Gli anticorpi monoclonali nel trattamento del mieloma multiplo recidivato/refrattario: anti CD38 e SLAMF7

Alessandro Corso

Immunological aspects of cancer chemotherapy

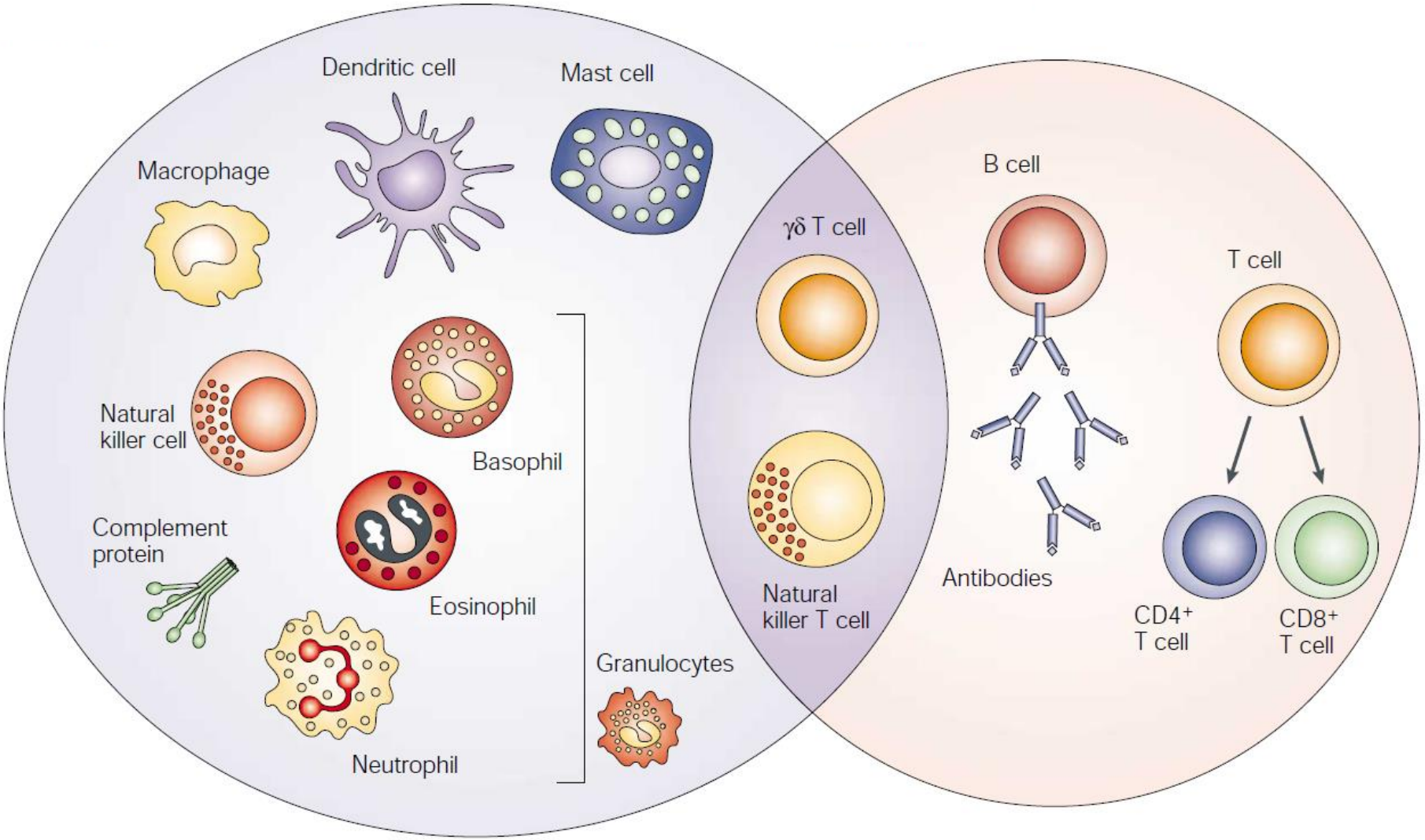
Zitvogel et al. Nature Reviews Immunology, 8:59-73; 2008

To win the fight against cancer, it is necessary not only to develop strategies to **kill all cancer (stem) cells** efficiently, by using the correct combination and schedule of chemotherapeutic agents, but also to attempt to **stimulate an immune response** so that the **immune system can keep residual tumour cells in check**

The complex network of anti-tumor immunity

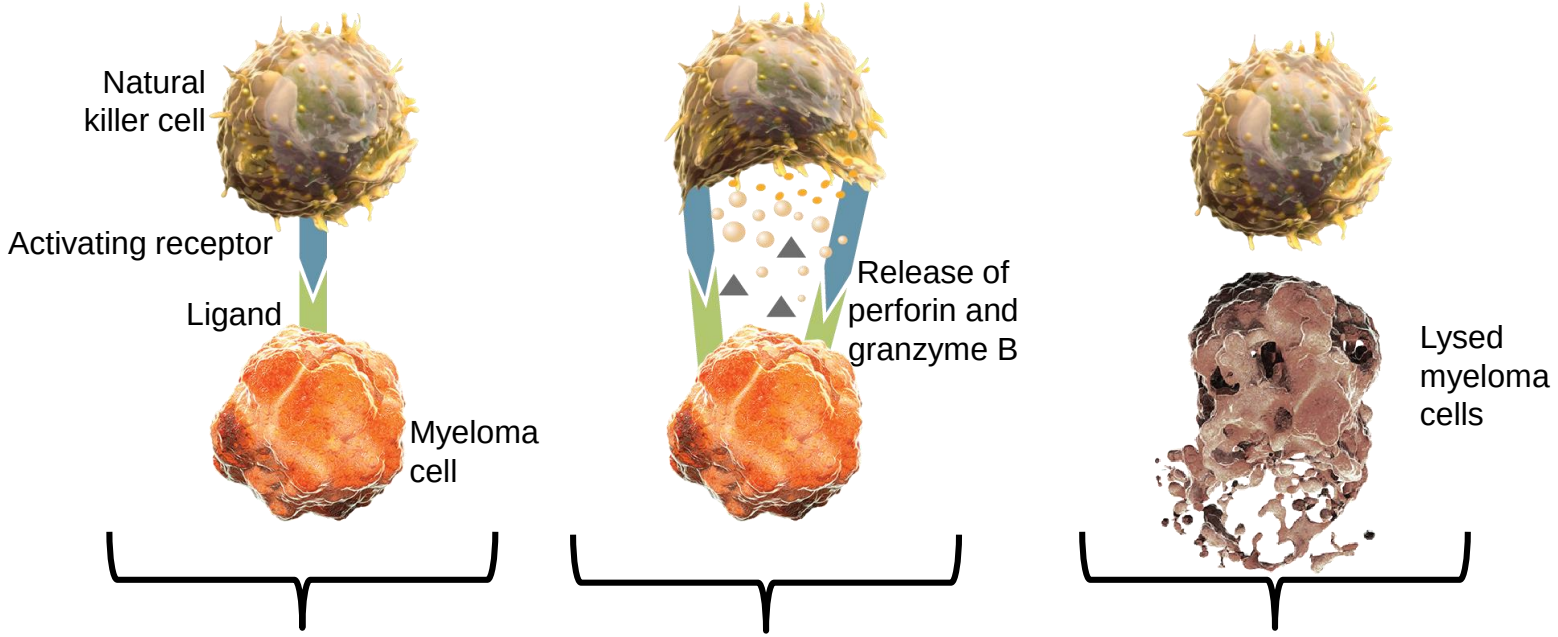
INNATE IMMUNITY
Immediate antigen-independent activity

ADAPTIVE IMMUNITY
Delayed antigen-specific **memory a**ctivity



Natural Killer Cells in the Immune Response to Multiple Myeloma

- **NK cell cytotoxicity is a highly regulated, stepwise process that occurs via the formation of a lytic immune synapse[1]**



Key steps include:
- Adhesion
- Signaling for cell activation via activating receptors

Key steps include:
- Actin reorganization
- Receptor clustering
- Polarization of the MTOC
- Lytic granule docking
- Lytic granule release

Key steps include:
- Receptor downregulation
- Inactivity
- Detachment

Adapted from Orange JS et al. 2008.[2]

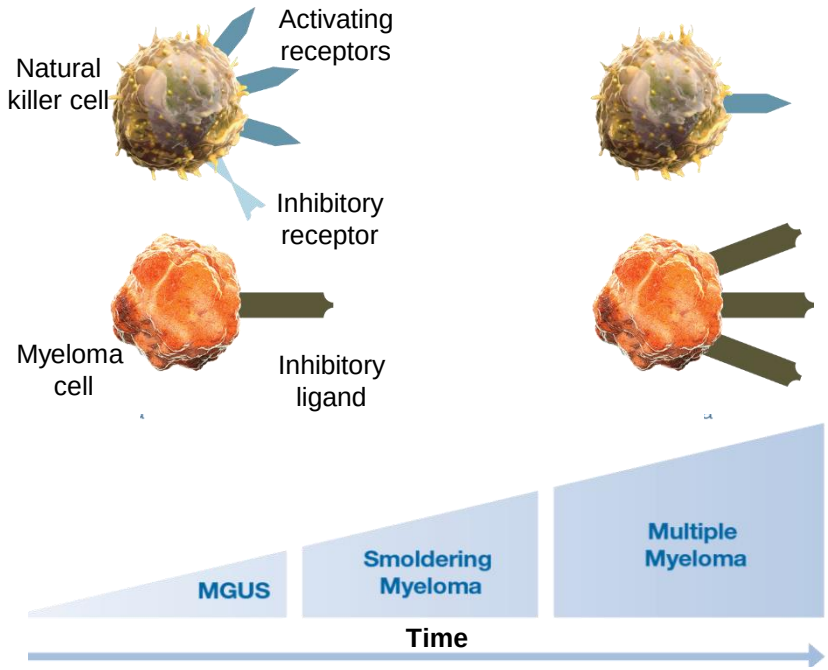
MTOC, microtubule-organizing center; MM, multiple myeloma; NK, natural killer.

1. Mace EM et al. *Immunol Cell Biol.* 2014;92:245-255. 2. Orange JS. *Nat Rev Immunol*. 2008;8:713-725.

Immune Escape in Multiple Myeloma

- While the immune system is well-equipped to identify and eliminate myeloma cells, they can escape immune-mediated destruction via immune evasion and immunosuppressive strategies[1]

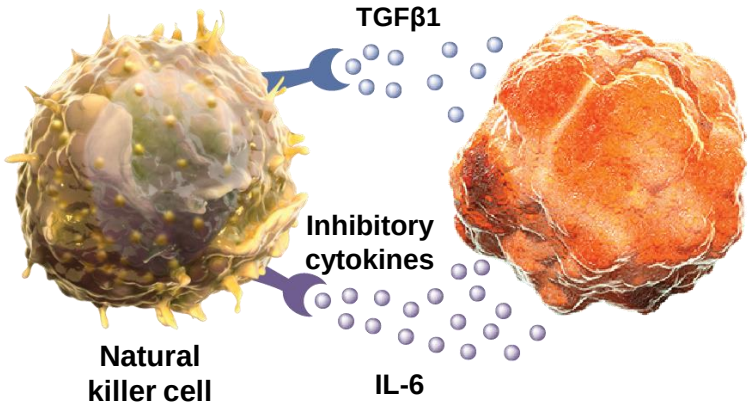
Immune Evasion



NKG2D receptors on NK cells[2]

Expression of inhibitory molecules, including MHC class I ligands[2]

Immunosuppression



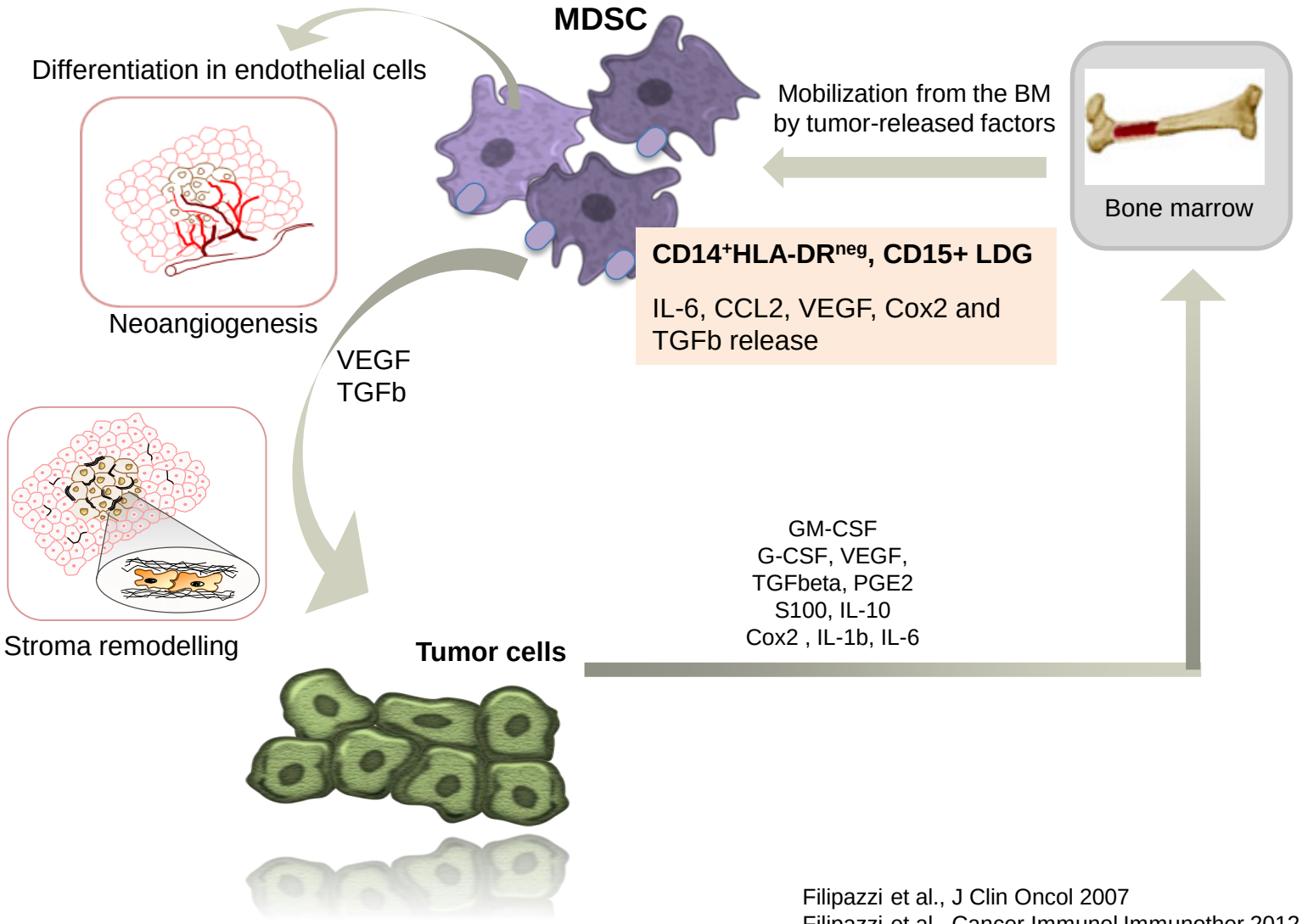
- TGF-β has substantial inhibitory effects on NK cell cytolytic activity[3]
- IL-6 induces NK cell dysfunction[4] and has been proposed as an autocrine growth factor for MM[4,5]

Images adapted from Godfrey J and Benson DM Jr. 2012.[2]

IL-6, interleukin 6; MGUS, monoclonal gammopathy of undetermined significance; MHC, major histocompatibility complex; MM, multiple myeloma; NK, natural killer; NKG2D, natural-killer group 2, member D; TGFβ1, transforming growth factor beta 1.

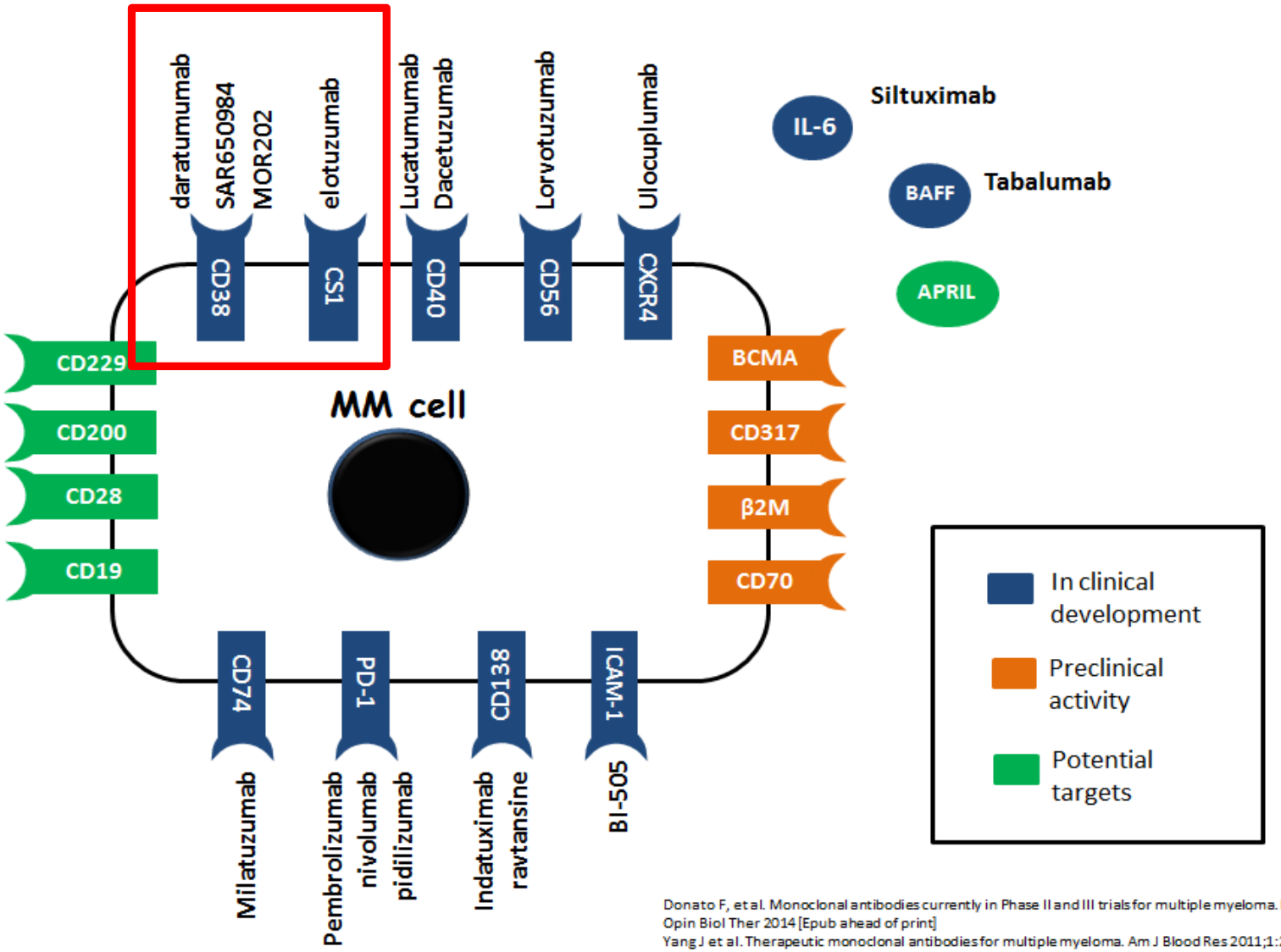
1. Vesely MD et al. *Ann Rev Immunol.* 2011;29:235-271. 2. Godfrey J, Benson DM Jr. *Leuk Lymphoma.* 2012;53:1666-1676. 3. Rook AH et al. *J Immunol*.1986;136:3916-3920. 4. Urashima M et al. *Blood*. 1996;87:1928-1938. 5. Tanner J. *J Clin Invest*. 1991;88:239-247.

Myeloid-derived suppressor cells: best tumor allies



Filipazzi et al., J Clin Oncol 2007
Filipazzi et al., Cancer Immunol Immunother 2012

Targets for mAbs in MM



Donato F, et al. Monoclonal antibodies currently in Phase II and III trials for multiple myeloma. Expert Opin Biol Ther 2014 [Epub ahead of print]

Yang J et al. Therapeutic monoclonal antibodies for multiple myeloma. Am J Blood Res 2011;1:22–33

Mateo G, et al. Prognostic value of immunophenotyping in multiple myeloma: A study by the PETHEMA/GEM Coopereatvie study groups on patients uniformly treated with high-dose therapy

Atanackovic D, et al. Surface molecule CD229 as a novel target for the diagnosis and treatment of multiple myeloma. Haematologica 2014;96:1512–20.

Lonial et al, Leukemia 2015

Daratumumab

16 Novembre 2015: Approvazione FDA

"Darzalex is indicated for the treatment of patients with multiple myeloma who have received ***at least three prior lines of therapy including a proteasome inhibitor (PI) and an immunomodulatory agent or who are double-refractory*** *to a PI and an immunomodulatory agent."*

http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm472875.htm

23 Aprile 2016: Approvazione EMA

"Darzalex as ***monotherapy*** *is indicated for the treatment of adult patients with* ***relapsed and refractory*** *multiple myeloma, whose* ***prior therapy included a proteasome inhibitor and an immunomodulatory*** *agent and who have demonstrated* ***disease progression on the last therapy****."*

Elotuzumab

30 Novembre 2015: Approvazione FDA

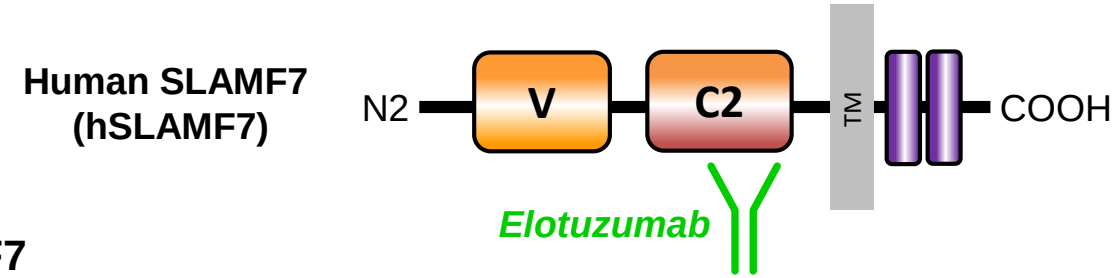
"Empliciti is indicated in combination with Lenalidomide and Dexamethasone for the treatment of patients with multiple myeloma who have received ***one or three prior lines of therapy****."*

http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm474684.htm

29 Gennaio 2016: Approvazione EMA

"Empliciti received the the granting of a marketing authorisation for the treatment of multiple myeloma in combination with Lenalidomide and dexamethasone for the treatment of patients who have received at least one prior therapy."

Elotuzumab: anti-hSLAMF7 antibody



SLAMF7

- Highly expressed on the surface of >95% of myeloma cells[1,2]
- Expressed on other lymphocytes including natural killer (NK) cells[2]

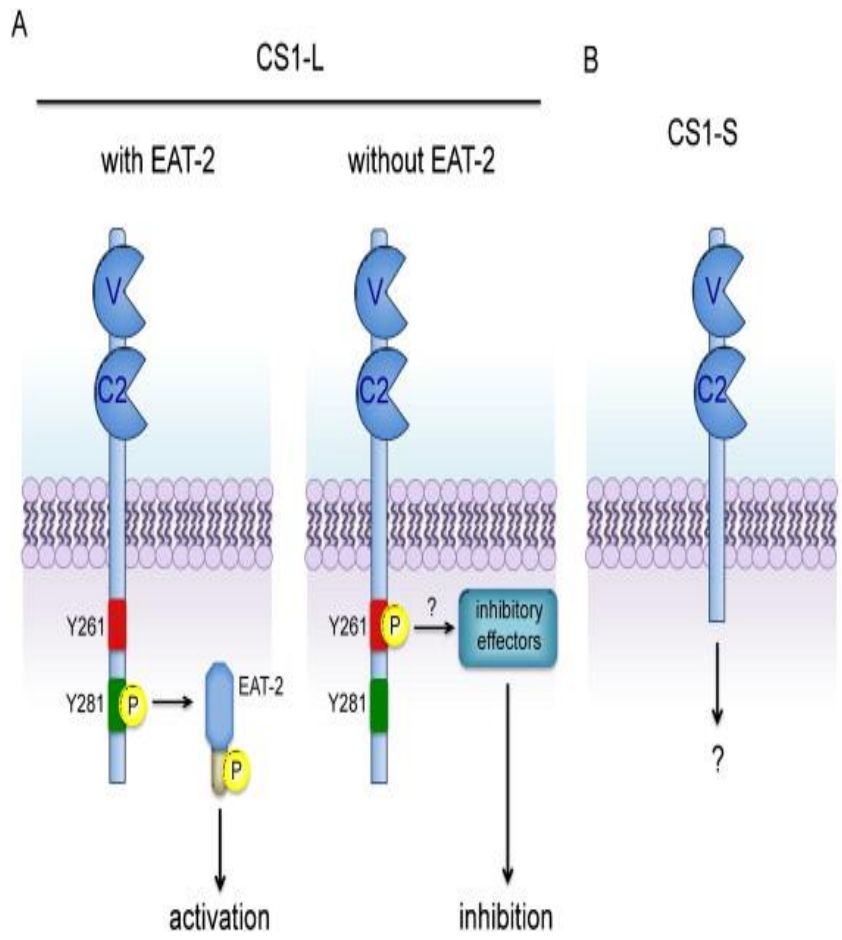
Elotuzumab

- Humanized, IgG1 isotype immunostimulatory monoclonal antibody (mAb), specific for human SLAMF7[2,3]
  - No cross-reactivity with mouse homologue
- Dual mechanism of action to kill myeloma cells
  - Directly activates NK cells[4]
  - Binds to SLAMF7 on myeloma cells, mediating antibody-dependent cell-mediated cytotoxicity (ADCC)[2]

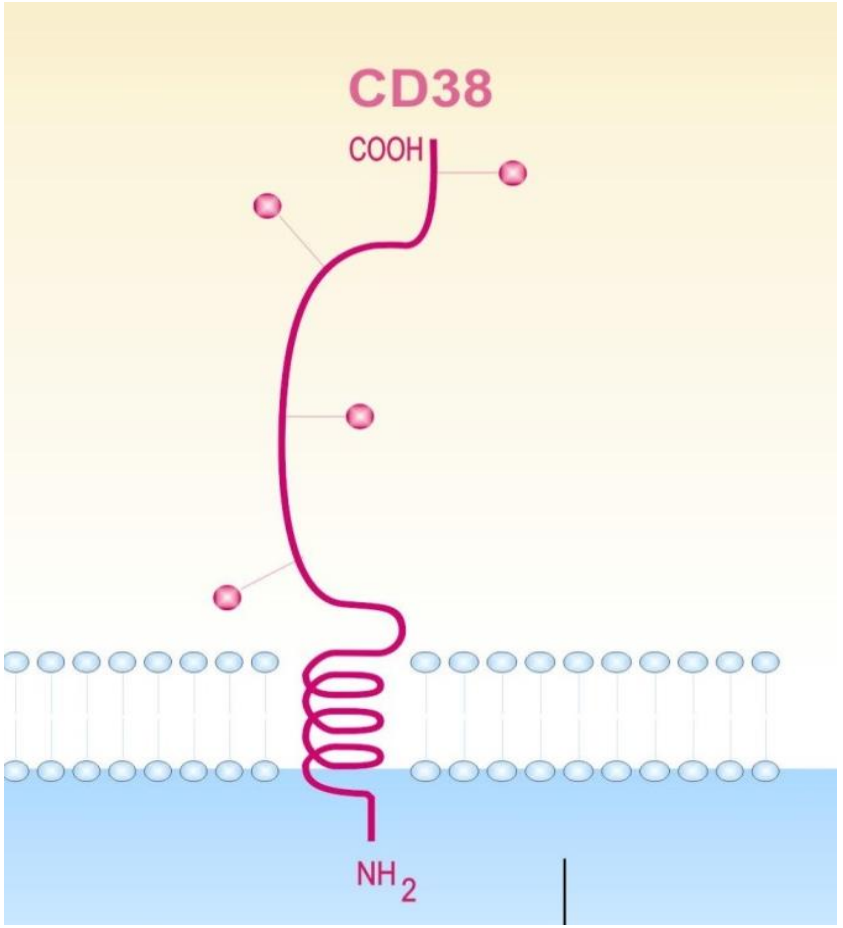
1. Hsi ED et al. *Clin Cancer Res* 2008;14:2775–84; 2. Tai YT et al. *Blood* 2008;112:1329–37; 3. Kumaresan PR et al. *Mol Immunol* 2002;39:1–8; 4. Collins SM et al. *Cancer Immunol Immunother* 2013;62:1841–9

Cell Surface Targets

CS1 (SLAMF7)



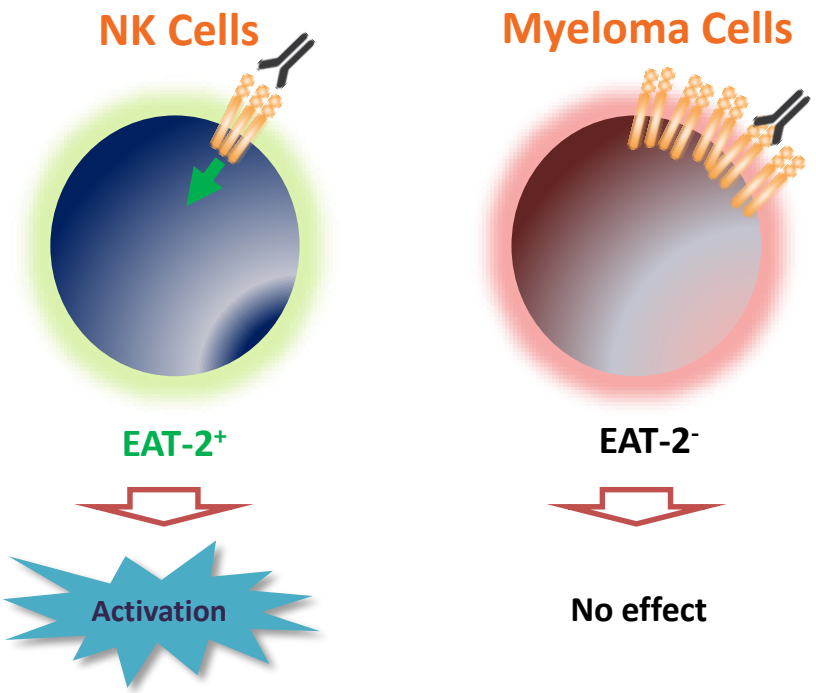
CD38



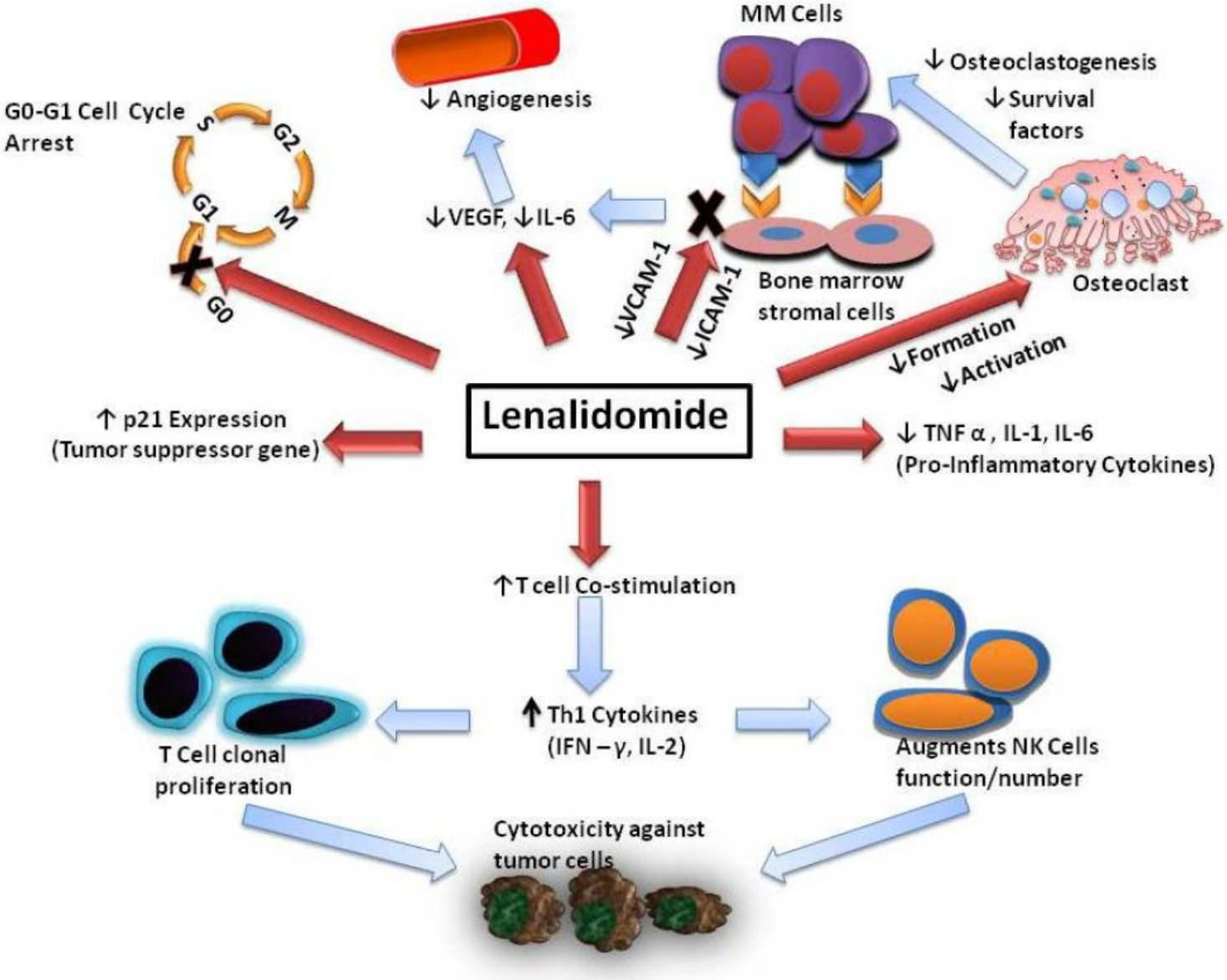
SLAMF7: Signalling lymphocytic activation molecule F(amily)7.

Vellette et al. Crit Rev Oncol/Hem. 2013 168-77; Malavasi et al., Physiol Rev. 2008;88(3):841-86.

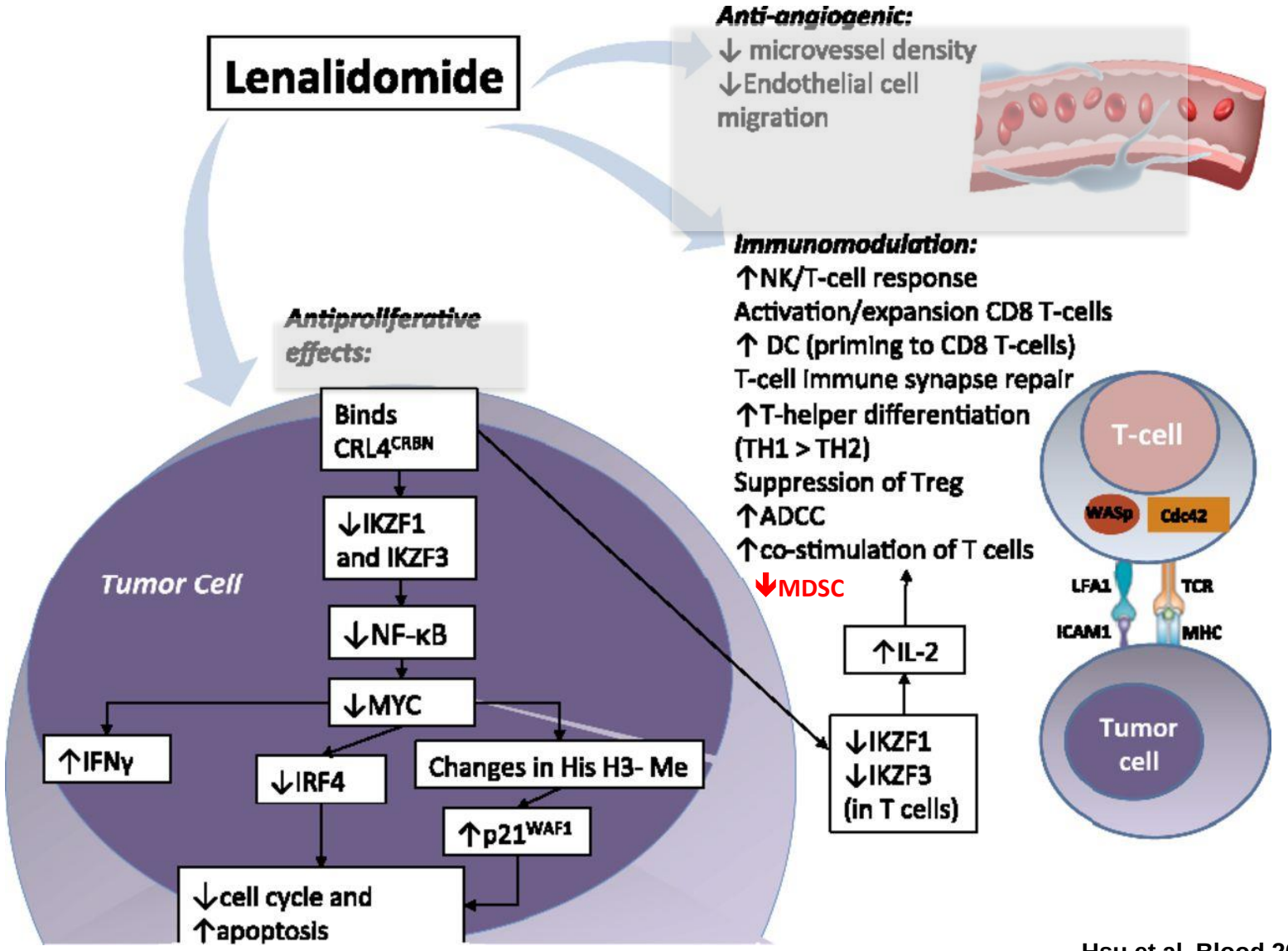
Differential SLAMF7 signalling: Elotuzumab activates NK cells but not myeloma cells



Guo et al (*Mol Cell Bio*), 2015: Phosphorylation of SLAMF7 is mediated by Src kinases. Inhibitory mechanism (in $EAT2^-/CD45^+$ cells) is mediated by SHIP-1. MM cells are deficient for EAT-2 and CD45, therefore SLAMF7 does not mediate activating or inhibitory effects in these cells.
EAT-2 = Ewing's Sarcoma associated transcript 2; SLAMF7 = Signalling Lymphocyte Activation Molecule Family 7.


MM Cells
↓ Osteoclastogenesis
↓ Survival factors
↓ Angiogenesis
G0-G1 Cell Cycle Arrest
S
G2
M
G1
G0
↓VEGF, ↓IL-6
↓VCAM-1
↓ICAM-1
Bone marrow stromal cells
Osteoclast
↓Formation
↓Activation
Lenalidomide
↑ p21 Expression (Tumor suppressor gene)
↓ TNF α , IL-1, IL-6 (Pro-Inflammatory Cytokines)
↑T cell Co-stimulation
↑ Th1 Cytokines (IFN – γ, IL-2)
T Cell clonal proliferation
Augments NK Cells function/number
Cytotoxicity against tumor cells

Immunomodulating properties of lenalidomide



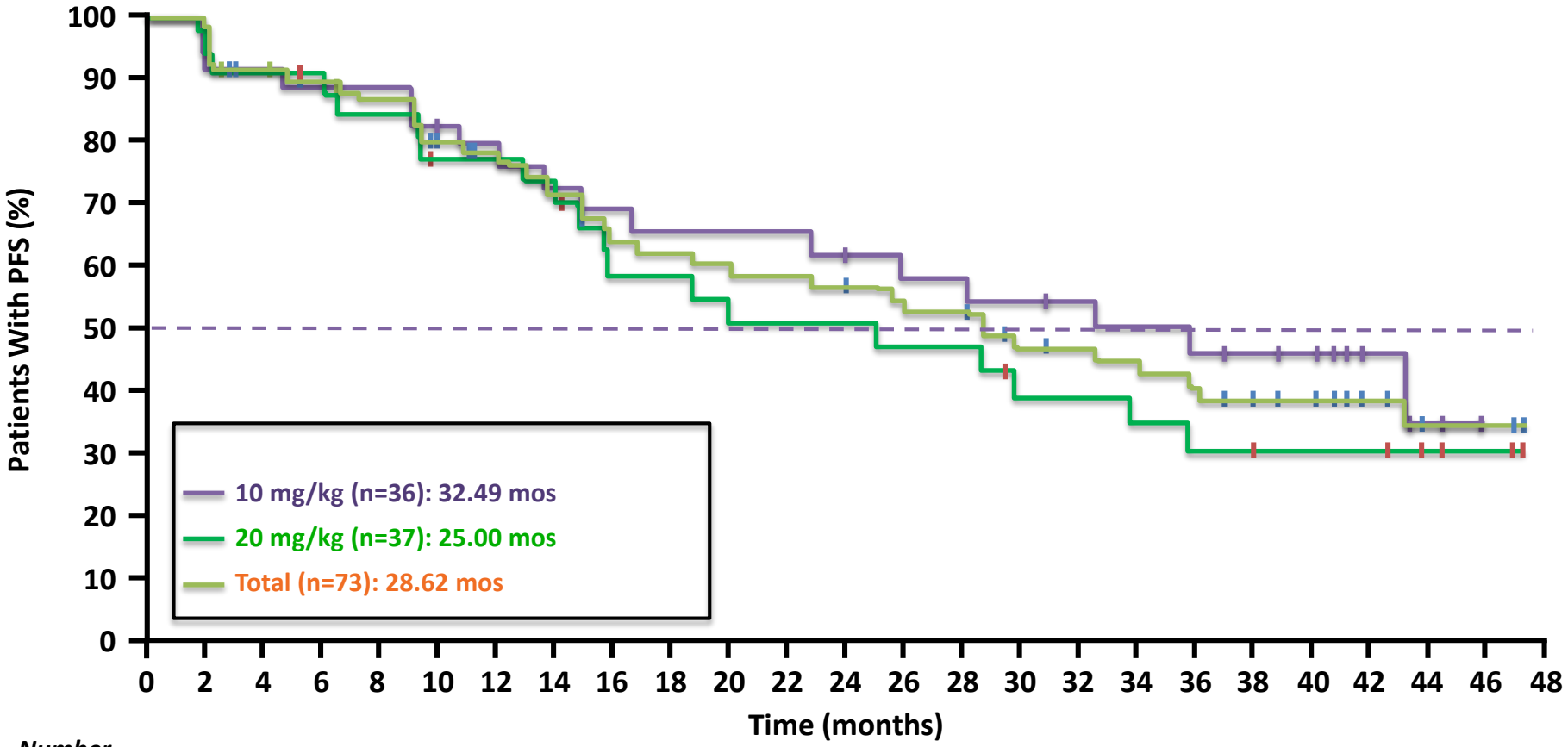
Hsu et al. Blood 2011

1703: Phase 2 Efficacy (Response rate)

Assessment	Elo 10 mg/kg (n=36)	Elo 20 mg/kg (n=37)	Total (n=73)
Overall response*, n (%)	33 (92)	28 (76)	61 (84)
Best confirmed response, n (%)			
Stringent complete response (sCR)	2 (6)	1 (3)	3 (4)
Complete response (CR)	4 (11)	3 (8)	7 (10)
Very good partial response (VGPR)	17 (47)	14 (38)	31 (43)
Partial response (PR)	10 (28)	10 (27)	20 (27)
Stable disease (SD)	3 (8)	7 (19)	10 (14)
Missing	0	2 (5)	2 (3)
Median time to first response, mos	1.0	1.7	1.0
Median duration of response, mos	23.0	18.0	20.8

Richardson PG et al. ASH 2014: Abstract 2273

1703: Phase 2 Progression-Free Survival



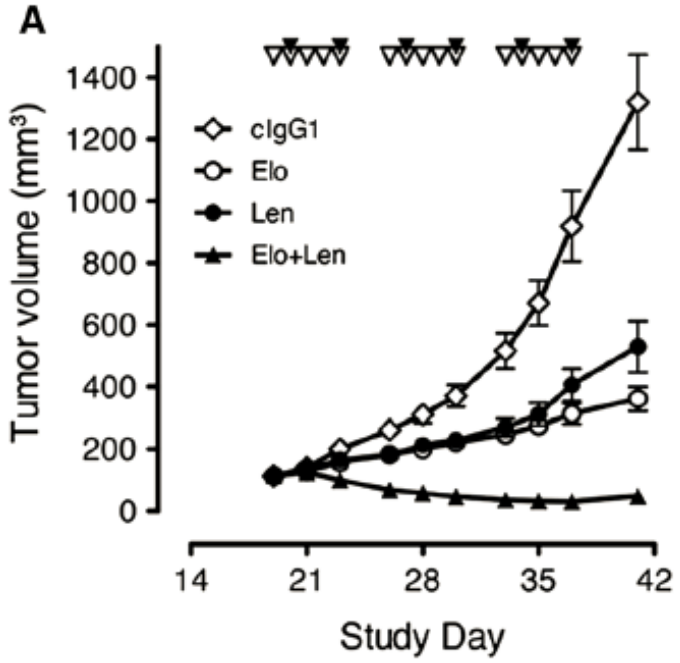
Number at risk:																									
10 mg/kg	36	33	32	30	29	26	23	21	19	18	18	18	16	15	15	14	13	12	11	10	8	4	2	0	0
20 mg/kg	37	32	27	26	24	21	21	19	15	15	13	13	13	12	12	9	9	8	7	7	6	6	4	2	0
Total	73	65	59	56	53	47	44	40	34	33	31	31	29	27	27	23	22	20	18	17	14	10	6	2	0

Relative dose intensity was 96% for elo, 77% for len, and 75% for dex.
PFS, progression-free survival.

1. Richardson P et al. Presented at the Annual Meeting of the American Society of Hematology 2014:Abstract 302.

Elotuzumab in combination with Lenalidomide enhanced anti-myeloma activity

- In MM xenograft mouse model, the combination of Elo + Len significantly reduced tumor volume compared with either agent alone

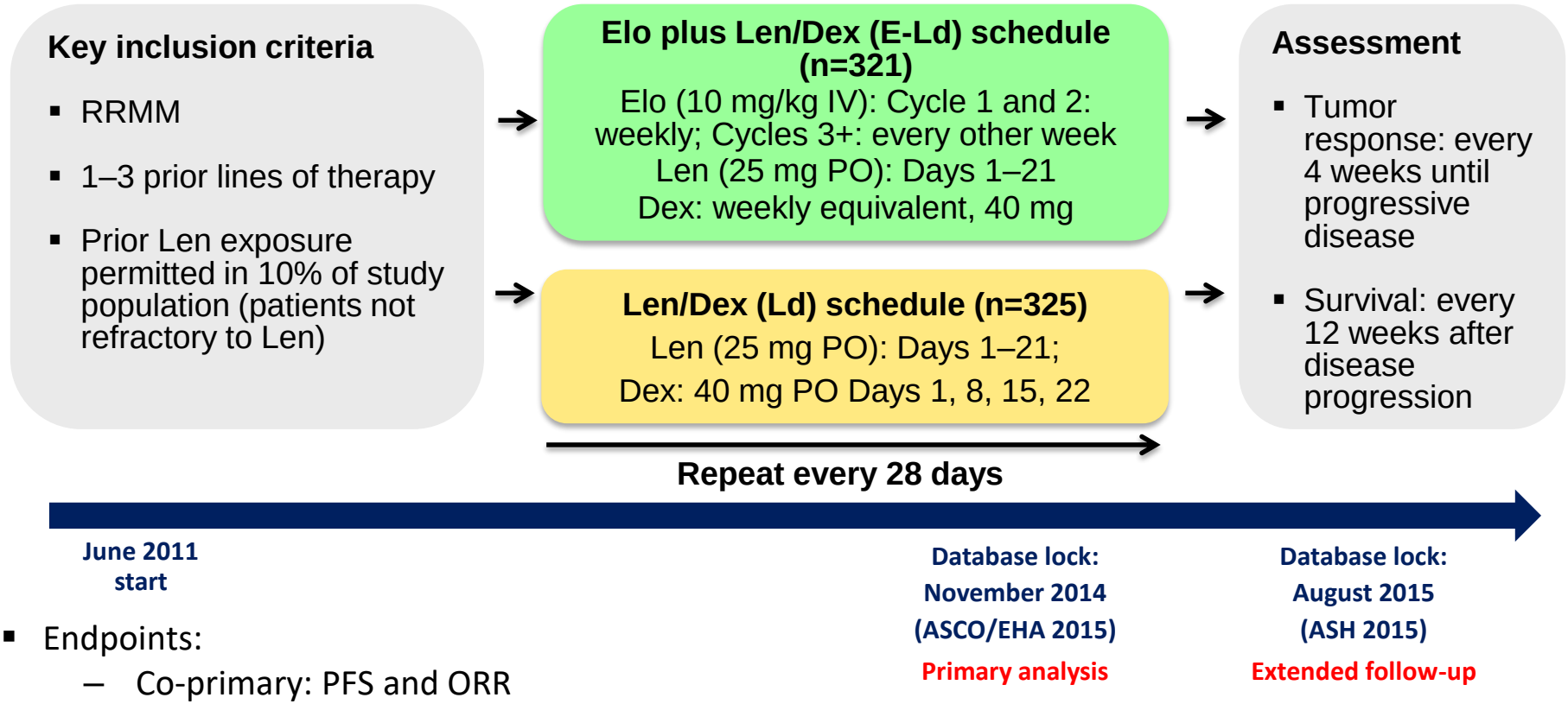


- Lenalidomide enhances T-cell activation and cytokine production leading to natural killer cell stimulation
- Lenalidomide also exhibits direct anti-myeloma activity, which enhances the cell's sensitivity to natural killer cell–mediated killing

Balasa B et al. *Cancer Immunol Inmmunother*. 2014 Oct 7 [Epub ahead of print].

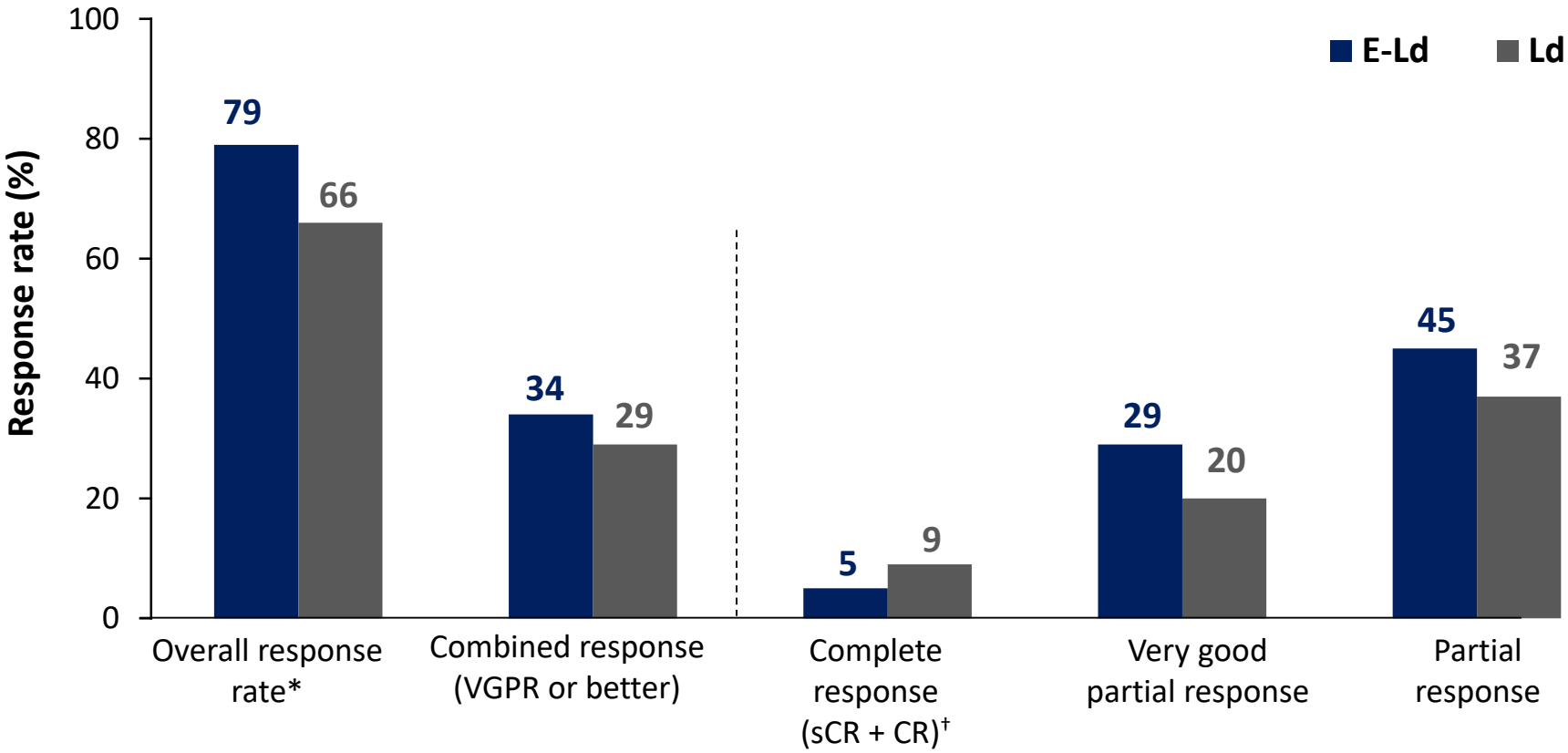
ELOQUENT-2 Study Design

- Open-label, international, randomized, multicenter, phase 3 trial (168 global sites)



- Endpoints:
  - Co-primary: PFS and ORR
  - Other: OS, DOR, quality of life, safety
- All patients received premedication to mitigate infusion reactions prior to elotuzumab administration; Elotuzumab IV infusion administered ~ 2–3 hours

Co-Primary Endpoint: Overall Response Rate

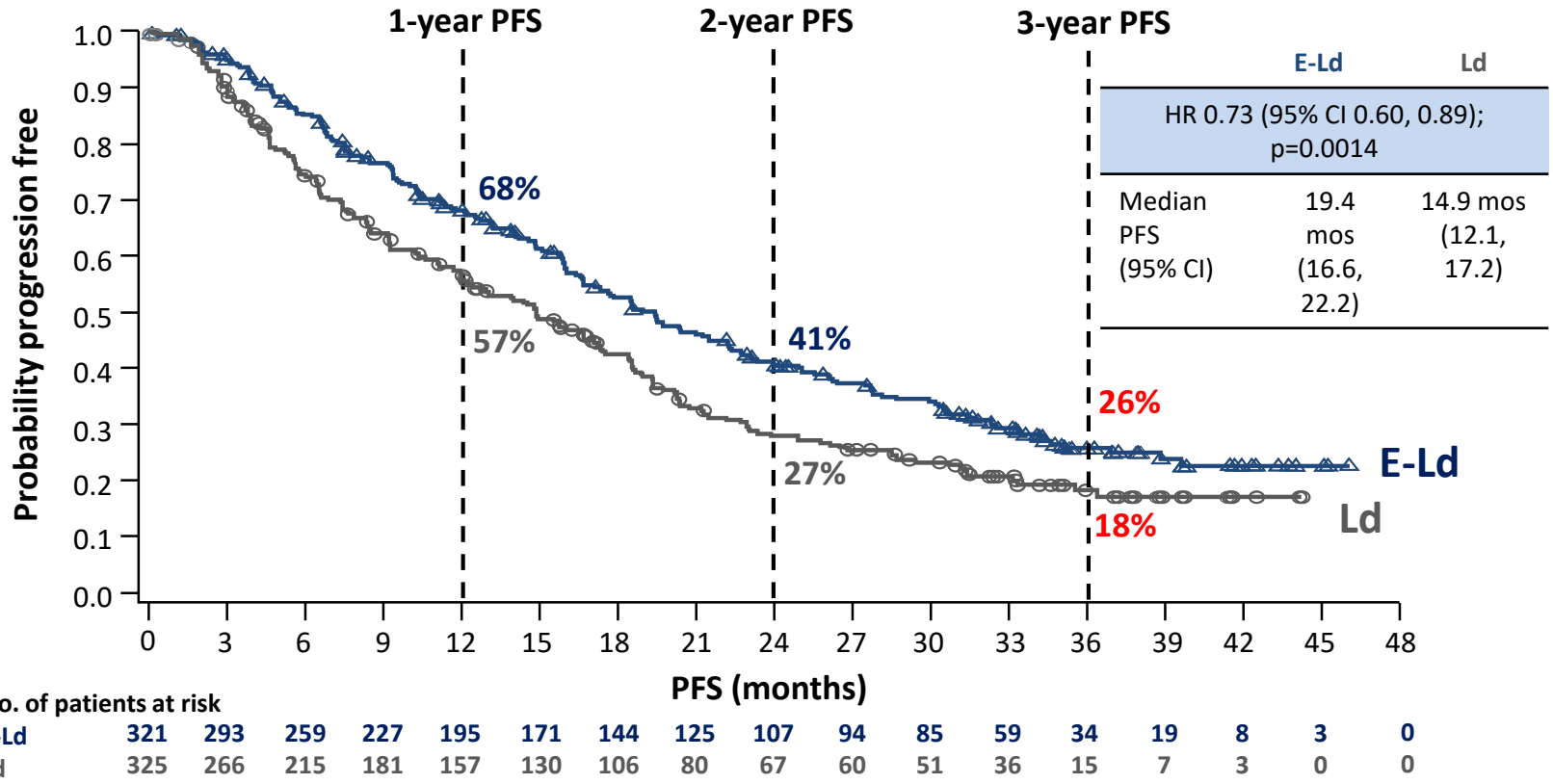


*Defined as partial response or better

†Complete response rates in the E-Ld group may be underestimated due to interference from therapeutic antibody in immunofixation and serum protein electrophoresis assay

Dimopoulos MA et al , ASH 2015 (Abstract 28) *Oral presentation*

Co-Primary Endpoint: Extended Progression-Free Survival

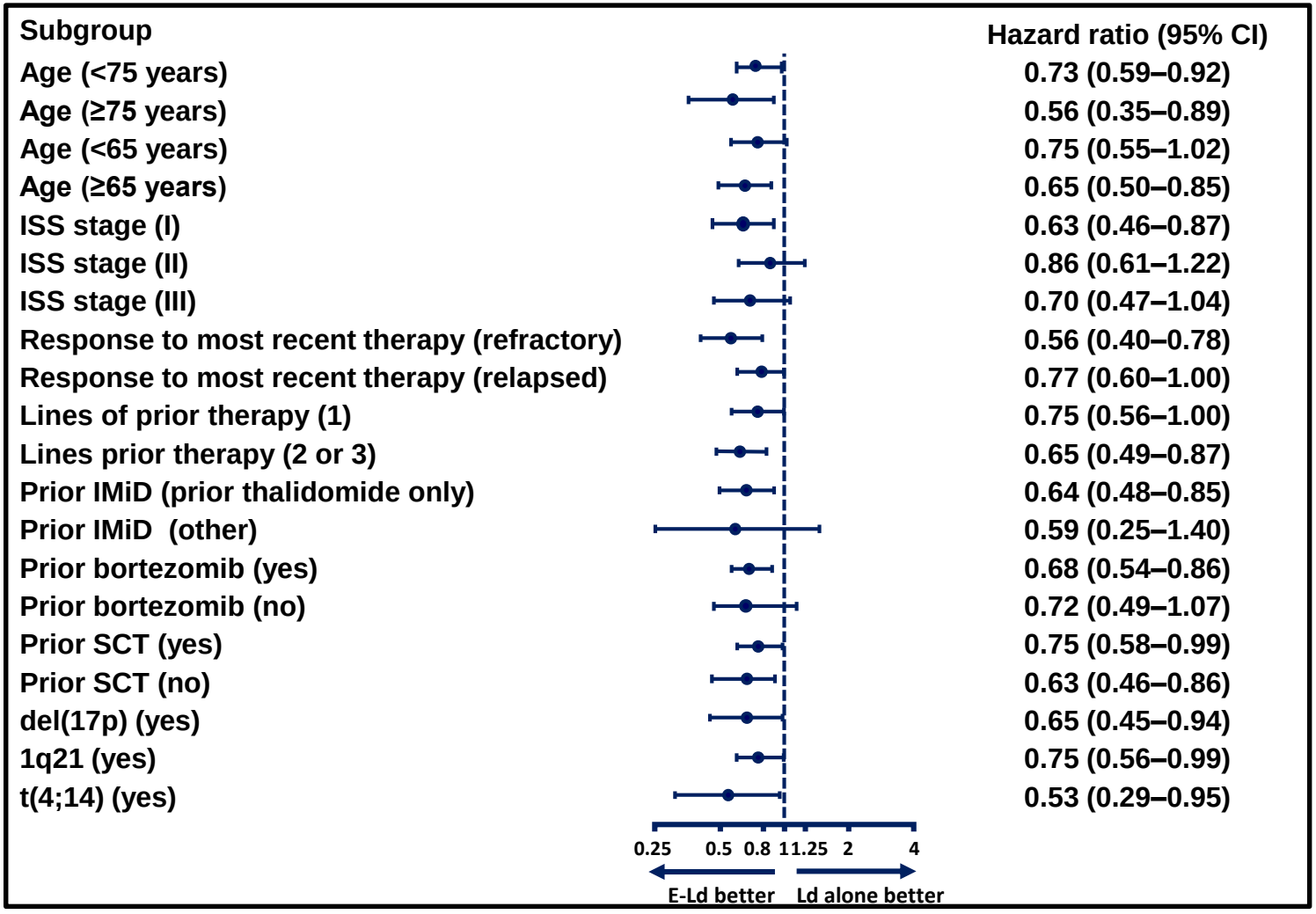


	E-Ld	Ld
HR 0.73 (95% CI 0.60, 0.89); p=0.0014		
Median PFS (95% CI)	19.4 mos (16.6, 22.2)	14.9 mos (12.1, 17.2)

PFS benefit with E-Ld was maintained over time (vs Ld):
E-Ld–treated patients had a **27% reduction in the risk of disease progression or death**; treatment difference at 1, 2 and 3 years was 11%,14% and 8% respectively relative improvement in PFS of 44% at 3 years

Dimopoulos MA et al, ASH 2015 (Abstract 28) Oral presentation

Progression-Free Survival: Subgroup Analysis

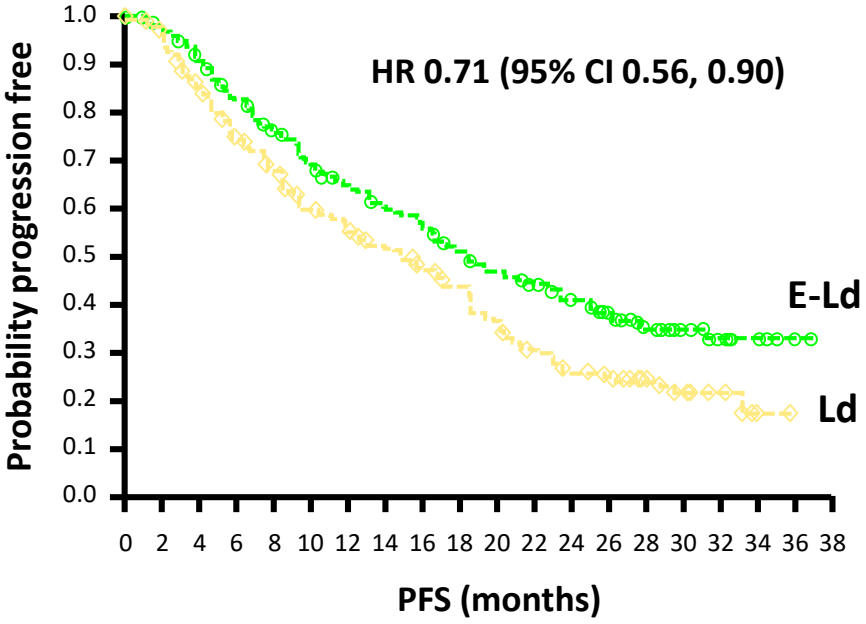


PFS benefit in E-Ld group was consistently better across key subgroups

IMiD = immunomodulatory drug; ISS = International Staging System

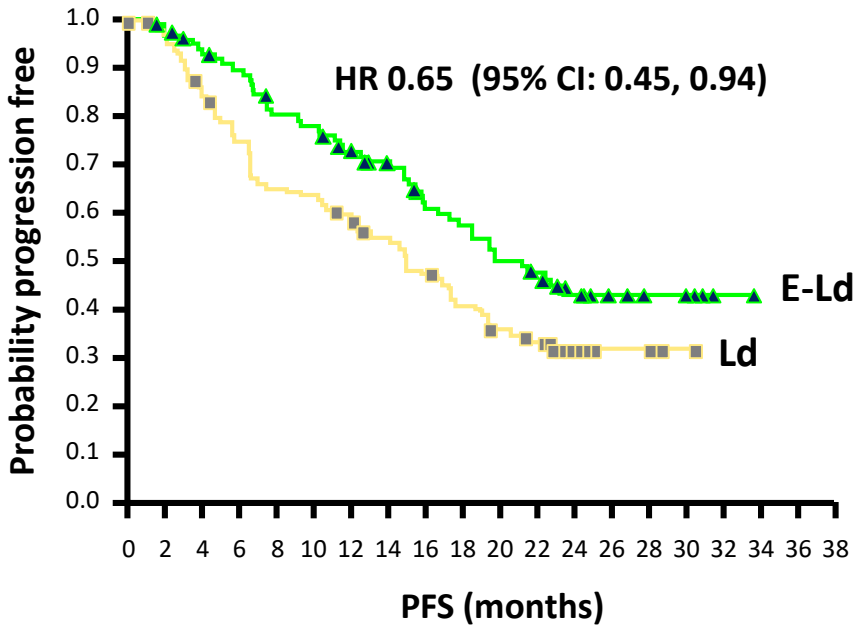
Progression-Free Survival Without and With del(17p)

del(17p)-



E-Ld: median (95% CI): 18.46 (15.84, 22.77)
Ld: median (95% CI): 14.85 (11.86, 18.43)

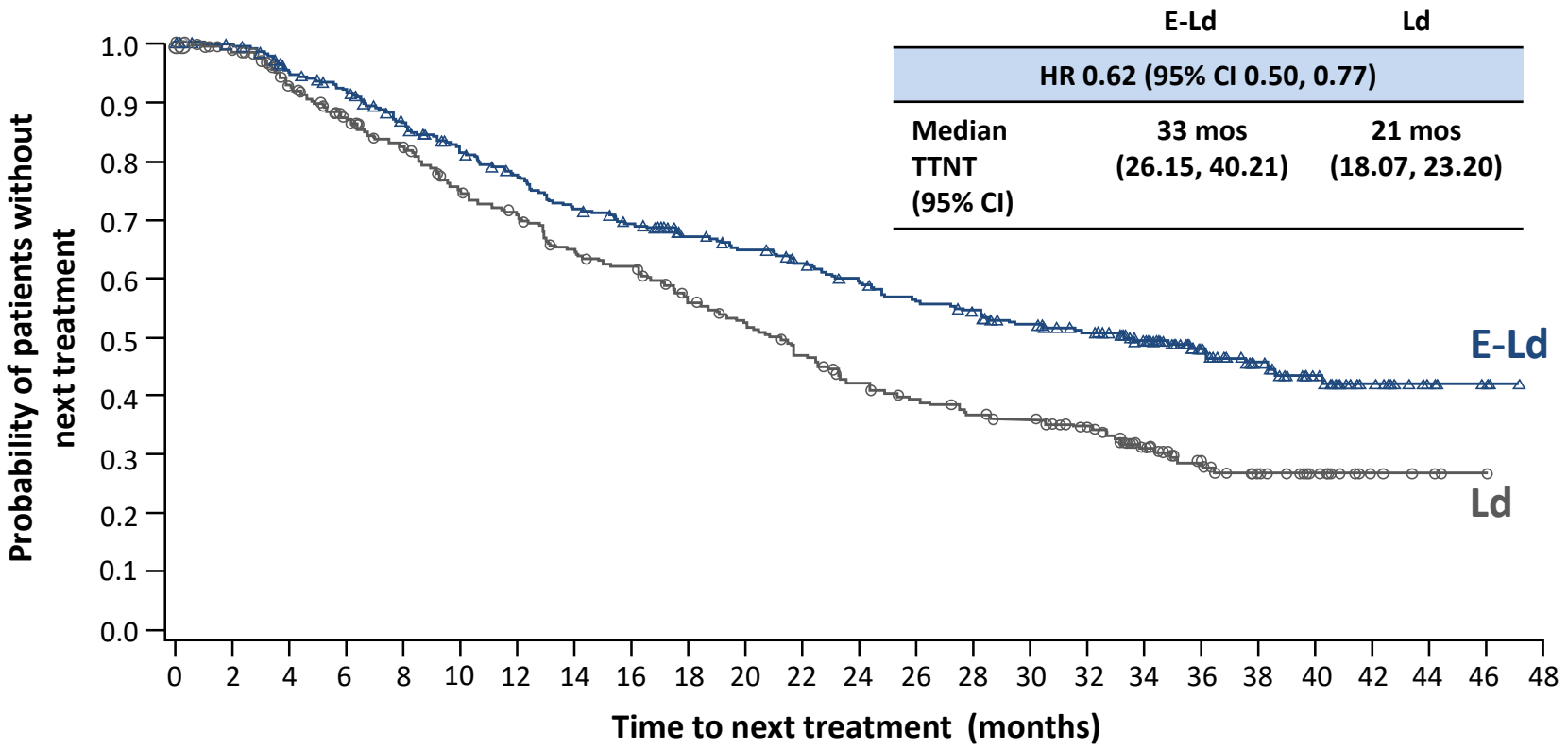
del(17p)+



E-Ld: median (95% CI): 21.19 (16.62, NE)
Ld: median (95% CI): 14.92 (10.61, 18.50)

Lonial S et al ASCO 2016 (Abstract 8037) *Poster presentation*

Time to Next Treatment

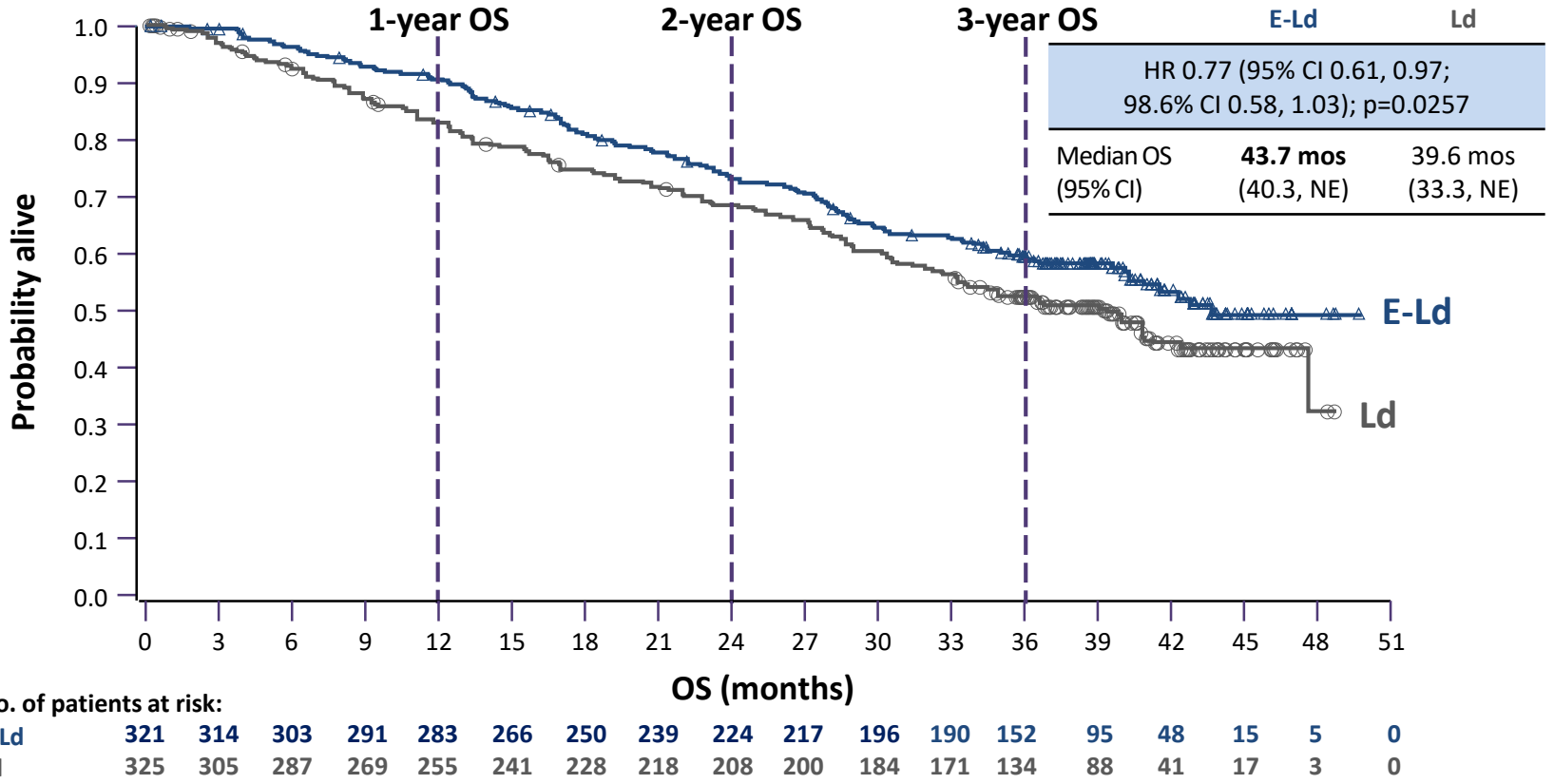


No. of patients at risk

E-Ld	321	315	294	282	259	239	225	208	198	182	174	165	153	144	138	126	118	94	65	46	32	14	6	3	0
Ld	325	305	276	251	232	206	193	174	166	148	135	120	105	96	89	85	76	46	30	20	13	5	3	1	0

E-Ld-treated patients had a median delay of 1 year in the time to next treatment vs Ld-treated patients

Interim Overall Survival



Prespecified interim analysis for overall survival indicates a strong trend (p=0.0257) with early separation sustained over time for E-Ld vs Ld

Dimopoulos MA et al , ASH 2015 (Abstract 28) *Oral presentation*

Adverse Events Reported in ≥30% of Patients

Adverse event, n (%)	E-Ld (n=318)		Ld (n=317)	
	Any grade	Grade 3 to 4	Any grade	Grade 3 to 4
Common non-hematologic adverse events				
Fatigue	149 (47)	27 (9)	123 (39)	26 (8)
Pyrexia	119 (37)	8 (3)	78 (25)	9 (3)
Diarrhea	149 (47)	16 (5)	114 (36)	13 (4)
Constipation	113 (36)	4 (1)	86 (27)	1 (0.3)
Muscle spasms	95 (30)	1 (0.3)	84 (27)	3 (1)
Cough	100 (31)	1 (0.3)	57 (18)	0
Common hematologic toxicities				
Lymphopenia	316 (99)	244 (77)	311 (98)	154 (49)
Anemia	306 (96)	60 (19)	301 (95)	67 (21)
Thrombocytopenia	266 (84)	61 (19)	246 (78)	64 (20)
Neutropenia	260 (82)	107 (34)	281 (89)	138 (44)
Infections	259 (81)	89 (28)	236 (74)	77 (24)

- The exposure-adjusted* infection rate was 198 in the E-Ld arm and 192 in the Ld arm
- Exposure-adjusted* second primary malignancy rate was 5 and 3 in the E-Ld and Ld arms
- **Infusion reactions of any grade were experienced by 10% of patients**
  - Most infusion reactions were Grade 1 or 2 and occurred (70%) during the first treatment cycle
  - There were no Grade 4 or 5 infusion reactions

Dimopoulos MA et al , ASH 2015 (Abstract 28) *Oral presentation*

Infusion Reactions

Events, n (%)	E-Ld (n=318)		
	Grade 1/2	Grade 3	Grade 4/5
Infusion reaction	**29 (9)**	**4 (1)**	**0**
Pyrexia	10 (3)	0	0
Chills	4 (1)	0	0
Hypertension	3 (1)	1 (<1)	0

- Infusion reactions occurred in 10% of patients
- 70% of infusion reactions occurred with the first dose
- No Grade 4 or 5 infusion reactions
- Elotuzumab infusion was interrupted in 15 (5%) patients due to an infusion reaction (median interruption duration 25 minutes)
- 2 (1%) patients discontinued the study due to an infusion reaction

Daratumumab

16 Novembre 2015: Approvazione FDA

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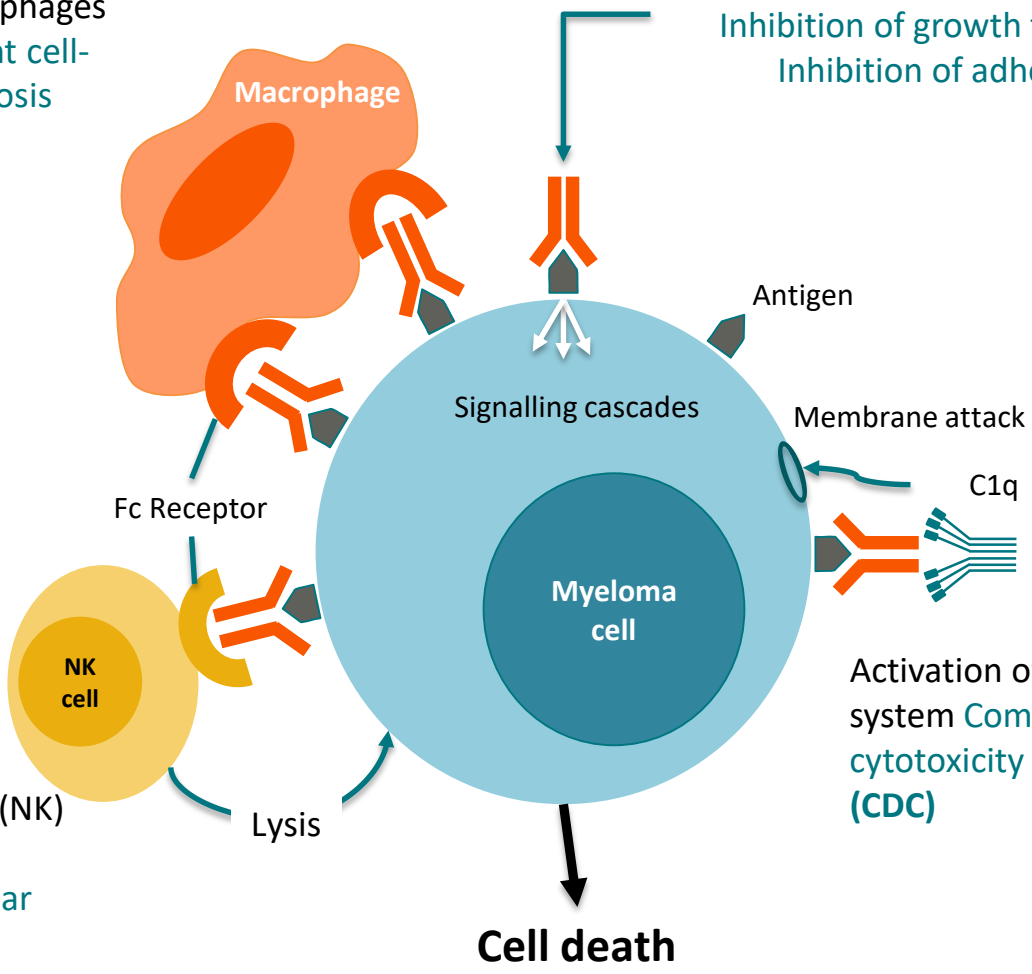
Monoclonal antibodies act through different modes of action in MM

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)

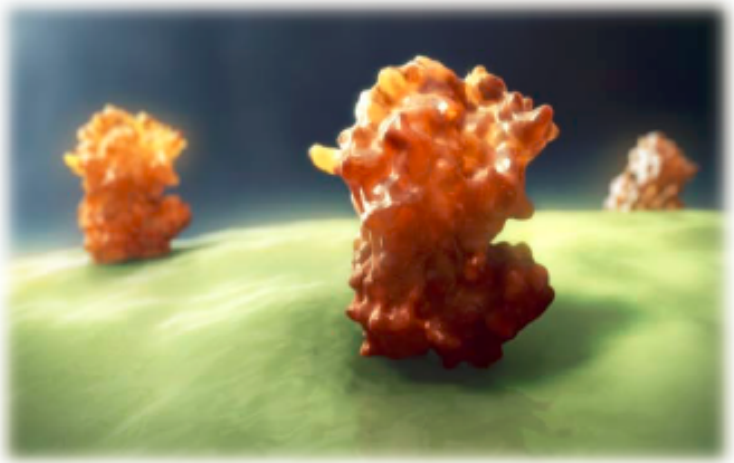


Activation of the complement system Complement-dependent cytotoxicity
(CDC)

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

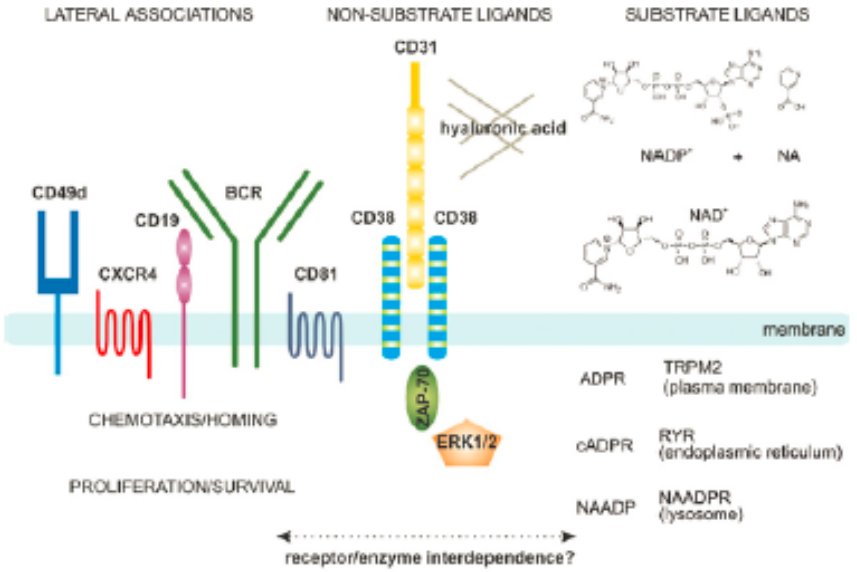
van de Donk NW et al. Blood 2016;127:681-95

CD38, cell surface receptor and an ectoenzyme, is a rational therapeutic target for treatment of myeloma



- Type II transmembrane protein (m.w. ≈45 kDa)
- Highly and uniformly expressed on myeloma cells
  - CD38 present on CD4, CD8, NK cells and B lymphocytes at a relatively low level
  - Also some CD38 expression on tissues of non-hematopoietic origin

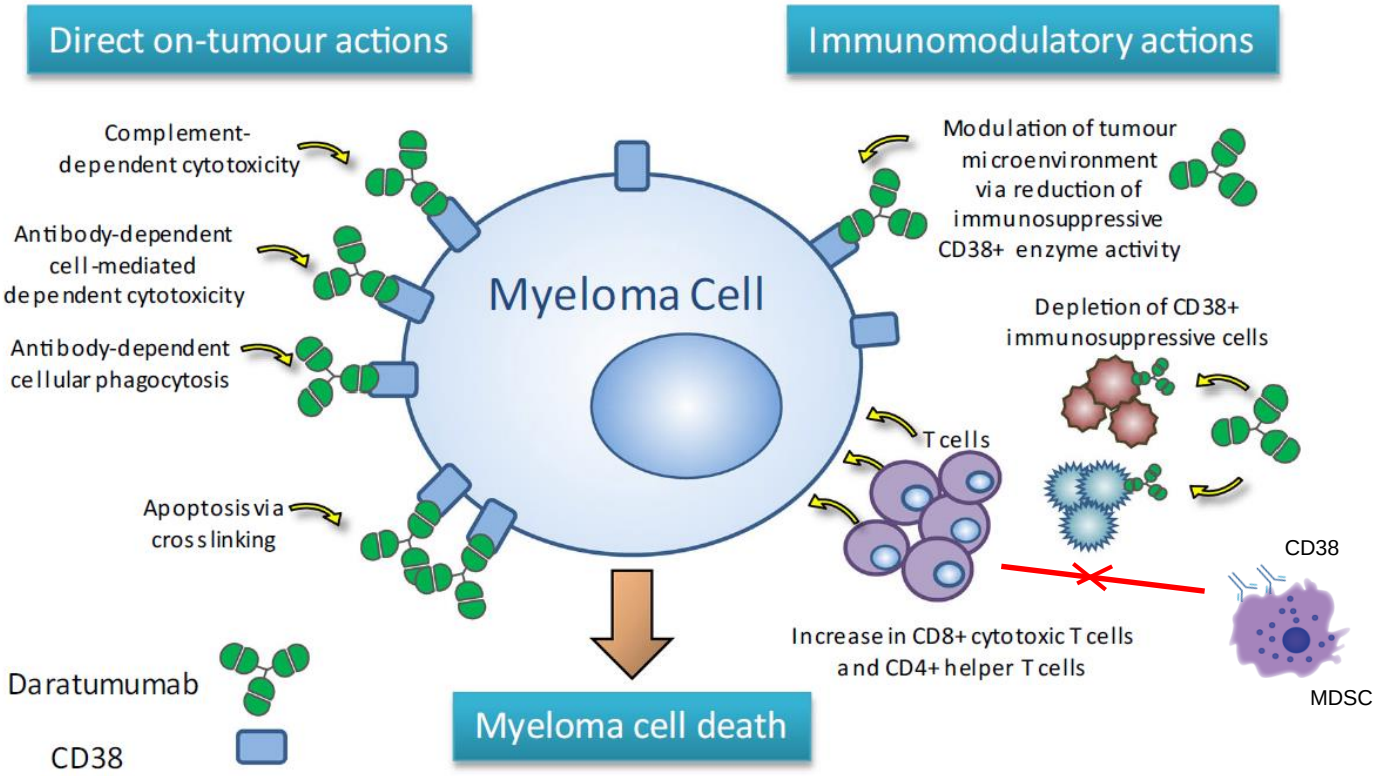
- CD38 has several intracellular functions
  1. Regulates signaling, homing and adhesion in close contact with BCR complex and CXCR4
  2. Regulates activation and proliferation of human T lymphocytes
  3. As an ectoenzyme, CD38 interacts with NAD+ and NADP+, which are converted to cADPR, ADPR, and NAADP in intracellular Ca2+-mobilization



Malavasi et al Blood 2011, 118:3470-3478

Daratumumab – 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.



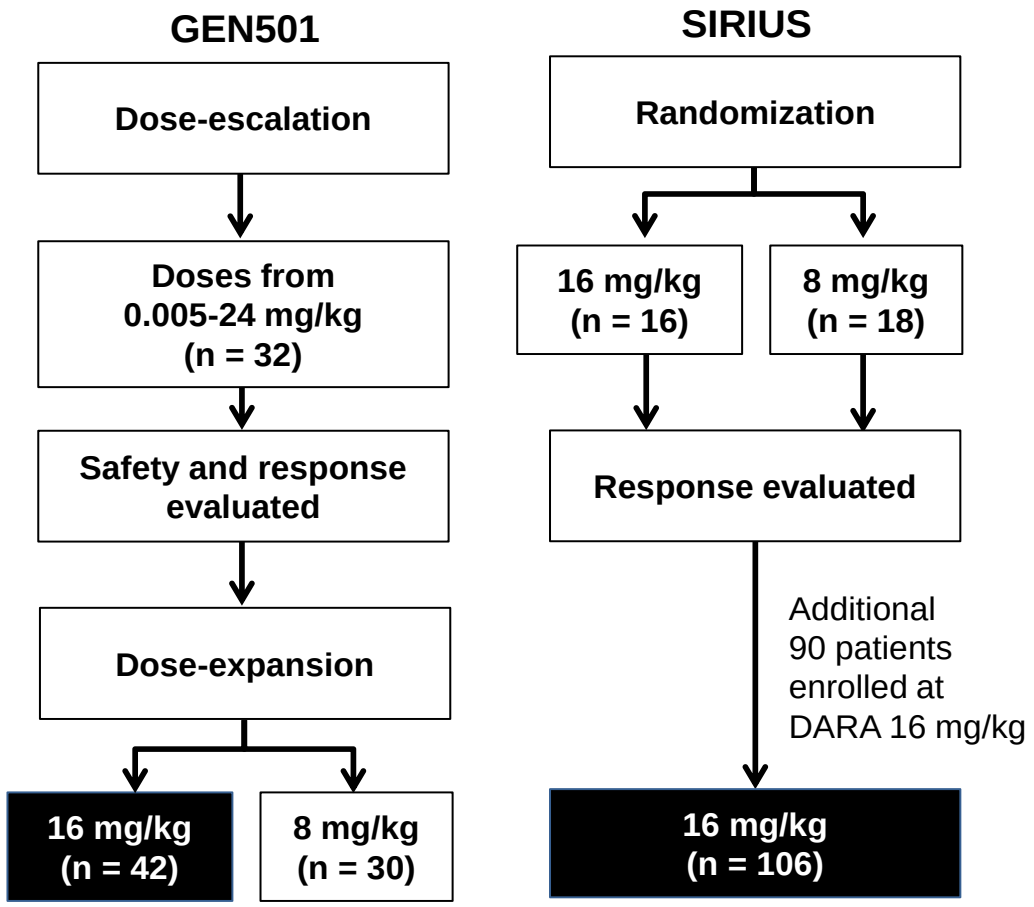
By combining direct on-tumor actions of traditional antibody therapy with systemic modulation of the immune system, daratumumab provides a multifaceted approach.

MDSC: myeloid-derived suppressor cell

McKeage et al- Drugs Ther Perspect DOI 10.1007/s40267-016-0346-x

Daratumumab Monotherapy GEN501 and *Sirius* study - Pooled analysis

- **Daratumumab as single agent** approved by FDA and EMA in Relapsed/Refractory Multiple Myeloma
- Treated patients (total 149) received **a median of 5 prior lines of therapy 86%** of patients were double refractory to PI and IMiDs; **39%** and **55%** of patients were refractory to Karfilzomib and Pomalidomide



16 mg/kg
N = 148

Usmani, SZ. Blood. 2016. http://dx.doi.org/10.1182/blood-2016-03-705210.

Daratumumab Monotherapy

Efficacy in Combined Analysis

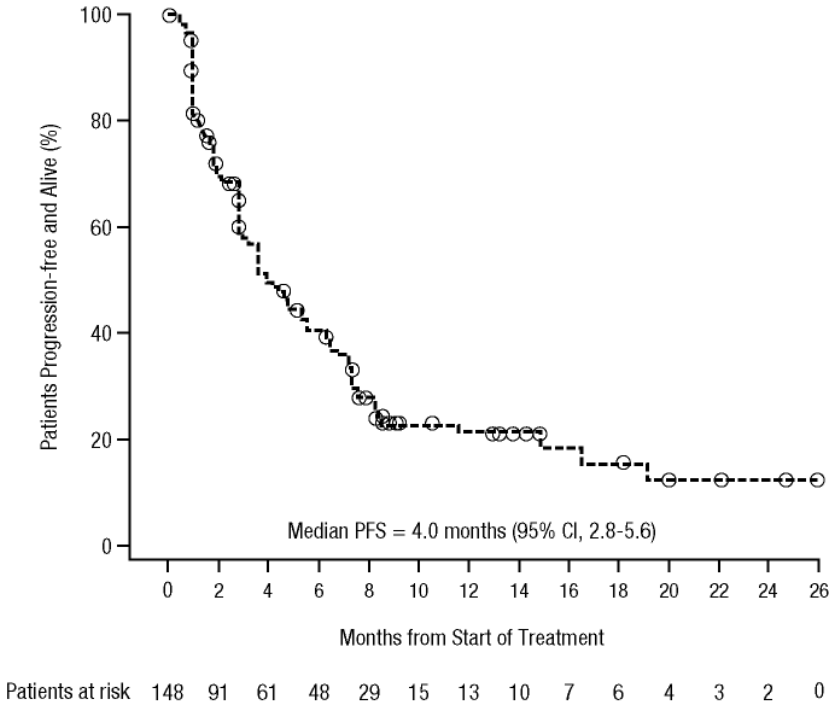
	16 mg/kg (N = 148)	
Response	n (%)	95% CI
ORR	46 (31.1)	23.7-39.2
Clinical benefit (ORR + MR)	55 (37.2)	29.4-45.5
VGPR or better (sCR+CR+VGPR)	20 (13.5)	8.5-20.1
CR or better (sCR+CR)	7 (4.7)	1.9-9.5
sCR	3 (2.0)	0.4-5.8
CR	4 (2.7)	0.7-6.8
VGPR	13 (8.8)	4.8-14.6
PR	26 (17.6)	11.8-24.7
MR	9 (6.1)	2.8-11.2
SD	68 (45.9)	37.7-54.3
PD	18 (12.2)	7.4-18.5
NE	7 (4.7)	1.9-9.5

CI, confidence interval; ORR, overall response rate; MR, minimal response; VGPR, very good partial response; CR, complete response; sCR, stringent complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluable.

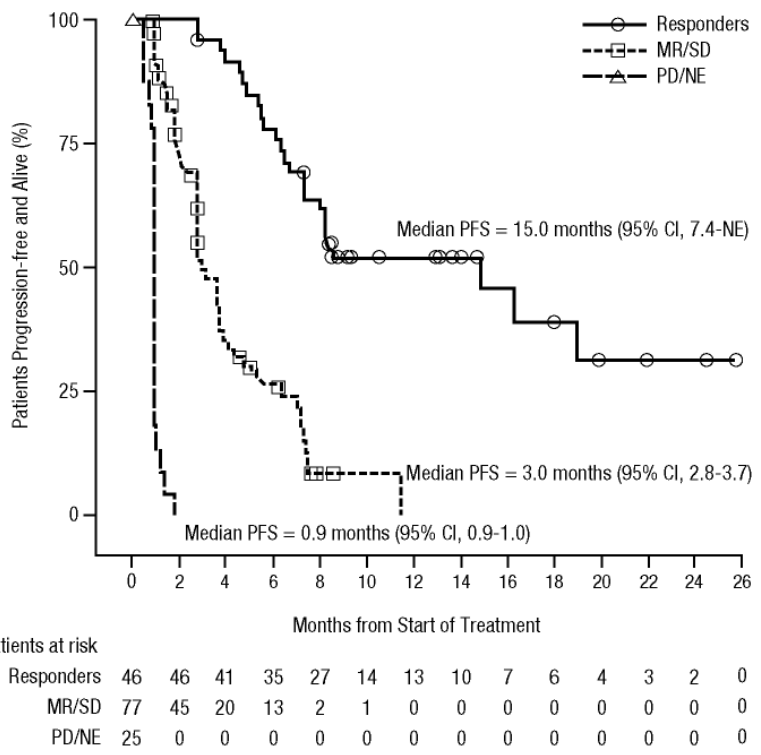
- The **ORR** for the combined dataset was **31.1%** (95% CI, 23.7%-39.2%)
- Median (range) **TTR: 0.95** (0.5-5.6) months
- Median **DOR = 7.6** (95% CI, 5.6-NE) months; responses deepened with continued treatment (7/10 PR → VGPR; 3 PR → CR - 1 patient - sCR - 2 patients)
- Responses were seen across all subgroups (regardless of prior lines of therapy, refractory status, renal function, baseline % of pc in the bone marrow

Usmani S, et al. Clinical Efficacy of Daratumumab Monotherapy in Patients With Heavily Pretreated Relapsed or Refractory Multiple Myeloma – Blood Prepublished online May 23, 2016

Daratumumab Monotherapy – PFS



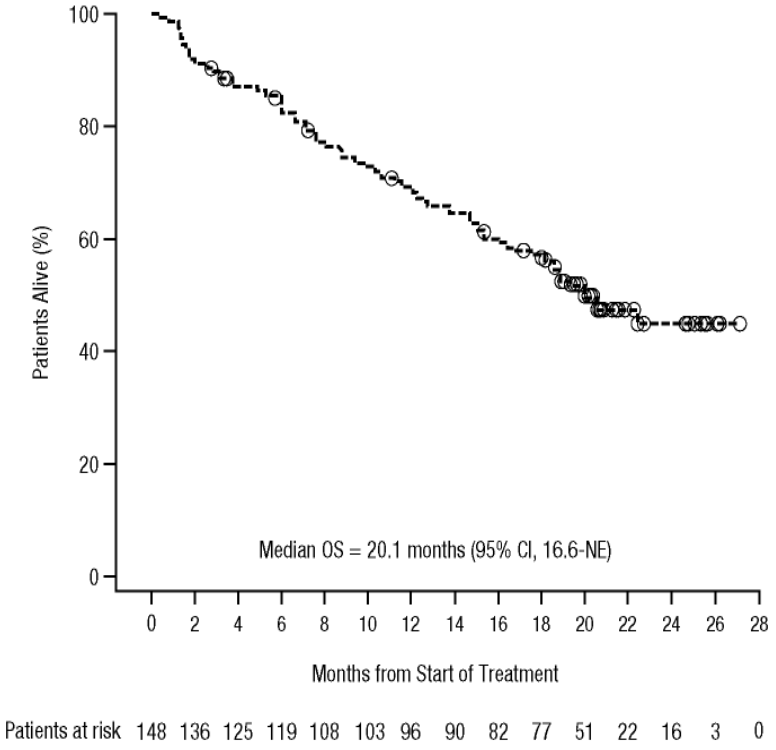
- After a **median follow-up of 20.7 months** (0.5-27.1 months), the **median PFS** was **4.0 months** (95% CI, 2.8-5.6 months)
- Overall, 12-month PFS rate was 21.6% (95% CI, 14.4%-29.8%)



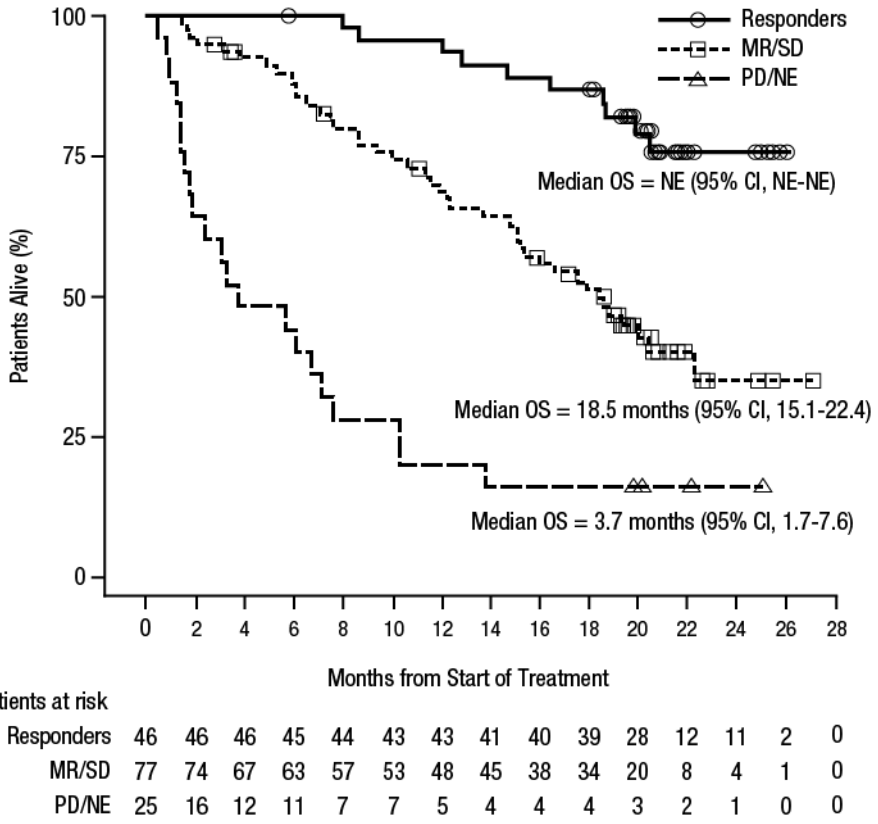
- **Median PFS** for **≥PR** vs MR/SD vs PD/NE (**15.0 months** [95% CI, 7.4-NE months] vs 3.0 months [95% CI, 2.8-3.7 months] vs 0.9 months [95% CI, 0.9-1.0 months])

Usmani, SZ. Blood. 2016. http://dx.doi.org/10.1182/blood-2016-03-705210.

Daratumumab Monotherapy – OS



- The **median OS** (combined study) **20.1 months** (95% CI, 16.6-NE months)
- The **18-month** and **24-month OS** rates **56.5%** (95% CI, 47.9%-64.2%) and **45.0%** (95% CI, 35.5%-54.1%)



Patients at risk

Responders	46	46	46	45	44	43	43	41	40	39	28	12	11	2	0
MR/SD	77	74	67	63	57	53	48	45	38	34	20	8	4	1	0
PD/NE	25	16	12	11	7	7	5	4	4	4	3	2	1	0	0

- Median OS for **≥PR** vs MR/SD vs PD/NE (**NE months** [95% CI,NE -NE] vs **18.5** [95% CI,15.1-22.4] vs 3.7 [95% CI, 1.7-7.6 months])

Usmani S, et al. Clinical Efficacy of Daratumumab Monotherapy in Patients With Heavily Pretreated Relapsed or Refractory Multiple Myeloma – Blood Prepublished online May 23, 2016

Daratumumab Monotherapy

Incidence and Severity of Most Common (≥20%) TEAEs

	16 mg/kg N = 148		
Event, n (%)	All grades	Grade ≥3	Grade 4
Fatigue	62 (41.9)	3 (2.0)	0
Nausea	44 (29.7)	0	0
Anemia	42 (28.4)	26 (17.6)	0
Back pain	40 (27.0)	4 (2.7)	0
Cough	38 (25.7)	0	0
Thrombocytopenia	32 (21.6)	13 (8.8)	8 (5.4)
Upper respiratory tract infection	32 (21.6)	1 (0.7)	0
Neutropenia	31 (20.9)	11 (7.4)	4 (2.7)

TEAE, treatment-emergent adverse event.

- AEs were consistent with the individual GEN501 and SIRIUS studies; no new safety signals were identified

Usmani, SZ. Blood. 2016. http://dx.doi.org/10.1182/blood-2016-03-705210.

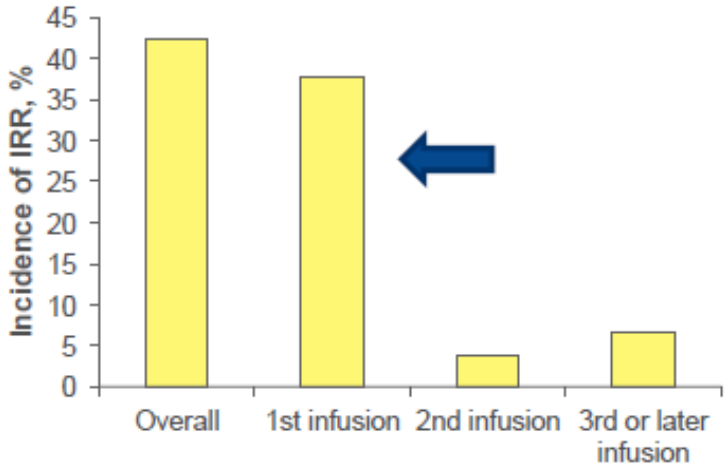
Infusion related reactions (IRRs) ≥ 5%

	16 mg/kg N = 148	
Event, n (%)	**All grades**	**Grade ≥3**
Nasal congestion	17 (11.5)	0
Cough	12 (8.1)	0
Rhinitis allergic	10 (6.8)	0
Chills	10 (6.8)	0
Throat irritation	9 (6.1)	0
Dyspnea	8 (5.4)	1 (0.7)
Nausea	8 (5.4)	0

IRR, infusion-related reaction.

IRRs were observed in 48% of patients and those observed in ≥ 5% of patients were mainly respiratory conditions

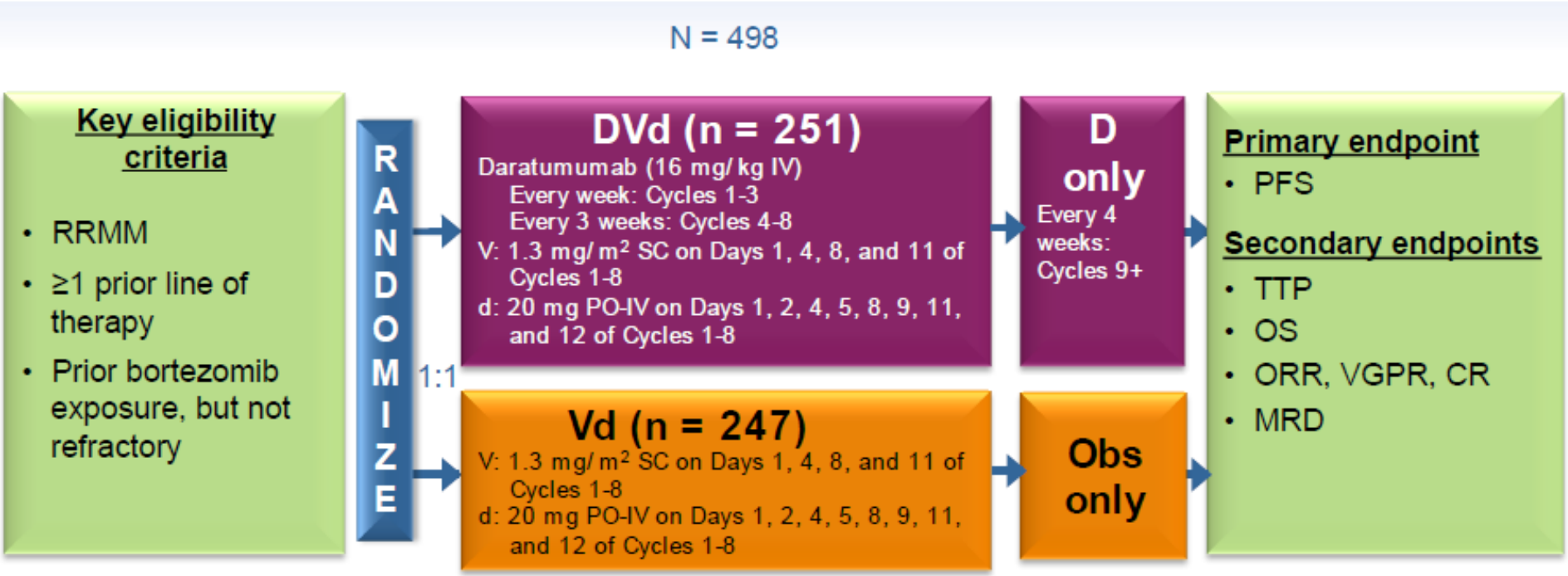
- **4 (2.7%) patients had grade ≥3 IRRs** (bronchospasm [n = 2]; dyspnea, hypoxia, and hypertension [n = 1 each])
- **95.8% of IRRs were observed during the first infusion** and the incidence of IRRs decreased during the second (7.0%) and subsequent (7.0%) infusions
- **IRRs were managed** with pre- and post-infusion medications, (antihistamines, corticosteroids, and paracetamol/acetaminophen)
- Supportive care treatment with **G-CSF** was required by 12 patients **(8.1%)**
- **46 (31.1%) patients received transfusions** during the study: red blood cell and platelet transfusions received by 44 (29.7%) and 14 (9.5%) of patients, respectively, **without any AE related to hemolysis**.
- **No patients discontinued** treatment due to IRRs (in MMY2002 SIRIUS study)



Usmani, SZ. Blood. 2016. http://dx.doi.org/10.1182/blood-2016-03-705210.

Daratumumab
Combination therapy

VD plus DARATUMUMAB in relapsed myeloma
Results of CASTOR phase 3 trial



Stratification factors

- ISS (I, II, and III)
- Number of prior lines (1 vs 2 or 3 vs >3)
- Prior bortezomib (no vs yes)

- Cycles 1-8: repeat every 21 days
- Cycles 9+: repeat every 28 days

Statistical analyses

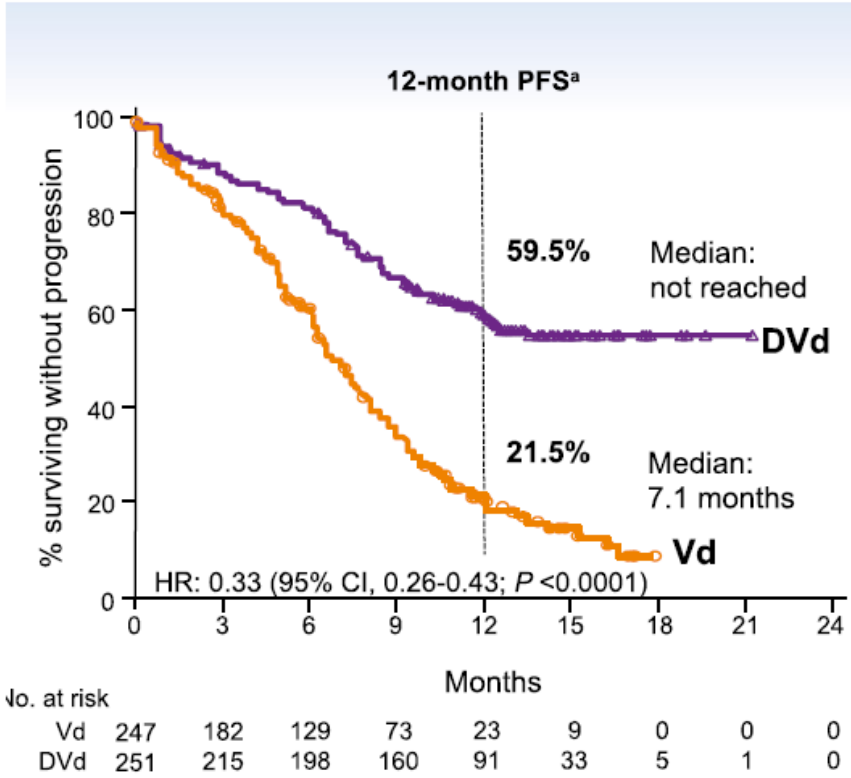
- Planned to enroll 480 patients
- Primary analysis: ~177 PFS events

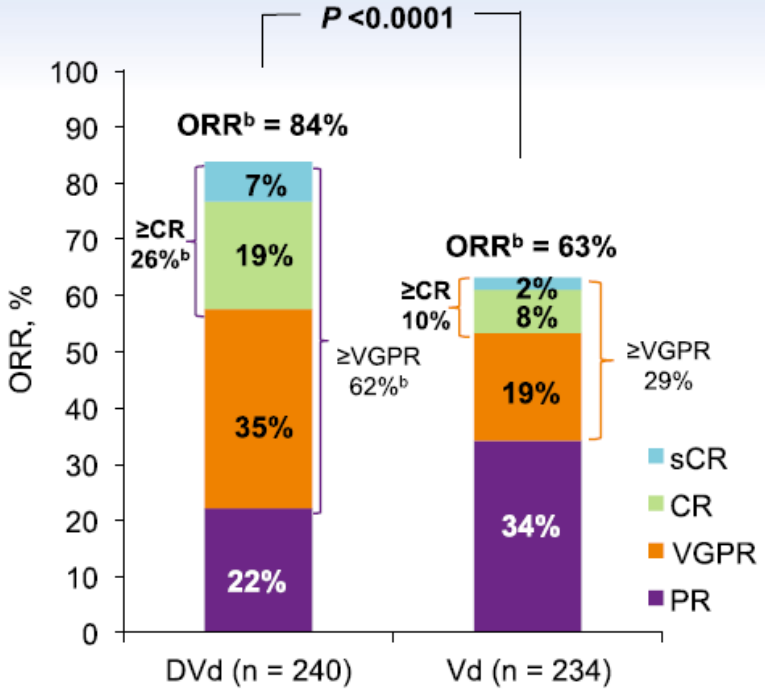
Premedication for the DVd treatment group consisted of dexamethasone 20 mg, acetaminophen, and an antihistamine

Palumbo, NEJM 2016
Mateos, ASH 2016

VD plus DARATUMUMAB in relapsed myeloma
Results of CASTOR phase 3 trial

UPDATED EFFICACY



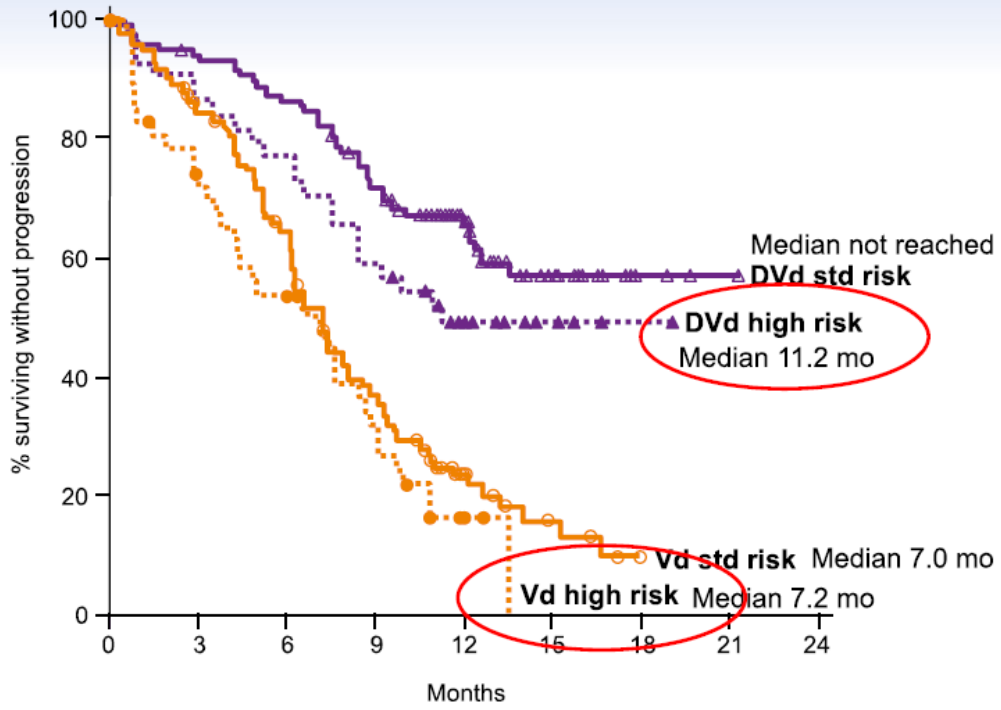


- Median (range) follow-up: 13.0 (0-21.3) months
- Responses continue to deepen in the DVd group with longer follow-up
  - An additional 7% achieved ≥CR with longer follow-up

Palumbo, NEJM 2016
Mateos, ASH 2016

VD plus DARATUMUMAB in relapsed myeloma
Results of CASTOR phase 3 trial

PFS BY CYTOGENETICS



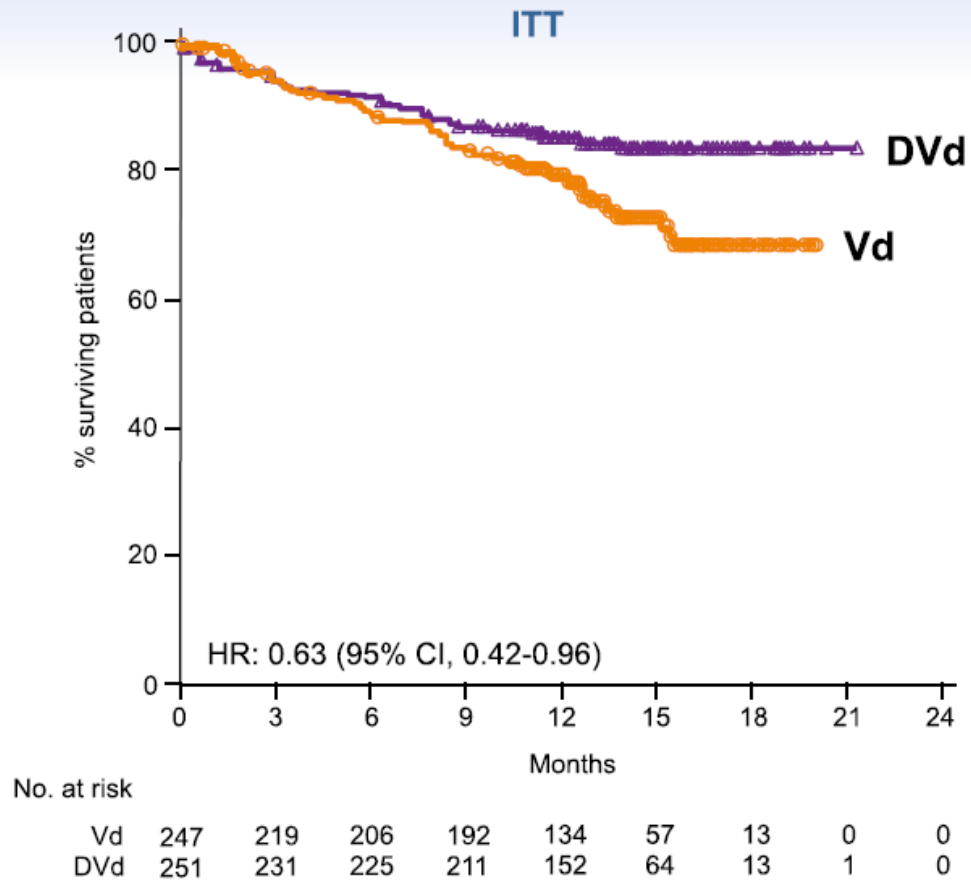
High risk[b]	DVd n = 44	Vd n = 51
Median PFS, mo	**11.2**	**7.2**
HR (95% CI)	0.49 (0.27-0.89)	
P value	0.0167	

Standard risk	DVd n = 123	Vd n = 135
Median PFS, mo	**NR**	**7.0**
HR (95% CI)	0.29 (0.20-0.43)	
P value	<0.0001	

Palumbo, NEJM 2016
Mateos, ASH 2016

VD plus DARATUMUMAB in relapsed myeloma
Results of CASTOR phase 3 trial

OVERALL SURVIVAL



- OS events
  - 37 (15%) in DVd
  - 58 (24%) in Vd
- Curves are beginning to separate, but OS data are immature
- OS HR for DVd versus Vd by prior lines:
  - 1 prior line = HR: 0.42 (95% CI, 0.19-0.93)
  - 1-3 prior line = HR: 0.54 (95% CI, 0.34-0.84)

Palumbo, NEJM 2016
Mateos, ASH 2016

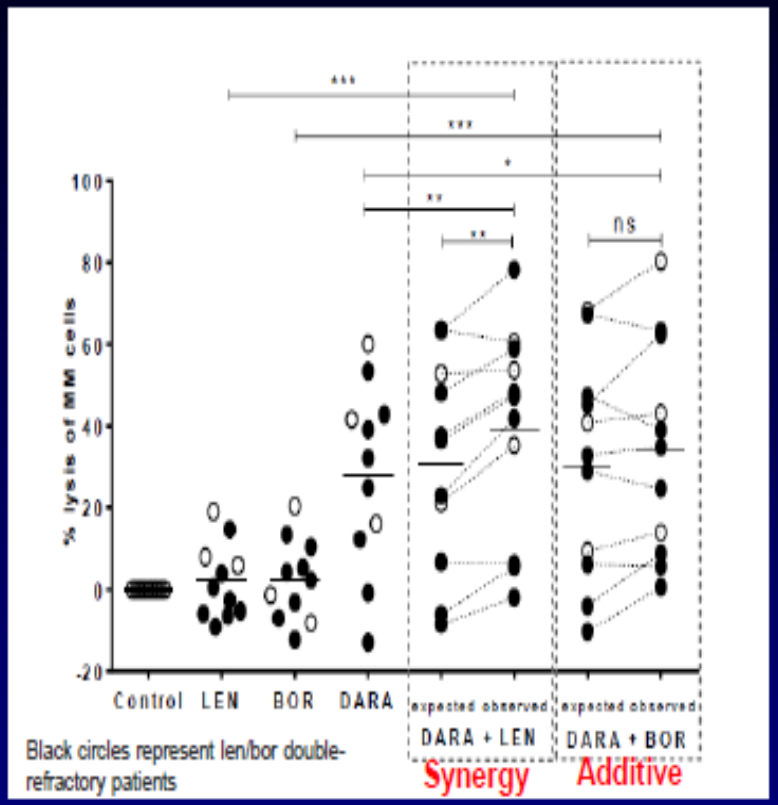
Most Common TEAEs: Updated Analysis

	DVd (n = 243)		Vd (n = 237)	
Hematologic, n (%)	All-grade ≥25%[a]	Grade 3/4 ≥5%[a]	All-grade ≥25%[a]	Grade 3/4 ≥5%[a]
Thrombocytopenia	145 (60)	110 (45)	105 (44)	78 (33)
Anemia	67 (28)	36 (15)	75 (32)	38 (16)
Neutropenia	45 (19)	32 (13)	23 (10)	11 (5)
Lymphopenia	32 (13)	24 (10)	9 (4)	6 (3)
Nonhematologic, n (%)				
Peripheral sensory neuropathy	120 (49)	11 (5)	90 (38)	16 (7)
Diarrhea	83 (34)	9 (4)	53 (22)	3 (1)
Upper respiratory tract infection	72 (30)	6 (3)	43 (18)	1 (0.4)
Cough	66 (27)	0	30 (13)	0
Fatigue	53 (22)	12 (5)	58 (25)	8 (3)
Pneumonia	33 (14)	22 (9)	28 (12)	23 (10)
Hypertension	22 (9)	16 (7)	8 (3)	2 (0.8)

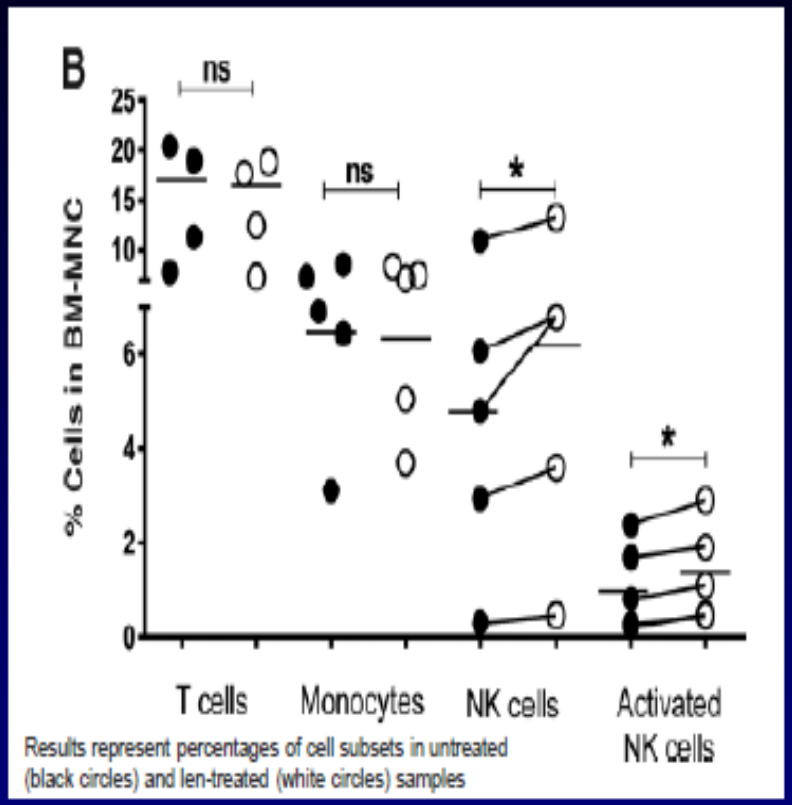
- Grade 3/4 TEAEs: 79% of DVd patients versus 63% of Vd patients
- Discontinuations due to TEAEs: 9% of DVd patients versus 9% of Vd patients[b]
- No new IRRs; incidence remains stable with longer follow up (45%)

TEAE, treatment-emergent adverse event; IRR, infusion-related reaction.
[a]Common TEAEs listed are either ≥25% all grade OR ≥5% grade 3/4.
[b]Vd arm treated for 8 cycles and DVd arm treated until progressive disease, per protocol.

Is there any rationale for the combination with PIs/IMiDs?



Black circles represent len/bor double-refractory patients



Results represent percentages of cell subsets in untreated (black circles) and len-treated (white circles) samples

- Len and bor significantly improves DARA-mediated lysis of primary MM cells from len and bor-refractory patients
- Synergy between lenalidomide and daratumumab appeared to be due to the action of lenalidomide on the effector cells present in bone marrow mononuclear cells and not via direct effects on the tumor cells

*P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant

Nijhof et al. Clin Cancer Res. 2015;21(12):2802-10.

RD plus DARATUMUMAB in relapsed myeloma
Results of POLLUX phase 3 trial

Multicenter, randomized (1:1), open-label, active-controlled, phase 3 study

Key eligibility criteria

- RRMM
- ≥1 prior line of therapy
- Prior lenalidomide exposure, but not refractory
- Creatinine clearance ≥30 mL/min

Stratification factors

- No. of prior lines of therapy
- ISS stage at study entry
- Prior lenalidomide

RANDOMIZE 1:1

DRd (n = 286)

Daratumumab 16 mg/kg IV
- Qw in Cycles 1 to 2, q2w in Cycles 3 to 6, then q4w until PD

R 25 mg PO
- Days 1 to 21 of each cycle until PD

d 40 mg PO
- 40 mg weekly until PD

Rd (n = 283)

R 25 mg PO
- Days 1 to 21 of each cycle until PD

d 40 mg PO
- 40 mg weekly until PD

Cycles: 28 days

Primary endpoint

- PFS

Secondary endpoints

- TTP
- OS
- ORR, VGPR, CR
- MRD
- Time to response
- Duration of response

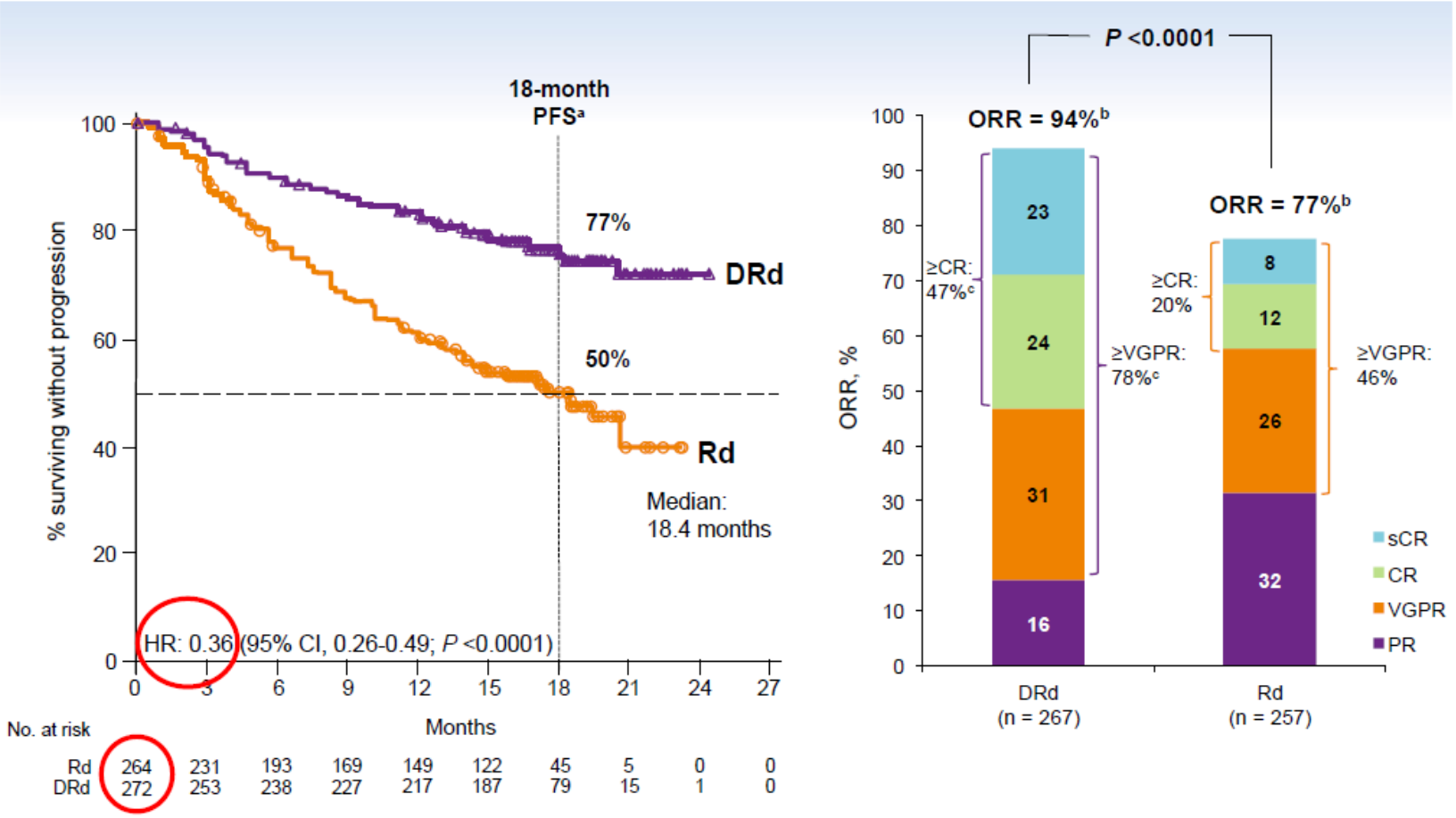
Statistical analyses

- Primary analysis: ~177 PFS events

Dimopoulos, NEJM 2016
Moreau, ASH 2016

RD plus DARATUMUMAB in relapsed myeloma
Results of POLLUX phase 3 trial

UPDATED EFFICACY

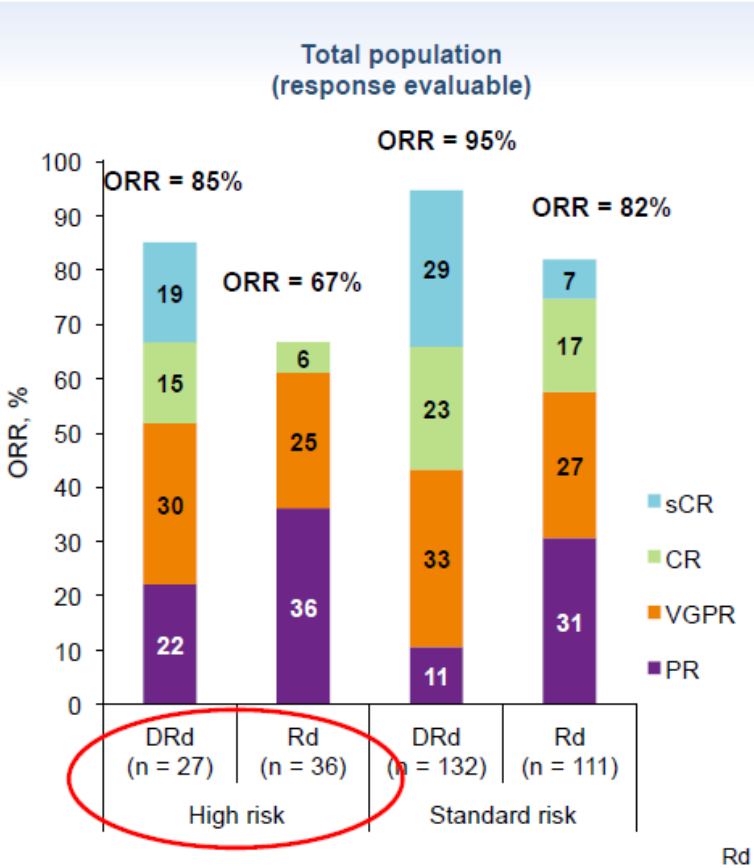


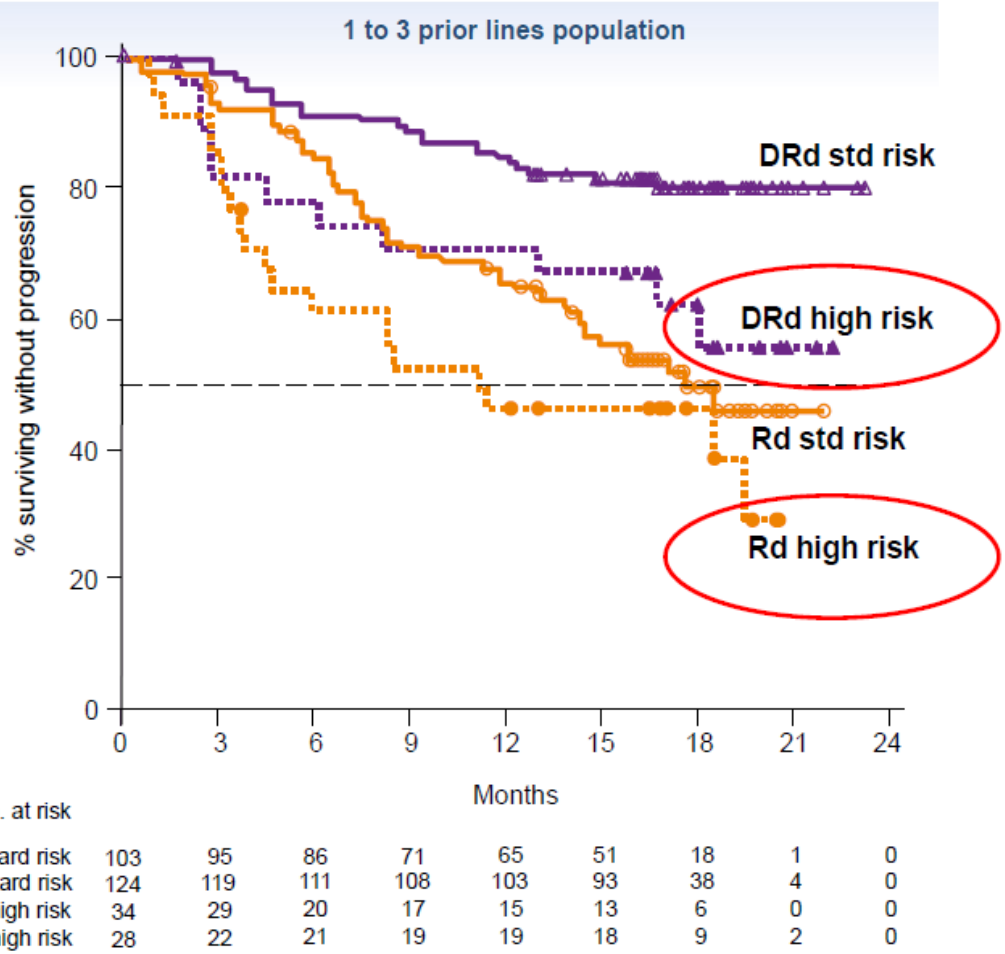
Responses continue to deepen in the DRd group with longer follow-up

Dimopoulos, NEJM 2016
Moreau, ASH 2016

RD plus DARATUMUMAB in relapsed myeloma
Results of POLLUX phase 3 trial

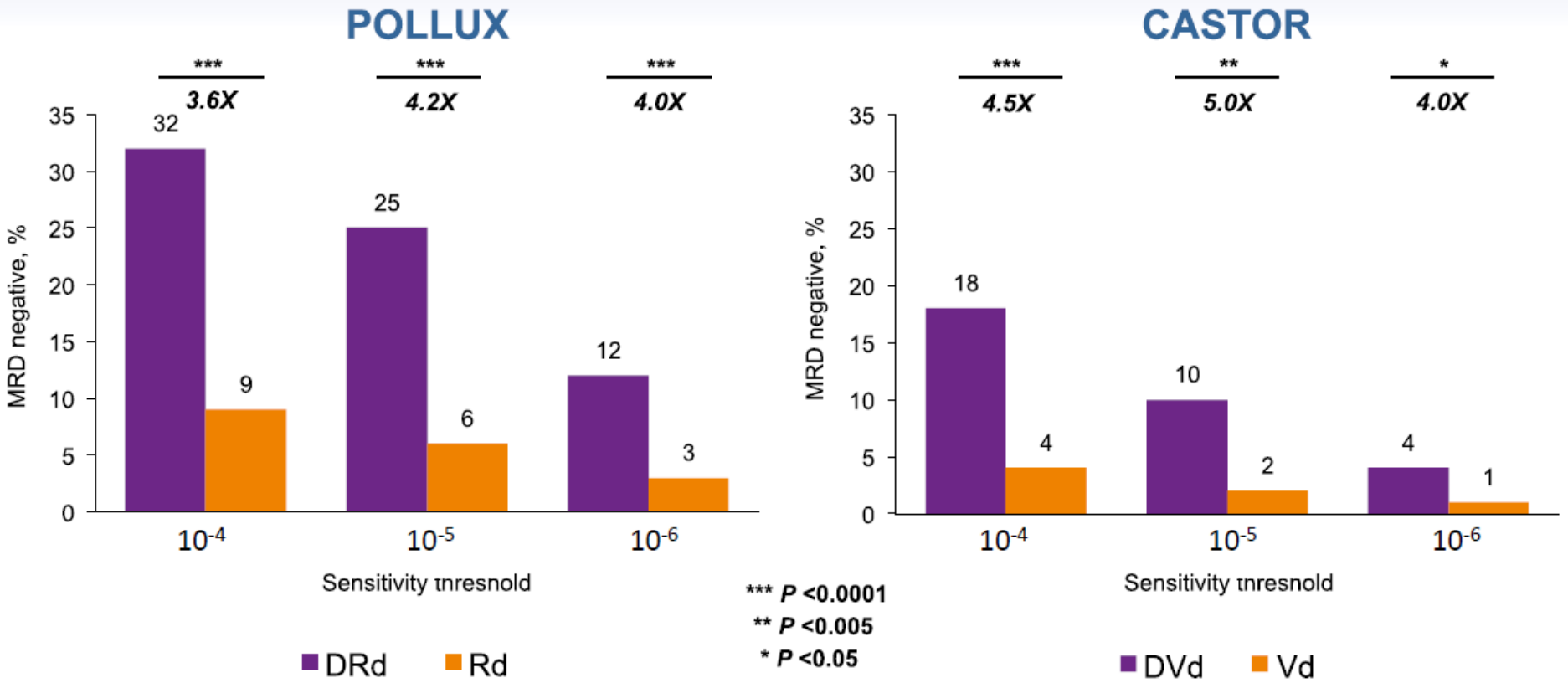
RESPONSE AND PFS BY CYTOGENETICS





Dimopoulos, NEJM 2016
Moreau, ASH 2016

CASTOR and POLLUX phase 3 trials
Impact of daratumumab on MRD assessed by NGS

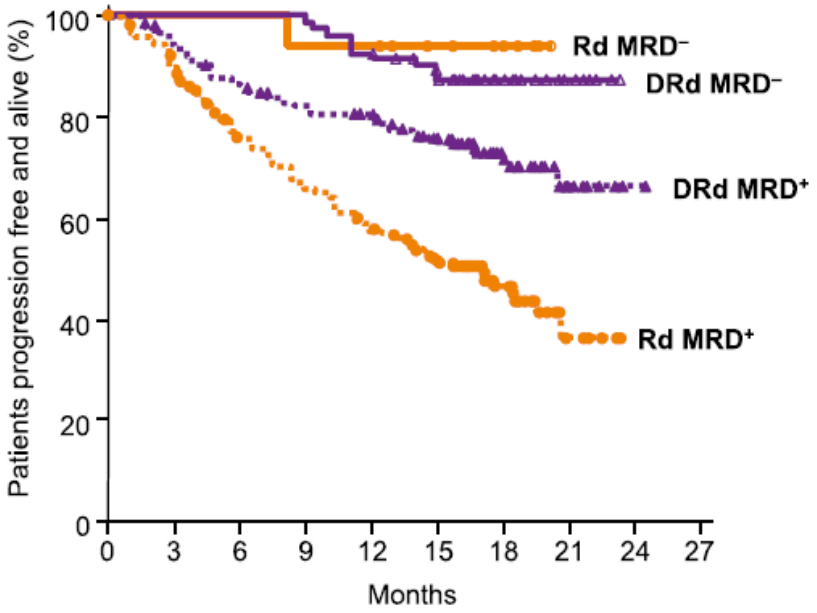


The proportion of patients with MRD negative was found to be 4 times higher in the daratumumab arm in CASTOR and POLLUX trials

Avet-Loiseau, ASH 2016

CASTOR and POLLUX phase 3 trials
Impact of daratumumab on MRD assessed by NGS

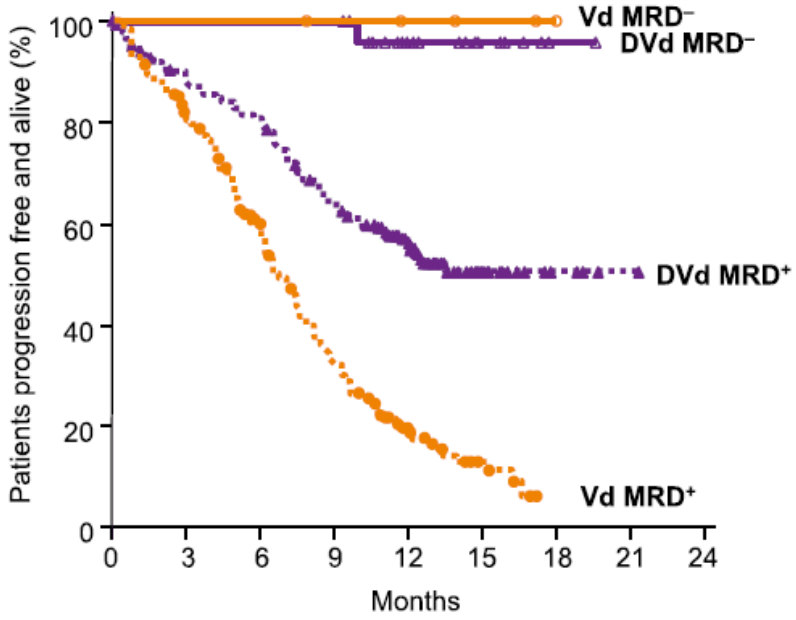
POLLUX



Patients at risk

Rd MRD negative	16	16	16	15	15	12	10	0	0	0
DRd MRD negative	71	71	71	70	66	57	28	6	0	0
Rd MRD positive	267	233	190	166	144	120	38	5	0	0
DRd MRD positive	215	195	178	167	161	137	54	9	1	0

CASTOR



Patients at risk

Vd MRD negative	6	6	6	5	3	2	0	0	0
DVd MRD negative	26	26	26	26	15	7	1	0	0
Vd MRD positive	241	176	123	68	20	7	0	0	0
DVd MRD positive	225	189	172	134	76	26	4	1	0

- Impressive PFS in MRD-negative patients (18 months PFS > 80%)

- PFS benefit in MRD-positive patients who reveived dara versus control arm

Avet-Loiseau, ASH 2016

Most Common AEs

	DRd (n = 283)		Rd (n = 281)	
Hematologic AEs	All-grade (%) ≥25%	Grade 3/4 (%) ≥5%	All-grade (%) ≥25%	Grade 3/4 (%) ≥5%
Neutropenia	**59**	**52**	**43**	**37**
Febrile neutropenia	**6**	**6**	**3**	**3**
Anemia	31	12	35	20
Thrombocytopenia	27	13	27	14
Lymphopenia	6	5	5	4
Nonhematologic AEs				
Diarrhea	43	5	25	3
Fatigue	35	6	28	3
Upper respiratory tract infection	32	1	21	1
Constipation	29	1	25	0.7
Cough	29	0	13	0
Muscle spasms	26	0.7	19	2
Pneumonia	14	8	13	8

Infections and infestations:

- Grade 3 or 4: 28% patients in DRd vs 23% patients in Rd
- The most common grade 3 or 4 infections/infestations AE was pneumonia (8% vs 8%)
- Incidence of IRRs reported as in the other trials

Prevention of IRR

- Administer Pre-medication 1 hour prior every Dara Infusion
  - Intravenous Corticosteroid (Methylprednisolone 100 mg or equivalent long acting steroid)
  - Oral anti pyretic (paracetamol 1000 mg)
  - Oral or Intravenous antihstamine (dyphenidramine 25 – 50 mg or equivalent)
- **Post Medication Corticosteroid on 1° and 2 nd day after all Dara infusions**

	Dilution volume	Initial rate (first hour)	Rate increment	Maximum rate
First infusion	1000 mL	50 mL/hour	50 mL/hour every hour	200 mL/hour
Second infusion[a]	500 mL	50 mL/hour	50 mL/hour every hour	200 mL/hour
Subsequent infusions[b]	500 mL	100 mL/hour	50 mL/hour every hour	200 mL/hour

[a] Escalate only if there were no Grade 1 (mild) or greater infusion reactions during the first 3 hours of the first infusion.

[b] Escalate only if there were no Grade 1 (mild) or greater infusion reactions during a final infusion rate of ≥100 mL/hr in the first two infusions.

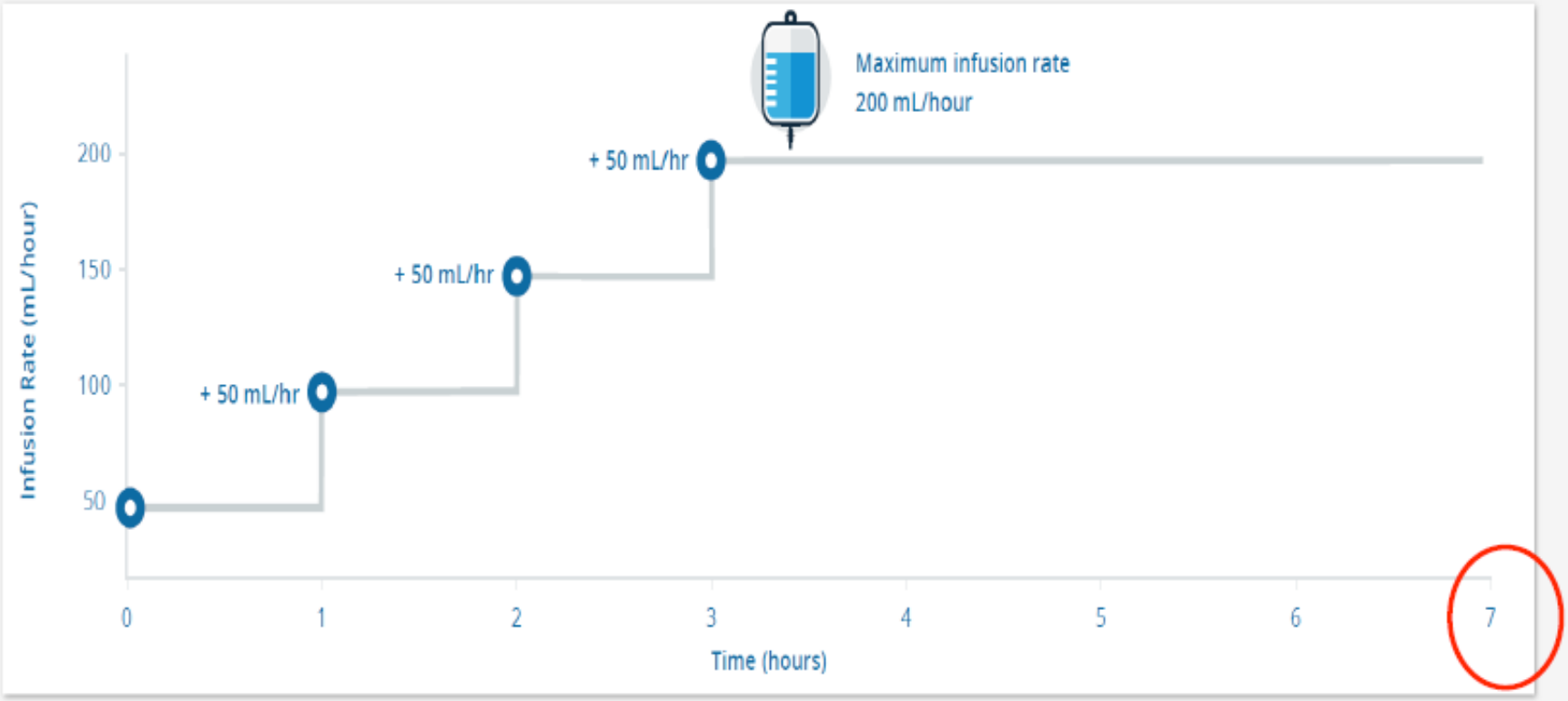
First infusion

Dilution volume
1,000 mL

Initial infusion rate (first hour)
50 mL/hour

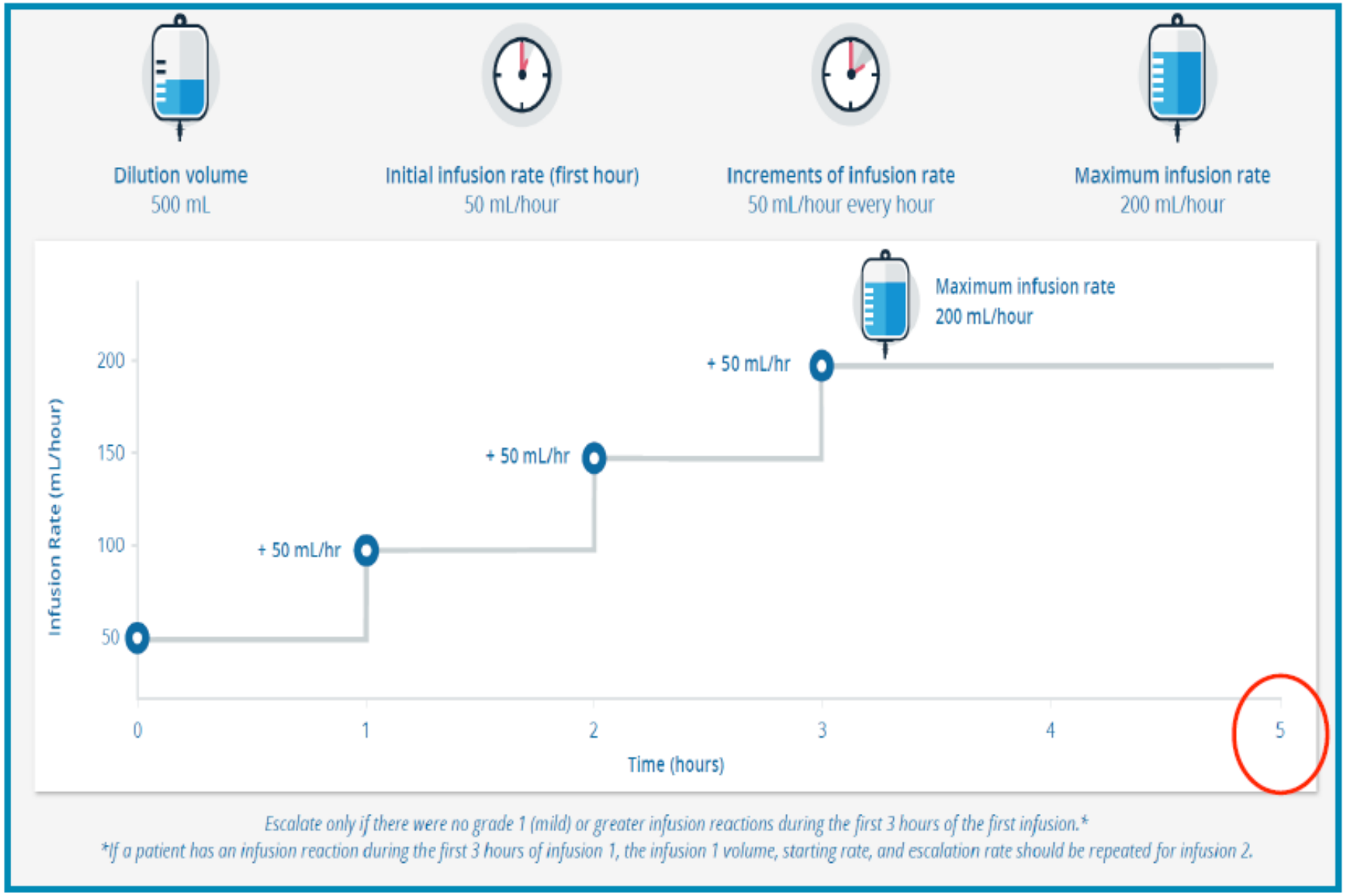
Increments of infusion rate
50 mL/hour every hour

Maximum infusion rate
200 mL/hour



**If a patient has an infusion reaction during the first 3 hours of infusion 1, the infusion 1 volume, starting rate, and escalation rate should be repeated for infusion [2].*

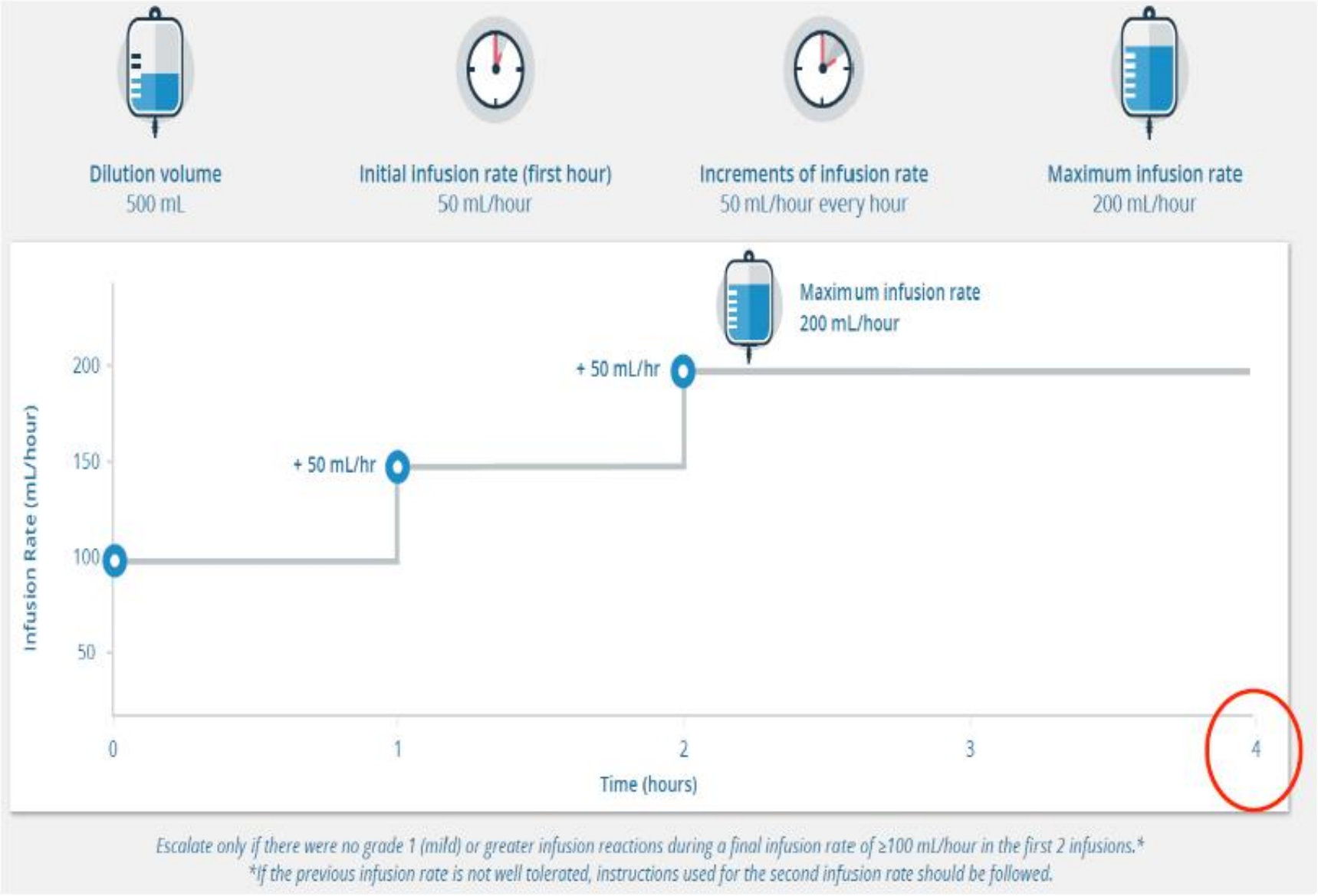
Second infusion



*Escalate only if there were no grade 1 (mild) or greater infusion reactions during the first 3 hours of the first infusion.**

**If a patient has an infusion reaction during the first 3 hours of infusion 1, the infusion 1 volume, starting rate, and escalation rate should be repeated for infusion 2.*

Subsequent infusions



*Escalate only if there were no grade 1 (mild) or greater infusion reactions during a final infusion rate of ≥100 mL/hour in the first 2 infusions.**

**If the previous infusion rate is not well tolerated, instructions used for the second infusion rate should be followed.*

I pazienti devono ricevere una adeguata pre-medicazione per ridurre il rischio di IRRs

Medicazione pre-infusione

Durante i giorni di infusione di datatumumab, i pazienti riceveranno la seguente pre-medicazione prima dell'infusione:

- Acetaminofene (paracetamolo) 650-1000 mg orale (PO) circa 1 ora prima dell'infusione
- Un antistaminico (difenidramina 25-50 mg IV o PO,o equivalente)
- Metilprednisolone 100 mg IV per la prima e seconda infusione di daratumumab; a partire dalla terza infusione il metilprednisolone può essere ridotto a 60 mg IV

Approssimativamente 1 ora prima di ogni infusione di Daratumumab la pre-medicazione dovrebbe essere somministrata a tutti i pazienti

Corticosteroide per via intravenosa

Antipiretico orale

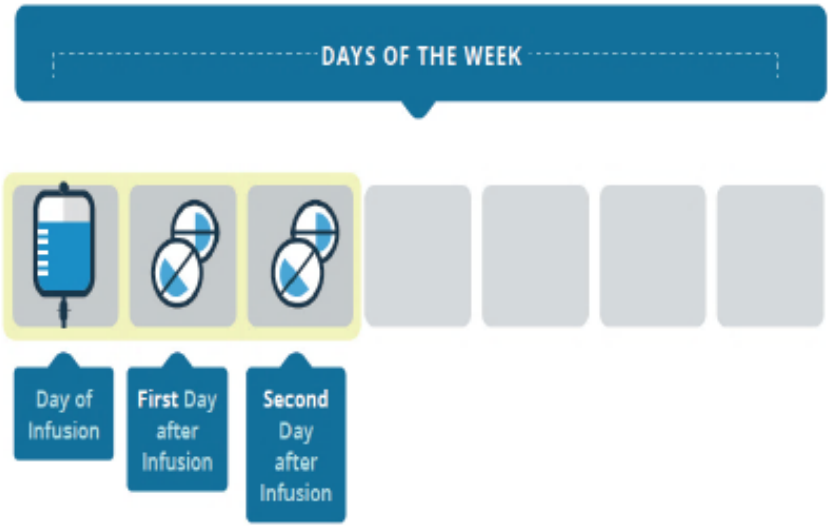
Antistaminico orale o per via intravenosa

EMEA SmPc: Daratumumab US Prescribing Information, last accessed April 2016.

I pazienti devono ricevere anche una adeguata medicazione post trattamento per ridurre il rischio di IRRs

Medicazione post-infusione

- Durante ciascuno dei due giorni seguenti tutte le infusioni di Daratumumab (iniziando il giorno dopo l'infusione) i pazienti riceveranno Metilprednisolone 20 mg PO
- In pazienti con una storia di malattia polmonare ostruttiva dovrebbero essere considerate medicazioni aggiuntive post-infusione comprendenti broncodilatatori e corticosteroidi inalatori.
- Dopo le prime quattro infusioni, se il paziente non ha IRR serie, questi farmaci inalatori post-infusione possono essere interrotti a discrezione del medico.
- Iniziare la profilassi antivirale per prevenire la riattivazione di herpes zoster entro 1 settimana dall'inizio del daratumumab e proseguire per 3 mesi dopo il trattamento



US PI: Daratumumab US Prescribing Information, last accessed April 2016.

Montelukast as Prevention of IRRs

- Use of Montelukast (an Inhibitor of Leucotriene Receptors) to Reduce Infusion Reactions in an Early Access Program (EAP) of Daratumumab in United States Patients With Relapsed or Refractory Multiple Myeloma:
- 10 mg of montelukast >30 min prior to the first daratumumab infusion

Table 5. Observed IRRs in Patients With and Without Montelukast Therapy

	Montelukast 10 mg as Pre-Infusion (n=50)	No Montelukast Given as Pre-Infusion (n=298)
IRR rate at first infusion	38.0%	58.5%
Respiratory symptoms	20%	32%
Gastrointestinal symptoms	4%	11%
Chills	14%	14%
Median time for first infusion (hours)	6.7	7.6

- A total of 24 subjects experienced infusion related reactions that were considered SAEs but no subject discontinued the study due to an infusion related reaction

- The observed IRR rate during the first daratumumab infusion **was one-third lower** in patients who received montelukast than in patients who did not receive it
- **Respiratory and gastrointestinal symptoms were lower** in patients who received montelukast, whereas chills were observed at a similar rate in both groups
- The **median time for the first infusion was 0.9 hours shorter** in patients who received montelukast

Adapted from: Chari et al, Abs n.2142 ASH 2016

Management of IRRs

- In case of occurrence of IRRs
  - React early to mild signs of symptoms and immediately stop the infusion
  - Manage symptoms appropriately, consider e.g. antihistamines, corticosteroids
  - Once symptoms have resolved, treatment may be resumed at half the infusion rate
  - In case of grade 4 IRRs permanently discontinue treatment

Adapted from: Protocol for: Lokhorst et al. N Engl J Med 2015 Aug 26 [Epub]

Open-label, Multicenter, Dose-escalation Phase 1b Study to Assess the Subcutaneous Delivery of Daratumumab in Patients (Pts) With Relapsed or Refractory Multiple Myeloma (PAVO)

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ClinicalTrials.gov Identifier: NCT02519452

Usmani SZ, et al. Presented at ASH 2016 (Abstract 1149), oral presentation.

PAVO: Study Design

Phase 1b, open-label, multicenter, dose-finding, proof of concept study

Key eligibility criteria
- RRMM with measurable disease
- ≥2 prior lines of treatment
- Not received anti-CD38 therapy

Group 1 (n = 8)
DARA: 1,200 mg
rHuPH20: 30,000 U

→

Group 2[a] (n = 45)
DARA: 1,800 mg
rHuPH20: 45,000 U

Primary endpoints
- C_{trough} of DARA at Cycle 3/Day 1
- Safety

Secondary endpoints
- ORR
- CR
- Duration of response
- Time to response

Dosing schedule
- Approved schedule for IV
- 1 Cycle = 28 days

Infusion time
- 1,200 mg: 20-min infusion (60 mL)
- 1,800 mg: 30-min infusion (90 mL)

Pre-[b]/post-infusion medication
- Acetaminophen, diphenhydramine, montelukast, and methylprednisolone

RRMM, relapsed or refractory multiple myeloma; QW, weekly; Q2W, every 2 weeks; Q4W, every 4 weeks; C_{trough}, trough concentration; ORR, overall response rate; CR, complete response; PK, pharmacokinetic.

[a]Group 2 comprises 4 distinct cohorts, each treated with DARA 1,800 mg and rHuPH20 45,000 U. C_{trough} on Cycle 3/Day 1 in Group 1 supported dose selection for Group 2. The study evaluation team reviewed safety after Cycle 1 and PK after Cycle 3/Day 1 for each group.

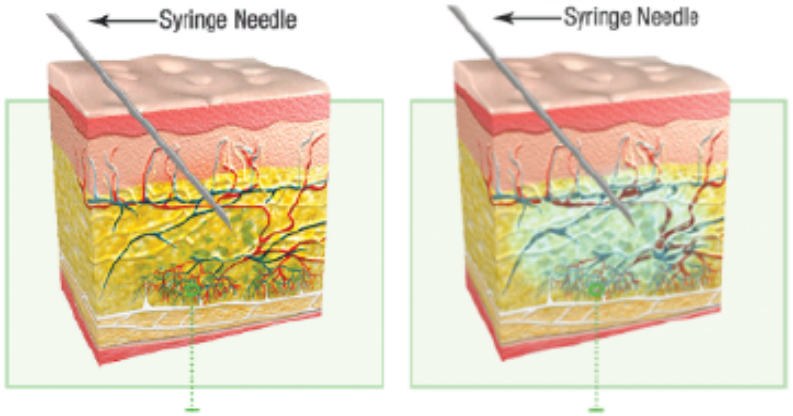
[b]Administered 1 hour prior to infusion.

Usmani SZ, et al. Presented at ASH 2016 (Abstract 1149), oral presentation.

Recombinant Human Hyaluronidase

- The ENHANZE™ platform of recombinant human hyaluronidase (rHuPH20) temporarily breaks down the hyaluronan barrier, allowing rapid absorption of injected drugs[1]
- Herceptin SC® and MabThera SC® are approved in Europe as co-formulate products with rHuPH20[2,3]
  - Dosing time is 5 to 8 minutes with subcutaneous (SC) administration versus 0.5 to 6 hours with IV[4-6]

Schematic of rHuPH20[1]

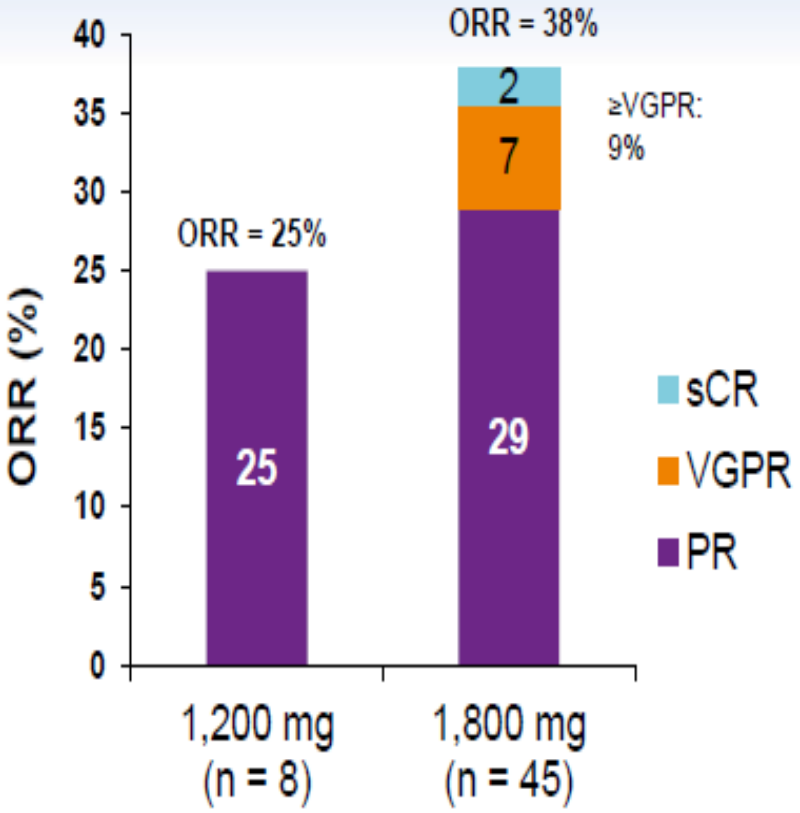


Aim: To determine the safety, pharmacokinetics, and efficacy of DARA as SC administration

1. Halozyme Therapeutics. Mechanism of action for Hylenex recombinant (hyaluronidase human injection). www.hylenex.com/mechanism-of-action. Accessed November 8, 2016.
2. European Medicines Agency. Herceptin: EPAR – product information. 2016.
3. European Medicines Agency. MabThera: EPAR – product information. 2016.
4. Ismael G, et al. *Lancet Oncology*. 2012;13(9):869-878.
5. Shpilberg O, et al. *Br J Cancer*. 2013;109(6):1556-1561.
6. De Cock E, et al. *PLoS One*. 2016;11(6):e0157957.

ORR

Response	1,200 mg n = 8	1,800 mg n = 45
ORR, % (n)	25 (2)	38 (17)
sCR	0 (0)	2 (1)
CR	0 (0)	0 (0)
VGPR	0 (0)	7 (3)
PR	25 (2)	29 (13)
MR	13 (1)	11 (5)
SD	50 (4)	38 (17)
PD	13 (1)	13 (6)



- Responses to DARA-PH20 were observed across both groups

Deeper responses were observed in the 1,800-mg group

Response-evaluable set.

sCR, stringent complete response; VGPR, very good partial response; PR, partial response; MR, minimal response; SD, stable disease; PD, progressive disease.

Usmani SZ, et al. Presented at ASH 2016 (Abstract 1149), oral presentation.

Grade 3/4 TEAEs

Grade 3/4 TEAEs (>1 patient)	1,200 mg n = 8	1,800 mg n = 45
Hematologic, % (n)		
Anemia	13 (1)	13 (6)
Thrombocytopenia	13 (1)	7 (3)
Neutropenia	13 (1)	7 (3)
Lymphopenia	0 (0)	7 (3)
Nonhematologic, % (n)		
Hypertension	25 (2)	4 (2)
Fatigue	25 (2)	2 (1)
Device-related infection	0 (0)	4 (2)
Hyponatremia	0 (0)	4 (2)

AE profile of DARA-PH20 was consistent with IV DARA

Usmani SZ, et al. Presented at ASH 2016 (Abstract 1149), oral presentation.

IRRs

	1,200 mg n = 8	1,800 mg n = 45
IRR, % (n)	13 (1)	24 (11)
Chills	13 (1)	9 (4)
Pyrexia	0 (0)	9 (4)
Pruritus	0 (0)	4 (2)
Dyspnea	13 (1)	0 (0)
Flushing	0 (0)	2 (1)
Hypertension	0 (0)	2 (1)
Hypotension	0 (0)	2 (1)
Nausea	0 (0)	2 (1)
Non-cardiac chest pain	13 (1)	0 (0)
Oropharyngeal pain	0 (0)	2 (1)
Paresthesia	0 (0)	2 (1)
Rash	0 (0)	2 (1)
Sinus headache	0 (0)	2 (1)
Tongue edema	0 (0)	2 (1)
Vomiting	0 (0)	2 (1)
Wheezing	0 (0)	2 (1)

- All IRRs in the 1,800-mg group were grade 1 or 2
- One grade 3 IRR of dyspnea in the 1,200-mg group
- No grade 4 IRRs were observed
- All IRRs occurred during or within 4 hours of the first infusion
- No IRRs occurred during subsequent infusions in either group
- Abdominal wall SC injections were well tolerated

Low IRR incidence and severity with DARA SC

Usmani SZ, et al. Presented at ASH 2016 (Abstract 1149), oral presentation.

Clinical assessment of M-protein response in MM and interference through mAbs

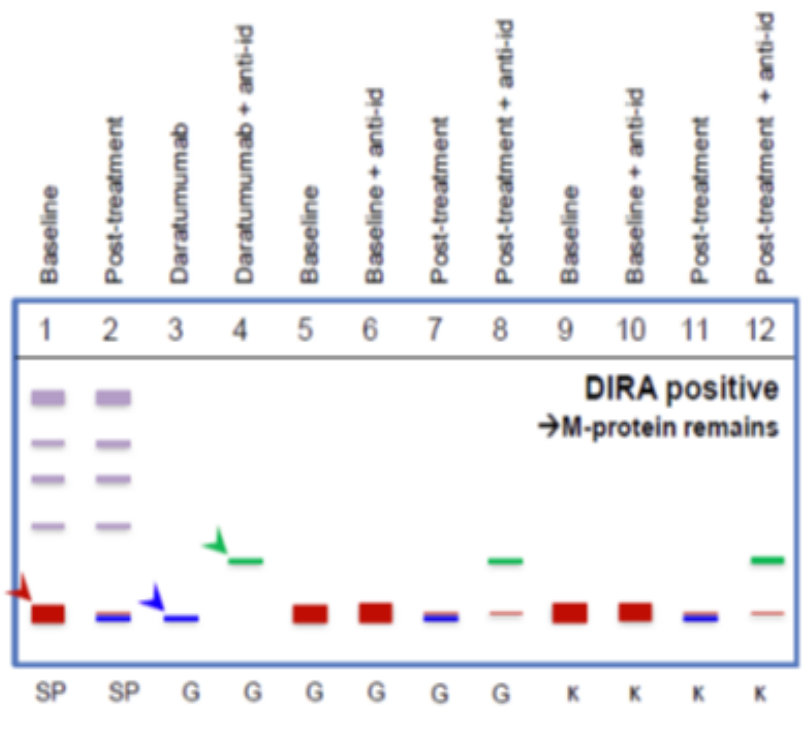
- All therapeutic mAbs may interfere with serum electrophoresis and immunofixation

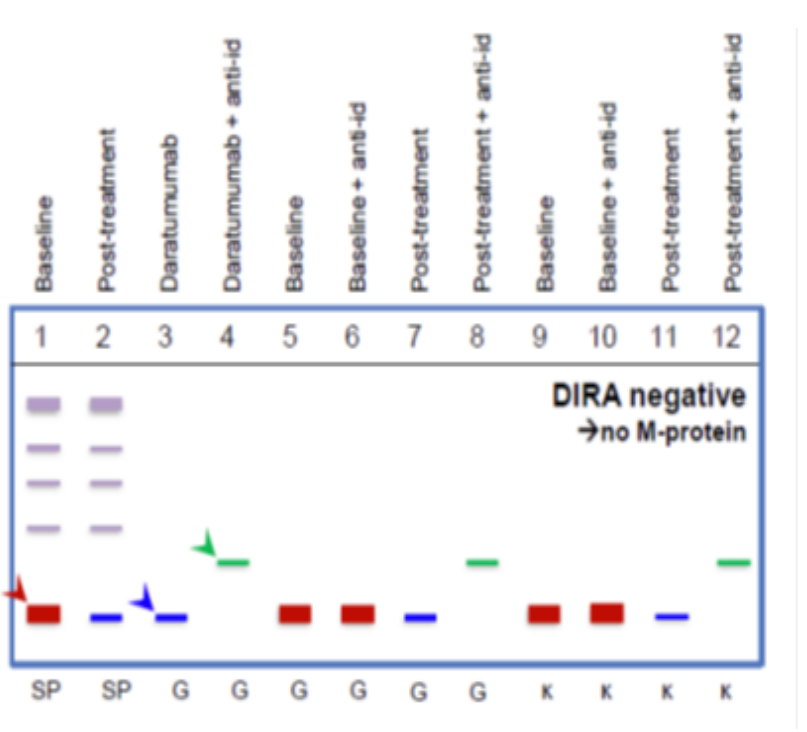
  → Difficult to discern between therapeutic antibody and the patient's clonal immunoglobulin

- **Class effect of mAbs in myeloma**
- Interference depends on isotype of the patient
- Daratumumab, Elotuzumab, Isatuximab (SAR650984) and MOR202 are IgG mAb

Durie et al. Leukemia. 2006;20(9):1467-1473.
McCudden et al. Clin Chem. 2010;56(12):1897-1899.
Axel et al. AACR 2014 (Abstract 2583); poster presentation

Daratumumab specific IFE Reflex Assay (DIRA) separates therapeutic antibody from M-protein





DIRA positive
→M-protein remains

DIRA negative
→no M-protein

SP = total serum protein fix
G = anti-IgG antisera
K = kappa antisera

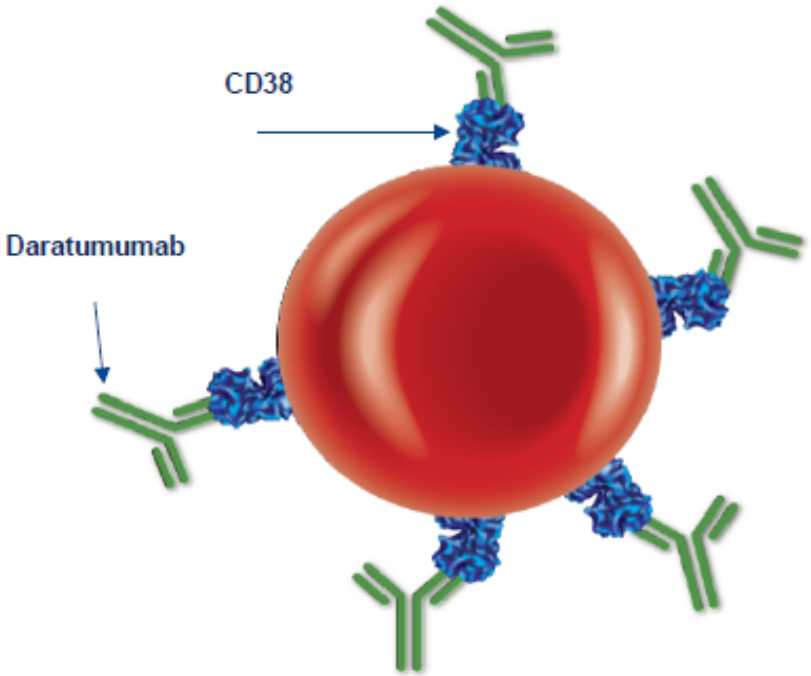
→ Daratumumab
→ Dara + anti-id complex
→ M-protein

McCudden C, et al. ASCO 2015 (abstract 8590); poster presentation

Interferences of monoclonal antibodies in MM: with blood compatibility testing

Blood compatibility testing for patients receiving anti-CD38 mAbs

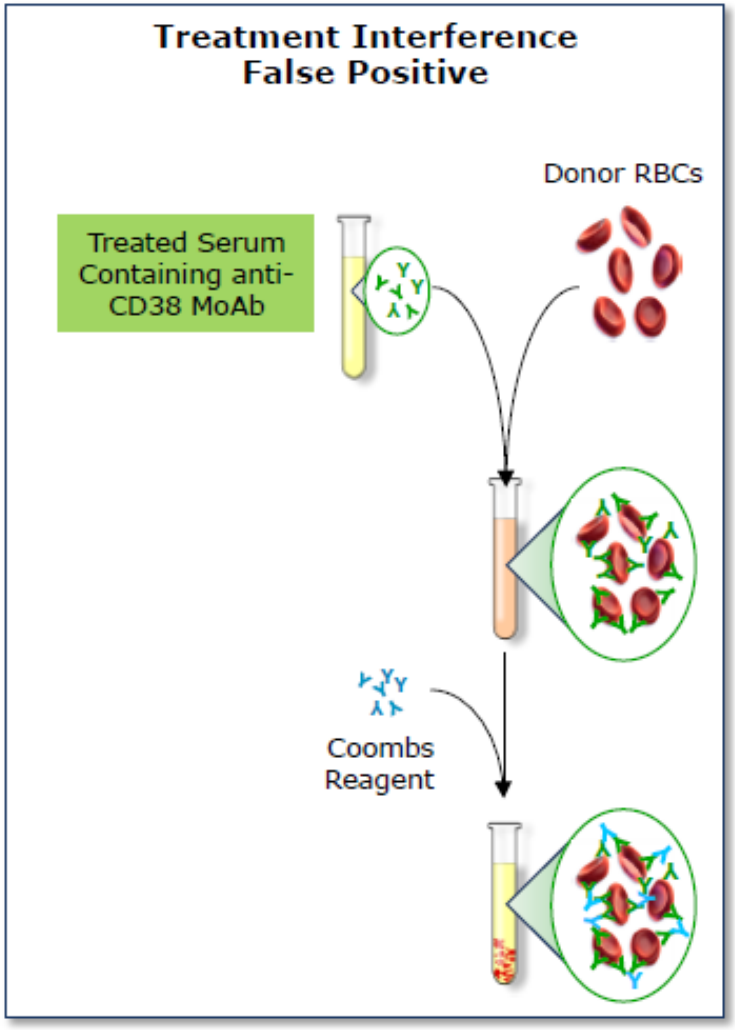
- CD38 is weakly expressed on human red blood cells (RBCs)
- Daratumumab binds to CD38 on RBCs → false positive results in the Indirect Antiglobulin Test (indirect Coombs test)

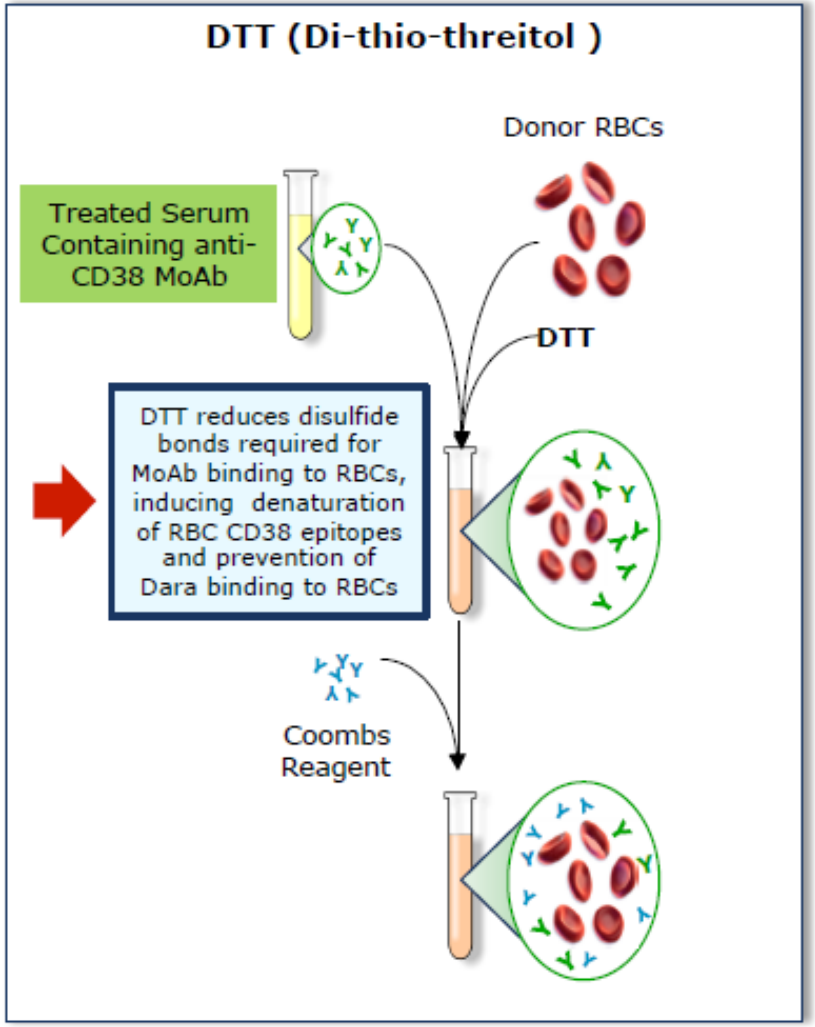


Daratumumab binds to CD38 on RBCs[2]

Chapuy et al. Transfusion. 2015;55(6 Pt 2):1545-54
Oostendorp et al. Transfusion. 2015;55(6 Pt 2):1555-62

Methods for Mitigating Monoclonal Antibody Therapy Assay Interference





DTT treatment of CD38+ cells reduced Daratumumab binding by 92%.

van de Donk Blood 2016;27(6):681–695; 2. van de Donk Immunol Rev 2016;270: 95–112

DARA interference with blood typing: What impact in the clinical practice?

- To date, **neither clinically significant hemolysis, nor transfusion reactions** after RBC and whole blood transfusions have occurred in patients receiving 16 mg/kg Daratumumab

- Daratumumab **does not interfere with ABO/RhD typing** but with minor ones; therefore blood products for transfusion can be identified for Daratumumab-treated patients by blood banks performing routine compatibility tests or by using genotyping

- If an emergency transfusion is required, **non-crossmatched, ABO/ RhD–compatible RBCs can be given,** per local blood bank practices

- To avoid unnecessary delays, **blood bank should be informed,** preferably before MoAb is started, that they will receive a sample from a Daratumumab-treated patient, so that appropriate protocols for typing and screening procedures can be applied

- Patients should carry a **blood transfusion card** indicating that they receive anti-CD38 MoAb therapy

Conclusions

- Although MM is an immunogenic tumor it is associated with a profound immune dysfunction
- NK cells have a central role in controlling myeloma cells and continue to remain an important target for immunotherapy
- Monoclonal antibodies are very effective therapies acting through different mechanisms including an immune modulating function
- Lenalidomide seem to be the ideal partner for these drugs since is able to enhance their activity not increasing significantly the toxicity
- A new learning curve is needed to prevent and manage the IRRs of MoAbs in order to take the maximum advantage of their unprecedented efficacy