

## ORIGINAL ARTICLE

## Changes in uninvolved immunoglobulins during induction therapy for newly diagnosed multiple myeloma

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Little is known about the impact of multiple myeloma (MM) treatment on uninvolved immunoglobulins (Ig). We identified 448 patients who received high-dose dexamethasone (HD-DEX), lenalidomide and dexamethasone (RD), bortezomib and dexamethasone (VD), bortezomib, cyclophosphamide and dexamethasone (VCD) or bortezomib, lenalidomide and dexamethasone (VRD) for newly diagnosed MM at our institution between 2000 and 2013, and who had available data on absolute lymphocyte count (ALC) and quantitative uninvolved Ig at baseline and at the end of four cycles of therapy. Changes in ALC and uninvolved Ig were significantly different across treatments, with VCD and HD-DEX producing reductions in uninvolved Ig, and RD, VD and VRD leading to increases in uninvolved Ig. In addition, treatment with RD, VD and VRD was independently associated with higher odds of achieving a  $\geq 25\%$  increase in or normalization of the primary uninvolved Ig on multivariate analysis. Although achievement of a humoral response in the primary uninvolved Ig was associated with a higher odds of achieving VGPR or better after four cycles of therapy, it was not associated with improved overall survival. These data highlight the different mechanisms of action of MM drugs and point toward a possible role for the use of VCD in treating antibody-mediated autoimmune disease.

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## INTRODUCTION

Multiple myeloma (MM) is a clonal plasma cell proliferative disorder that is predicted to account for  $> 15\%$  of all hematologic malignancies diagnosed in the United States this year.<sup>1</sup> The majority of patients present with an elevated monoclonal protein, and a reduction in one or more uninvolved, polyclonal immunoglobulin (Ig) is seen in  $> 90\%$  of patients, especially in individuals with IgA myeloma.<sup>2</sup>

Immunoparesis has been shown to have prognostic impact in MM, with inferior progression-free and overall survival (OS) seen in those with suppression of at least one uninvolved Ig compared with those in whom all uninvolved Ig are preserved;<sup>3</sup> In addition, suppression of the uninvolved (polyclonal) Ig of the same isotype as the tumor is associated with a poorer prognosis.<sup>4</sup> Similar findings have also been reported in MM-related disorders, including monoclonal gammopathy of undetermined significance,<sup>5</sup> smoldering MM<sup>6</sup> and light chain amyloidosis.<sup>7</sup>

There are, however, limited data on the changes that occur in uninvolved Ig during MM therapy. A pooled analysis of the phase 3 MM-009 and MM-010 lenalidomide trials showed that an increase in IgA occurred in  $> 50\%$  of patients with non-IgA MM and that these 'humoral responders' enjoyed longer progression-free and OS.<sup>8</sup> A similar increase in progression-free survival among patients with an increase in uninvolved Ig after 6 months of treatment with lenalidomide has also been reported.<sup>9</sup> However, these data are from patients with relapsed/refractory disease, which makes it difficult to discern the impact of the treatment regimen on immune function owing to prior exposure to other therapies, and there are also no data on agents such as bortezomib.<sup>10</sup>

We therefore sought to evaluate the changes in uninvolved Ig that occur during the first few months of therapy in a contemporary cohort of individuals with a new diagnosis of MM, and determine whether these changes are impacted by the treatment regimen, as well as the impact of such changes on treatment response and survival.

## SUBJECTS AND METHODS

## Study cohort

The study population was derived from an electronic database containing records of consecutive patients who were newly diagnosed with MM at Mayo Clinic between January 2000 and December 2013 ( $n = 1155$ ). Patients in whom Ig levels (IgA, IgG and IgM) were available both at the time of diagnosis as well as at the end of approximately four cycles of first-line therapy were included; this was chosen as it typically represents the end of induction therapy in transplant-eligible patients.<sup>11</sup> When Ig levels at the end of four cycles were unavailable, levels at the end of three ( $n = 44$ ), five ( $n = 22$ ) or six ( $n = 15$ ) cycles were used instead. Only those receiving the following first-line therapy were included: single-agent high-dose dexamethasone (HD-DEX), lenalidomide and dexamethasone (RD), bortezomib and dexamethasone (VD), bortezomib, cyclophosphamide and dexamethasone (VCD), and bortezomib, lenalidomide and dexamethasone (VRD). HD-DEX was included as it was used in the other treatments (RD, VD, VCD, VRD), and could therefore act as a useful comparator or control, whereas patient receiving thalidomide were excluded as it is not commonly used in contemporary induction regimens. Other exclusion criteria included biclonal or non-secretory MM, use of any plasma cell-directed therapy before the diagnosis of MM, and discontinuation or change of treatment prior to the end of cycle 4 of therapy.

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### Outcomes and definitions

After obtaining Institutional Review Board approval, baseline data on various parameters (Ig, difference in free light chains, white blood cell count, absolute lymphocyte count (ALC), percentage of bone marrow plasma cells), as well as the Ig and FLC levels at the end of four cycles of therapy were abstracted from the electronic medical record. Early transplant was defined as receipt of an autologous stem cell transplant after a maximum of six cycles of therapy. To assess change in uninvolved Ig, the mean of the percentage differences between baseline and four cycles for each uninvolved Ig (IgA and IgM for IgG myeloma, IgG and IgM for IgA myeloma, IgG and IgA for IgM and IgD myeloma, and IgG, IgM and IgA for light chain-only myeloma) was calculated. Change in the primary uninvolved Ig was determined by calculating the percentage change between baseline and four cycles in IgA (in cases of IgG myeloma) and IgG (for all other myeloma subtypes). Ig levels were categorized as 'low' or 'normal' based upon the reference ranges used in our laboratory (IgA: 61–356 mg/dl, IgM: 37–286 mg/dl, IgG: 767–1590 mg/dl). A humoral response in the primary uninvolved Ig constituted a conversion from a 'low' level to at least the lower limit of normal and/or a  $\geq 25\%$  increase in levels between baseline and four cycles.<sup>8</sup>

Treatment response at the end of four cycles of therapy was determined according to International Myeloma Working Group criteria.<sup>12</sup> Time to treatment failure (TTF) was defined as the duration from the start of initial therapy to the start of the next line of therapy (for disease progression) or death (if no additional treatment was received), or censored at the date of last follow-up. OS was calculated in a landmark fashion, as the duration between the date of follow-up after four cycles of therapy and the date of last follow-up or death, or censored at the date of last follow-up.

### Statistical analysis

The Kruskal–Wallis and  $\chi^2$ -tests were used to detect differences in medians and proportions, respectively, and logistic regression used to test the association between variables and outcomes of interest. OS and TTF analyses were performed using the Kaplan–Meier method, and the log-rank test used to make comparisons between groups; a *P*-value of  $< 0.05$  was considered to be significant. Statistical analysis was performed using SPSS v.20 (IBM Corp., Armonk, NY, USA).

### RESULTS

A total of 448 patients were included in this analysis (Table 1). The median age of the cohort was 63 years (range 33–91), and the majority of patients (58%) were male. The monoclonal Ig subtype was IgG (59%), IgA (23%) or light chain-only (16%) in the majority of cases, and the most common first line of therapy was RD ( $n = 221$ , 49%), followed by HD-DEX ( $n = 82$ , 18%), VCD ( $n = 77$ , 17%), VRD ( $n = 46$ , 10%) and VD ( $n = 22$ , 5%). Median OS was 7.8 years (95% confidence interval 7.0–8.5), and median TTF was 2.1 years (1.9–2.3); median follow-up was 6.4 years (5.7–7.0).

### Changes in uninvolved Ig between baseline and four cycles of therapy

The levels of white blood cell count, ALC and difference in free light chains after four cycles, and the changes in white blood cell count, ALC and uninvolved Ig between baseline and four cycles are shown in Table 2. Treatment with VCD was associated with the greatest median decline in ALC, with the smallest reduction in ALC seen with the use of HD-DEX (HD-DEX:  $-4\%$  ( $-71$  to  $+376$ ); RD:  $-11\%$  ( $-84$  to  $+719$ ); VD:  $-21\%$  ( $-70$  to  $+386$ ); VCD:  $-46\%$  ( $-93$  to  $+112$ ); VRD:  $-21\%$  ( $-76$  to  $+141$ ),  $P < 0.001$ ). Changes in uninvolved Ig were significantly different across treatments, with a median reduction in uninvolved Ig seen with the use of HD-DEX ( $-2\%$  ( $-78$  to  $+1094$ )) and VCD ( $-14\%$  ( $-75$  to  $+244$ )), and median increases observed with RD ( $+14\%$  ( $-71$  to  $+564$ )), VD ( $+29\%$  ( $-63$  to  $+329$ )) and VRD ( $+81\%$  ( $-65$  to  $+590$ ),  $P < 0.001$ ), with VCD producing the largest decline in uninvolved Ig. Similar trends were seen when change in only the primary uninvolved Ig was considered, with HD-DEX, VD and VCD producing median reductions in the primary uninvolved Ig (HD-DEX:  $-27\%$  ( $-80$  to  $+630$ ); VD:  $-2\%$  ( $-87$  to  $+320$ ); VCD:  $-18\%$  ( $-88$  to  $+271$ )), and

**Table 1.** Baseline demographics prior to starting first-line therapy

	N
Age, median (range)	63 (33–91)
Sex (%)	
Male	259 (58)
Female	189 (42)
Monoclonal Ig subtype (%)	
IgG	265 (59)
IgA	105 (23)
IgM	5 (1)
IgD	2 (0.5)
Light chain-only	71 (16)
BMPCs (%)	
$< 10\%$	25 (6)
10–59%	267 (60)
$\geq 60\%$	148 (33)
Not available	8 (2)
Uninvolved Ig, median (range), mg/dl	
IgG myeloma	
IgA	36 (2–355)
IgM	24 (5–237)
IgA myeloma	
IgG	398 (129–1210)
IgM	23 (5–110)
IgM/D myeloma	
IgG	336 (109–740)
IgA	22 (8–104)
Light chain-only myeloma	
IgG	444 (75–1060)
IgA	44 (3–306)
IgM	23 (5–100)
WBC, median (range), $\times 10^9/l$	5.2 (1.1–13.8)
ALC, median (range), $\times 10^9/l$	1.5 (0.2–5.2)
dFLC, median (range), mg/dl	49 (0–6620)
First line of therapy (%)	
HD-DEX	82 (18)
RD	221 (49)
VD	22 (5)
VCD	77 (17)
VRD	46 (10)

Abbreviations: ALC, absolute lymphocyte count; BMPCs, bone marrow plasma cells; dFLC, difference in free light chains; HD-DEX, high-dose dexamethasone; Ig, immunoglobulin; RD, lenalidomide and dexamethasone; VCD, bortezomib, cyclophosphamide and dexamethasone; VD, bortezomib and dexamethasone; VRD, bortezomib, lenalidomide and dexamethasone; WBC, white blood cells.

increases seen with RD and VRD (RD:  $+3\%$  ( $-69$  to  $+788$ ); VRD:  $+9\%$  ( $-81$  to  $+683$ ),  $P < 0.001$ ).

The same pattern of change in white blood cell count, ALC, uninvolved and primary Ig across treatment regimens was seen when only patients achieving VGPR or better after four cycles were considered (Supplementary Table 1). A dot-plot of the change in uninvolved Ig across different treatments and stratified by response after four cycles is shown in Figure 1.

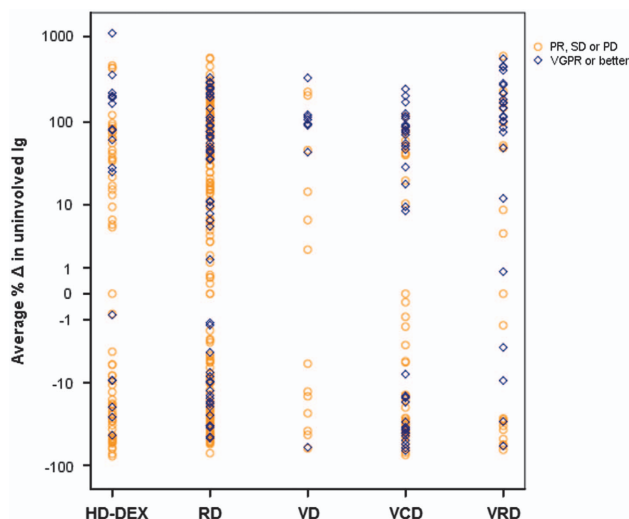
### Pattern of change in primary uninvolved Ig and humoral response

The pattern of change in the primary uninvolved Ig is shown in Table 3. Normalization of a low primary uninvolved Ig occurred in 43 patients overall (10%), and was more frequently seen with the RD (14%) and VRD (13%) compared with HD-DEX (5%), VD (5%) and VCD (1%,  $P = 0.035$ ). A similar pattern emerged when considering only the subgroup of patients in whom the primary

**Table 2.** Parameters after four cycles (C4) of therapy, and change ( $\Delta$ ) between baseline and C4

	All patients (n = 448)	HD-DEX (n = 82)	RD (n = 221)	VD (n = 22)	VCD (n = 77)	VRD (n = 46)	P-value
C4 WBC, median (range), $\times 10^9/l$	4.8 (1.2–33.9)	6.5 (3.5–19.1)	4.3 (1.2–13.4)	6.0 (2.7–33.9)	4.7 (2.0–21.2)	4.6 (2.4–10.4)	–
C4 ALC, median (range), $\times 10^9/l$	1.2 (0.2–7.4)	1.2 (0.4–7.4)	1.3 (0.3–4.3)	1.2 (0.5–2.3)	0.7 (0.2–2.3)	1.1 (0.4–1.8)	–
C4 dFLC, median (range), mg/dl	3 (0–1779)	5 (0–606)	5 (0–1779)	4 (0–211)	1 (0–169)	1 (0–150)	–
Response after C4 (%)							
VGPR or better	152 (34)	18 (22)	62 (28)	9 (41)	37 (48)	26 (57)	–
PR	193 (43)	30 (37)	112 (51)	8 (36)	25 (33)	18 (39)	–
SD or PD	101 (23)	33 (40)	47 (21)	4 (18)	15 (19)	2 (4)	–
Not evaluable	2 (0.5)	1 (1)	0 (0)	1 (5)	0 (0)	0 (0)	–
Early transplant(%)							
Yes	223 (50)	66 (81)	77 (35)	12 (55)	51 (66)	17 (37)	–
No	225 (50)	16 (20)	144 (65)	10 (46)	26 (34)	29 (63)	–
Median % $\Delta$ WBC (range)	–5 (–78 to +281)	+32 (–44 to +275)	–11 (–71 to +253)	–12 (–35 to +281)	–11 (–78 to +231)	–3 (–66 to +122)	< <b>0.001</b>
Median % $\Delta$ ALC (range)	–18 (–93 to +719)	–4 (–71 to +376)	–11 (–84 to +719)	–21 (–70 to +386)	–46 (–93 to +112)	–21 (–76 to +141)	< <b>0.001</b>
Median % $\Delta$ uninvolved Ig(range)	+9 (–78 to +1094)	–2 (–78 to +1094)	+14 (–71 to +564)	+29 (–63 to +329)	–14 (–75 to +244)	+81 (–65 to +590)	< <b>0.001</b>
Median % $\Delta$ primary uninvolved Ig (range)	–6 (–88 to +788)	–27 (–80 to +630)	+3 (–69 to +788)	–2 (–87 to +320)	–18 (–88 to +271)	+9 (–81 to +683)	< <b>0.001</b>

Abbreviations: ALC, absolute lymphocyte count; dFLC, difference in free light chains; HD-DEX, high-dose dexamethasone; Ig, immunoglobulin; PD, progressive disease; PR, partial response; RD, lenalidomide and dexamethasone; SD, stable disease; VCD, bortezomib, cyclophosphamide and dexamethasone; VD, bortezomib and dexamethasone; VRD, very good partial response; VRD, bortezomib, lenalidomide and dexamethasone; WBC, white blood cells. Bold indicates significant P values.



**Figure 1.** Dot-plot of average percentage change in uninvolved Ig for each treatment regimen, stratified by response after four cycles of therapy.

uninvolved Ig remained low ( $n=314$ ); within this cohort, an increase (but not to normal levels) was seen more frequently with the use of RD (49%), VD (44%) and VRD (53%) compared with HD-DEX (20%) and VCD (36%,  $P=0.001$ ).

A humoral response in the primary uninvolved Ig occurred in 111 patients (25%), and was observed more frequently with RD (28%), VD (32%) and VRD (41%) in comparison with HD-DEX (11%) and VCD (18%,  $P=0.001$ ). On multivariate analysis, adjusting for age, sex and monoclonal Ig subtype, treatment with RD (OR = 3.06 (1.42 – 6.60),  $P=0.004$ ), VD (OR = 3.91 (1.23 – 12.46),  $P=0.021$ ) and VRD (OR = 6.34 (2.49 – 16.14)),  $P < 0.001$ ) was associated with significantly higher odds of producing a humoral response in the primary uninvolved Ig (Table 4).

**Changes in uninvolved Ig, treatment response and survival**

On multivariate analysis adjusting for age, sex, treatment regimen and monoclonal Ig subtype, a humoral response in the primary uninvolved Ig was associated with a significantly greater odds of being in VGPR or better after four cycles of therapy (OR = 3.30 (1.91–5.69),  $P < 0.001$ , Table 5). Median TTF was longer in patients in whom a humoral response was seen, compared with those without a humoral response (2.5 years vs 2.0 years,  $P=0.034$ ), but no differences in OS were seen between the two groups (7.6 years vs 7.8 years,  $P=0.440$ , Figure 2). There were no significant differences in either TTF (1.9 years vs 2.2 years,  $P=0.513$ ) or OS (7.8 years vs 8.2 years,  $P=0.748$ ) between patients in whom the average percentage change in uninvolved Ig between baseline and four cycles was  $< 0$  and those in whom it was  $\geq 0$  (Figure 3).

**DISCUSSION**

MM is inextricably linked to immune dysfunction as it is a disorder of plasma cells, whose role is to produce antibodies during the adaptive immune response. Treatment of MM comprises of glucocorticoids, alkylating agents, thalidomide analogs and proteasome inhibitors, along with targeted therapies such as daratumumab<sup>13</sup> and elotuzumab,<sup>14</sup> which have recently been developed. It is apparent that studying the immune impact of MM treatment can generate information on the mechanism of action of these drugs, as well as providing evidence on the potential uses of these agents in immunologic disorders, where modulation of plasma cells and lymphocytes may be useful.

**Table 3.** Changes in primary uninvolved Ig between baseline and four cycles

	All patients (n = 448)	HD-DEX (n = 82)	RD (n = 221)	VD (n = 22)	VCD (n = 77)	VRD (n = 46)	P-value
$\Delta$ in primary uninvolved Ig (%)							<b>0.035<sup>a</sup></b>
Low $\rightarrow$ low	314 (70)	64 (78)	140 (63)	18 (82)	58 (75)	34 (74)	<b>0.001<sup>b</sup></b>
$\leq$ 0% change	185 (59)	51 (80)	71 (51)	10 (56)	37 (64)	16 (47)	
> 0% change	129 (41)	13 (20)	69 (49)	8 (44)	21 (36)	18 (53)	
Low $\rightarrow$ normal/high	43 (10)	4 (5)	31 (14)	1 (5)	1 (1)	6 (13)	
Normal $\rightarrow$ low	35 (8)	6 (7)	16 (7)	2 (9)	7 (9)	4 (9)	
Normal $\rightarrow$ normal/high	56 (13)	8 (10)	34 (15)	1 (5)	11 (14)	2 (4)	
Humoral response (%)							<b>0.001</b>
No	337 (75)	73 (89)	159 (72)	15 (68)	63 (82)	27 (59)	
Yes	111 (25)	9 (11)	62 (28)	7 (32)	14 (18)	19 (41)	

Abbreviations: HD-DEX, high-dose dexamethasone; RD, lenalidomide and dexamethasone; VD, bortezomib and dexamethasone; VCD, bortezomib, cyclophosphamide and dexamethasone; VRD, bortezomib, lenalidomide and dexamethasone. <sup>a</sup> $\chi^2$  for comparison across all categories. <sup>b</sup> $\chi^2$  for comparison within low–low category only. Bold indicates significant P values.

**Table 4.** Predictors of achieving a humoral response in primary uninvolved Ig

	OR (95% CI)	P-value
Age	0.99 (0.97–1.01)	0.396
Male sex	0.65 (0.41–1.02)	<b>0.060</b>
<i>Treatment regimen</i>		
HD-DEX	Ref.	–
RD	3.06 (1.42–6.60)	<b>0.004</b>
VD	3.91 (1.23–12.46)	<b>0.021</b>
VCD	1.78 (0.71–4.46)	0.218
VRD	6.34 (2.49–16.14)	< <b>0.001</b>
IgG myeloma	2.22 (1.37–3.60)	<b>0.001</b>

Abbreviations: HD-DEX, high-dose dexamethasone; RD, lenalidomide and dexamethasone; VD, bortezomib and dexamethasone; VCD, bortezomib, cyclophosphamide and dexamethasone; VRD, bortezomib, lenalidomide and dexamethasone. Bold indicates significant P values.

**Table 5.** Factors associated with achievement of VGPR or better after four cycles of therapy

	OR (95% CI)	P-value
Age	1.01 (0.98–1.03)	0.691
Male sex	1.93 (1.22–3.08)	<b>0.005</b>
<i>Treatment regimen</i>		
HD-DEX	Ref.	–
RD	1.28 (0.66–2.46)	0.463
VD	1.98 (0.65–6.06)	0.229
VCD	3.78 (1.77–8.06)	<b>0.001</b>
VRD	3.99 (1.65–9.61)	<b>0.002</b>
IgG myeloma	0.18 (0.11–0.28)	< <b>0.001</b>
Humoral response in uninvolved Ig	3.30 (1.91–5.69)	< <b>0.001</b>

Abbreviations: HD-DEX, high-dose dexamethasone; RD, lenalidomide and dexamethasone; VD, bortezomib and dexamethasone; VCD, bortezomib, cyclophosphamide and dexamethasone; VGPR, very good partial response; VRD, bortezomib, lenalidomide and dexamethasone. Bold indicates significant P values.

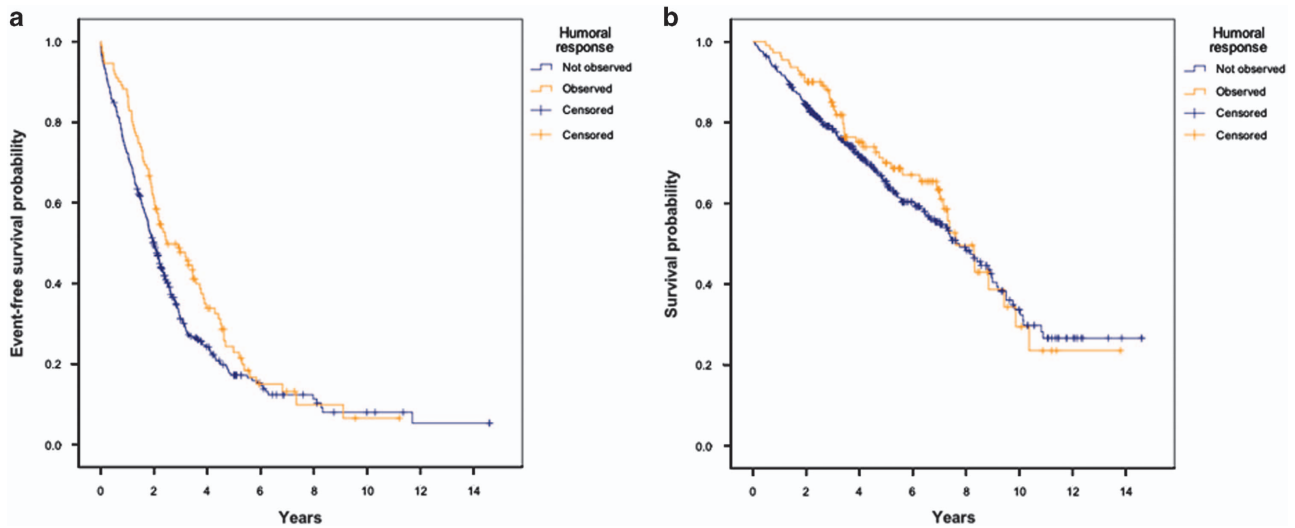
In this regard, our study has several noteworthy findings. First, our analysis has shown that MM treatments exert differential impacts on immune function in newly diagnosed patients, as measured by the change in ALC and uninvolved Ig during induction therapy. Treatment with VCD was associated with the greatest reductions in ALC and uninvolved Ig, whereas RD and VD

tended to produce increases in uninvolved Ig, with the triplet VRD combination leading to the highest increases in uninvolved Ig. Similarly, RD, VD and VRD were independently associated with greater odds of leading to humoral responses in the primary uninvolved Ig. These results were generally replicated when only considering patients who achieved VGPR or better after four cycles of therapy, confirming that these immunologic effects are not response-specific. To our knowledge, these are the first data comparing changes in uninvolved Ig and ALC between myeloma treatments and provide insight into the mechanisms of action of these drugs.

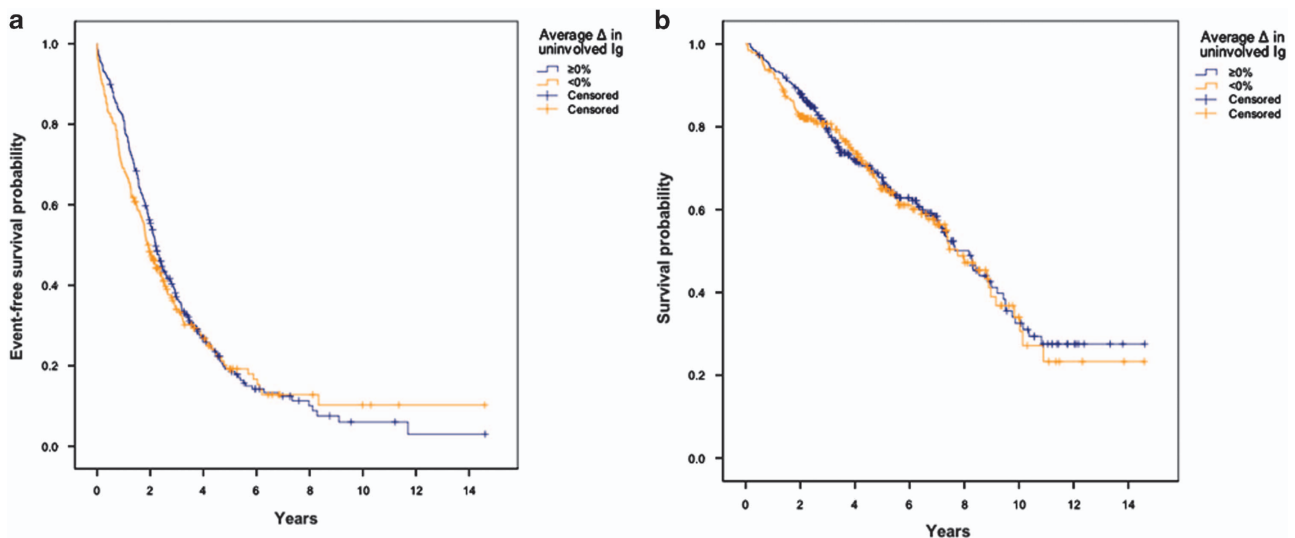
The molecular basis underlying the reduction in uninvolved Ig in MM is thought to be mediated by interactions between B-cell maturation antigen (BCMA) and its ligands, BAFF (B-cell activating factor) and (APRIL) a proliferation inducing ligand, which stimulate B-cell differentiation and antibody production.<sup>15</sup> Soluble BCMA has been identified in the serum of MM patients,<sup>16</sup> and recent work suggests that this may bind to circulating BAFF, thereby interfering with its role in stimulating antibody production and leading to a reduction in polyclonal, uninvolved Ig.<sup>17</sup> Pre-clinical data suggest that lenalidomide and bortezomib exert inhibitory effects on the BAFF/ARPIL/BCMA pathway,<sup>18</sup> with xenograft studies showing that BCMA levels decrease after treatment with the latter.<sup>16</sup> In addition, *in vitro* studies have shown that monoclonal antibodies directed against BCMA or APRIL augment the cytotoxicity of lenalidomide and dexamethasone against MM cells,<sup>19,20</sup> and such combinations are being investigated in Phase 1 trials.<sup>21</sup> Taken together, these mechanisms could explain our findings that RD, VD and especially VRD produced the largest rises in uninvolved Ig and greatest humoral responses, as lenalidomide and bortezomib may inhibit pathways that lead to immunoparesis in MM.

In contrast to RD, VD and VRD, treatment with VCD led to significant reductions in uninvolved Ig, with significantly smaller humoral responses rates. The most straightforward explanation is that the VCD regimen includes a classic cytotoxic agent (cyclophosphamide), leading to greater immunosuppression (by means of lymphopenia) in comparison with RD and VRD, which contain an immunomodulatory drug (lenalidomide) and produce an enhanced immune response. In the case of VCD, this effect seems to override the more tumor-specific action of bortezomib. Nevertheless, despite the different effects on uninvolved Ig, the odds of achieving VGPR or better after four cycles of therapy were very similar with VCD and VRD, suggesting that general suppression of lymphocytes and B cells (with VCD) and a more tumor-specific action (with VRD) may be roughly comparable treatment strategies. Unfortunately, head-to-head studies of these regimens are not available.





**Figure 2.** Time to treatment failure (a) and overall survival (b) stratified by presence or absence of a humoral response in the primary uninvolved Ig.



**Figure 3.** Time to treatment failure (a) and overall survival (b) stratified by average change in all uninvolved Ig.

We also found a greater suppression of uninvolved Ig with HD-DEX compared to RD, VD and VRD. This effect may be related to the dose intensity of dexamethasone; with HD-DEX, a total of 480 mg of dexamethasone is administered per month, whereas low-dose dexamethasone (120–160 mg per month) is the standard dose used in doublet and triplet regimens at our institution. Furthermore, the rates of achieving VGPR or better were lower with HD-DEX compared with combination therapy, and this may also limit recovery of uninvolved Igs.

In addition, we noted that the attainment of a humoral response in the primary uninvolved Ig was independently associated with higher odds of being in VGPR or better after four cycles of therapy, which translated into a marginal increase in TTF but had no impact on OS. Achieving better disease control likely enables recovery of normal plasma cells and restores the ability to mount a humoral immune response. The improvement in TTF in humoral responders is in keeping with previous studies showing improvement in progression-free survival among lenalidomide-treated patients in whom there were similar increases in uninvolved Ig;<sup>8,9</sup> nevertheless, the magnitude of the TTF benefit (~6 months) is smaller than

previously reported, whereas there was no OS benefit for humoral responders, in contrast to prior data.<sup>8</sup> Reasons for this include differences in patient cohort (we assessed newly diagnosed MM patients receiving induction therapy, whereas prior work was undertaken on either relapsed/refractory patients<sup>8</sup> or in patients receiving maintenance or post-induction therapy<sup>9</sup>) as well as the wider range of treatments in our study in comparison to previous data that pertained only to lenalidomide. Our results also suggest that the importance placed on uninvolved Ig as a marker of immunodeficiency, and subsequent risk of grave infectious complications, may be overstated.

There are limitations of our analysis aside from those inherent to a retrospective, observational study. First, there may have been selection bias in the choice of initial therapy, and the patient population was heterogeneous, albeit limited to only newly diagnosed MM patients who had not received prior therapy. The number of patients who received VD was small ( $n=22$ ), although the results of changes in ALC and uninvolved Ig seen with VRD seem to corroborate the findings with VD, given that the results with VRD were, to a degree, additive compared with RD and VD

alone. Finally, we were unable to capture data on infectious complications in parallel with the changes in Ig. However, the large number of patients studied, long follow-up, and evaluation of several treatments, all myeloma subtypes, and all uninvolved Ig serve to ensure that these results are a valuable addition to the literature.

In summary, this study highlighted that the changes in uninvolved Ig and ALC during MM therapy differ according to treatment regimen. VCD and HD-DEX produce reductions in uninvolved Ig, whereas RD, VD and VRD are associated with increases in uninvolved Ig, suggesting differential mechanisms of action among these agents. Moreover, these data point towards a possible role for VCD in the treatment of autoimmune disease, where reduction of antibodies production by non-malignant, polyclonal plasma cells is a therapeutic goal. Studies should examine the use of VCD in the treatment of these disorders, since a short course of VCD may be more tolerable than chronic treatment with steroids and other immunosuppressive agents, which is the typical therapy for such patients.

### CONFLICT OF INTEREST

SK has obtained research support for clinical trials from Celgene, Millennium, Novartis, Janssen and Sanofi. AD has received research support for clinical trials from Pfizer, Janssen, Millennium, Alnylam and Celgene. MAG has received research support from ISIS and Prothena, and honoraria from Celgene, Millennium Pharmaceuticals and Novartis. The remaining authors declare no conflict of interest.

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