PASSIVE IMMUNOTHERAPY: TARGETING TUMOR CELLS

CD38 in myeloma and beyond: groundwork & outlook

Alessandra Larocca, MD, PhD
Divisione di Ematologia 1
AOU Città della Salute e della Scienza
University of Torino, Italy, EU

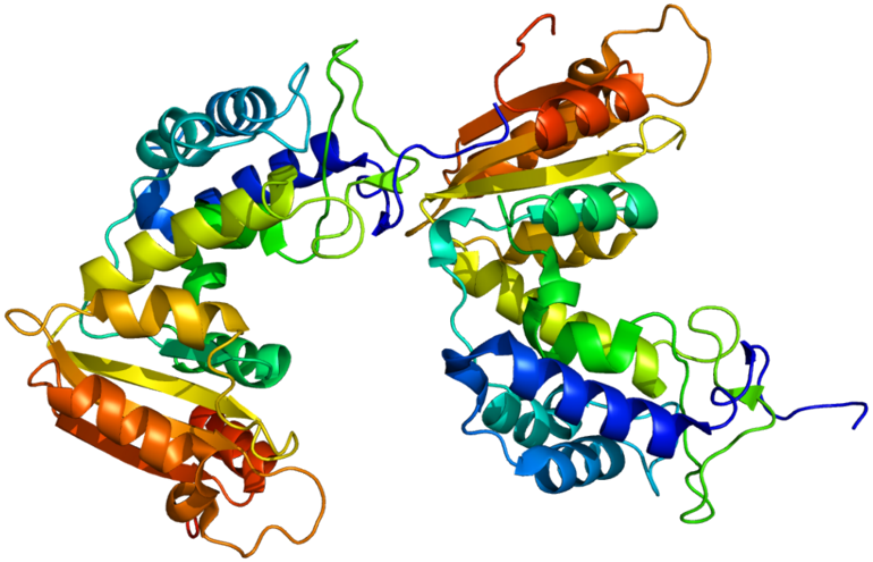
sclosures: A Larocca

search Support/P.I.	
nployee	
nsultant	
jor Stockholder	
eakers Bureau	
noraria	Janssen-Cilag, Celgene, BMS, Amgen
ientific Advisory Board	BMS

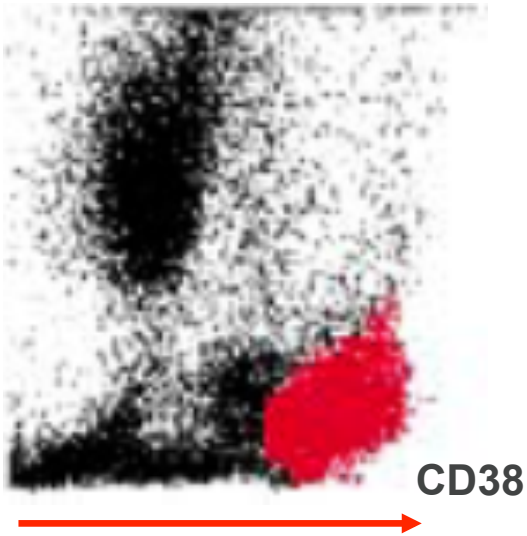
is presentation may contain unregistered products or indications of investigational drugs, please check the drug compendium or consult the company

CD38 as a Therapeutic Target

Structure of CD38



Flow cytometry of CD38
on myeloma cells



CD38 is a type II transmembrane glycoprotein, strongly expressed by myeloma cells

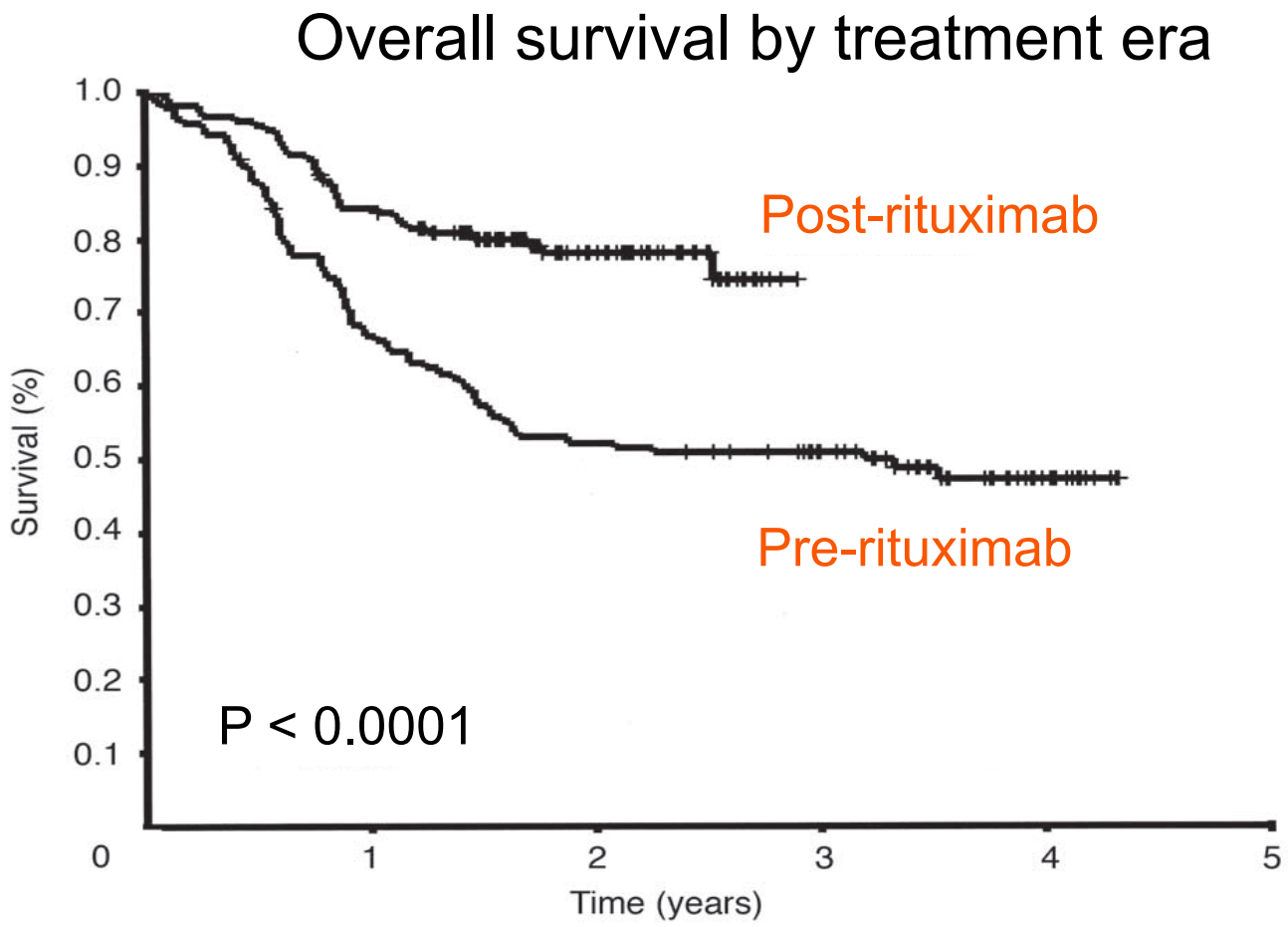
Role in cell signaling, cell adhesion, signal transduction, calcium homeostasis, production adenosine (immunosuppressive effect)

CD38 as a potential therapeutic antibody target for treatment of multiple myeloma (MM)

Lin P, et al. Am J Clin Pathol 2004

Rationale for moAbs in MM

The impact of rituximab in diffuse large B-cell lymphoma

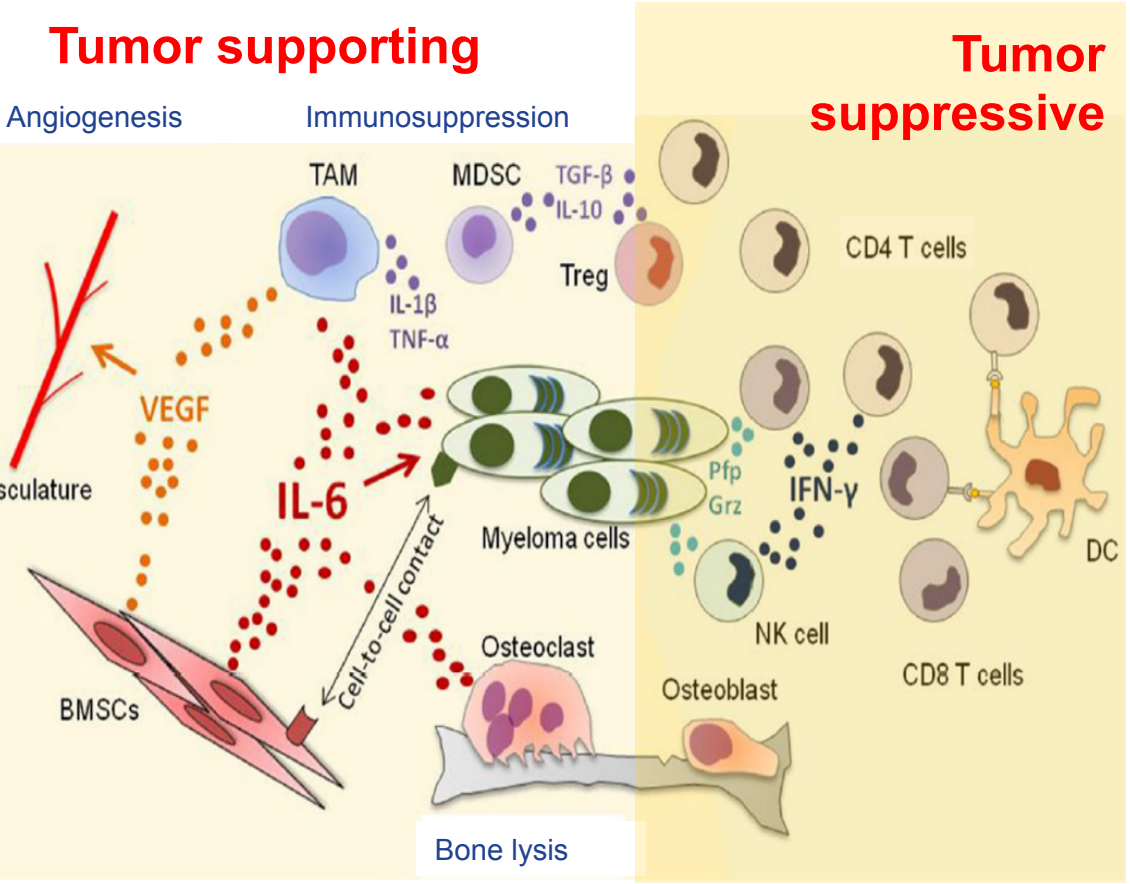


Can we find a monoclonal antibody that will change the course of myeloma in a similar way?

Sehn et al. J Clin Oncol 2005;23:50

he bone marrow microenvironment influences tumor growth in M

MM, the balance between tumor growth and tumor suppression is shaped by complex interactio
etween immune, non-immune, and malignant MM cells within the bone marrow microenvironme



, bone marrow stromal cells; DC, dendritic cell; Grz, granzyme; IFN, interferon; IL, interleukin; myeloid derived suppressor cell; MM, multiple myeloma; NK, natural killer; pfp, perforin; TAM, tumor ted macrophage; TGF, transforming growth factor; Th, T helper; TNF, tumor necrosis factor; Treg, ory T cell; VEGF, vascular endothelial growth factor.

Factors supporting tumor growth

- IL-6 released by BMSCs, tumor-associated macrophages, and osteoclasts promotes MM cell proliferation, survival, and drug resistance
- VEGF released by BMSCs and TAMs stimulates angiogenesis

Factors suppressing tumor growth

- CD8+ T cells and NK cells secrete IFN-ɣ and kill MM cells directly
- Th1 CD4+ T cells may inhibit tumor growth

Factors suppressing anti-MM immune cell activity

- MDSCs and Tregs secrete immunosuppressive factors such as IL-10 and TGF-β
- Th2 CD4+ T cells may promote tumor growth

Guillerey C et al. Cell Mol Life Sci. 2016;73:15

Immune Evasion Plays a Critical Role in Myeloma Pathogenesis

Immune System

Tumor

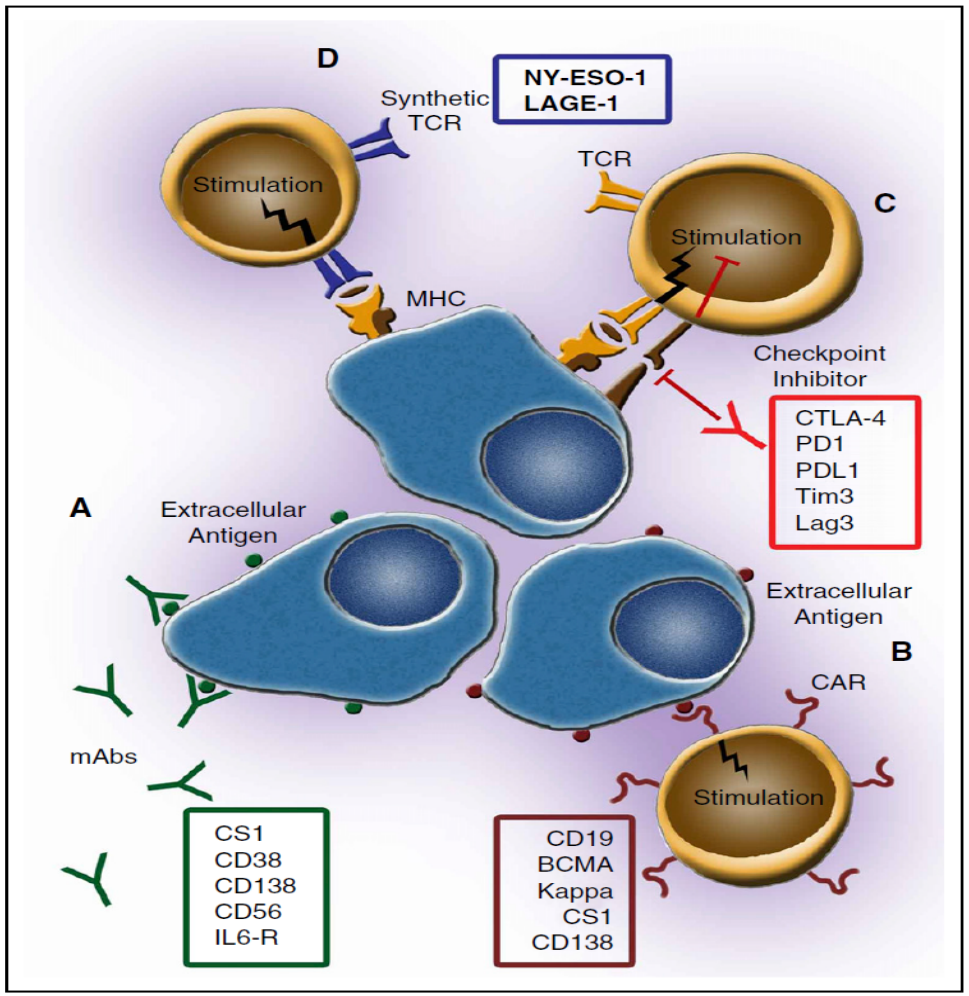
Immune dysregulation and immunosuppression

Microenvironment perturbation

Genetic alterations

Disease progression

Rationale for Immunotherapy in Multiple Myeloma



TARGETS FOR IMMUNOTHERAPY

- Targeting MM cell surface Ags
  - MoAb Anti-SLAMF7
  - MoAb Anti-CD38
- In MM patients, the normal immune syster favors tumor proliferation
  - Overcoming inhibitory immunosuppressi
  - Check-point inhibitors
  - IMIDs
  - Boosting immune effectors
  - Adoptive cell therapy

munomodulatory agent; MoAb, monoclonal antibody; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; SLAMF7, signaling lymphoc molecule family member 7.

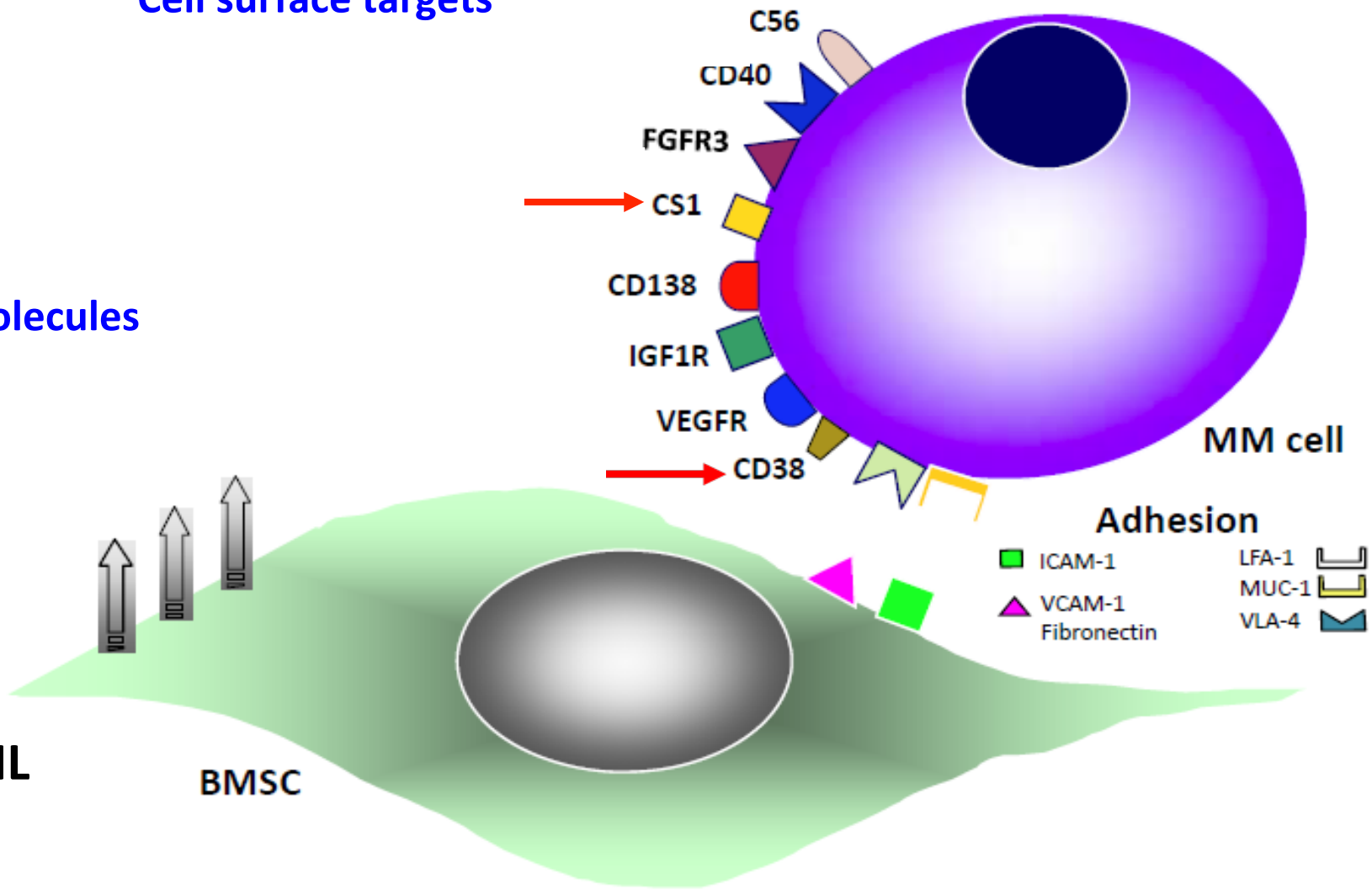
Hoyos V and Borrello I. Blood 2016; 128(13):

Targets for monoclonal antibody therapy in myeloma

Cell surface targets

Signalling molecules

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



Adapted from: Anderson KC. J Clin Oncol 2012;30

Three CD38 monoclonal antibodies

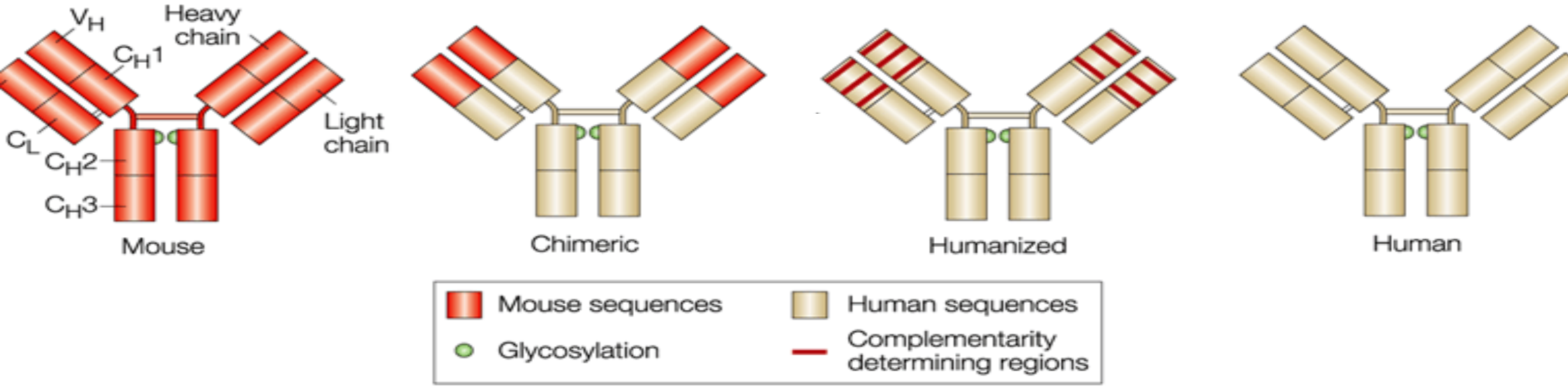
Chimeric:

Isatuximab (SAR650984)

Fully human:

Daratumumab (DARA)

MOR202 (MOR)



decreasing immunogenicity

Adapted from Imai & Takaoka. Nature Reviews Cancer 2006; 6

Anti-CD38-monoclonal antibodies act through different modes of action in MM

In vitro comparison of Daratumumab with analogs of CD38 antibodies

MoA	DARATUMUMAB	ISATUXIMAB	MOR202
Origin, isotype	Human IgG-kappa	Chimeric IgG1-kappa	Human IgG1-lambda
CDC	+++	+	+
ADCC	++	++	++
ADCP	+++	nd	++
PCD direct	-	++	-
PCD cross linking	+++	+++	+++
Modulation ectoenzyme function	+	+++	-

lement-dependent cytotoxicity
body-dependent cell-mediated cytotoxicity
mmed cell death
ody-dependent cell-mediated phagocytosis

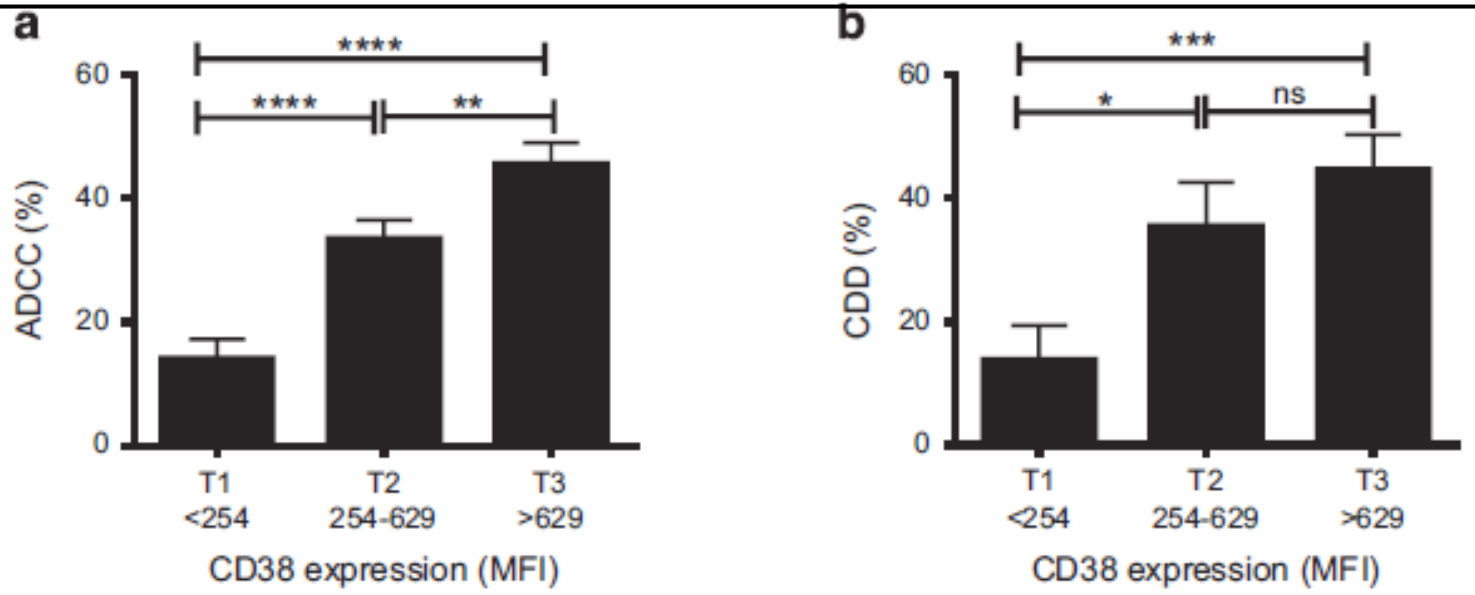
Van de Donk N, et al. Blood. 2018;13

Preclinical evidence for anti CD38-moAbs in MM

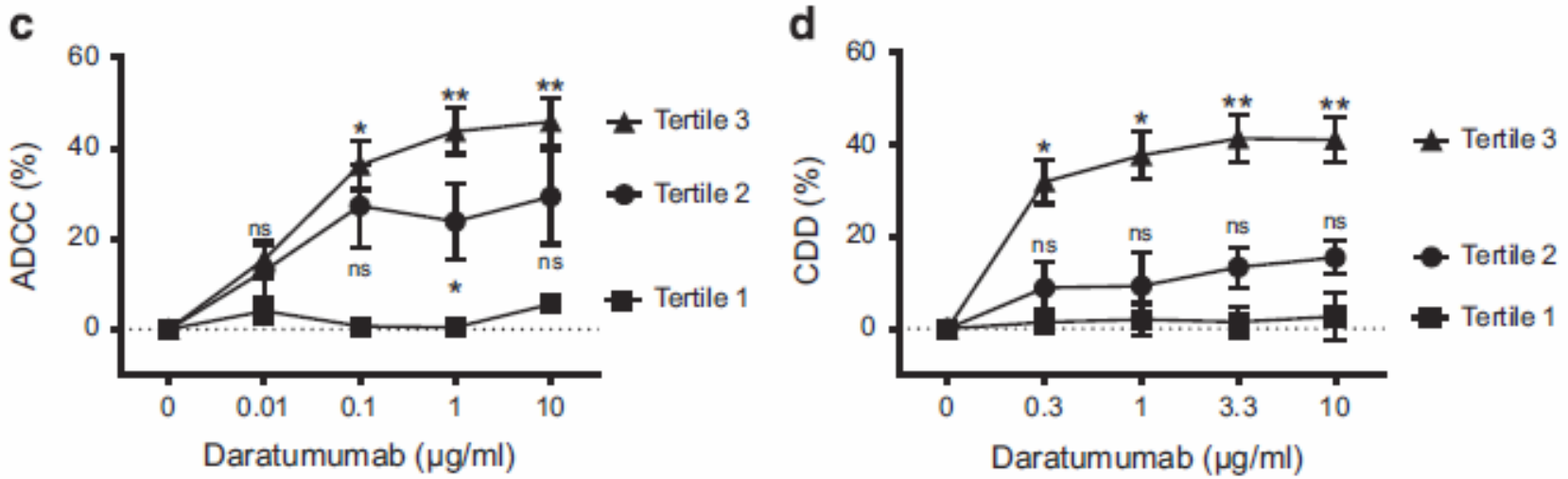
CD38 expression correlates with cell death (patient samples)

ients divided
tertiles
ording to CD38
ression on their
cells

127 patient samples)



se–response curve
cording to CD38
ression to evaluate
erent concentrations
daratumumab in
CC and CDC
says.

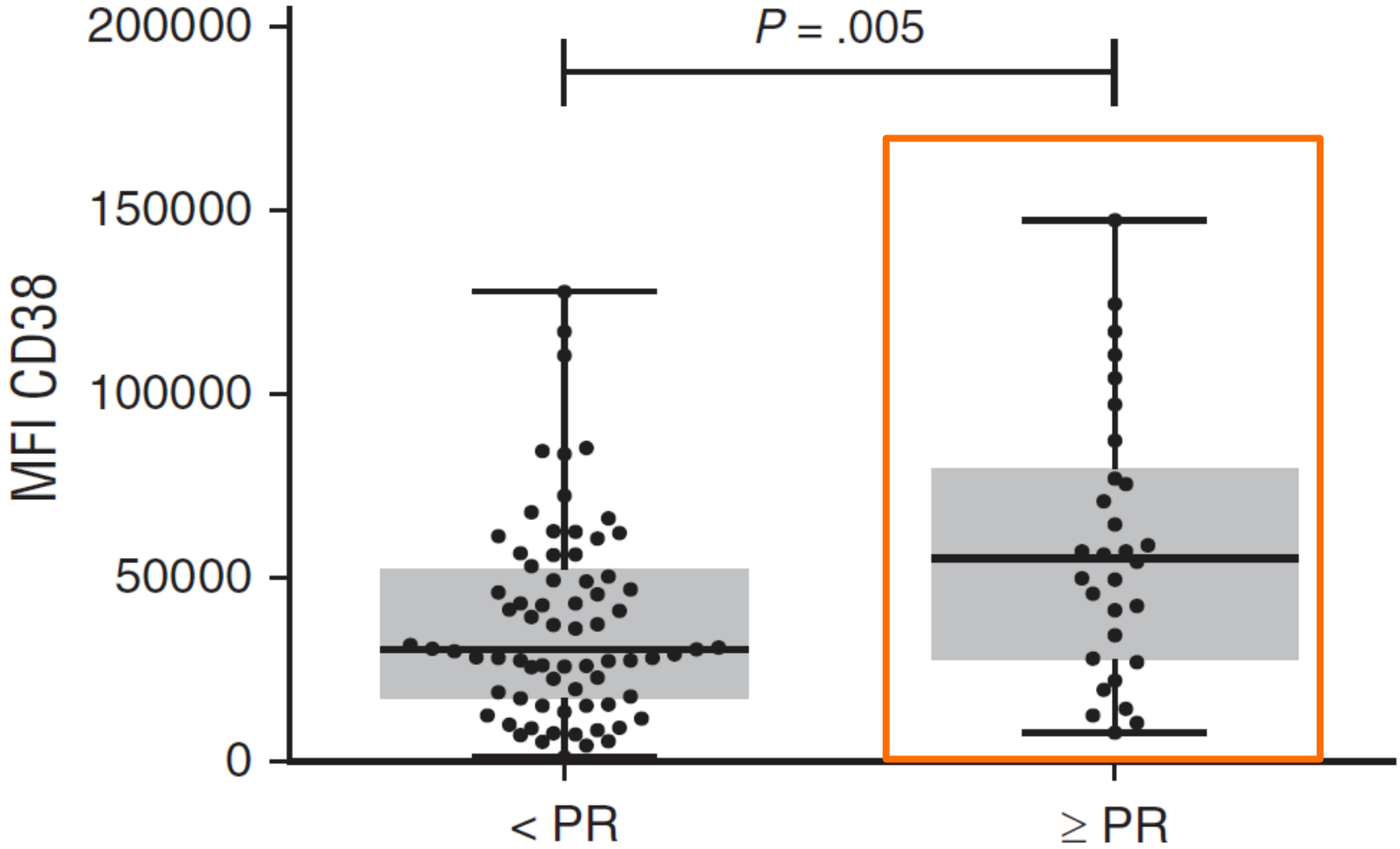


omplement-dependent cytotoxicity
Antibody-dependent cell-mediated cytotoxicity

*P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; ns, not significant

Nijhof et al. Leukemia 2015;29(10

CD38 expression: determinants of efficacy
Patients treated in GEN501 or SIRIUS (MMY2002)



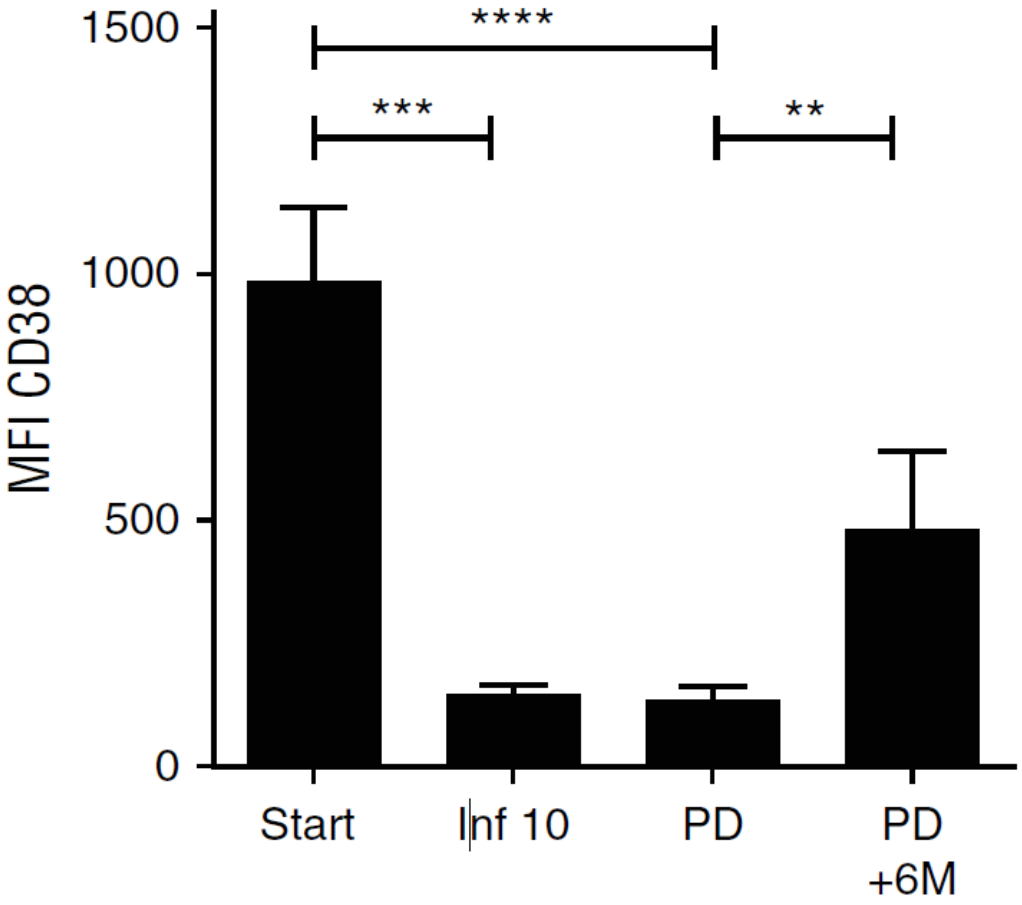
median fluorescence intensity CD38

artial response

Nijhof IS, et al. Blood 2016;128

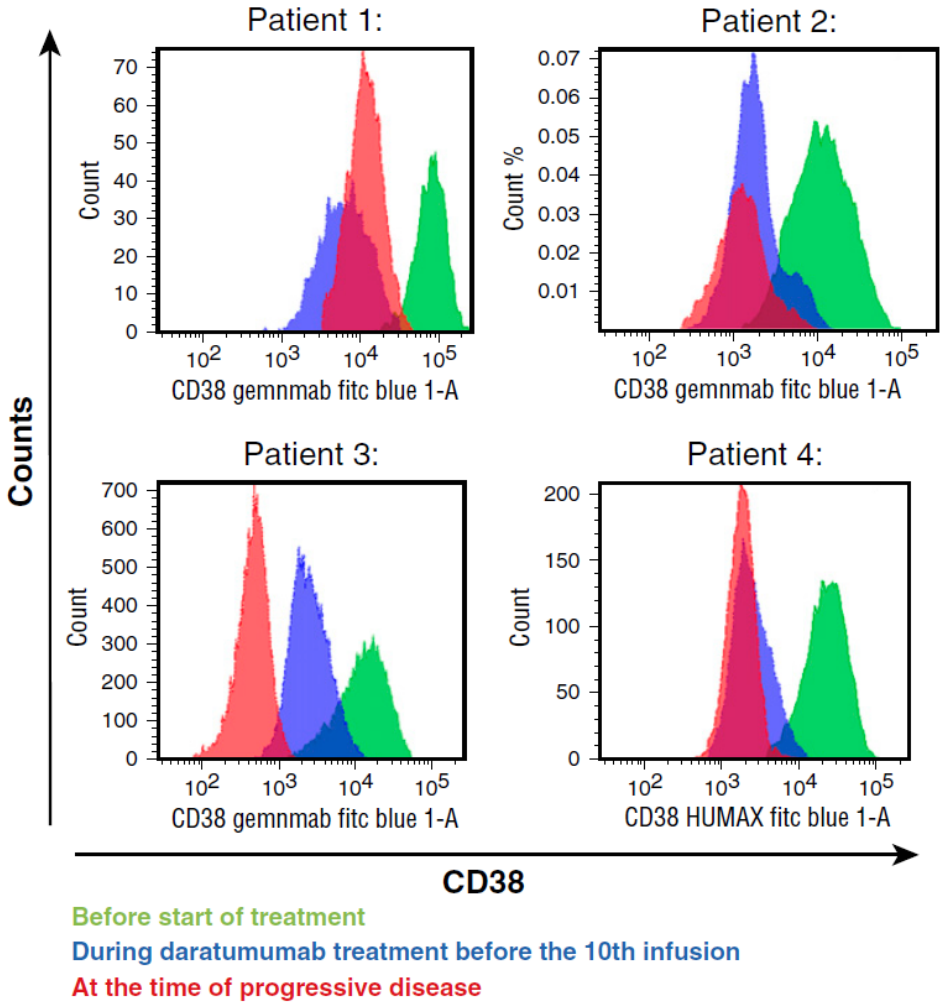
CD38 is rapidly reduced on MM cells from patients

038 expression on MM cells in BM samples obtained from 21 patients, who were subsequently treate
h daratumumab at a dose of 16 mg/kg in the GEN501 study.



median fluorescence intensity CD38

01; ***P < 0.001; ****P < 0.0001
gressive disease



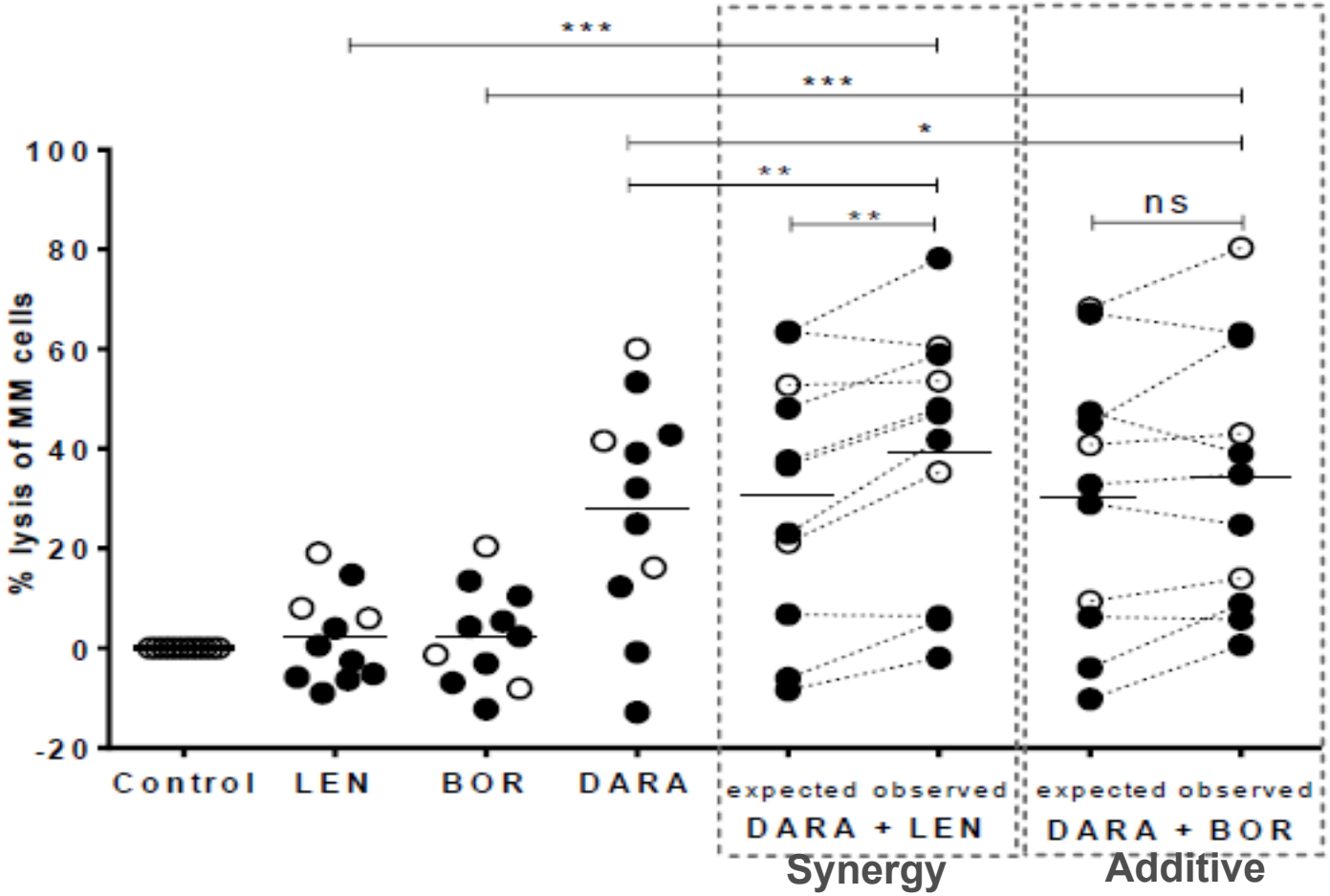
Nijhof IS, et al. Blood 2016;128

ara combined with LEN or BORT in BORT and LEN-refractory MM

aratumumab induced significant levels of MM cell lysis in the BM-MNC from refractory MM patients.

M cell lysis was significantly improved from 29.7% with daratumumab alone to 39.4% upon ombination of daratumumab-lenalidomide in patients were refractory to lenalidomide.

- ADCC assays
- 11/11 patients LEN-refractory
- 8/11 patients BORT-refractory
- The black circles represent the lenalidomide/bortezomib double-refractory patients



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

Nijhof IS, et al. Clin Cancer Res. 2015;21(12):280

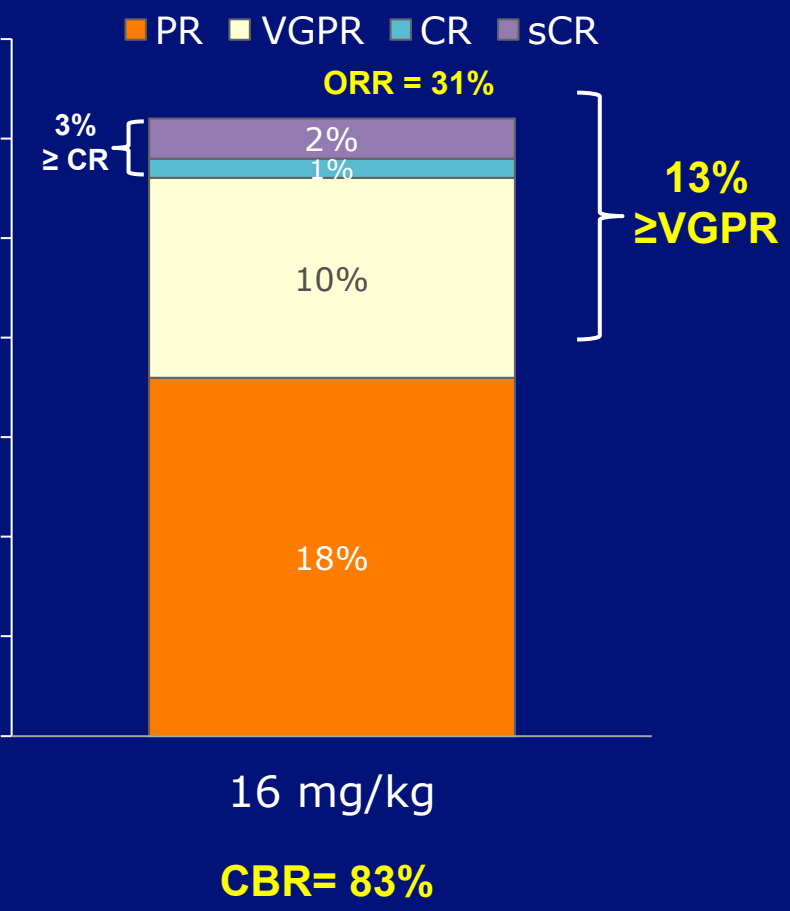
Clinical evidences for anti CD38-moAbs in MM

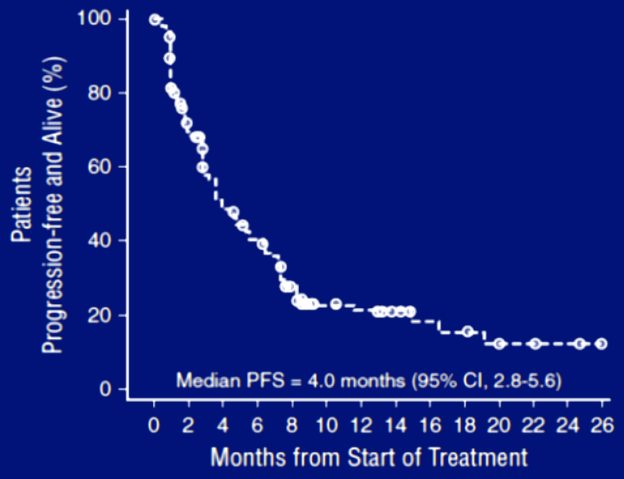
Daratumumab Single Agent (GEN501 and SIRIUS*)

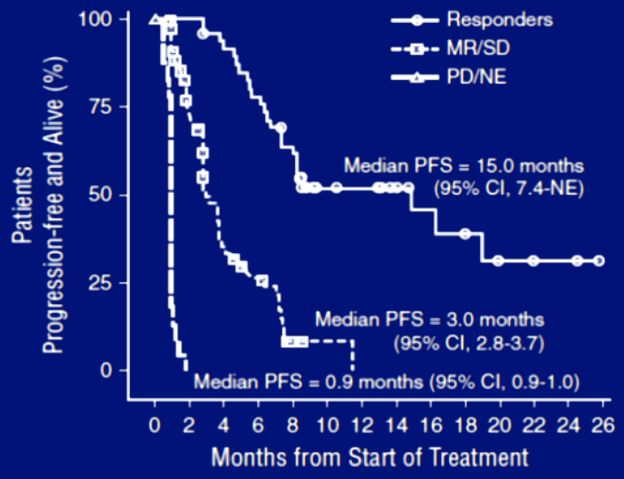
Median N prior lines: 5

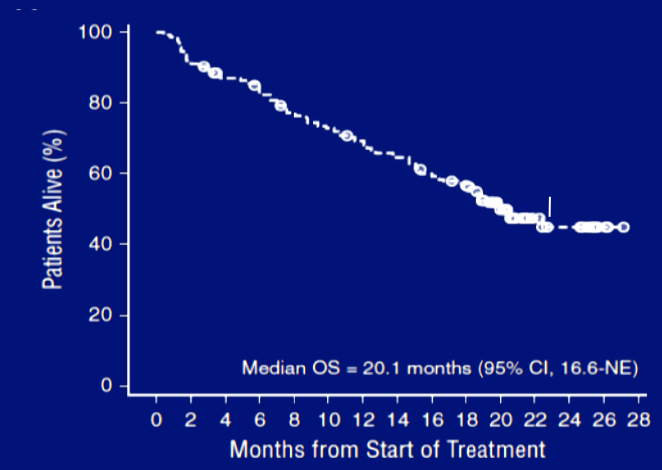
Refractory to Bortezomib and Lenalidomide: 87%; Refractory also to Pomalidomide: 55%

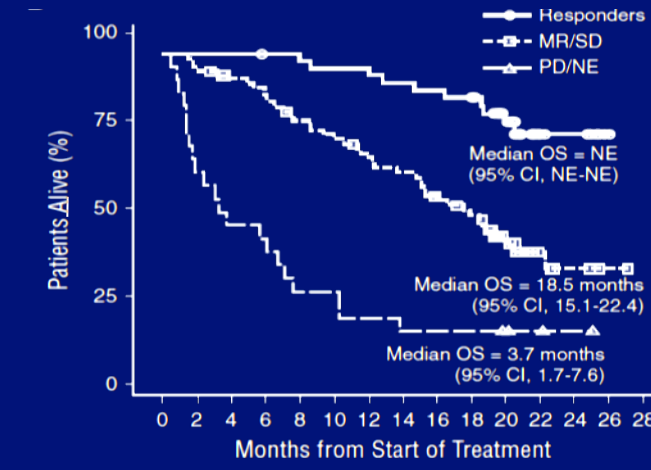
Creatinine clearence ≥ 30 (97%); age ≥75: 11%











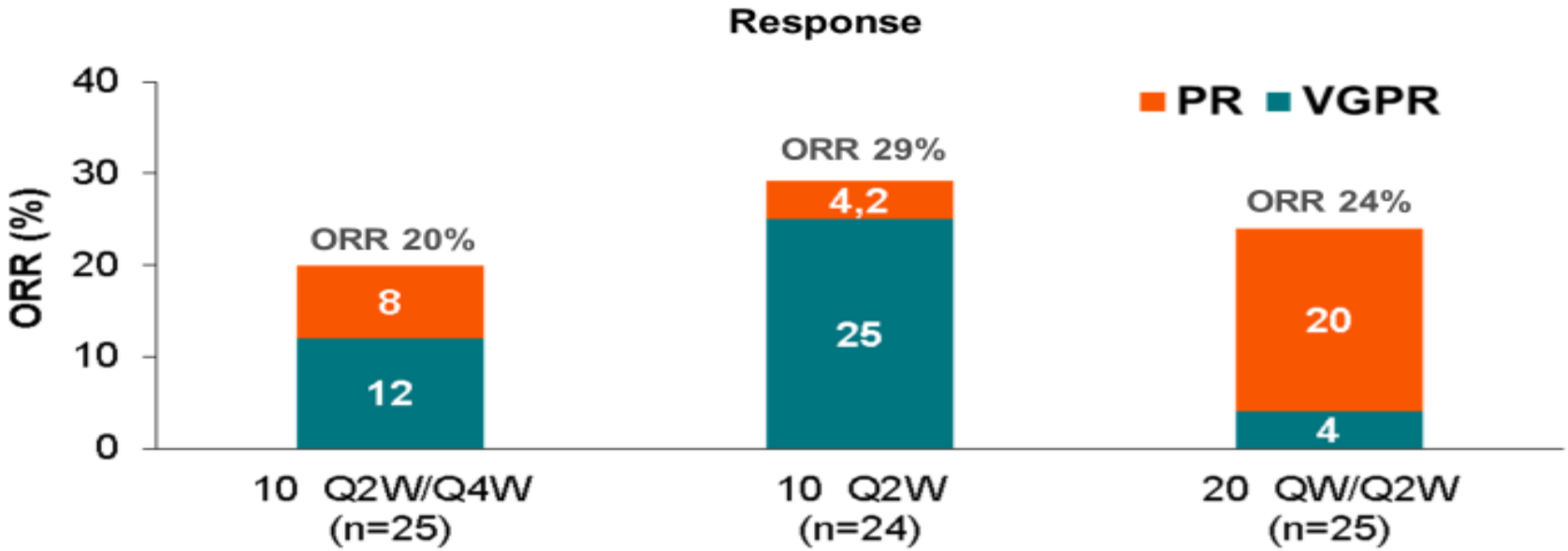
analysis
ce interval; OS, overall survival; PFS, progression-free survival; ORR, overall response rate; PR,
nse; VGPR, very good partial response; CR, complete response, sCR, stringent CR; CBR, clinical
onse (≥SD).

Usmani S, et al. Oral presentation: ASH 2015; Orlando, FL. Abst

Isatuximab monotherapy

TED10893 phase 2 study of isatuximab monotherapy in RRMM

Median prior lines of therapy 5



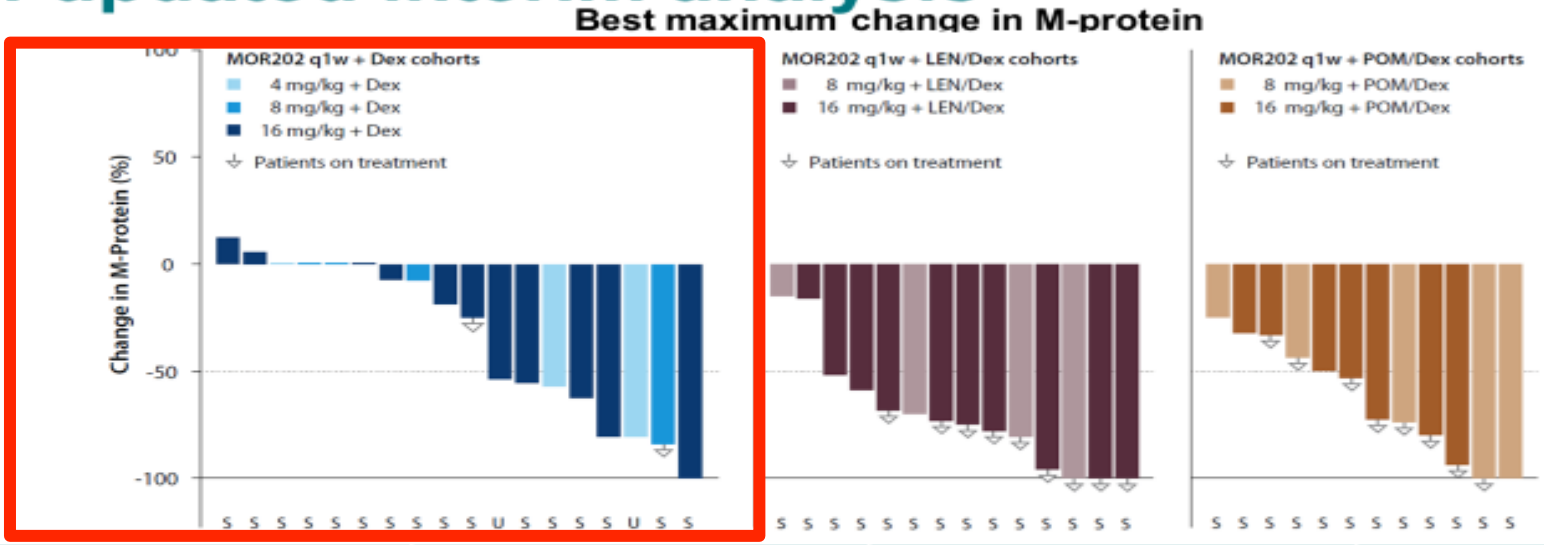
Time to first response, mo	2.0 (0.8– 2.1)	0.9 (0.9–1)	1.35 (0.9–2.8)

RRMM patients double refractory to PI and IMiDs or have received ≥3 prior lines of therapy

Vij R, et al. Presented at EHA 2016 (Abstract P274), poster presentatio

MOR202

Phase 1/2a study of MOR202 + POM-D or len-dex in RRMM: updated interim analysis



	MOR202 q1w + Dex (n=18)	MOR202 q1w + LEN/Dex (n=17)	MOR202 q1w + POM/Dex (n=13)
Median prior lines of therapy, n	3	2*	3
Refractory to any prior therapy, %	67	56	100
ORR, %	28	71	46
CR, %	0	6	15
Median (95% CI) PFS, months	4.7 (1.5, NE)	Not reached (5.1, NE)	17.5 (2.8, NE)
Median follow-up, months	22.1 months	7.5 months	8.5

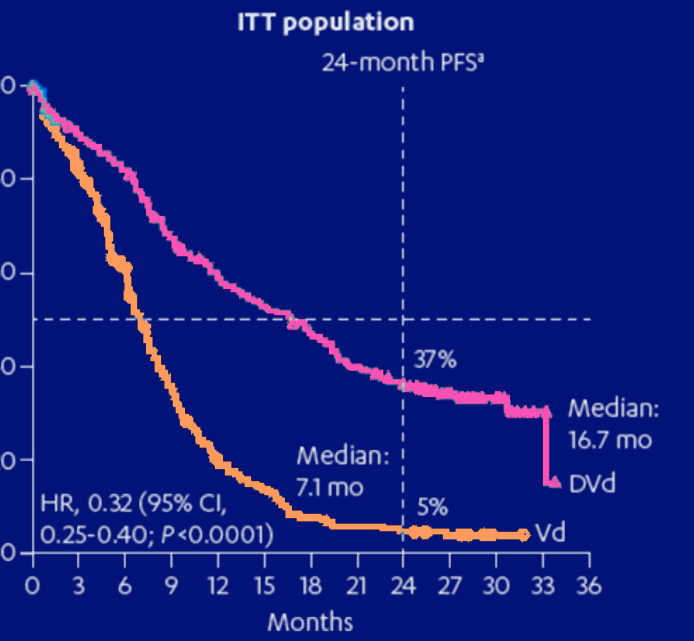
ne patient in the LEN/Dex cohorts data of prior therapies not yet available

Raab MS, et al. Poster presentation at ASCO 2017. Abstract 802

VD *versus* VD plus Daratumumab (CASTOR)

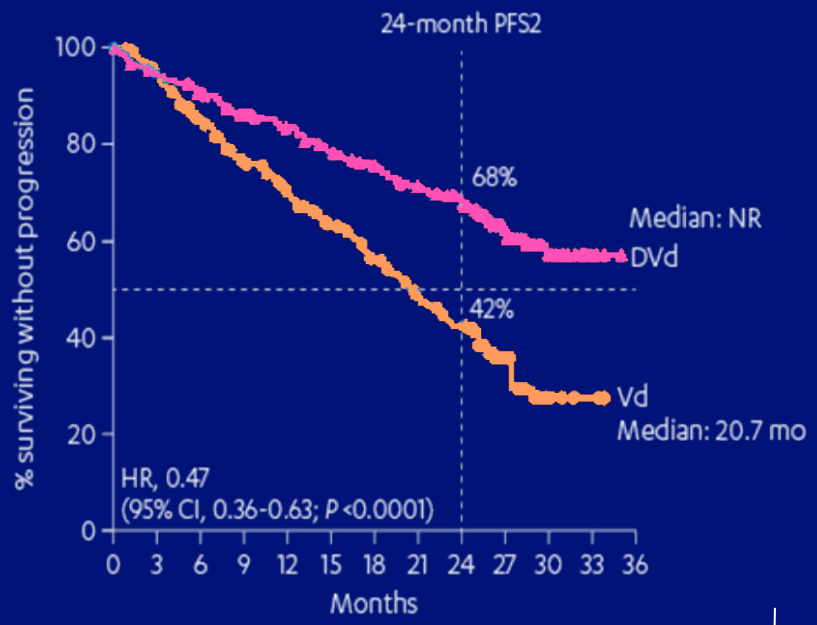
Median follow-up: 26.9 months

DVd vs Vd
Progression-free survival



	DVd (n=251)	Vd (n=247)
Median PFS, mo	16.7	7.1
HR (95% CI)	0.32 (0.25–0.40)	
P value	< 0.0001	

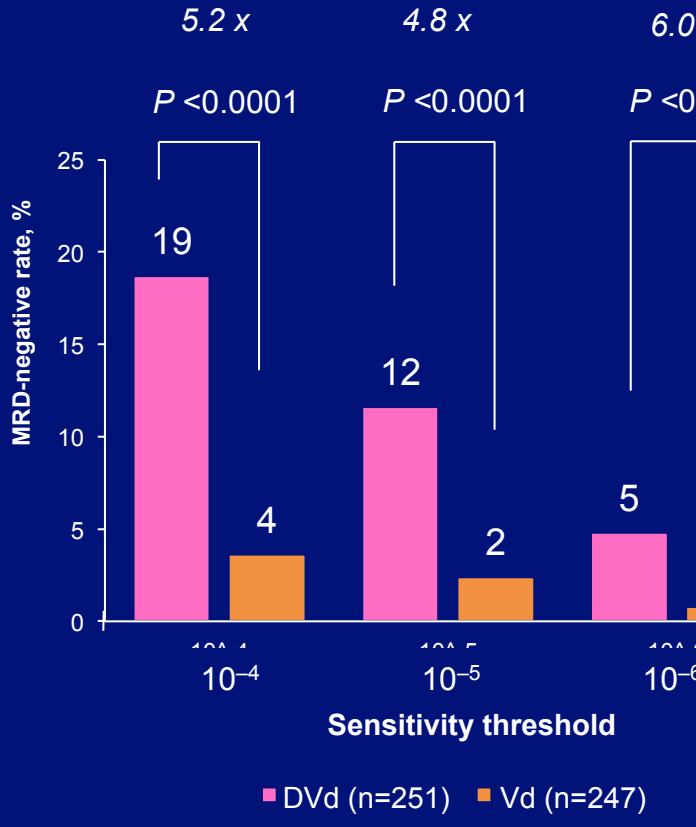
DVd vs Vd
Progression-free survival-2



	DVd (n=251)	Vd (n=247)
Median PFS2, mo	NR	20.7
HR (95% CI)	0.47 (0.36–0.63)	
P value	< 0.0001	

Median follow-up: 19.4 mont

DVd vs Vd
MRD negativity



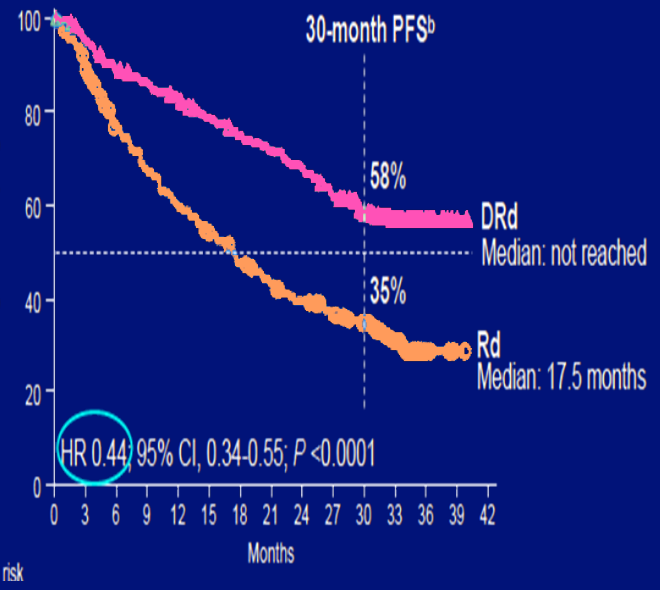
ogression-free survival; HR, hazard ratio, CI, confidence interval; m, months; d, low dose thasone; D, daratumumab; V, bortezomib; MRD, minimal residual disease

Spencer A, et al. Presented at ASH 2017 (Abstract 3145), poster prese
Lentzsch S, et al. Presented at ASCO 2017 (Abstract 8036), poster prese
Weisel K, et al. Presented at EHA 2017 (Abstract S459), oral prese

Rd *versus* Rd Daratumumab (POLLUX)

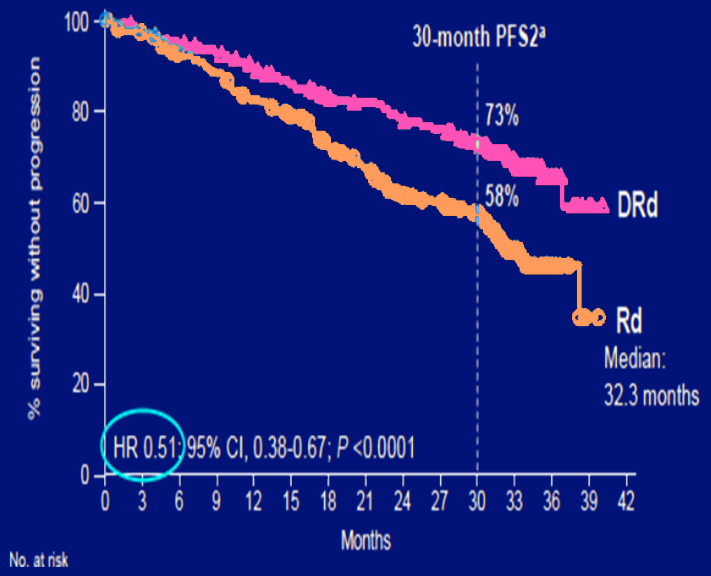
Median follow-up: 32.9 months

DRd vs Rd
Progression-free survival



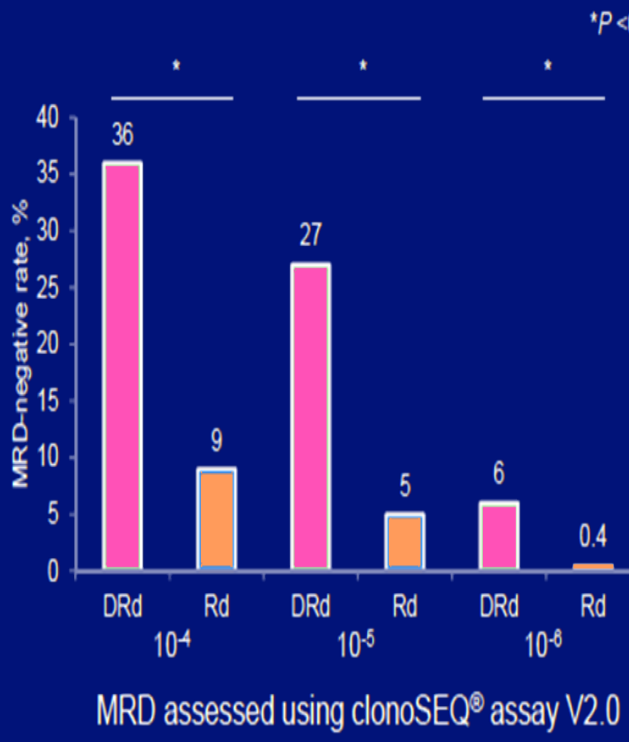
	DRd (n=286)	Rd (n=283)
dian PFS, mo	NR	17.5
(95% CI)	0.44 (0.34–0.55)	
alue	<0.0001	

DRd vs Rd
Progression-free survival- 2



	DRd (n=286)	Rd (n=283)
Median PFS2, mo	NR	32.3
HR (95% CI)	0.51 (0.38–0.67)	
P value	<0.0001	

DRd vs Rd
MRD negativity



MRD assessed using clonoSEQ® assay V2.0

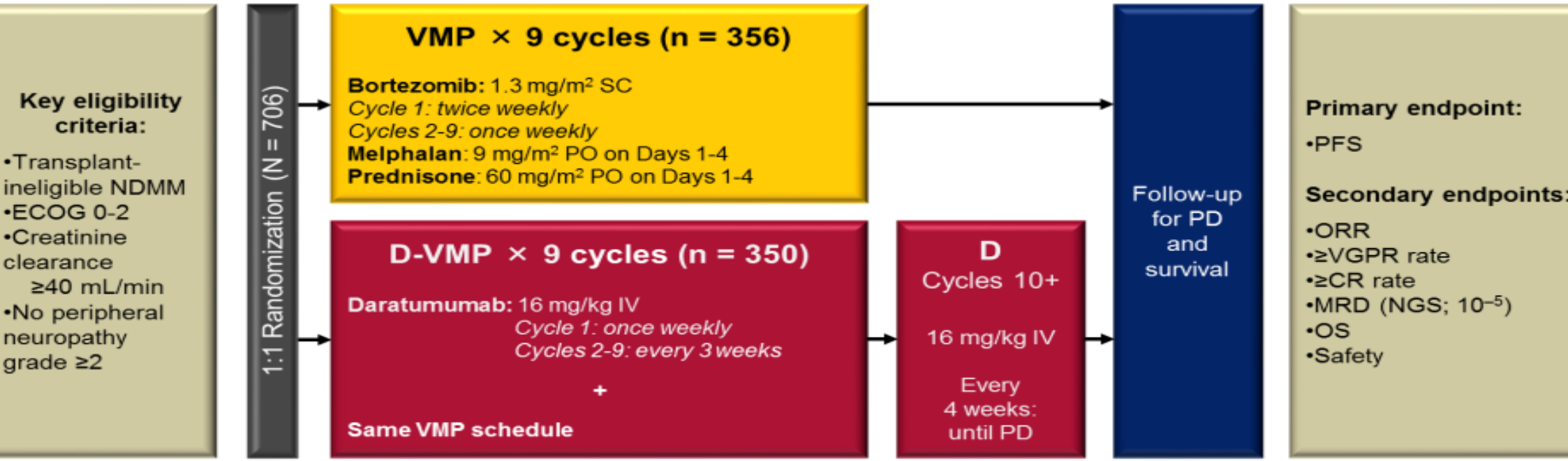
ogression-free survival; HR, hazard ratio, CI, confidence interval; P, P value; d, low
xamethasone; D, daratumumab; R, lenalidomide; MRD, minimal residual disease.

Dimopoulos MA, et al. Presented at ASH 2017 (Abstract 739), oral prese
Lentzsch S, et al. Presented at ASCO 2017 (Abstract 8036), poster prese
Weisel K, et al. Presented at EHA 2017 (Abstract S459), oral prese

Daratumumab-VMP *vs* VMP in NDMM transplant ineligibl

ALCYONE phase 3 study of daratumumab + VMP in NDMM

Design



Stratification factors

- SS (I vs II vs III)
- Region (EU vs other)
- Age (<75 vs ≥75 years)

- Cycles 1-9: 6-week cycles
- Cycles 10+: 4-week cycles

Statistical analyses

- 360 PFS events: 85% power for 8-month PFS improvement
- Interim analysis: ~216 PFS even

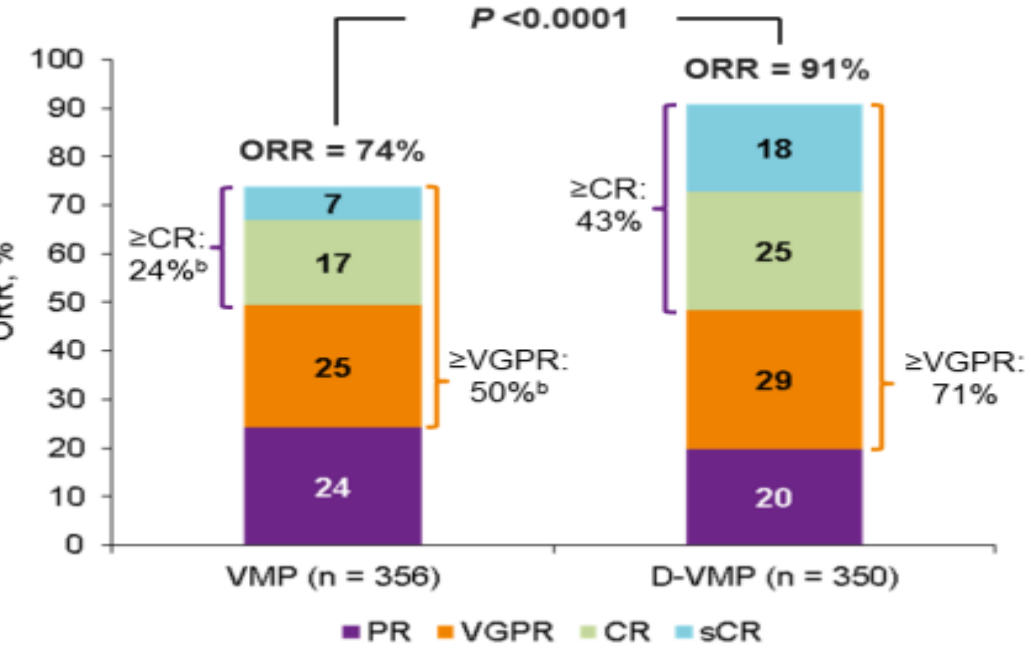
DMM, newly diagnosed multiple myeloma; ECOG, Eastern Cooperative Oncology Group; ISS, International Staging System; , European Union; VMP, bortezomib/melphalan/prednisone; SC, subcutaneously; PO, orally; D, daratumumab; IV, intravenously; , progressive disease; PFS, progression-free survival; ORR, overall response rate; VGPR, very good partial response; R, complete response; MRD, minimal residual disease; NGS, next-generation sequencing; OS, overall survival.

Mateos MV, et al. Presented at ASH 2017 (Abstract LBA-4), oral presentati

Daratumumab-VMP *versus* VMP

ALCYONE Efficacy: ORR[a]

- Median duration of response: 21.3 months in VMP versus not reached in D-VMP



	VMP (n = 263)[c]	D-VMP (n = 318)[c]
Median (range) time to **first** response, months	0.82 (0.7-12.6)	0.79 (0.4-15.5)
Median (range) time to **best** response, months	4.11 (0.7-20.5)	4.93 (0.5-21.0)

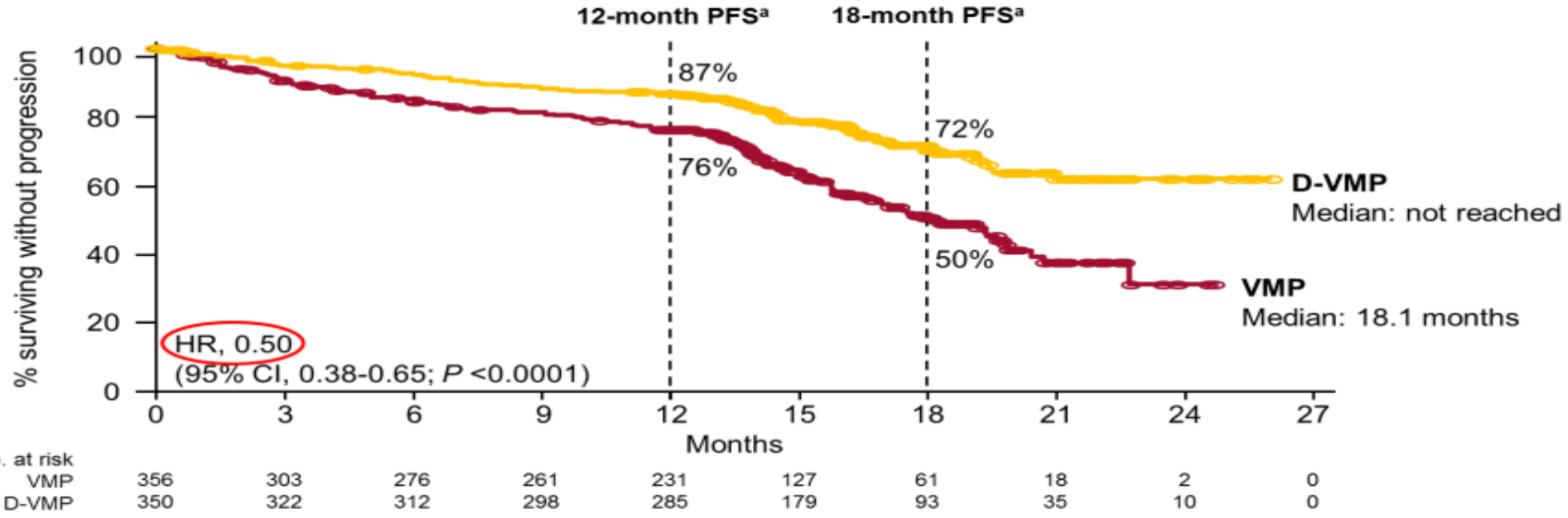
Significantly higher ORR, ≥VGPR rate, and ≥CR rate with D-VMP; >2-fold increase in rate of sCR with D-VMP

PR, partial response; sCR, stringent complete response.
[a]ITT population. [b]*P* <0.0001; *P* value was calculated with the use of the Cochran–Mantel–Haenszel chi-square test.
[c]Responders in response-evaluable population.

Daratumumab-VMP *versus* VMP

ALCYONE Efficacy: PFS

- Median (range) follow-up: 16.5 (0.1-28.1) months



50% reduction in the risk of progression or death in patients receiving D-VMP

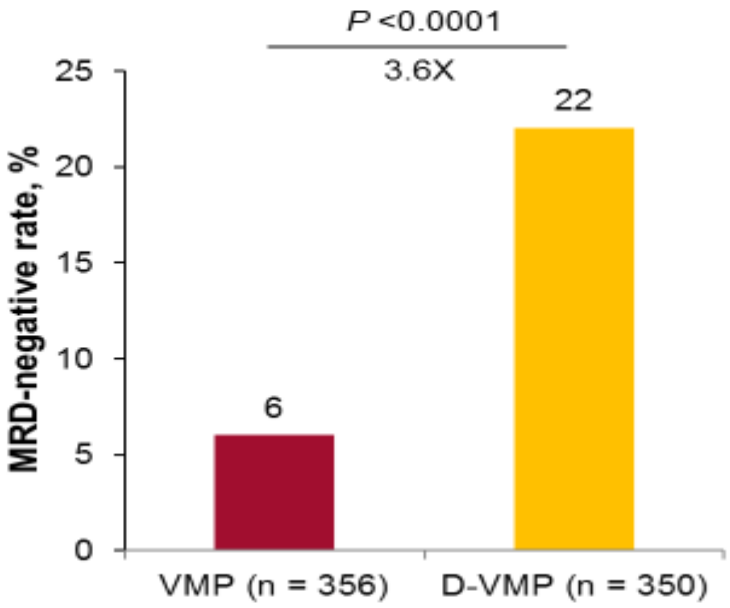
FS, progression-free survival; VMP, bortezomib/melphalan/prednisone; daratumumab; HR, hazard ratio; CI, confidence interval. aplan-Meier estimate.

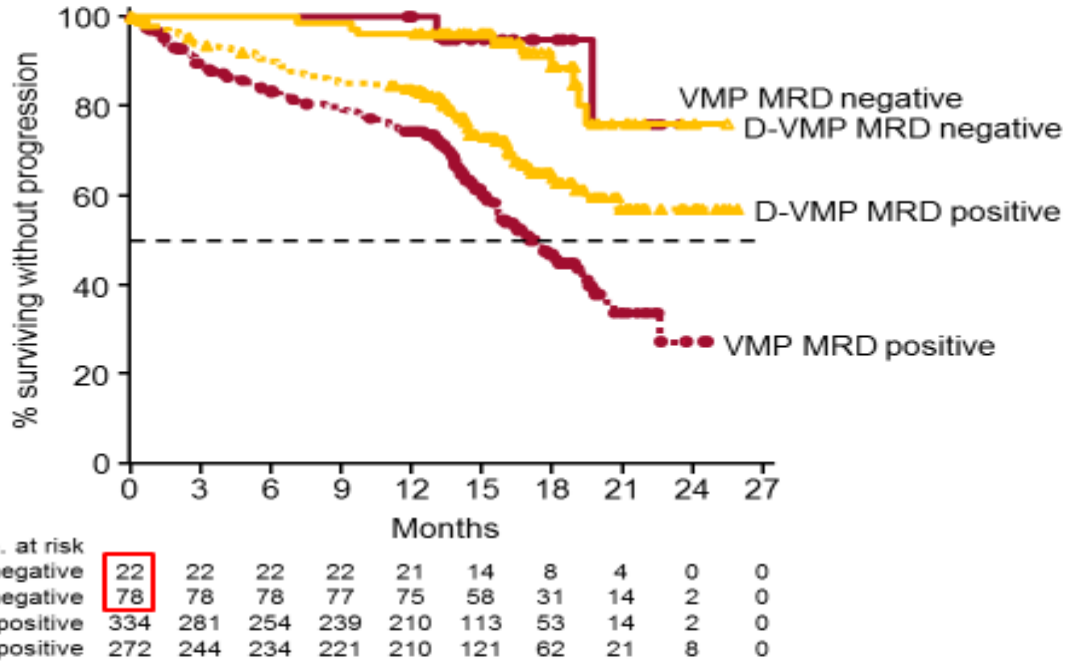
Mateos MV, et al. Presented at ASH 2017 (Abstract LBA-4), oral presentati

Daratumumab-VMP *versus* VMP

ALCYONE Efficacy: MRD[a] (NGS; 10^{-5} Sensitivity Threshold)

- Median (range) follow-up: 16.5 (0.1-28.1) months





No. at risk										
VMP MRD negative	22	22	22	22	21	14	8	4	0	0
D-VMP MRD negative	78	78	78	77	75	58	31	14	2	0
VMP MRD positive	334	281	254	239	210	113	53	14	2	0
D-VMP MRD positive	272	244	234	221	210	121	62	21	8	0

>3-fold higher MRD-negativity rate with D-VMP;
Lower risk of progression or death in all MRD-negative patients

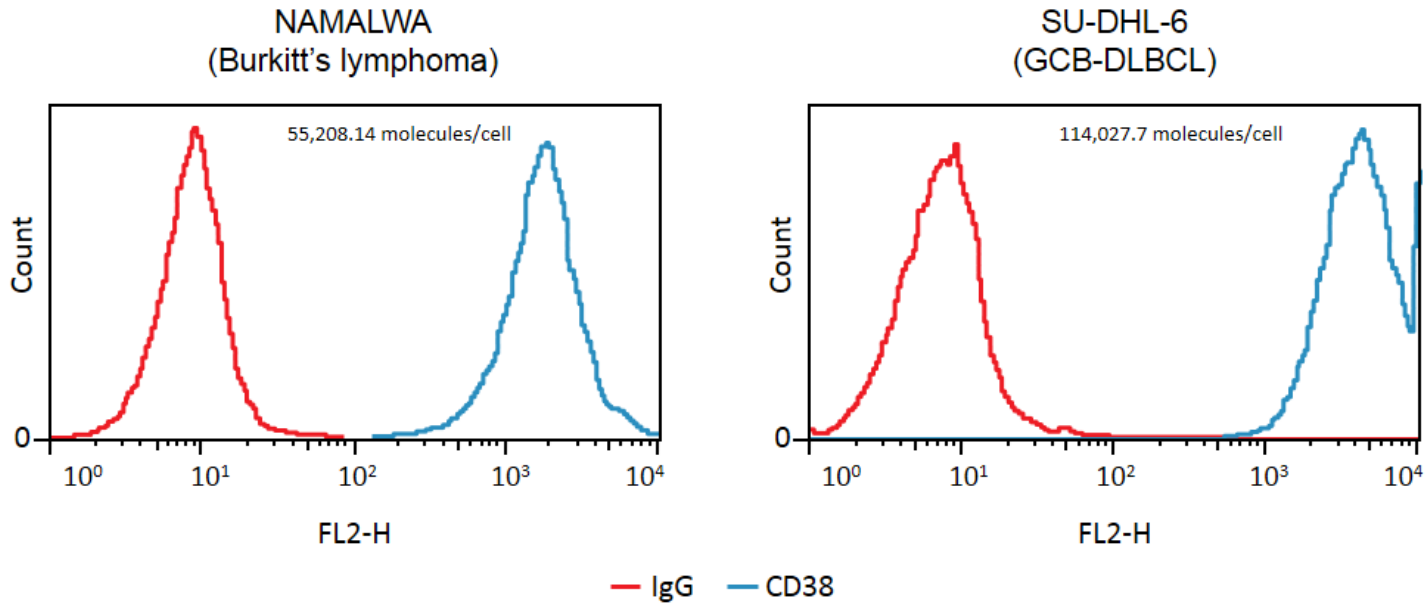
MRD, minimal residual disease; NGS, next-generation sequencing using clonoSEQ version 2.0 (Adaptive); VMP, bortezomib/melphalan/prednisone; D, daratumumab; CR, complete response ; sCR stringent complete response.
[a]Assessed at time of confirmation of CR/sCR, and if confirmed, 12, 18, 24, and 30 months after first dose.

Mateos MV, et al. Presented at ASH 2017 (Abstract LBA-4), oral presentation.

CD38 in myeloma and beyond: groundwork & outlook

Daratumumab + CHOP or R-CHOP: Preclinical Models of NHL

CD38 expression in representative NHL tumor cell lines



Evaluation of 16 NHL cell lines for CD38 expression suggested that CD38 expression levels varied among cell lines, but ***the majority (81%, n=13/16) had >1000 CD38 receptors per cell.***

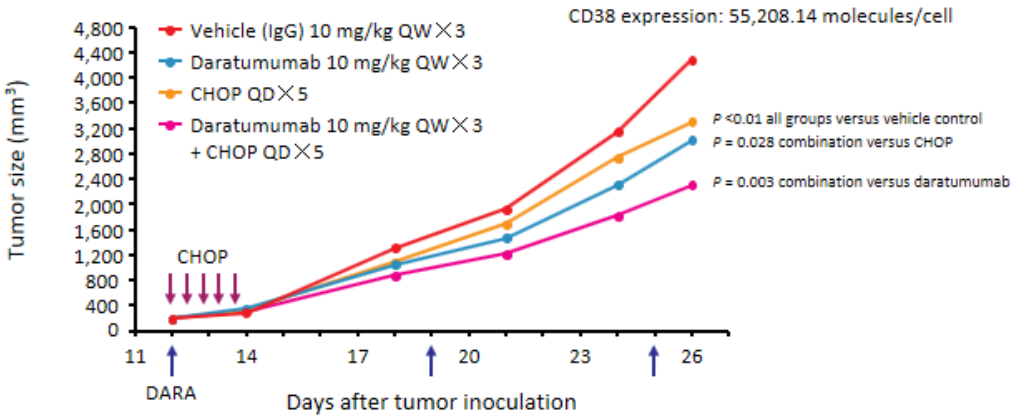
ected by flow cytometry using anti-CD38 antibody (blue curve); isotype control antibody (red curve) was used as a contr

Doshi P, et al. I

Daratumumab + CHOP or R-CHOP: Preclinical Models of NHL

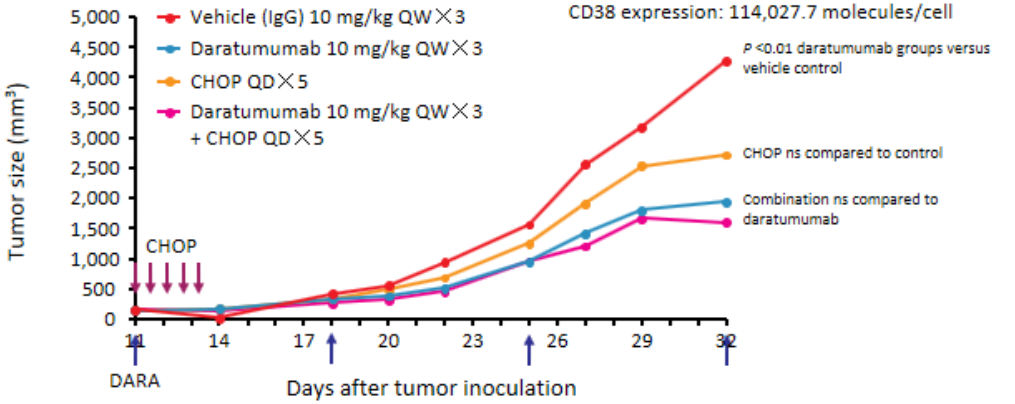
Effect of daratumumab ± CHOP in NAMALWA and SU-DHL-6 xenograft models

/SCID mice
ected with
NAMALWA
; treatment
ated when
s were ~200
mm^3



In NAMALWA in vivo model, DARA in combination with CHOP showed significant tumor growth inhibition (47%, p <0.001) compared to the control group on day 26.

/SCID mice
ected with
2×10^5
OHL-6 cells;
eatment
ated when
nors were
200 mm^3

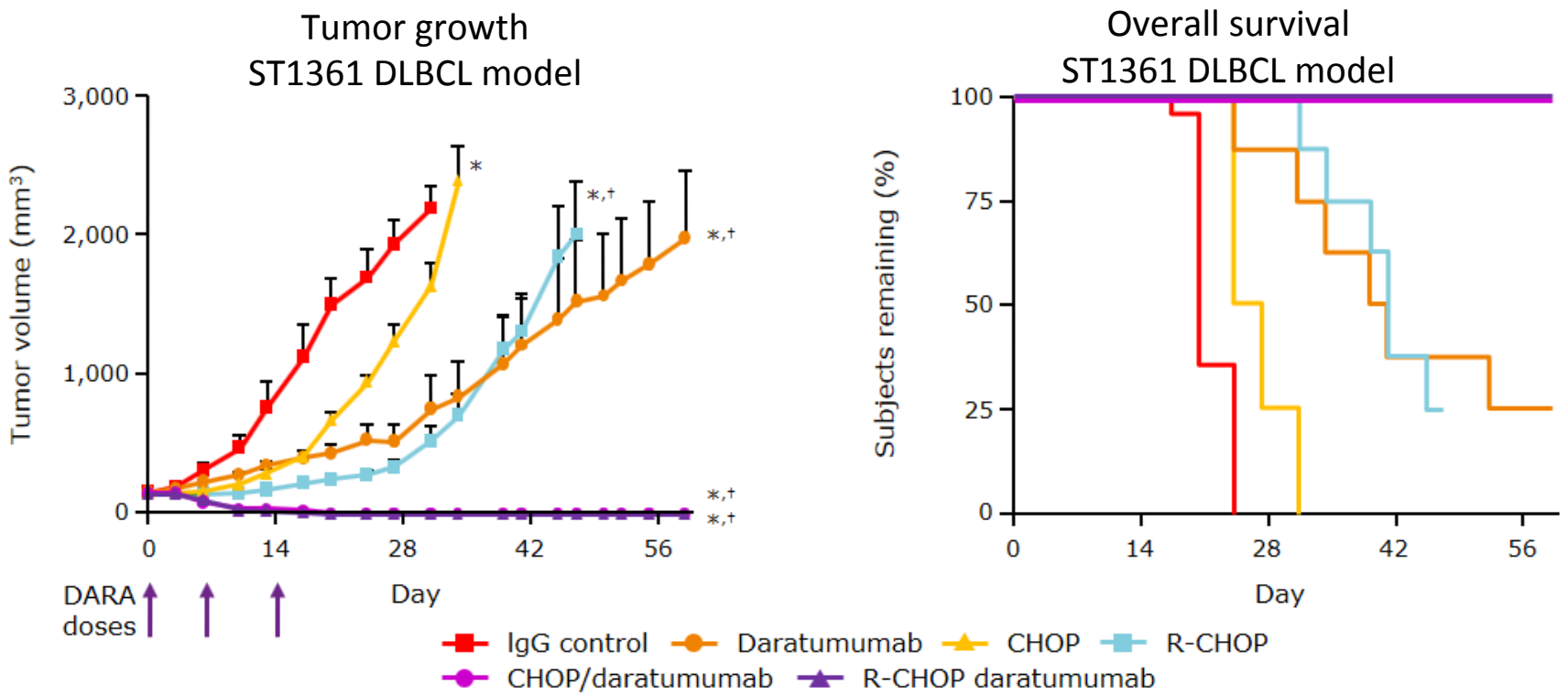


In SU-DHL-6 model, daratumumab either alone or in combination with CHOP showed significant tumor growth inhibition (55% an 63%, respectively, p<0.01) by day 32.

Doshi P, et al. I

Daratumumab + CHOP or R-CHOP: Preclinical Models of NHL

Effect of daratumumab on tumor growth in patient-derived DLBCL model



- SCID mice injected with fragments from ST1361 patient-derived tumor; assessment of tumor growth inhibition and overall survival was initiated when mean tumor volume was ~150-250 mm^3. Daratumumab was administered weekly at 20 mg/kg ± CHOP

In a patient-derived DLBCL model with high (3+) CD38 expression in 80% of tumor cells by IHC, daratumumab in combination with CHOP or R-CHOP showed tumor regression and the tumors did not regrow when the treatment with daratumumab was stopped after 3 doses.

s control; †*P* <0.05 vs CHOP.

Doshi P, et al. I

aratumumab in Mantle Cell Lymphoma and Follicular Lymphoma

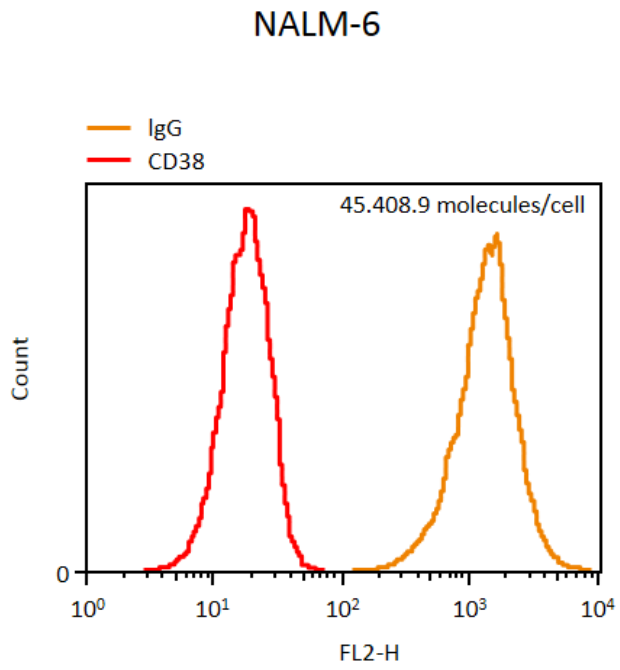
- **Daratumumab induces cell killing by ADCC and ADCP in MCL and FL cells**
- **Lenalidomide pretreatment of PBMCs significantly increases daratumumab ADCC activity**
- **Prophylactic daratumumab treatment inhibits MCL and FL tumor growth *in vivo* in subcutaneous tumor models**
- **Daratumumab improves overall survival in systemic mouse models of MCL and FL**
- **Combination of daratumumab with CHOP or R-CHOP are highly effective regimens in a tFL mouse model**

ibody-dependent cell-mediated cytotoxicity
rammed cell death
body-dependent cell-mediated phagocytosis

Pérez-Galán P, et al. Oral presentation at: 14th International Conference on Malignant Lymphom
June 14-17, 2017; Lugano, Sw

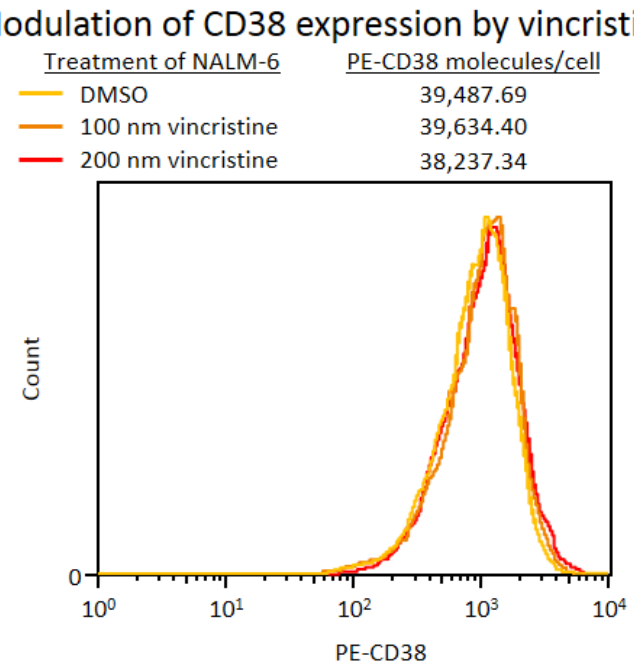
Daratumumab +/- Vincristine: Preclinical Models of Acute Lymphoblastic Leukemia (ALL)

CD38 expression in representative ALL tumor cell line and effect of vincristine on CD38 expression



FL2-H

CD38 expression in NALM-6 cells detected via flow cytometry using anti-CD38 antibody; isotype control antibody was used as a control



PE-CD38

NALM-6 cells incubated with either dimethyl sulfoxide or vincristine (100 or 200 nM) for 24 hours

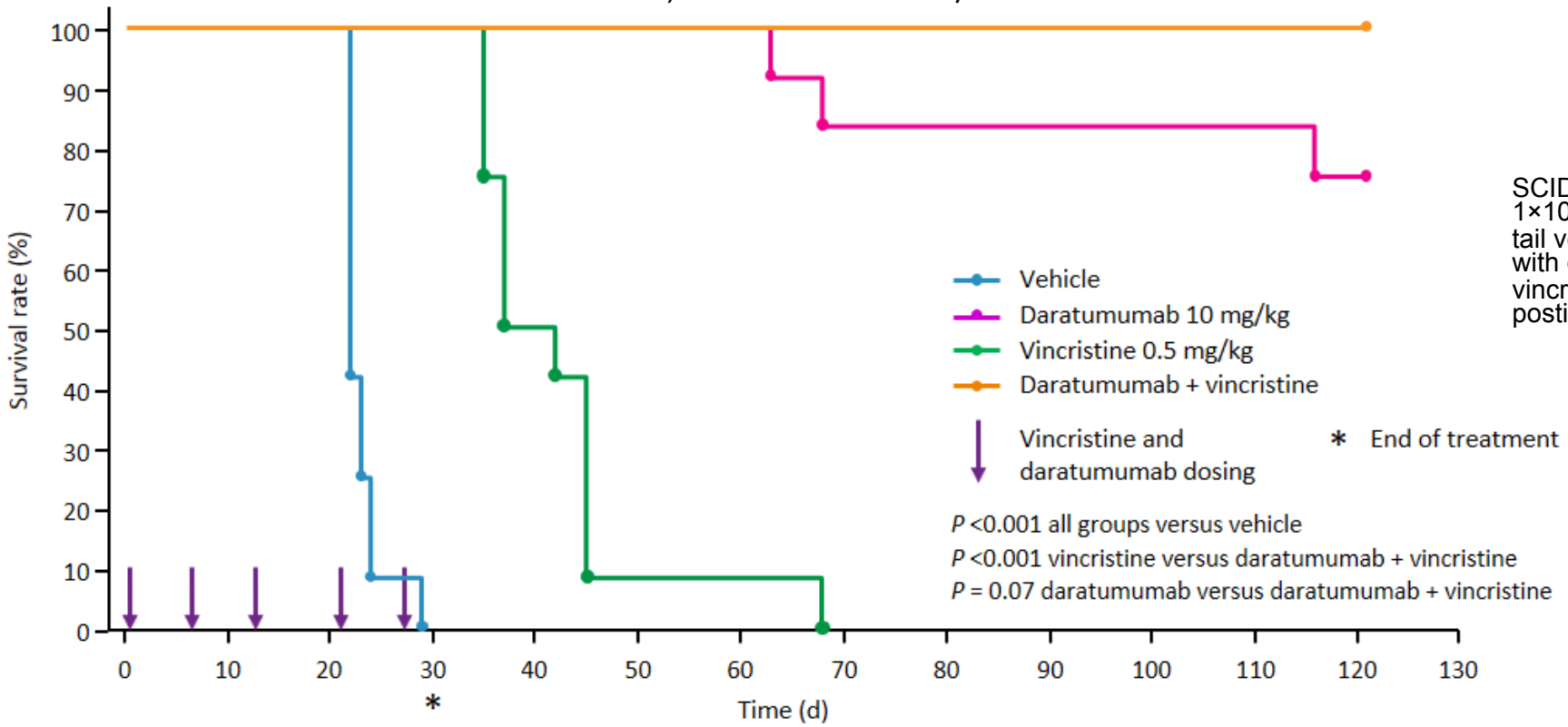
Evaluation of the expression of CD38 in 9 ALL cell lines suggested that **CD38 expression varied among different cell lines but the majority (88%, n=8/9) had >1000 CD38 receptors per cell.** Treatment with vincristine did not modulate CD38 expression.

Doshi P, et al. E

Daratumumab +/- Vincristine: Preclinical Models of Acute Lymphoblastic Leukemia (ALL)

Effect of daratumumab ± vincristine in NALM-6 xenograft model

NALM-6: 45,409 CD38 molecules/cell



SCID mice injected wit
1×10^5 NALM-6 cells vi
tail vein. Animals treat
with daratumumab and
vincristine on Day 2
postinoculation

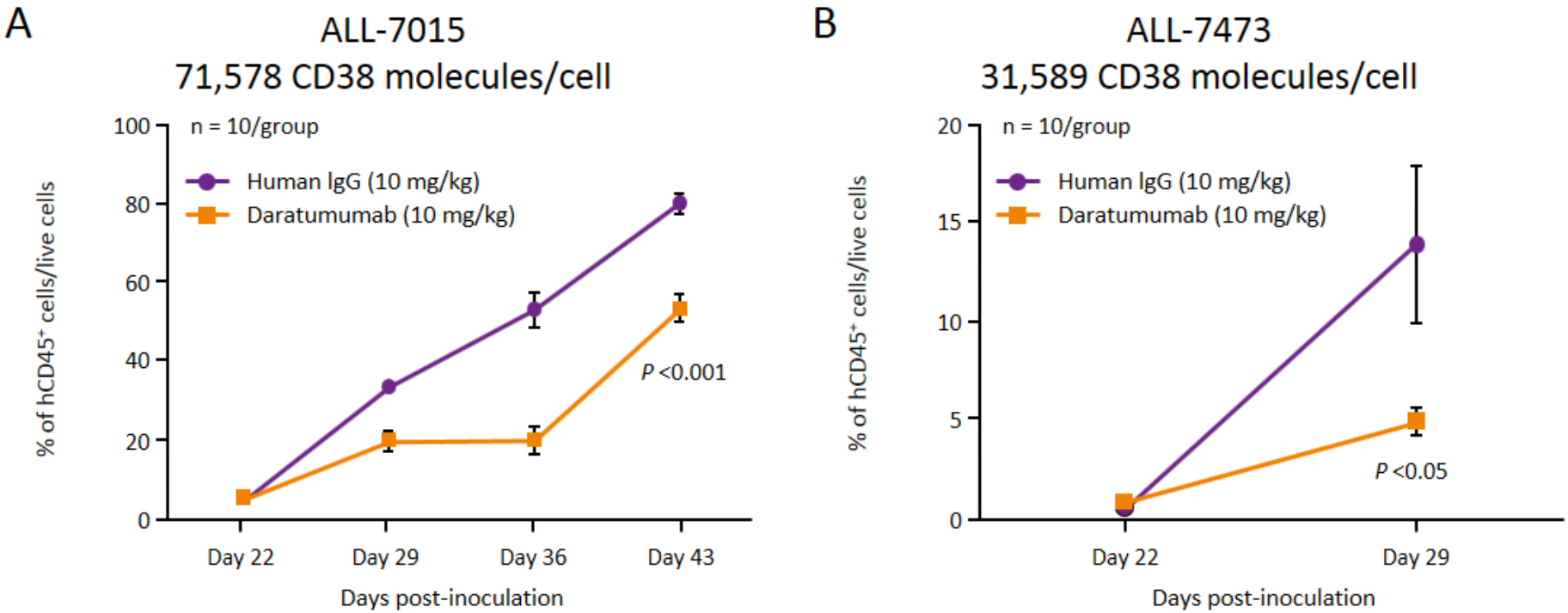
umumab either alone or with vincristine showed significant prolongation of survival.

nimals in the control group died by day 22 and the animals in vincristine group died by day 43, however, 80-100% of
ls in daratumumab alone or in combination with vincristine survived beyond day 88.

Doshi P, et al. E

Daratumumab +/- Vincristine: Preclinical Models of Acute Lymphoblastic Leukemia (ALL)

Effect of daratumumab on tumor growth in patient-derived models



Tumor burden measured for NOD/SCID mice injected with 2×10^6 ALL-7015 (n = 10) and ALL-7473 (n = 10) tumor cells. Dosing initiated when tumor burden in peripheral blood was 5%-10% $CD45^+$ cells; mice were treated weekly with control IgG or daratumumab

tient-derived CD38+/BCR-ABL+ **B-ALL**-7015 model, daratumumab treatment resulted in significant tumor grov
tion p<0.001) by day 22 compared to the control antibody.
38+/BCR-ABL- **T-ALL**-7473 model, treatment with daratumumab resulted in significant tumor growth inhibitio
(p<0.05) but the animals developed disease by day 14 and were sacrificed on day 21.

Doshi P, et al. E

Other hematological malignancies

- CD38 antibodies activity against lymphoma cell lines and mouse model.

- Daratumumab exerts significant cytotoxicity against ***chronic lymphocytic leukemia (CLL) cells*** via ADCC and ADCP, but without significant CDC, probably as a result of high complement inhibitor expression.
- Daratumumab interferes with CD38 signaling and reduces CLL adhesion, migration, and homing.

- CD38 is also strongly expressed on NK cells, which explains the sustained response with daratumumab in a patient with relapsed/refractory ***nasal-type extranodal NK-cell–T-cell lymphoma***

- Preclinical studies have also shown activity of CD38 antibodies against T-cell acute lymphoblastic leukemia (ALL) and B-cell ALL***, Waldenstrom macroglobulinemia.***

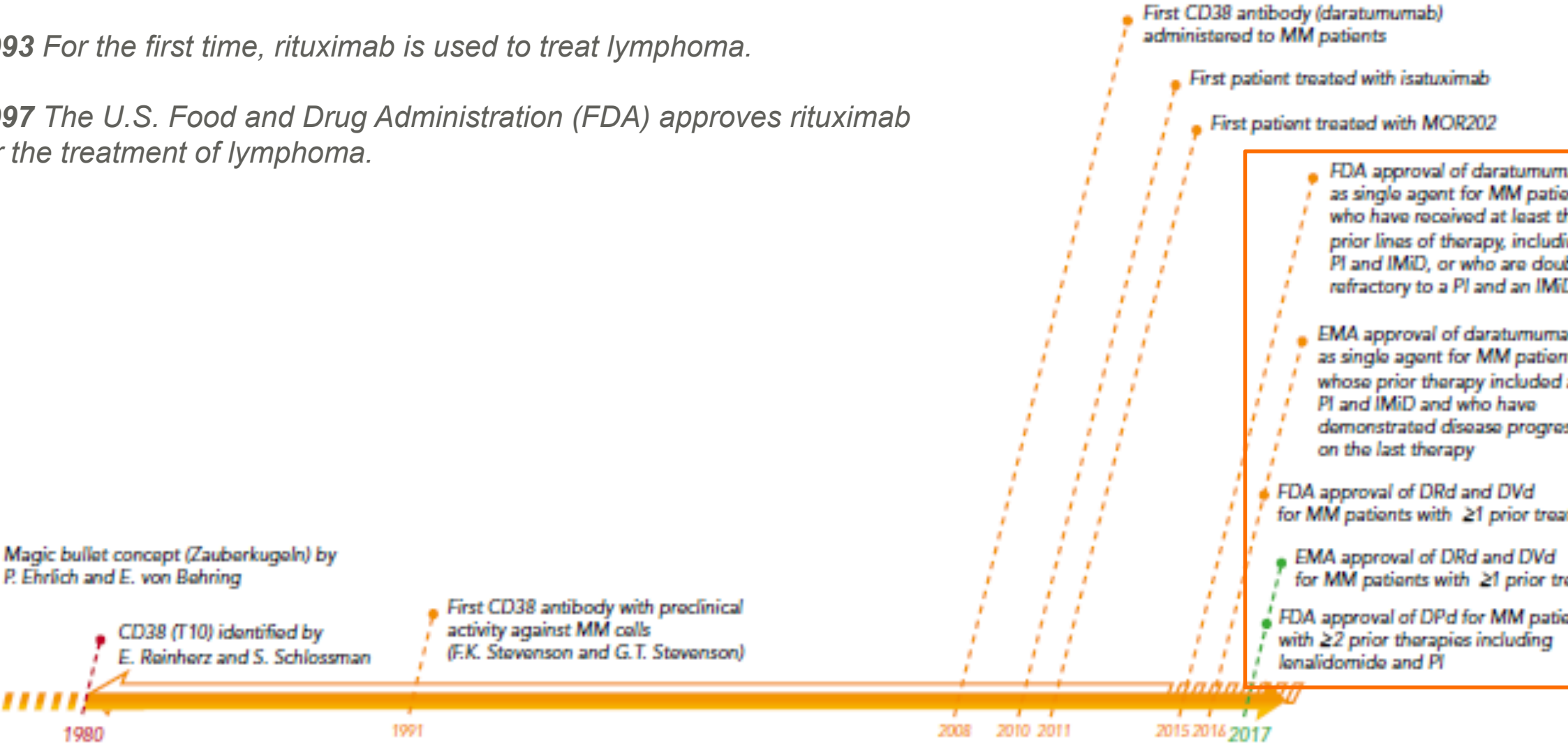
Niels W. C. J. van de Donk, Paul G. Richardson,Fabio Malavasi Blo

CD38 in myeloma and beyond: groundwork & outlook

History of CD38 antibodies

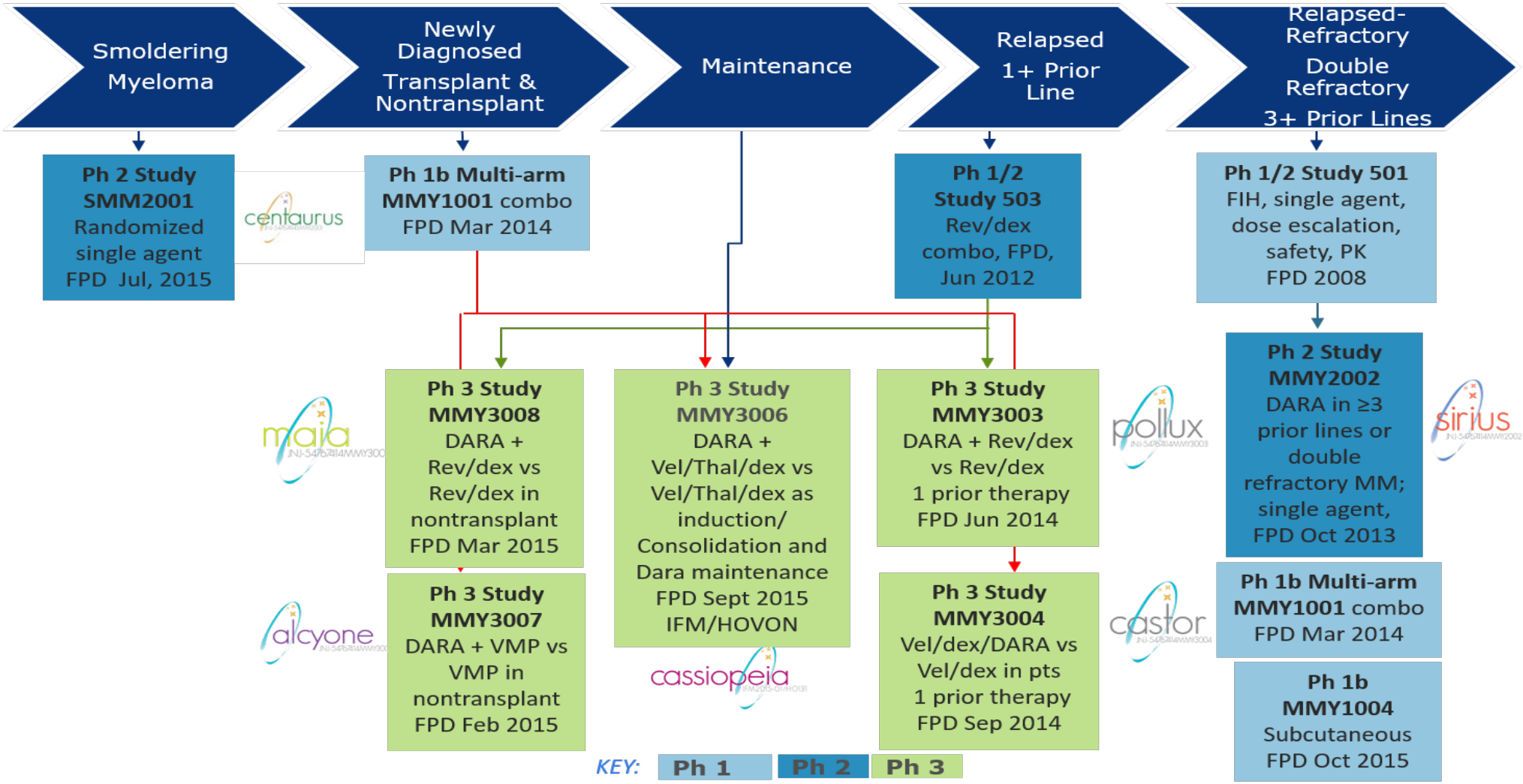
***93** For the first time, rituximab is used to treat lymphoma.*

***97** The U.S. Food and Drug Administration (FDA) approves rituximab r the treatment of lymphoma.*



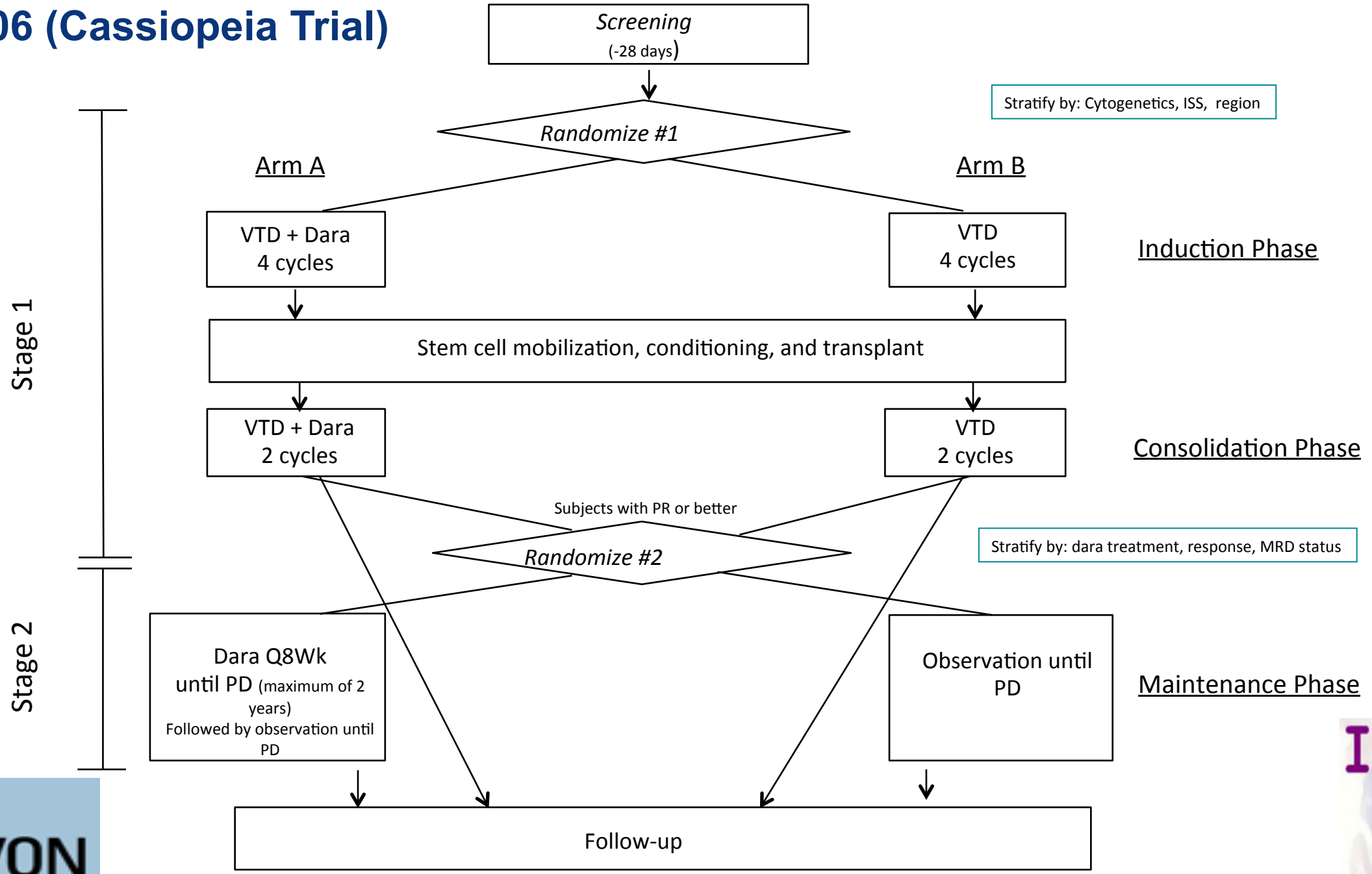
Niels W. C. J. van de Donk, Paul G. Richardson,Fabio Malavasi Blo

Daratumumab Development In MM Settings



Immunotherapeutic strategies are emerging as promising therapeutic approches in MM with several MoAb in advanced stages of clinical development.

Y3006 (Cassiopeia Trial)



Selected ongoing or planned studies with CD38-targeting antibody-containing regimens in other conditions

Study	Phase	Patients	Treatment
NCT02841033	1/2	Relapsed or refractory AL amyloidosis	Daratumumab as single agent
NCT02816476 (AMYDARA)	2	Patients with AL amyloidosis not in VGPR or better after previous treatment	Daratumumab as single agent
NCT03067571	2	AML or high-risk myelodysplastic syndrome (relapsed or refractory)	Daratumumab as single agent
NCT03011034	2	Transfusion-dependent patients with low or intermediate-1 risk myelodysplastic syndrome who are relapsed or refractory to erythropoiesis-stimulating agents	Daratumumab as single agent
NCT02413489 (Carina)	2	Relapsed/refractory CD38+ mantle cell lymphoma, diffuse large B-cell lymphoma, and follicular lymphoma	Daratumumab as single agent
NCT02927925	2	Relapsed/refractory NKTCL, nasal type	Daratumumab as single agent
NCT01084252	1/2	Relapsed/refractory CD38+ hematological malignancies such as B-cell non-Hodgkin lymphoma, MM, AML, B-ALL, and CLL	Isatuximab as single agent
NCT02999633	2	Relapsed or refractory T-ALL and T-LBL	Isatuximab as single agent

Niels W. C. J. van de Donk, Paul G. Richardson,Fabio Malavasi Blo

Conclusions
CD38

D38 antibodies have impressive single agent activity in heavily pretreated MM patients

D38 antibodies can also safely be added to backbone regimens, markedly increasing their
ficacy.

D38 antibodies will increasingly be incorporated into first-line anti MM regimens over the ne
ars given their efficacy and manageable toxicity profile.

arious studies are currently evaluating the role of CD38 antibodies in induction, consolidatio
d maintenance in both transplant-eligible and elderly patients with newly diagnosed MM.

D38 antibodies are also being evaluated in smoldering MM to prevent progression to active

rthermore, CD38 antibodies are currently also under investigation in other hematologic
alignancies, as well as in solid tumors.

Acknowledgments

Divisione di Ematologia U
Azienda Ospedaliero Universitaria Città della Salute e della Scienza
Torino, Italy

Prof. Mario Boccadoro

Dr. Sara Bringhen
Dr. Francesca Gay
Dr. Stefania Oliva
Dr Mariella Genuardi

Dr. Chiara Cerrato
Dr. Giusy Cetani
Dr. Mattia D'Agostino
Dr. Francesca Bonello
Dr. Roberto Mina
Dr. Marco Salvini

Dr. Paola Omedé &
Laboratory Staff

Dr. Benedetto Bruno &
Transplant Unit

Nurses

Data Managing Staff

Statistician Dr. Stefano Spada
Dr. Gianni Ciccone and CPO