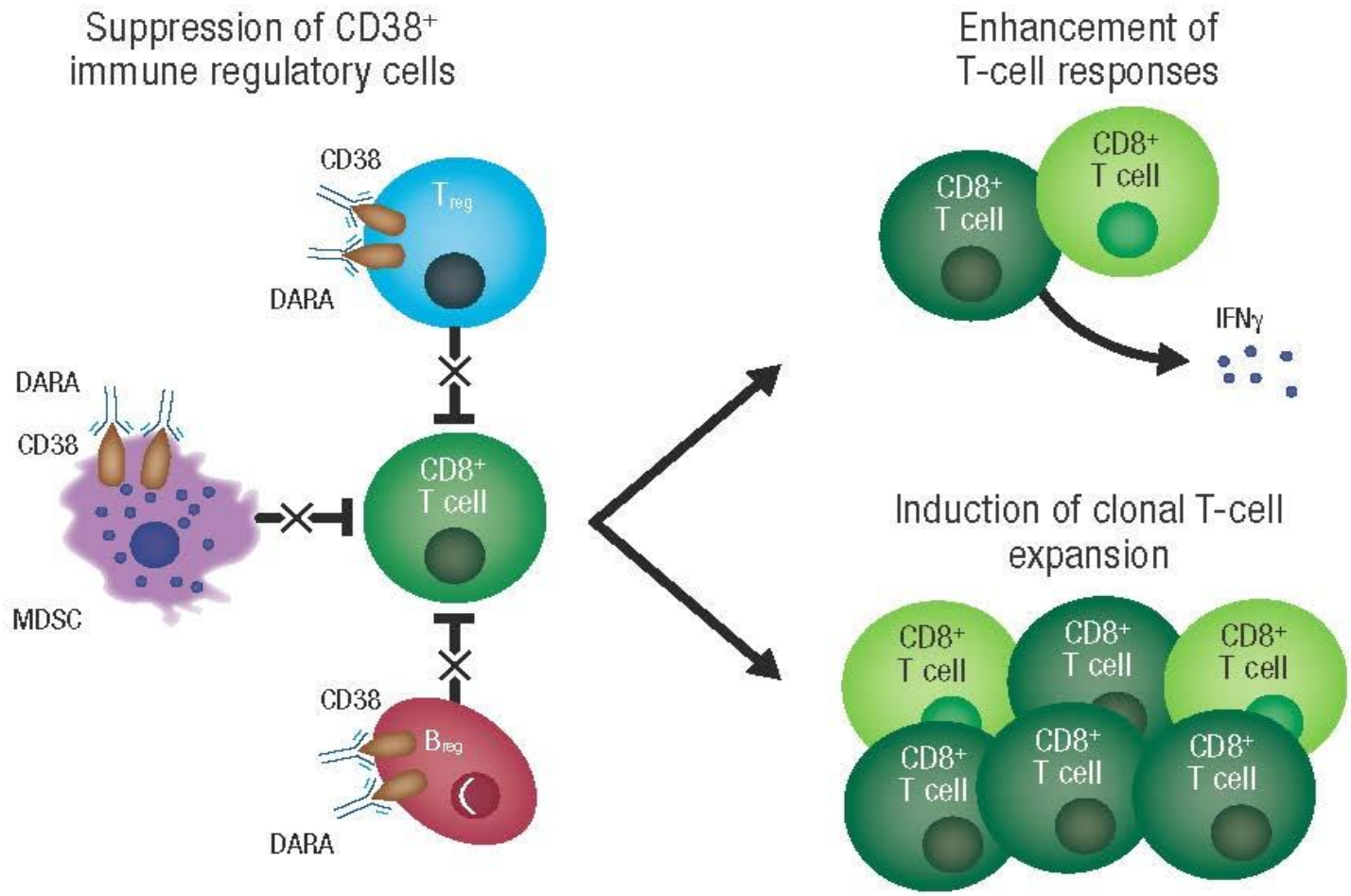
CD3+, CD4+, and CD8+ T-cell counts increase in bone marrow with DARA treatment

- Similar expansion was observed in bone marrow (BM; n = 58), with median maximum percent increases of 20%, 6%, and 27% for $CD3^+$, $CD4^+$, and $CD8^+$ T-cell counts, respectively

	Percent change from baseline at on-treatment visit (% of lymphocytes) n = 58		
	$CD45^+CD3^+$	$CD45^+CD3^+$ $CD4^+$	$CD45^+CD3^+$ $CD8^+$
Minimum	–40.40	–60.7	–10.89
1st quartile	12.13	–8.67	14.58
Median	19.95	5.66	26.99
Mean	29.28	13.42	39.09
3rd quartile	47.65	25.34	53.71
Maximum	121.6	125.5	187.9

Krejcik J et al. ASH, 2015; Orlando, FL. Abstract 3037.

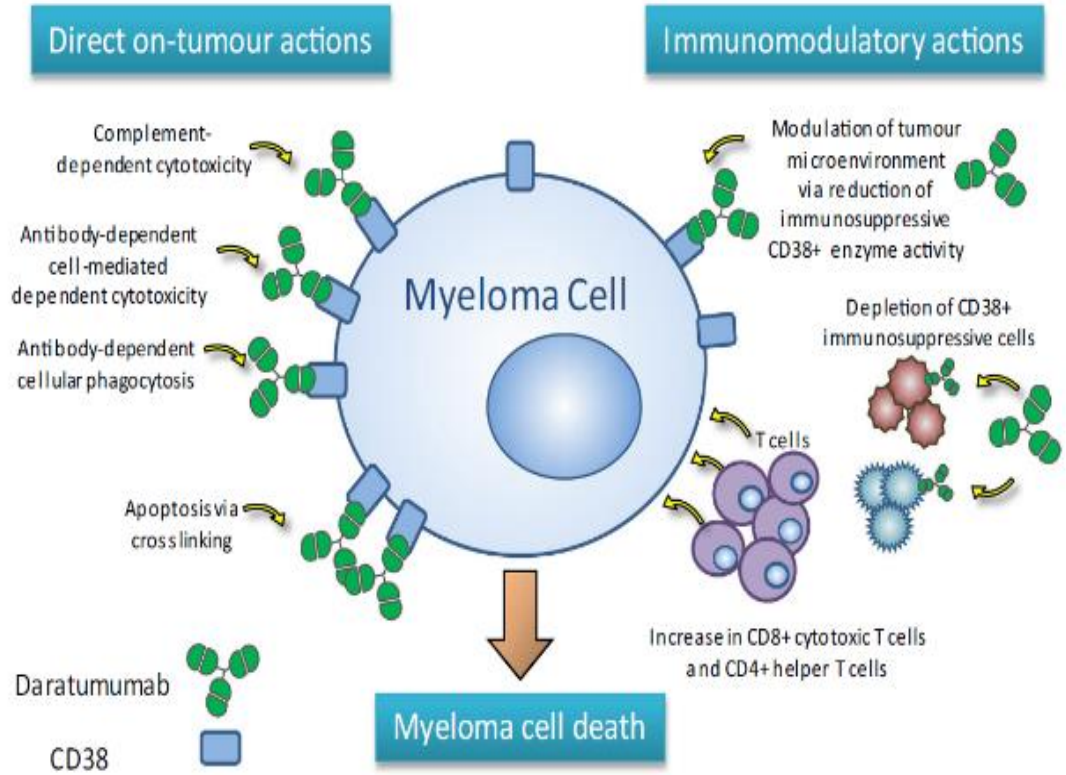
Potential immunomodulatory mechanism of action of DARA



Krejcik J et al. *Blood*, 2016.

DARA – Mechanisms of action

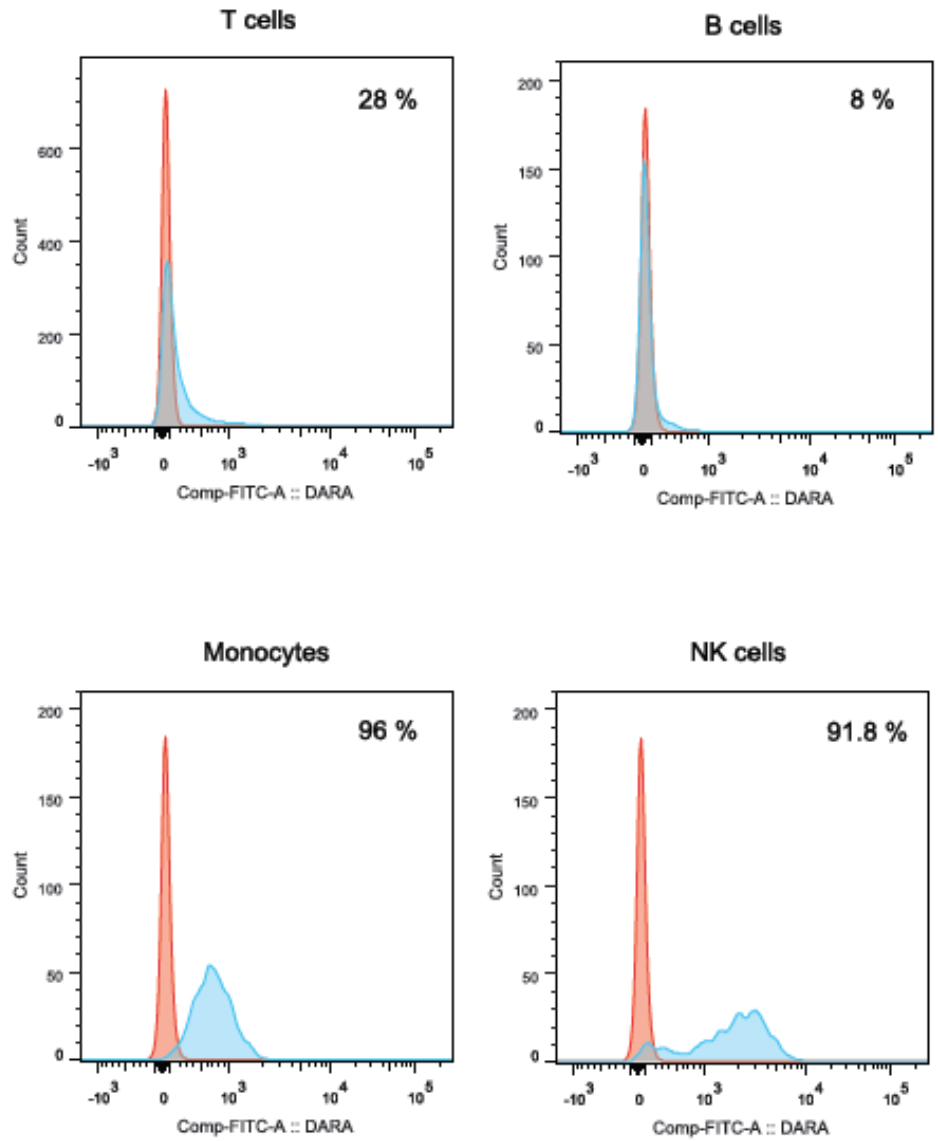
- **Direct on-tumour acvity** through CDC, ADCC, ADCP and direct apoptosis via cross-linking.
- **Immunomodulatory mechanisms**, through modulation of the tumor microenvironment.
- Depletion of immunosuppressive cell populations and increases in cytotoxic and helper T cells.



By combining direct on-tumor actions of traditional antibody therapy with systemic modulation of the immune system, daratumumab provides a multifaceted approach.

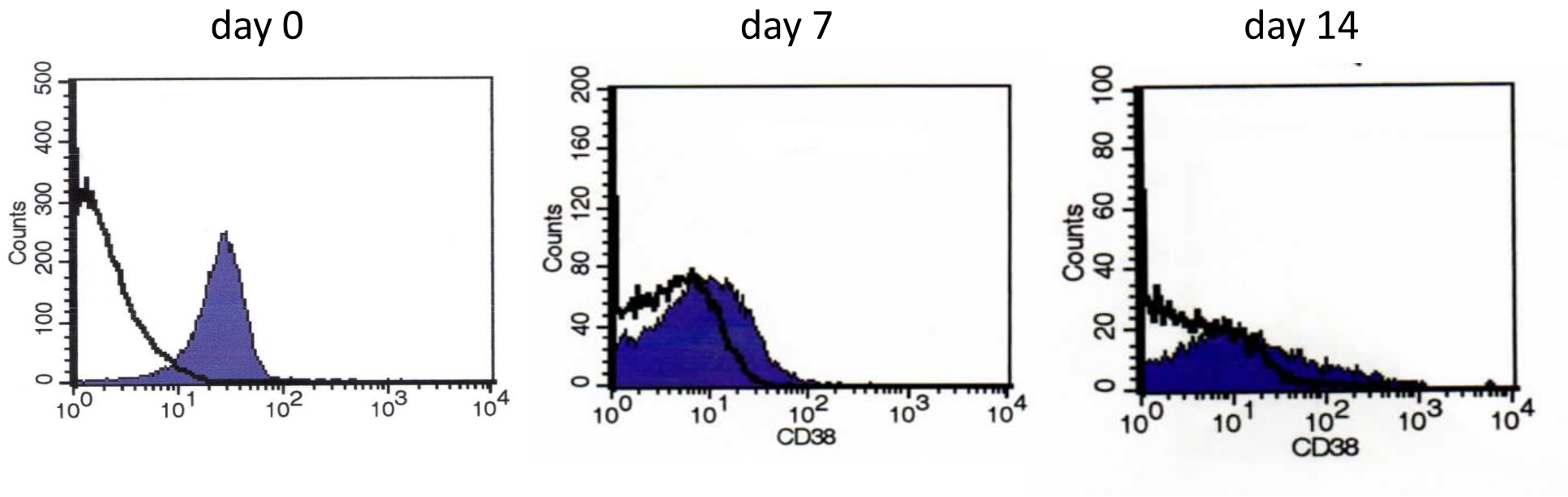
McKeage K & Lyseng-Williamson KA *Drugs Ther Perspect*, 2016.

Binding of DARA on different cell types



Toscani D et al. Poster presented at: 57th American Society of Hematology (ASH) 2015; Orlando, FL.

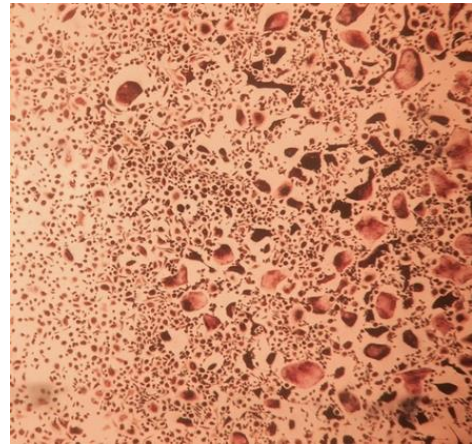
CD38 expression during *in vitro* osteoclastogenesis



From healthy donor purified monocytes

Toscani D et al. Poster presented at: 57th American Society of Hematology (ASH) 2015; Orlando, FL.

Dara effect on *in vitro* osteoclastogenesis from BM MNCs



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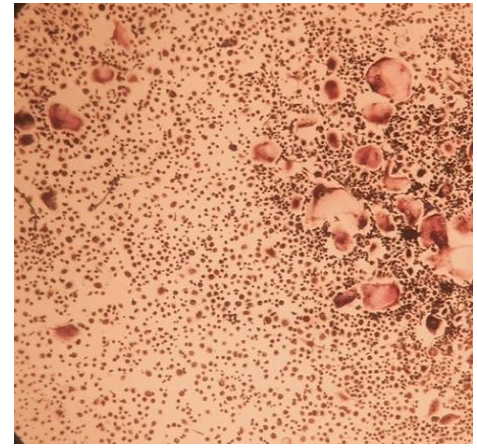
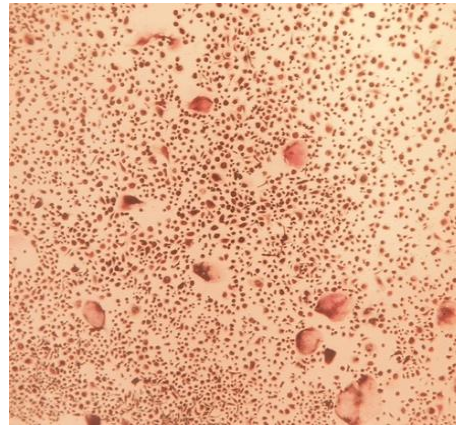
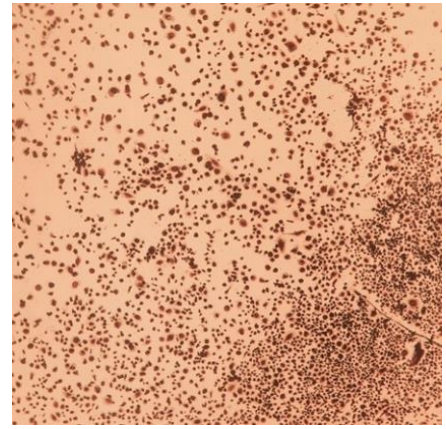


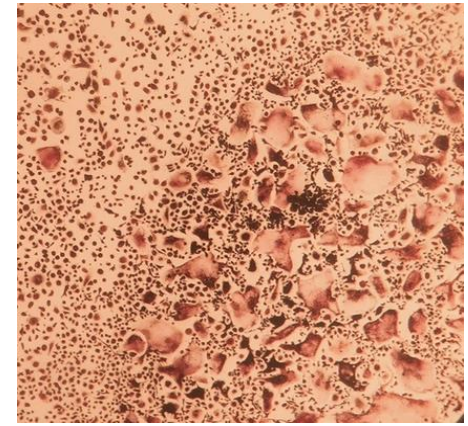
DARA 1ug/ml



DARA 10ug/ml



DARA 25ug/ml



IgG 25ug/ml

Toscani D et al. Poster presented at: 57th American Society of Hematology (ASH) 2015; Orlando, FL.

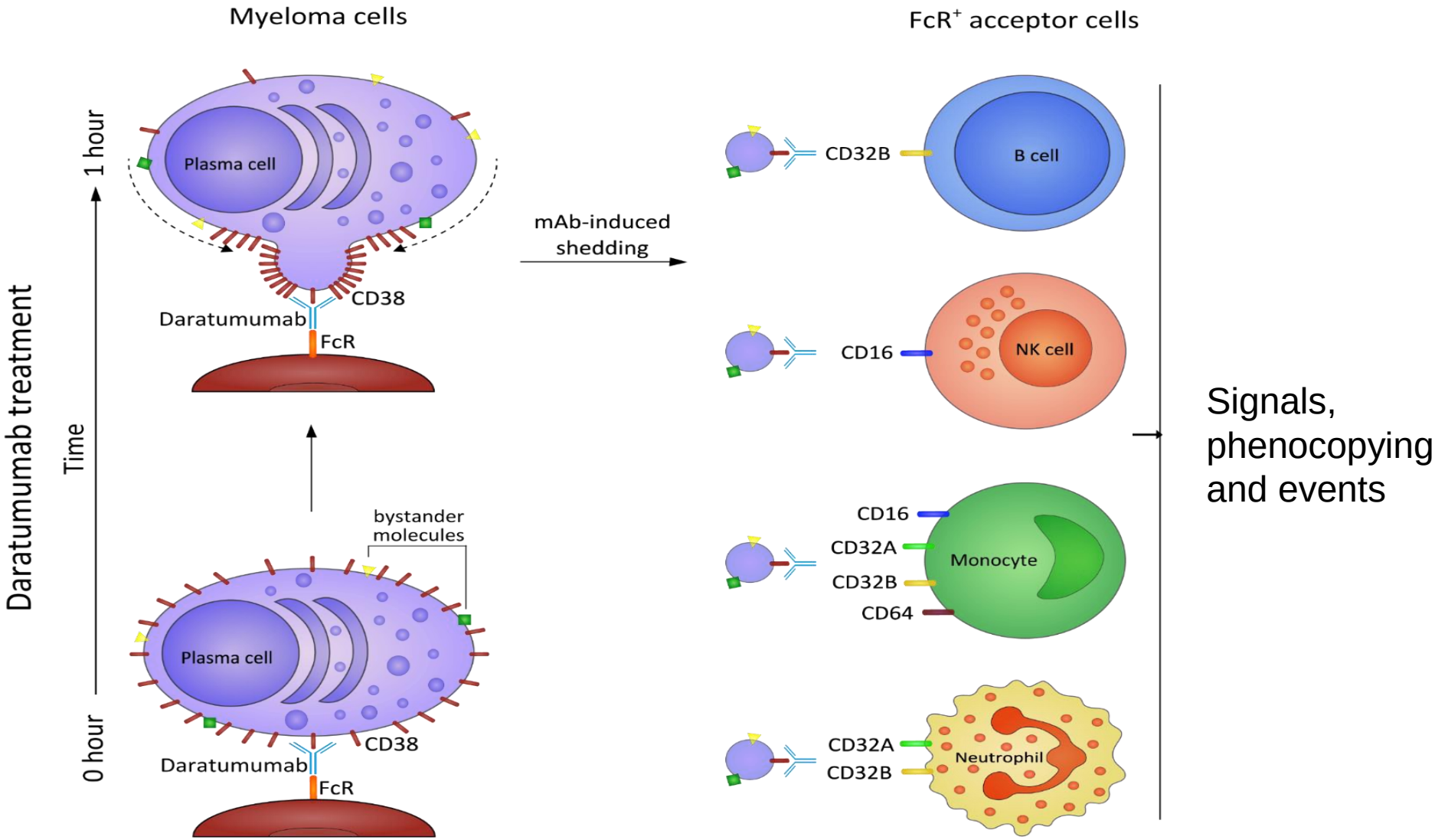
Anti-CD38 antibody-mediated therapy in MM: some unbeaten paths of potential application

- Do therapeutic anti-CD38 antibodies interfere with the enzymatic activities ruled by CD38?

- Do the products derived from the ectoenzymes operate outside the niche?

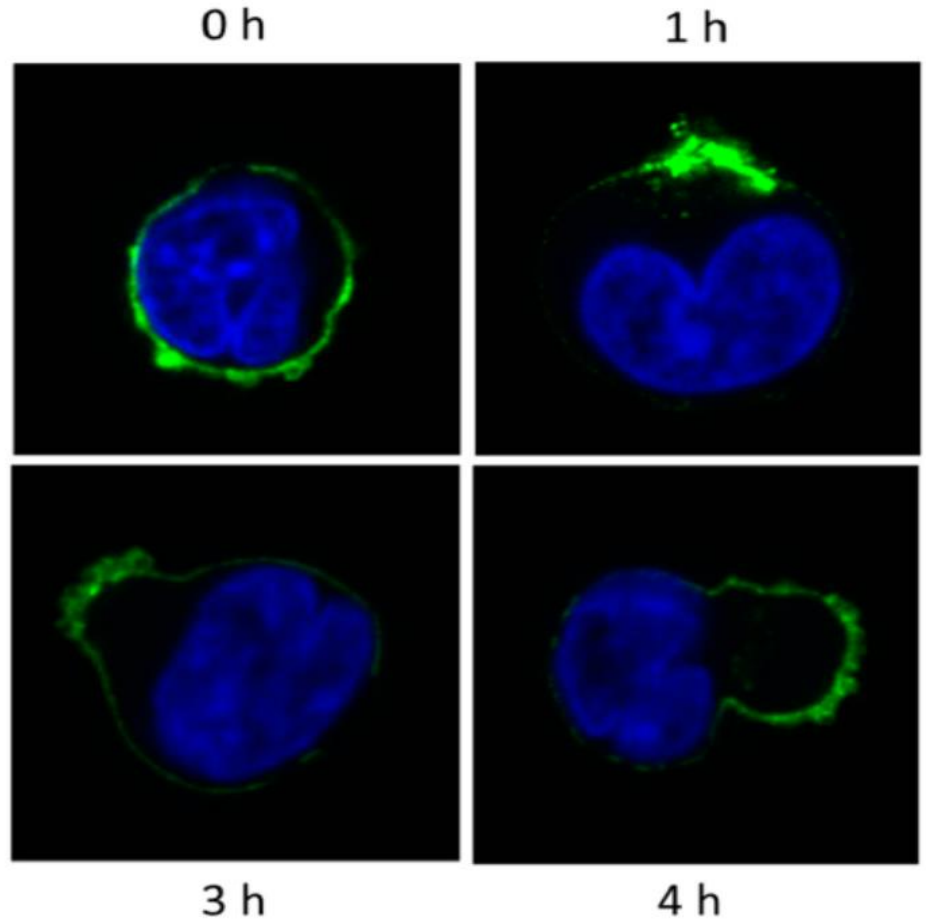
Malavasi F et al. ASH (2016) Abstract n. SCI-36; oral presentation.

In vivo events when a mAb reaches its MM target



Chillemi A et al. *Cells*, 2015.

DARA effect after CD38 ligation on MM cells

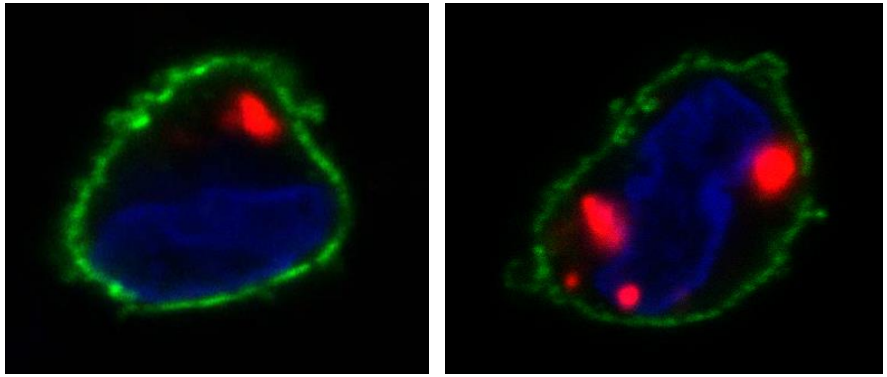


Confocal microscopy analysis of CD38/DARA interaction at 37°C on a human MM cell line

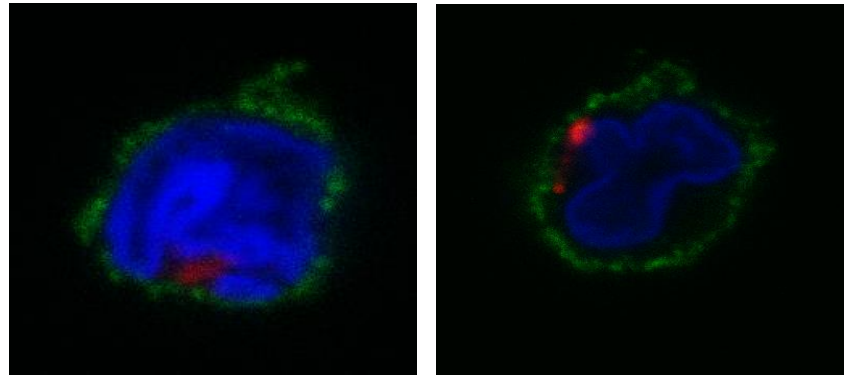
Malavasi F et al. ASH (2016) Abstract n. SCI-36; oral presentation.

DARA effect after CD38 ligation on effectors cells

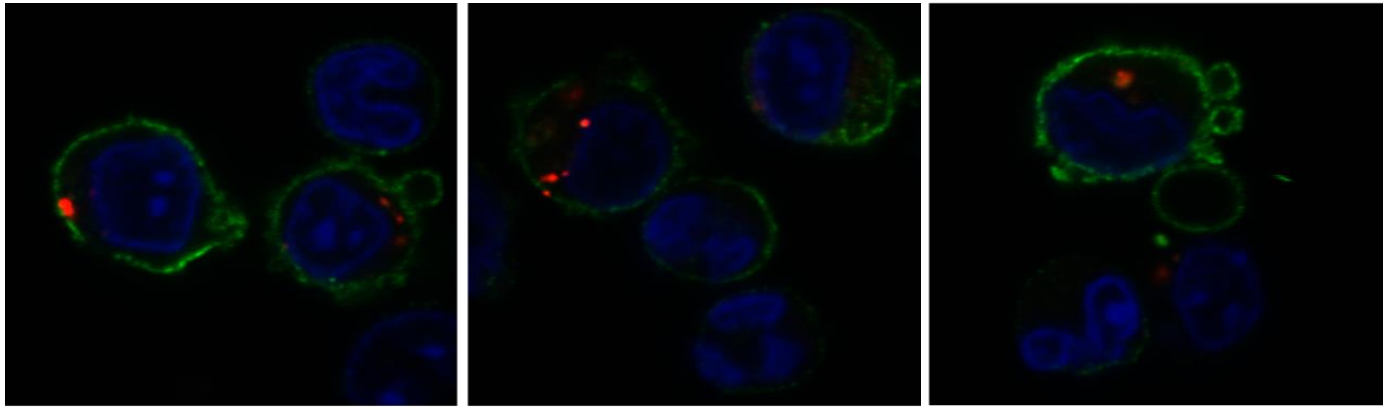
Whither MV from multiple myeloma:
Entering monocytes ($CD14^+$)



Whither MV from multiple myeloma:
Entering NK cells ($CD16^+$)

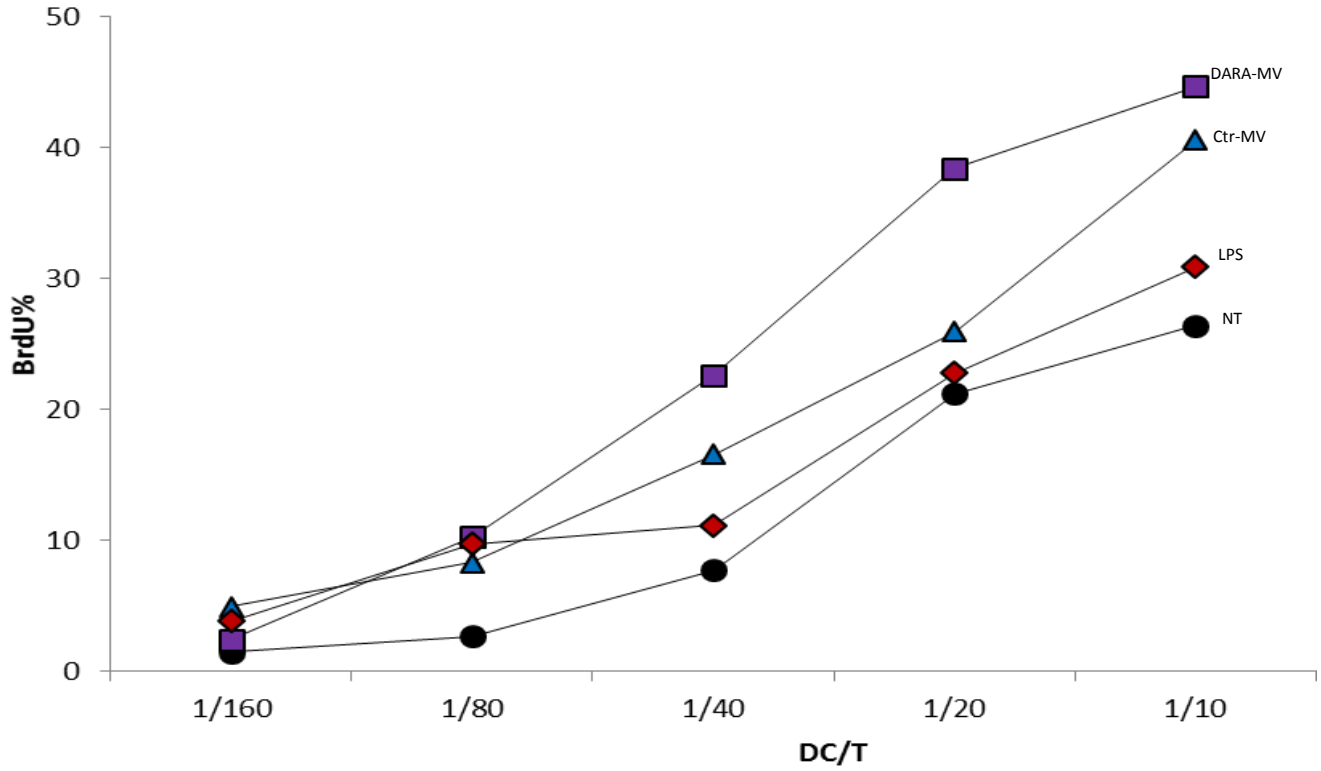


Whither MV from multiple myeloma:
Entering MDSC ($CD15^+/CD33^+/CD11b^+$)



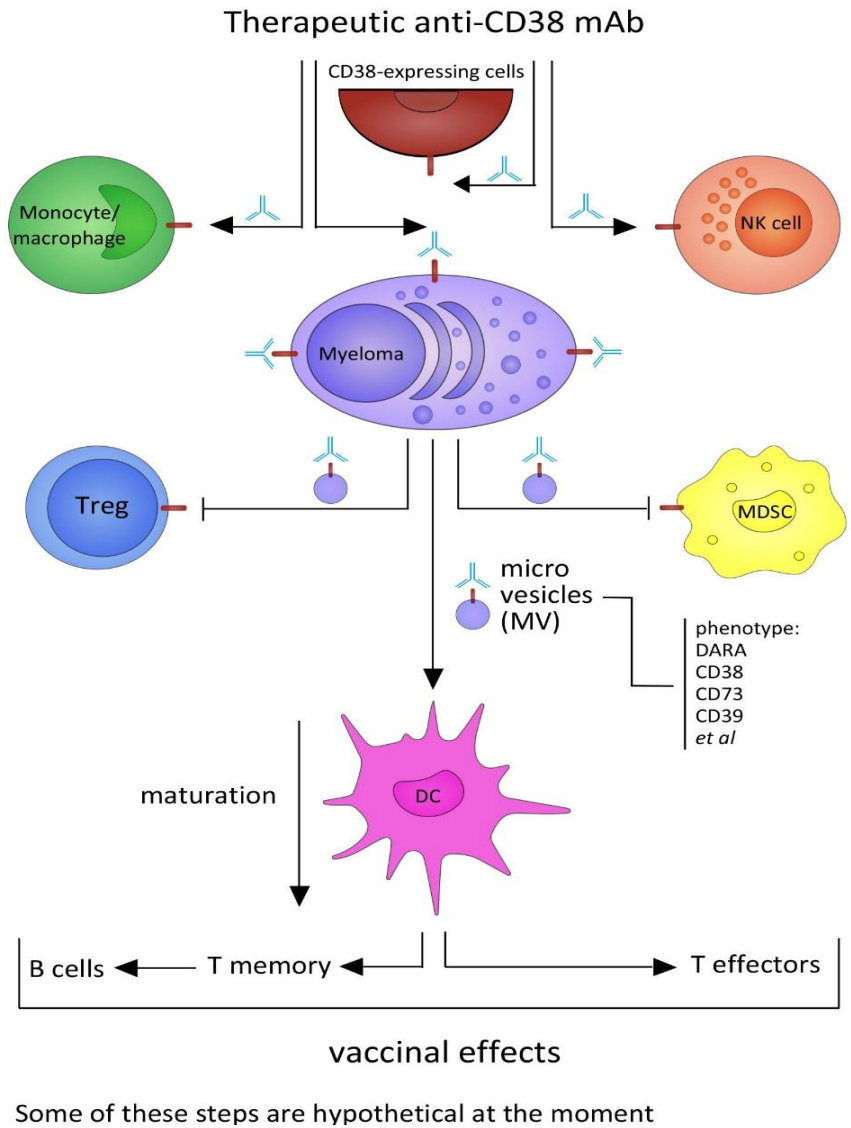
Malavasi F. et al ASH (2016) Abstract n. SCI-36; oral presentation.

APC activity of DCs treated with DARA-MV



Malavasi F. et al ASH (2016) Abstract n. SCI-36; oral presentation.

CD38 in the time of therapeutic mAbs



Malavasi F et al. ASH (2016) Abstract n. SCI-36; oral presentation.

DARA pharmacokinetics as intravenous infusion in Relapsed MM patients

Pharmacokinetics (PK) data were available from 100 patients in GEN501 and 123 patients in SIRIUS

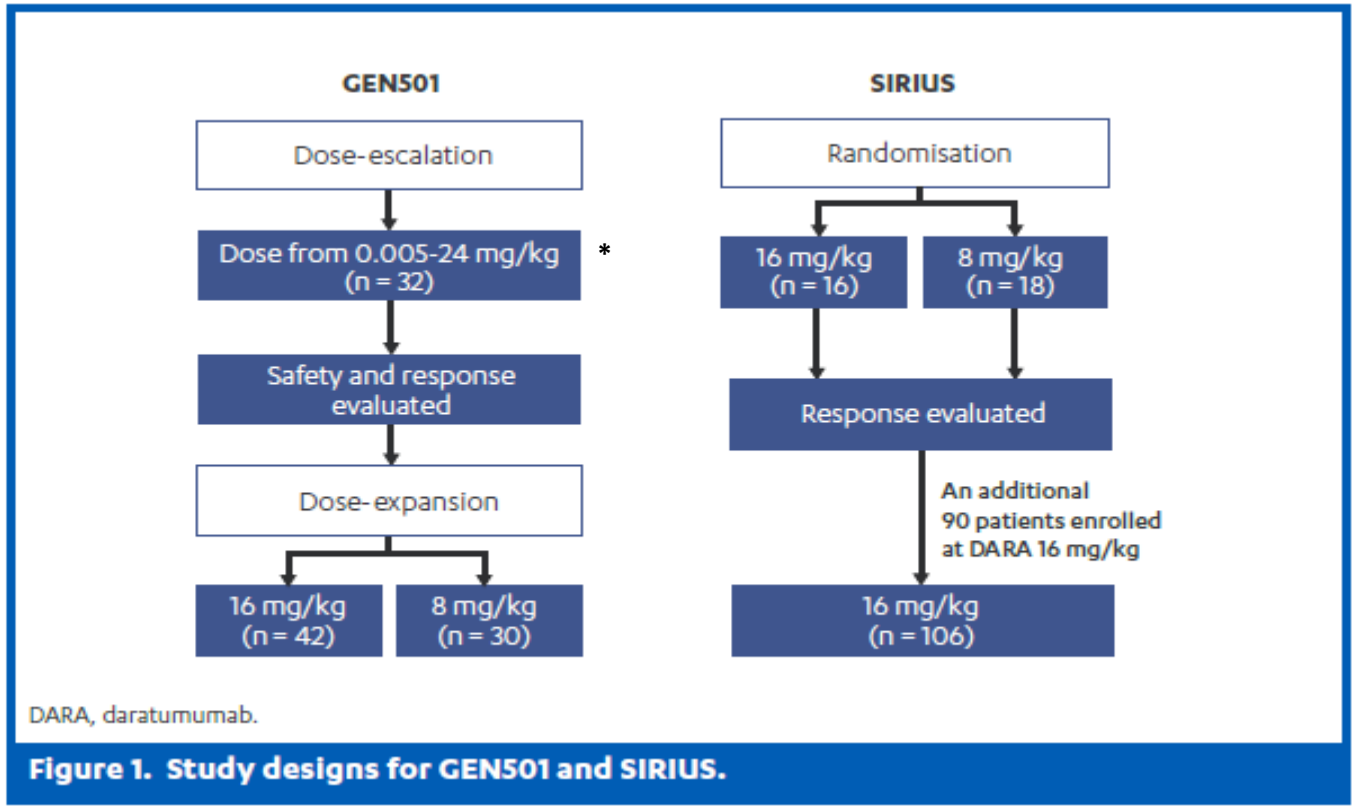
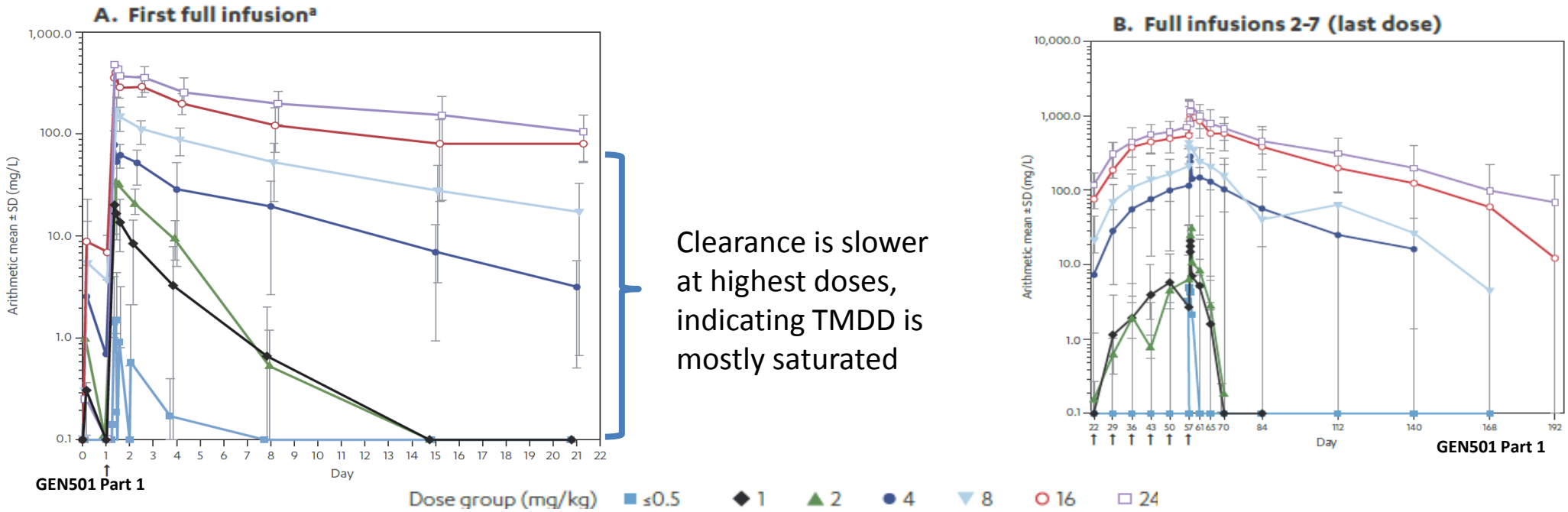


Figure 1. Study designs for GEN501 and SIRIUS.

*All concentrations for patients receiving DARA 0.005 mg/kg and 0.05 mg/kg were below the limit of quantification).

Clemens M et al., IMW 2015.

Mean serum DARA concentration after first full infusion

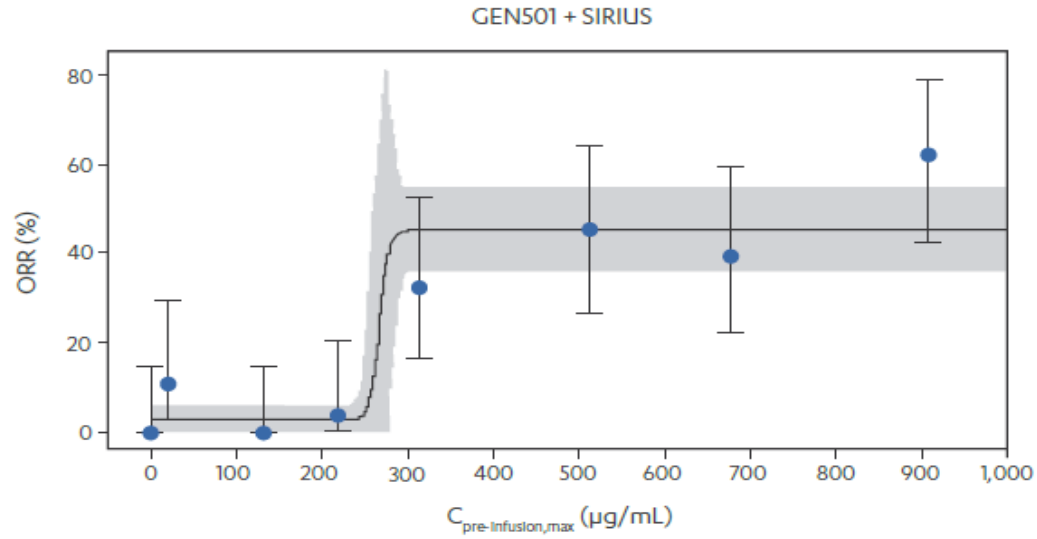


Clearance is slower at highest doses, indicating TMDD is mostly saturated

- Following the first full infusion, the maximum concentration (Cmax) was approximately dose proportional for doses of 1 mg/kg to 24 mg/kg and increased in a greater than dose-proportional manner after the last (7th) dose

Clemens M et al., IMW 2015.

Exposure-efficacy relationship



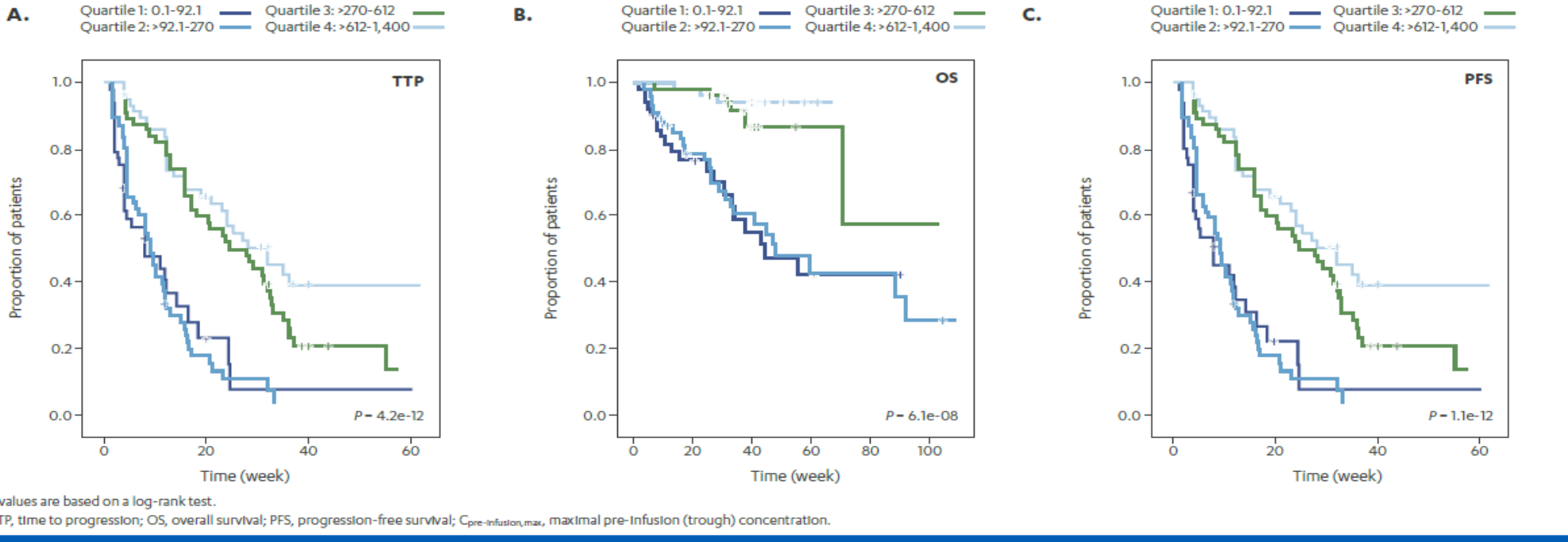
ORR, overall response rate; $C_{pre\text{-}Infusion,max}$, maximal pre-infusion (trough) concentration; CI, confidence interval. Solid blue dots represent the proportion of responders grouped by 8 quantiles of $C_{pre\text{-}Infusion,max}$ and plotted at the geometric mean for each group. The bars represent the 95% CI for the proportion in each group. Centered curves and shaded areas represent predicted values and the 95% CI of model-predicted response rate, respectively.

Figure 2. Logistic regression analysis of ORR and predicted $C_{pre\text{-}infusion,max}$ using an E_{max} model.

- 90% maximal effect on ORR (EC*ORR90*) was achieved when Cpre-infusion, max was equal to 274 µg/mL, which is expected to provide a target occupancy higher than 99%
- Predicted and observed Pk data suggest that approximately 80% of patients who completed ≥8 infusions at the 16-mg/kg dose had Cpre-infusion,max above the estimated EC*ORR*

Xu XS et al., IMW 2015.

Clinical endpoints and efficacy are correlated with DARA exposure



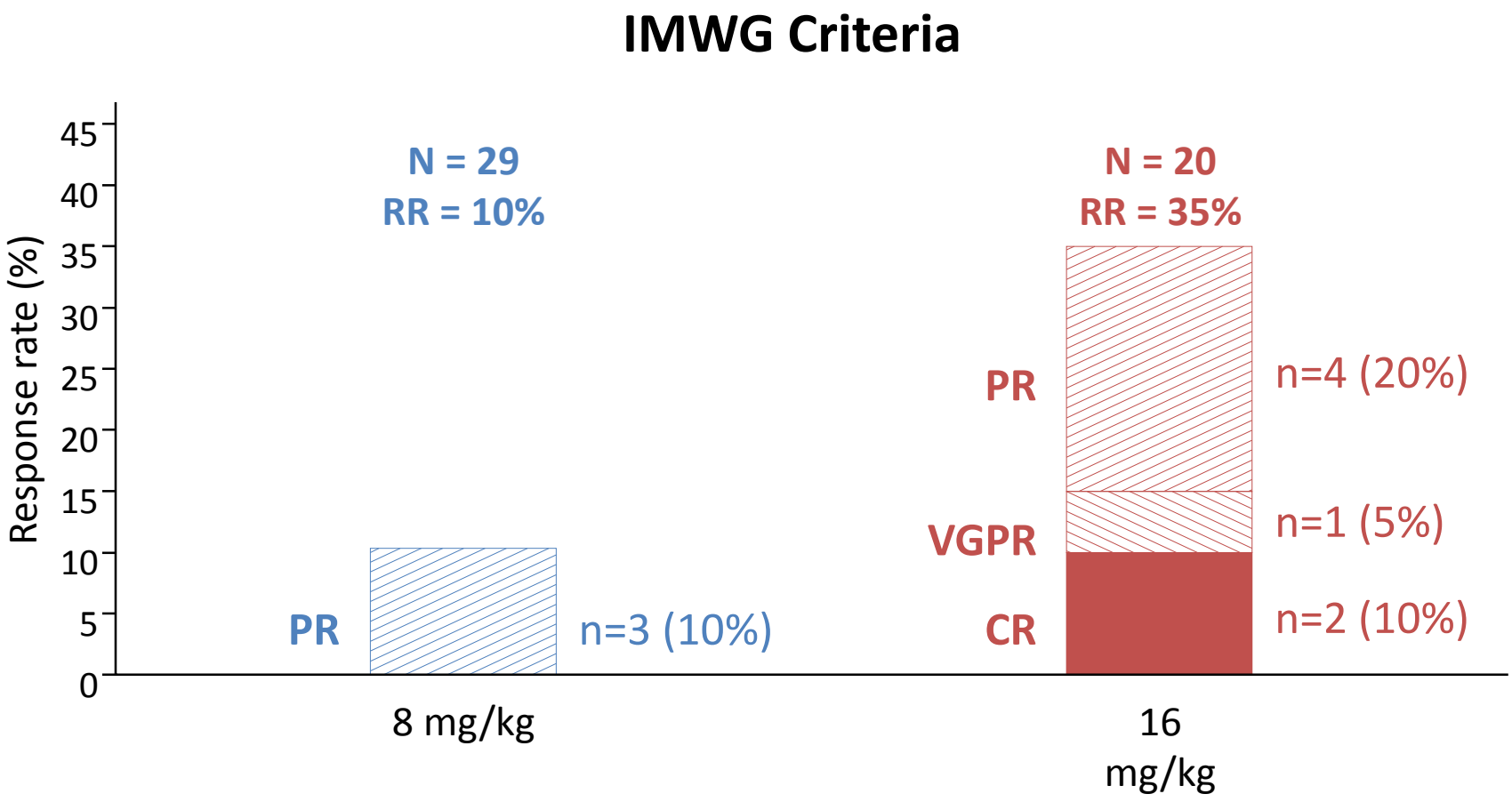
P values are based on a log-rank test.
TTP, time to progression; OS, overall survival; PFS, progression-free survival; $C_{pre\text{-}infusion,max}$, maximal pre-infusion (trough) concentration.

Figure 4. Kaplan-Meier curves of (A) TTP, (B) OS, and (C) PFS for the quartiles of predicted $C_{pre\text{-}infusion,max}$.

- Most analysed efficacy endpoints (i.e. ORR, TTP, OS, PFS, and MRP) were significantly correlated with DARA exposures
- These data are consistent with the clinical data in which no dose-related safety signal was observed

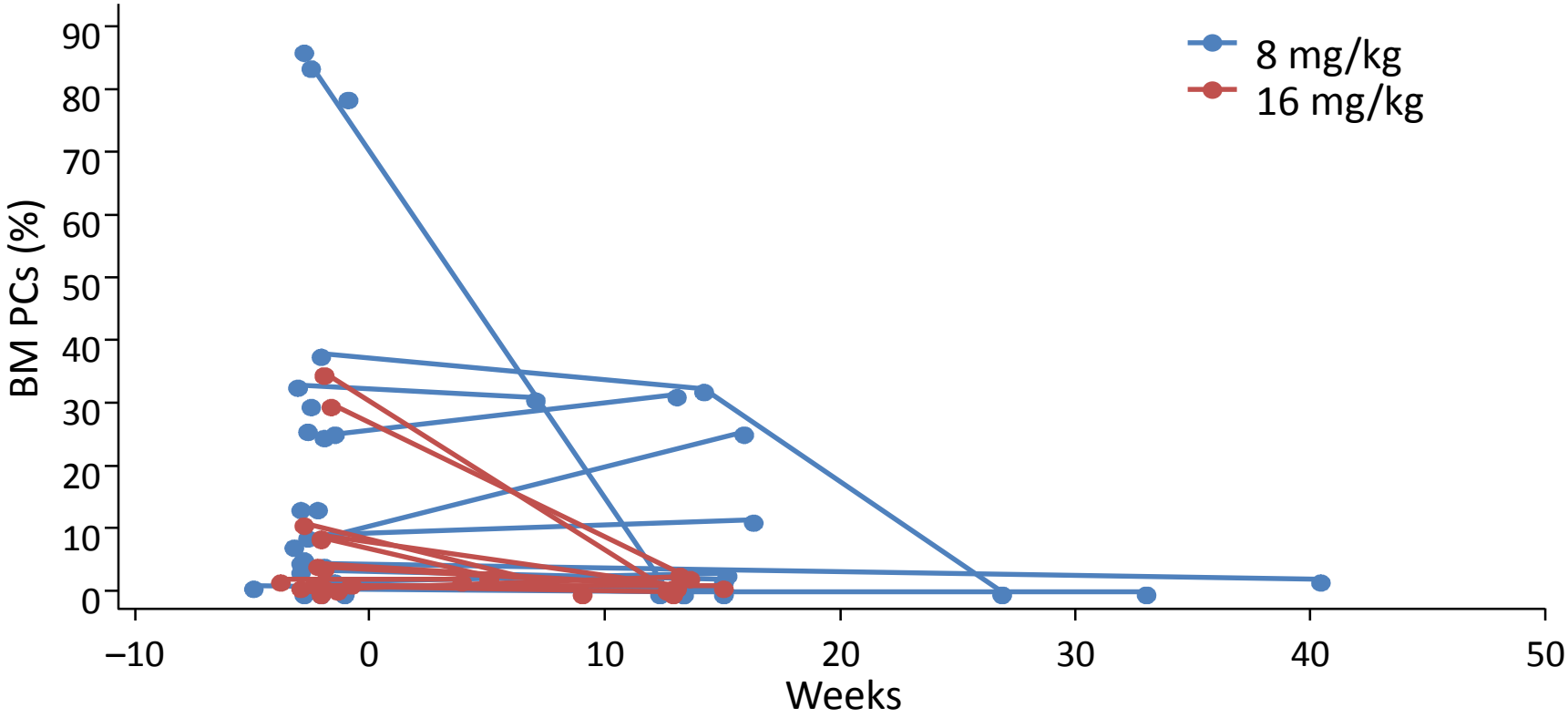
Xu XS et al., IMW 2015.

GEN501: Response



Lokhorst HM et al., ASCO 2014.
Lokhorst HM et al. *NEJM,* 2015.

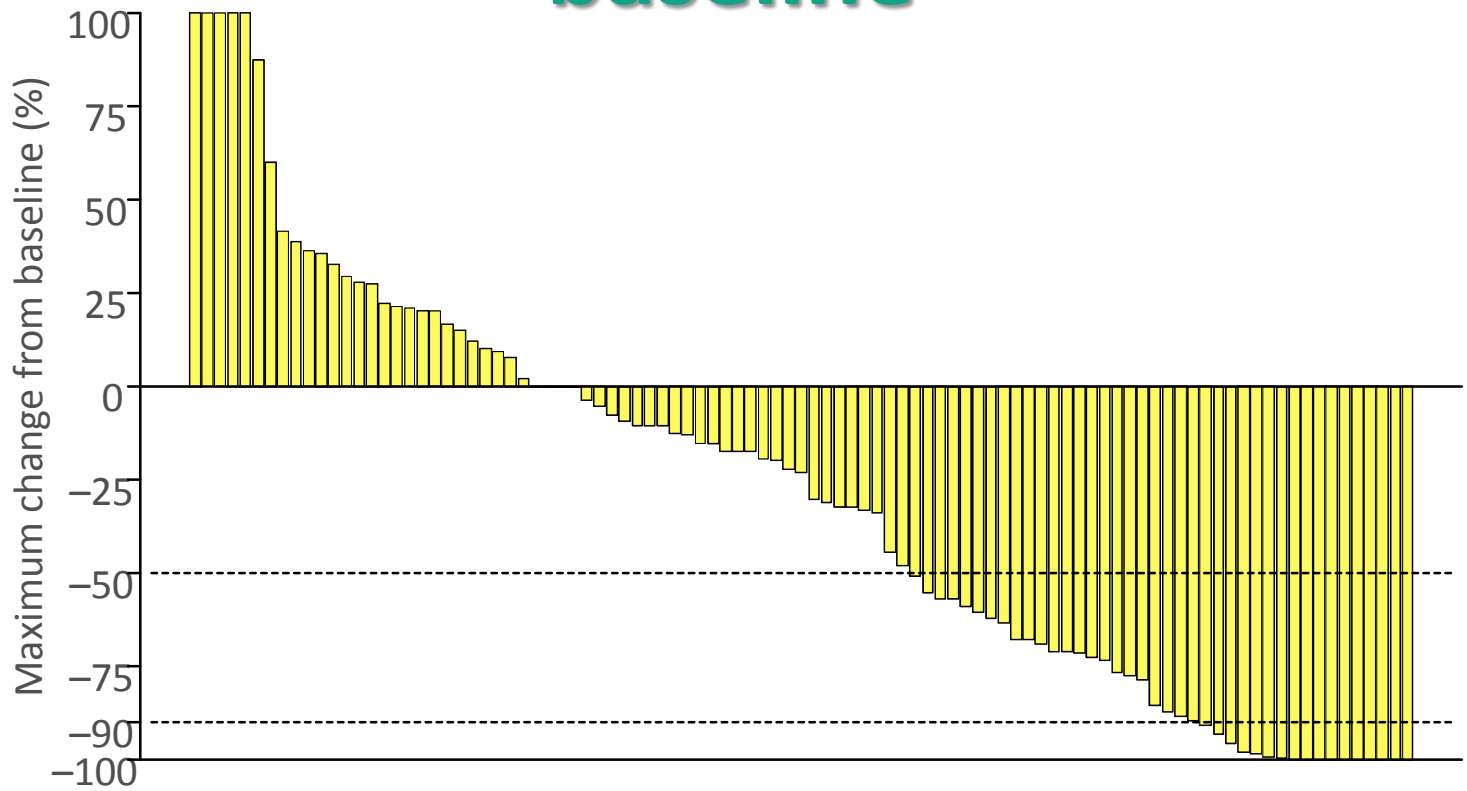
GEN501: Reduction in bone marrow plasma cells



All patients who achieved a PR or better at 16 mg/kg and who had bone marrow involvement cleared bone marrow plasma cells after DARA treatment (4/4 patients)

Lokhorst HM et al., ASCO 2014.
Lokhorst HM et al. *NEJM,* 2015.

SIRIUS study: change in paraprotein from baseline

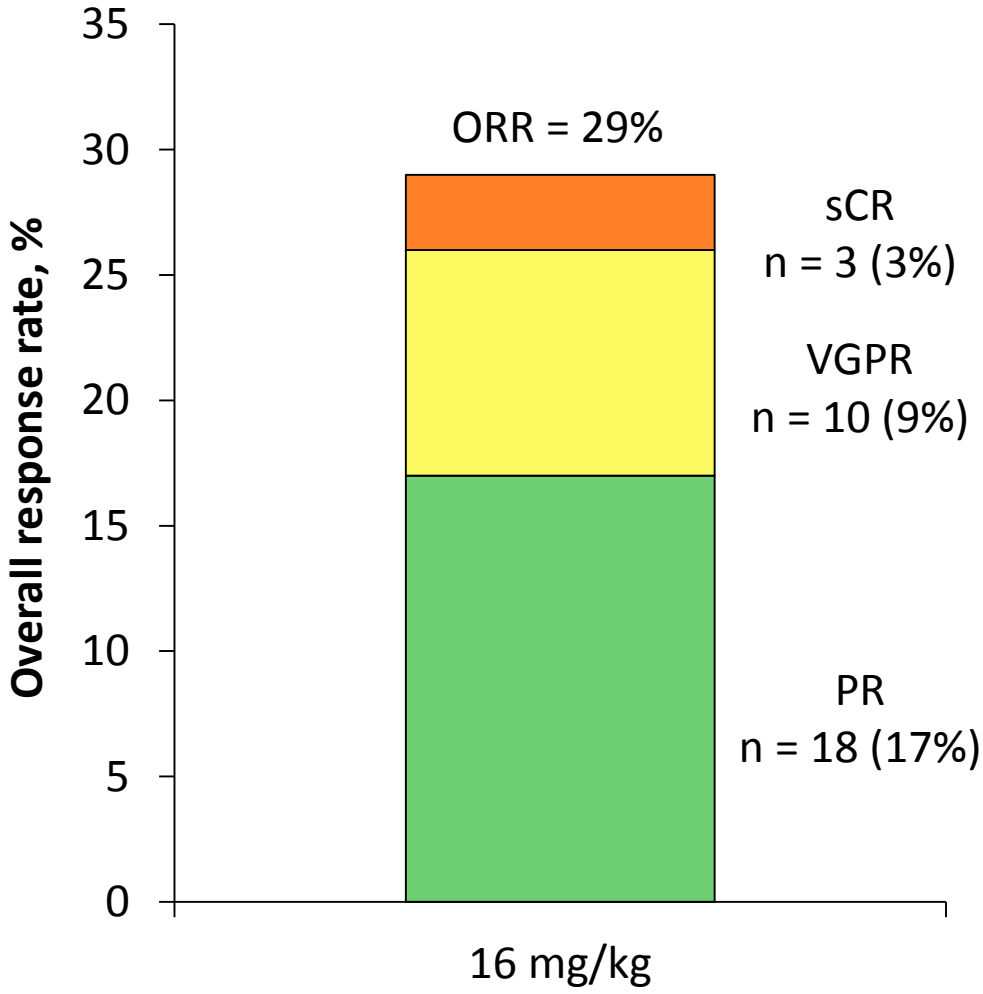


The majority of patients had reductions in paraprotein from baseline

- 40 patients (38%) had reductions >50%
- 17 patients (16%) had reductions >90%

Lonial S et al., ASCO 2015.

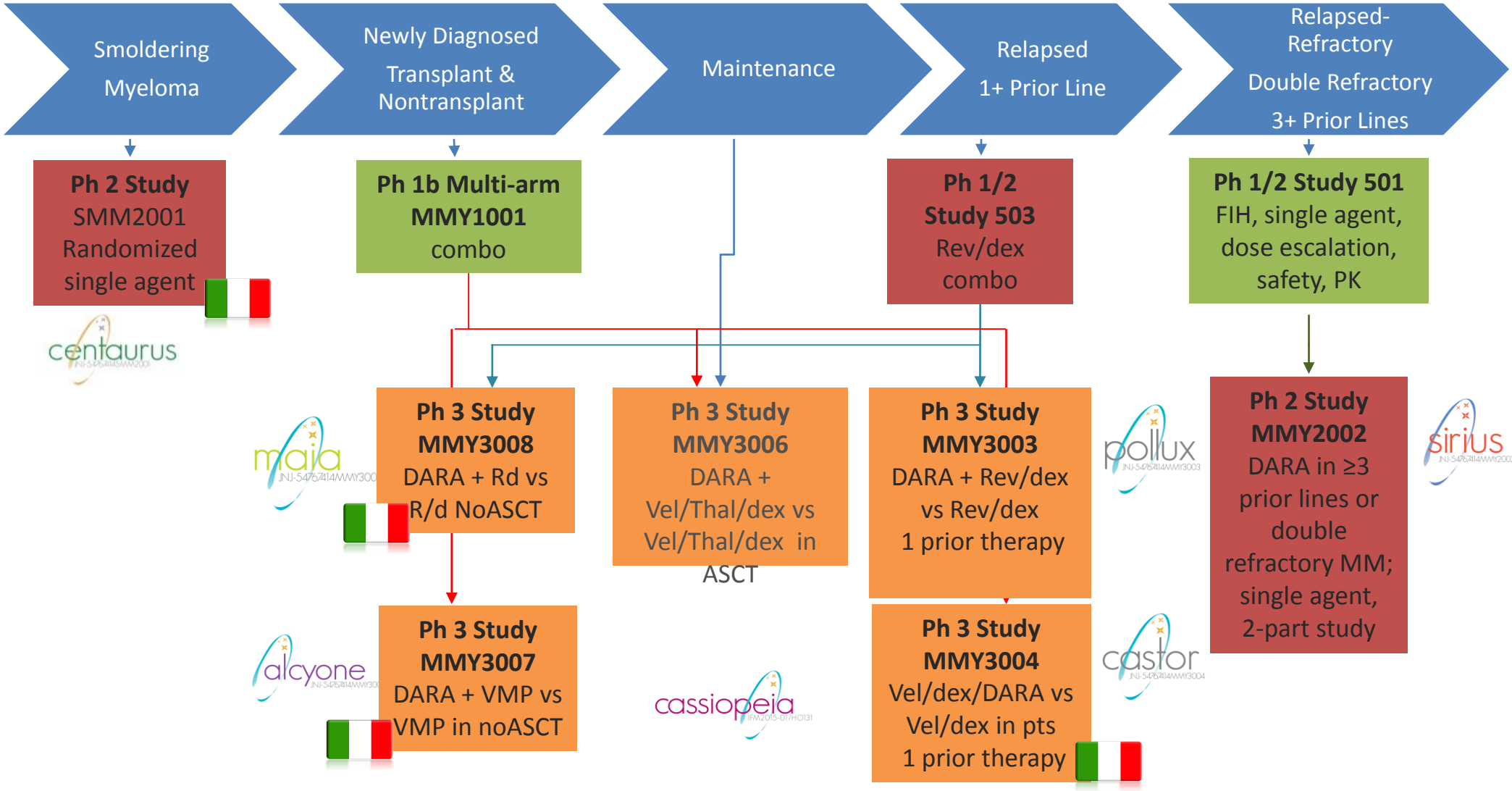
SIRIUS: Overall Response Rate



- **ORR was 29% (95% CI, 21–39) in patients receiving 16 mg/kg DARA**
- Stringent complete response (sCR) in 3% of patients (95% CI, 0.6–8.0)
- VGPR or better achieved in 12% (95% CI, 7–20) of patients
- Clinical benefit rate (ORR + MR) was 34% (95% CI, 25–44)

Lonial S et al., ASCO 2015.

DARA development in all MM settings



SLAMF7/CS1: expression profile on hemopoietic cells

- Cell surface glycoprotein receptor
- SLAM (Signaling Lymphocyte Activating Molecule) family:

  SLAM/CD150

  2B4

  CD84

  NTB-A

  Ly-9

Cell type	CS-1 expression
Non-hematopoietic cell	-
Activated monocytes	+
Immature dendritic cells	-
Mature dendritic cells	+
NK cells, NK-T cells	+
$CD8^+$ T lymphocytes	+
Activated B lymphocytes	+
Normal plasma cells	+
MM plasma cells	**++**

Veillette A et al. *Crit Rev Oncol Hematol,* 2013.

SLAMF7/CS1: an atypical SLAM family member

SLAM family receptors.

Receptor	Alternative name	Physiological ligand	Number of ITSMs	Expression pattern	Interaction with		Phenotypes knock-out mice
					SAP	EAT-2	
SLAM	CD150 SLAMF1	SLAM	2	T, B, DC, Mϕ, plat	+	+	T, Mϕ, plat, NK-T
Ly-9	CD229 SLAMF3	Ly-9	1	T, B, NK, DC, Mϕ	+	+	$CD4^+$ T, innate-like $CD8^+$ T, NK-T
2B4	CD244 SLAMF4	CD48	3	NK, $CD8^+$ T, DC, Mϕ, eos	+	+	NK
CD84	SLAMF5	CD84	2	T, B, NK, DC, Mϕ, gran, plat, mast, eos	+	+	T, B (GC)
NTB-A	Ly108 CD352 SLAMF6	NTB-A	2	T, B, NK, DC, neutro	+	+	T, B, neutro, NK-T
CS1	CRACC CD319 SLAMF7	CS1	1	Human: NK, NK-T, DC, B, PC, T Mouse: NK, NK-T, DC, Mϕ, B, T	−	+	NK

Veillette A et al. *Crit Rev Oncol Hematol,* 2013.

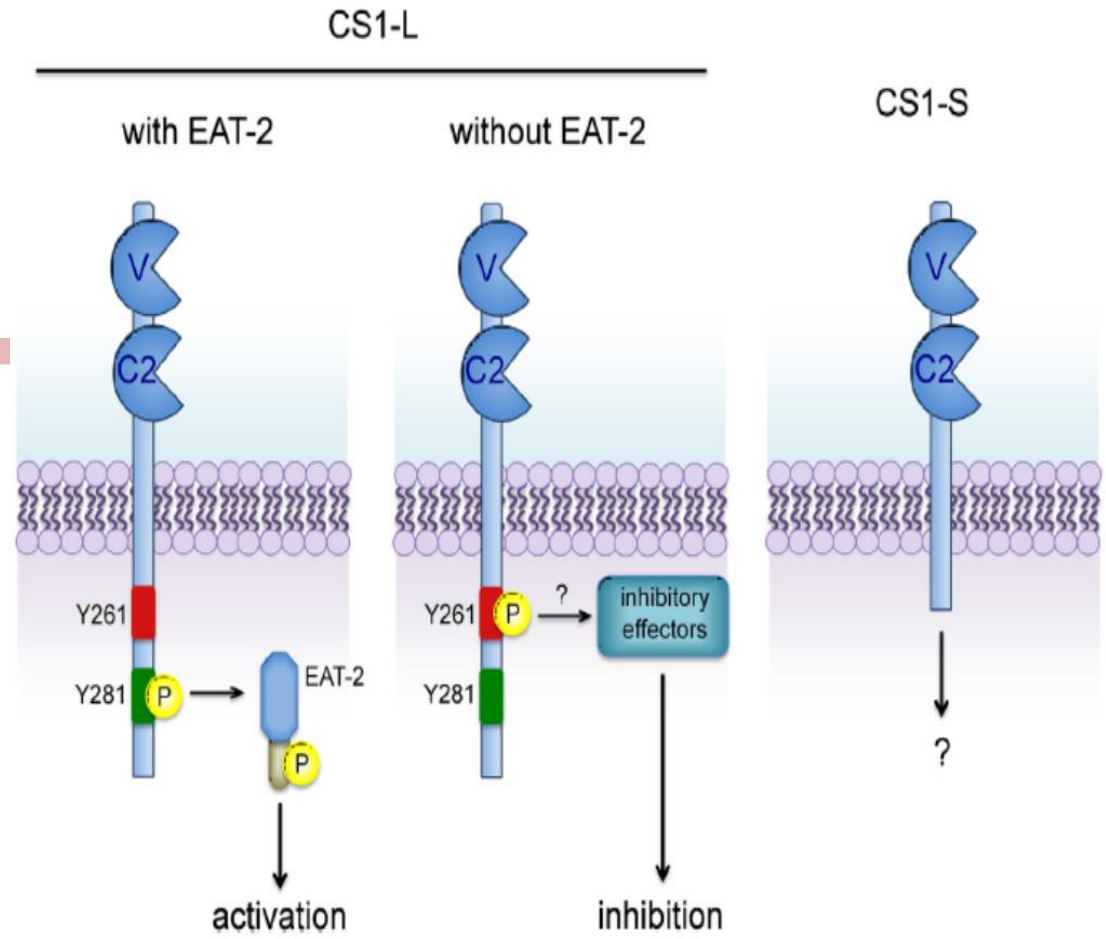
SLAMF7/CS1: functions

Cell type	EAT-2 expression	SLAMF7 function
Activated Monocytes	-	Decrease of proinflammatory cytokine secretion
NK cells	+	Increase of IFNγ production, cytotoxic activity
B cells	-	Proliferation and cytokine production
T cells	-	Inhibition of antigen-induced T cell proliferation and cytokine production
NK-T cells		unknown
Dendritic cells	+	unknown
MM plasma cells	-	Adhesion to BMSC

Veillette A et al. *Crit Rev Oncol Hematol,* 2013; Cruz-Munoz ME et al *Nat Immunol,* 2009.

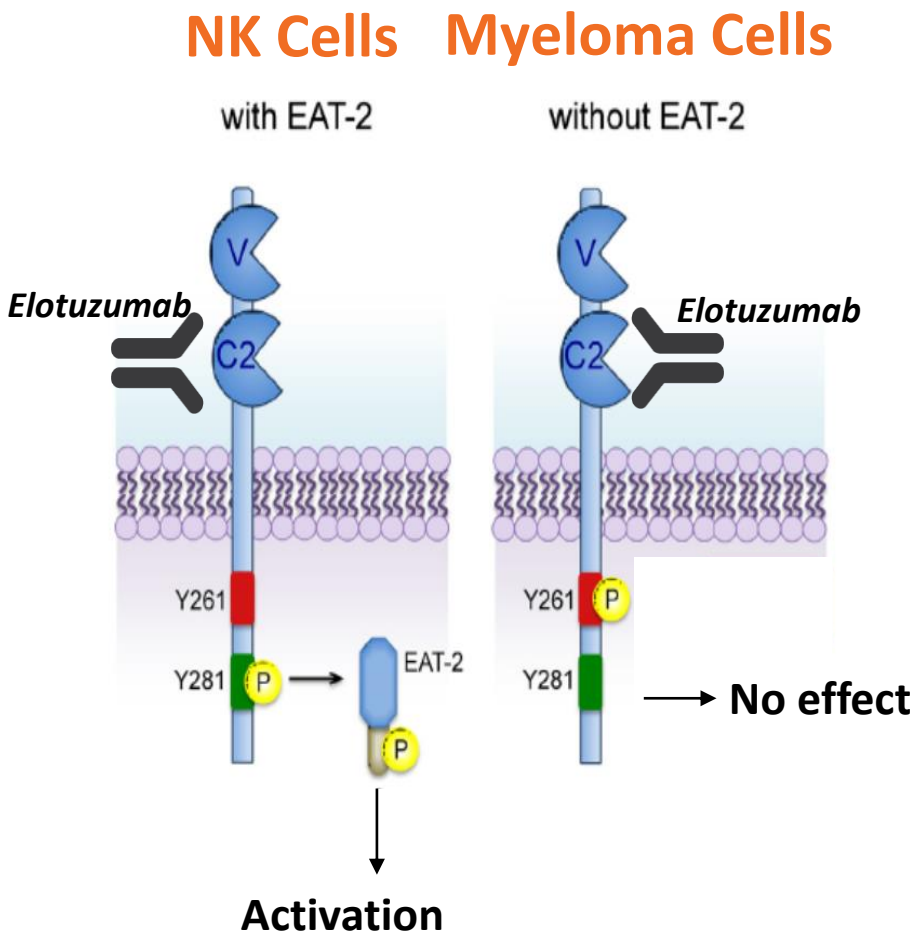
SLAMF7/CS1: structure and function interplay

mediates self-adhesion



Adapted from: Veillette A et al. *Crit Rev Oncol Hematol,* 2013.

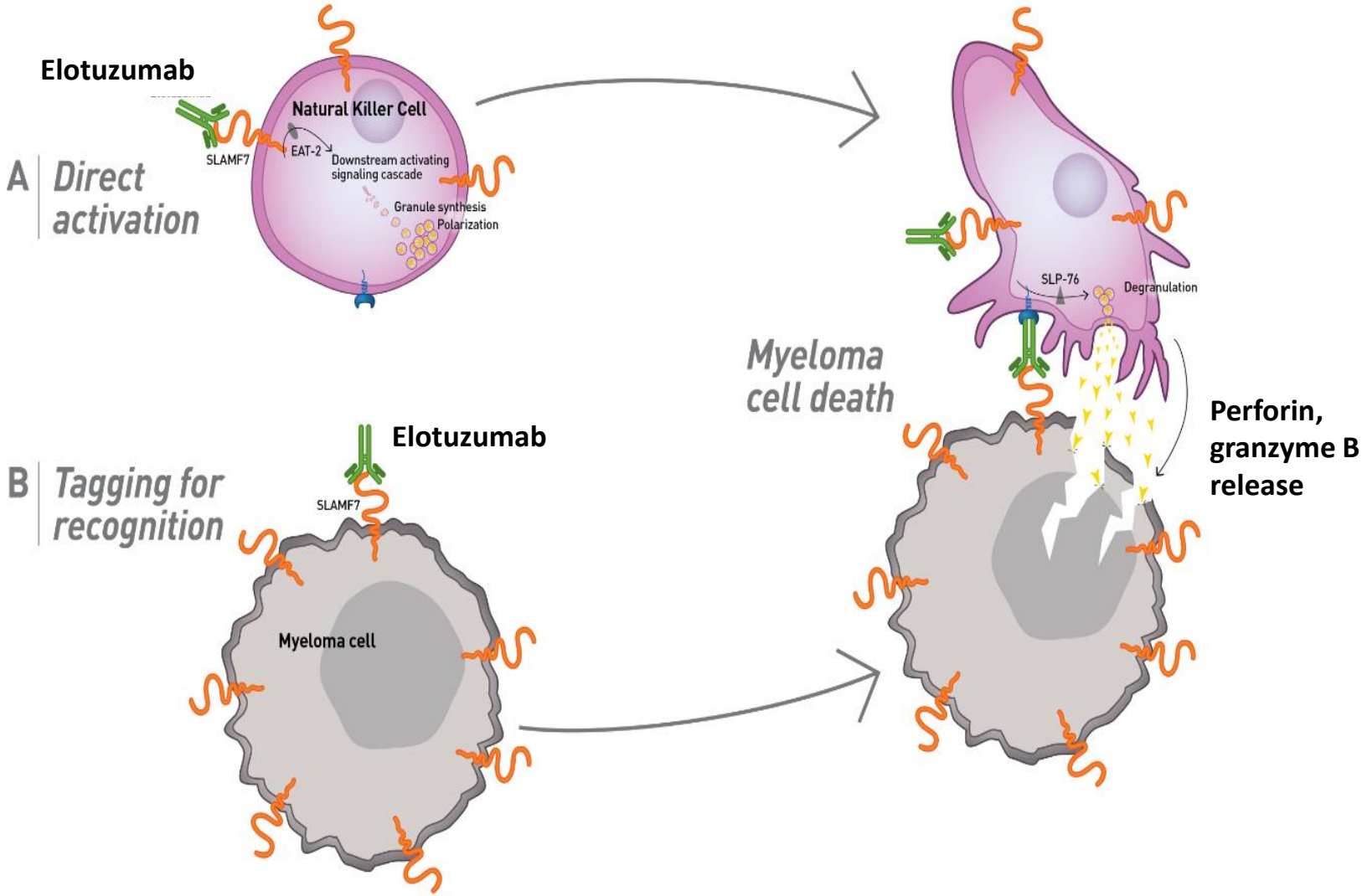
Elotuzumab: a monoclonal antibody targeting SLAMF7



- Humanized, IgG1 mab specific for human SLAMF7
  - —No cross-reactivity with non-human homologues or other SLAM family members
- Binds to a membrane-proximal motif of SLAMF7
  - —Critical for mediating killing of target cells (*in vitro*)

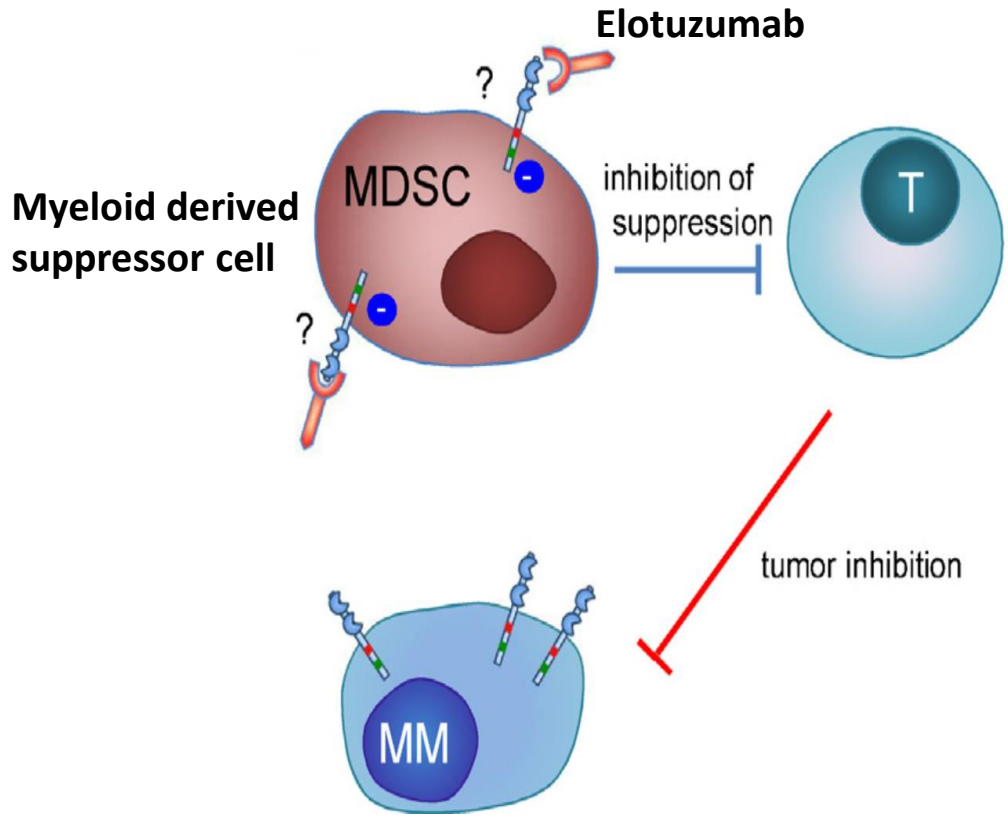
Veillette A et al. *Crit Rev Oncol Hematol,* 2013; Cruz-Munoz ME et al. *Nat Immunol* 2009*;* Guo H et al. *Mol Cell Bio, 2015*.

Elotuzumab: mechanisms of action in MM (I)



Veillette A et al. *Crit Rev Oncol Hematol,* 2013; Cruz-Munoz ME et al. *Nat Immunol,* 2009.

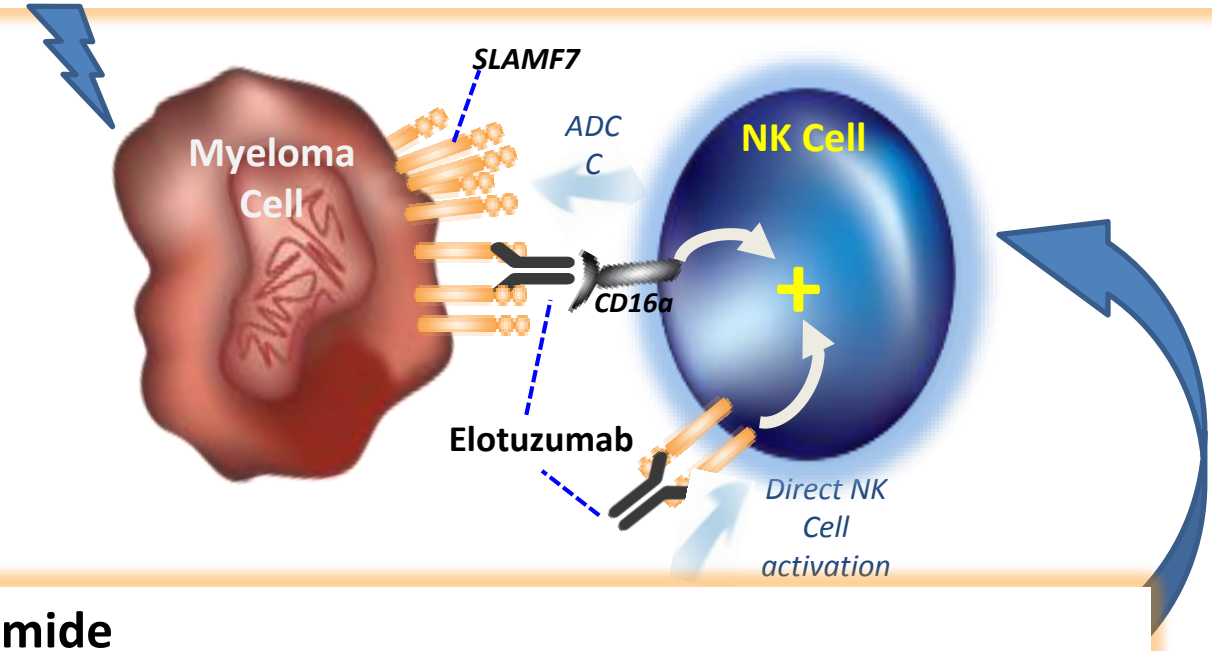
Elotuzumab: potential alternative mechanism of action in MM



Veillette A et al. *Crit Rev Oncol Hematol,* 2013.

Elotuzumab synergizes with Lenalidomide to enhance MM cell death

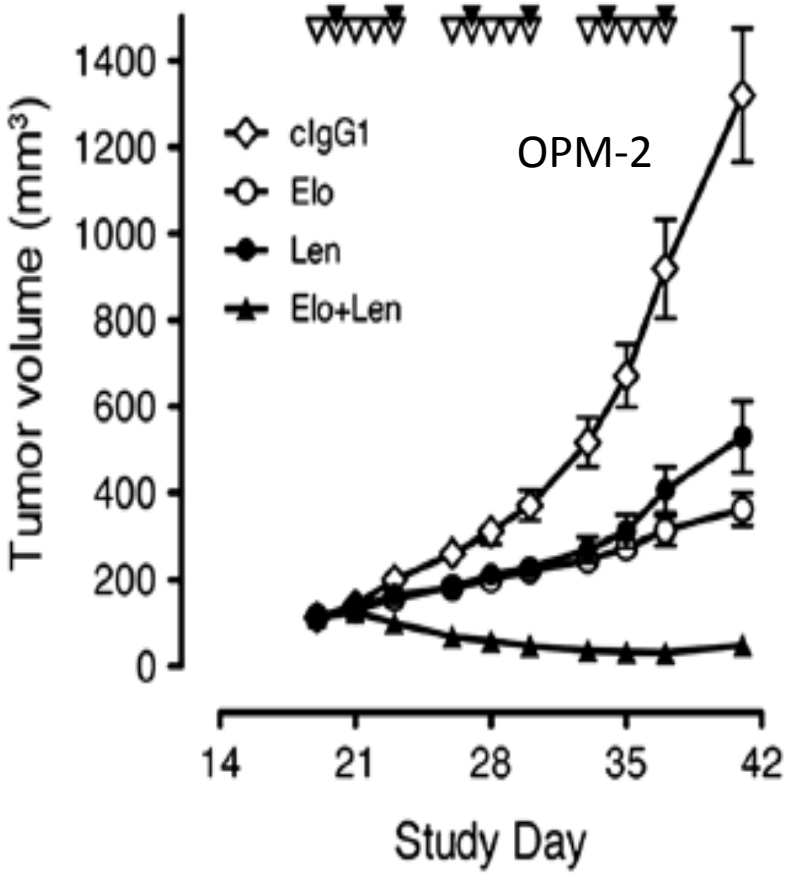
Lenalidomide
Induces myeloma cell injury and lowers threshold for NK cell-mediated killing of myeloma cells by Elotuzumab

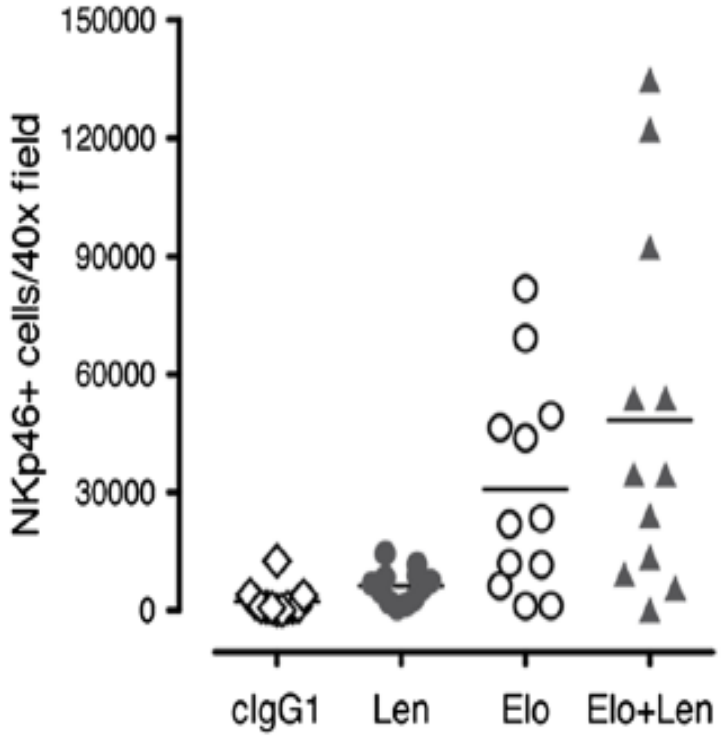


Lenalidomide
Enhances adaptive and innate immune system including production of IL2 to increase NK cell activity

Balasa B et al. *Cancer Immunol Immunother,* 2015.

Elotuzumab plus Lenalidomide: *in vivo* effects



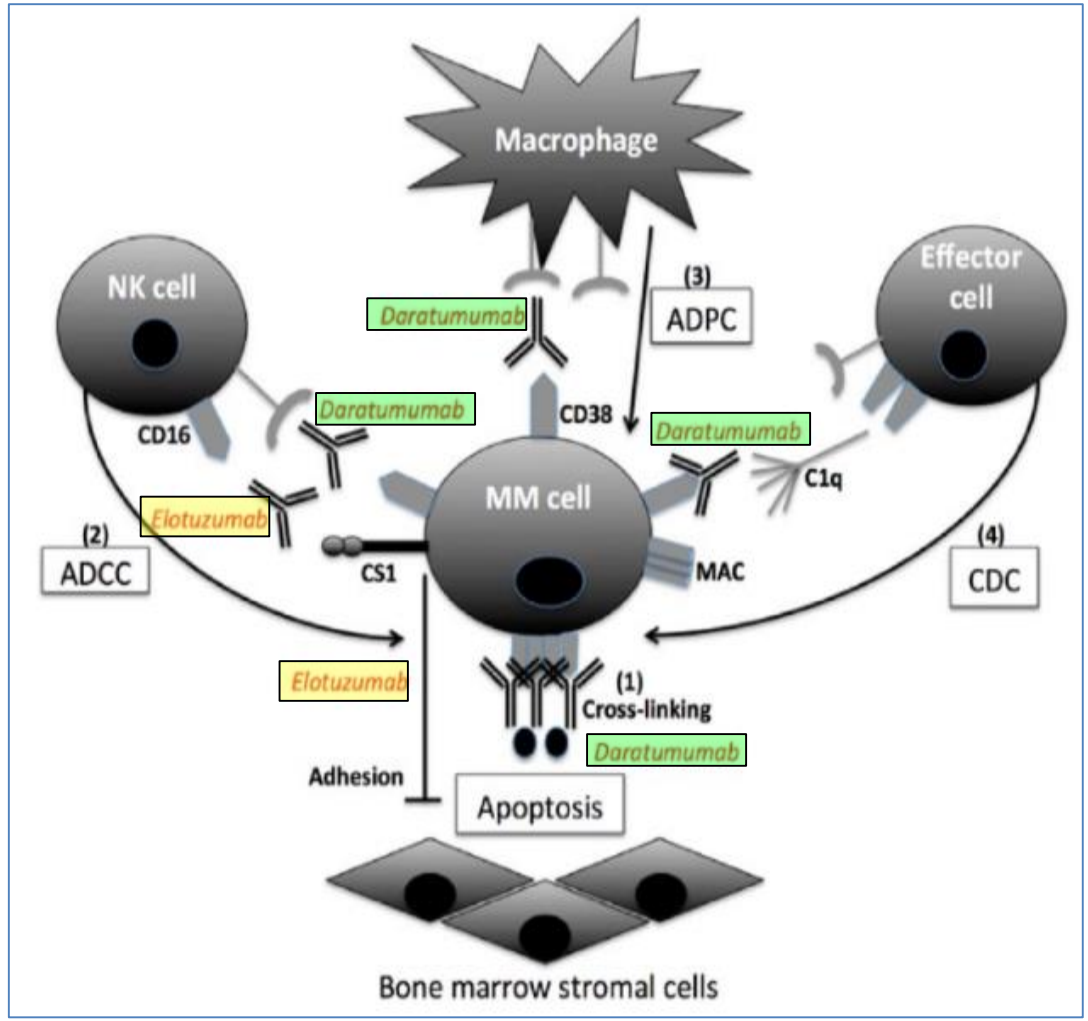


Elo= Elotuzumab, Len= Lenalidomide

Xenograft Mouse model: IcrTac:ICR-Prkdc^{scid}: lacks of T/B cells due to a defect in V(D)J recombination

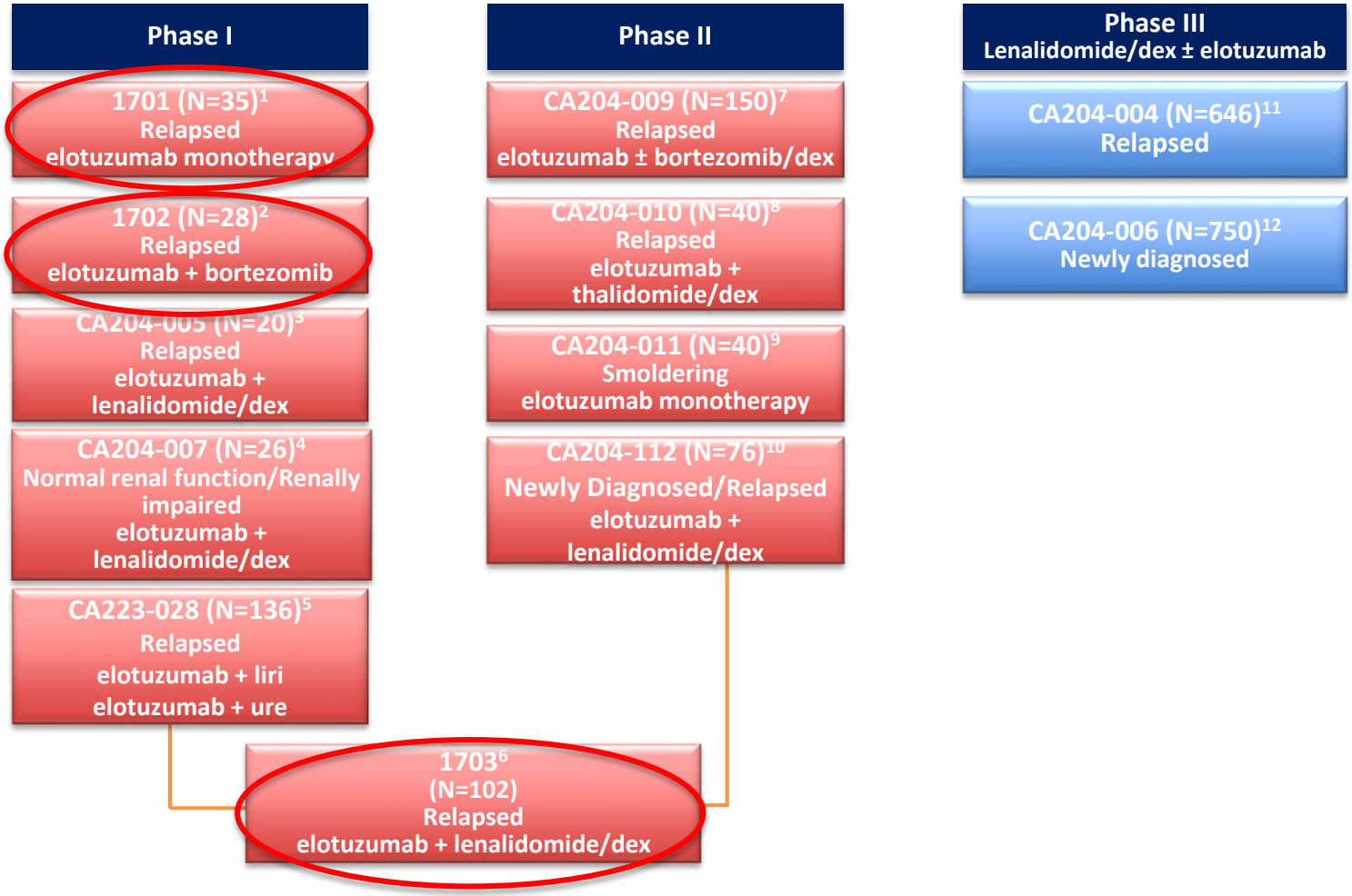
Balasa B et al. *Cancer Immunol Immunother,* 2015.

Differences in the mAbs between Elotuzumab and DARA



Salma A et al. Immunotherapy: A New Approach to Treating Multiple Myeloma Annals of Pharmacotherapy 1–14 April 20, 2016

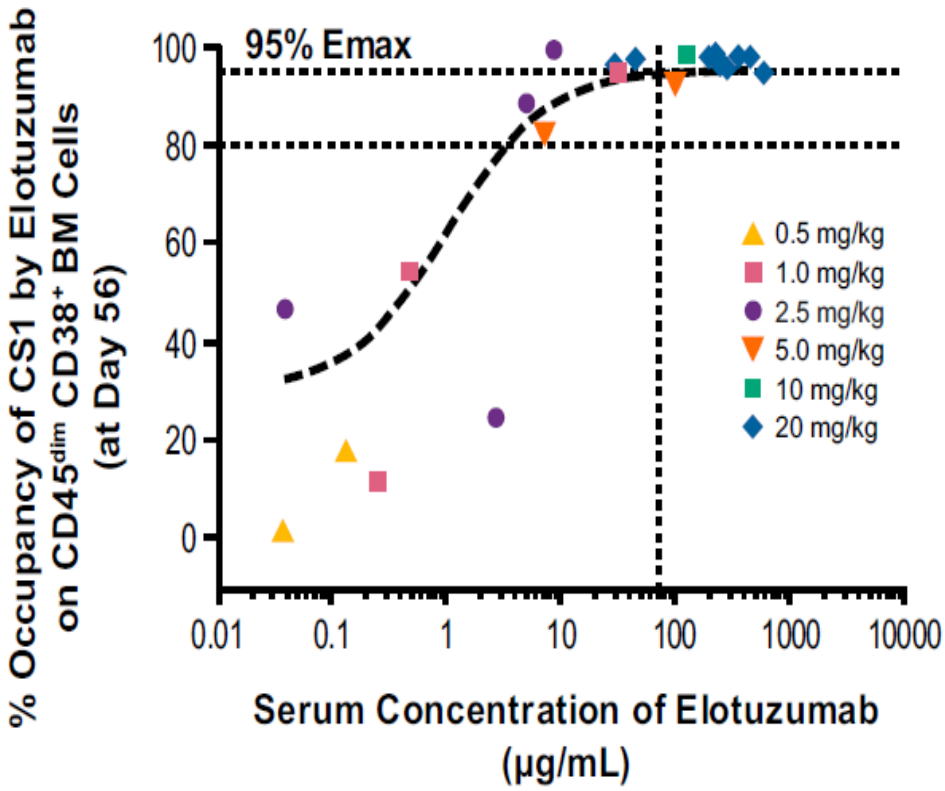
Elotuzumab Clinical Development Program



Dex= dexamethasone; liri= lirilumab; ure= urelumab.

1. Clinicaltrials.gov. NCT00425347. 2. Clinicaltrials.gov. NCT00726869. 3. Clinicaltrials.gov. NCT01241292. 4. Clinicaltrials.gov. NCT01393964. 5. Clinicaltrials.gov. NCT02252263. 6. Clinicaltrials.gov. NCT00742560. 7. Clinicaltrials.gov. NCT01478048. 8. Clinicaltrials.gov. NCT01632150. 9. Clinicaltrials.gov. NCT01441973. 10. Clinicaltrials.gov. NCT02159365. 11. Clinicaltrials.gov. NCT01239797. 12. Clinicaltrials.gov. NCT01335399.

1701: Pharmacodinamics



- Saturation of SLAMF7/CS1 by Elotuzumab on BM target cells increased as the dose of Elotuzumab increased.
- At dose of 10 mg/kg and 20 mg/kg Elotuzumab, SLAMF7/CS1 receptors on BM-derived myeloma cells were consistently saturated.
- Lower dose groups exhibited more variation in the level of target cell saturation achieved.

Zonder JA et al. *Blood,* 2012.

Phase 1 and 2 Elotuzumab Trials: Summary

Trial	Phase	Treatment	Sample Size	Efficacy (%)	Median PFS
1701	1	Elotuzumab monotherapy	35	SD=26.5	—
1702	1	Elotuzumab + Bortezomib	28	ORR=48	9.46 mo
1703	1	Elotuzumab + Lenalidomide/ Dexamethasone	28	ORR=82	33 months
1703	2	Elotuzumab + Lenalidomide/ Dexamethasone	73	ORR=84	29 months
009	2	Elotuzumab + Bortezomib/ Dexamethasone	152	ORR=65	9.7 months

Monoclonal antibodies in MM

Target	Antibody	Mechanisms of action	Activity as mono-therapy	Activity/under evaluation in combo
CS1/ SLAMF7	Elotuzumab (Humanized IgG1k)	• ADCC • Enhance NK activity • Interference with cell interaction	-	+ VD + Rd
CD38	Daratumumab (Fully human IgG1k)	• ADCC • CDC • ADCP • Direct induction of apopotosis • Modulation CD38 function	+	+ V-based + Rd + PomDex + VCD + Rd

Monoclonal antibodies in MM

Target	mAb		Stage of development
Surface molecules			
SLAMF7 (CS1)	Elotuzumab	Humanized	Phase 1/2/3
CD38	Daratumumab	Fully human	Phase 1/2/3/4
	Isatuximab (SAR650984)	Chimeric	Phase 1/2
	MOR202	Fully human	Phase 1/2
CD138	Indatuximab ravtansine (BT062)		Phase 1/2
BCMA	J6M0-mcMMAF (GSK2857916)		Phase 1
Signaling molecules			
IL-6	Siltuximab		Phase 2
RANKL	Denosumab		Phase 3
VEGF	Bevacizumab		Phase 2
DKK1	BHQ880		Phase 2
Immune checkpoint inhibitors			
PD-1	Pembrolizumab		Phase 1/2/3
	Nivolumab		Phase 1/2
	Pidilizumab		Phase 1/2
PD-L1	Durvalumab		Phase 1
CTLA4	Ipilimumab		Phase 1/2
KIR	Lirilumab		Phase 1

Bianchi G et al. *Blood,* 2015; van de Donk NW et al. *Blood*, 2016.

Grazie per l’attenzione…..