Predictive Biomarkers and Personalized Medicine

Clinical Cancer Research

Thalidomide in Total Therapy 2 Overcomes Inferior Prognosis of Myeloma with Low Expression of the Glucocorticoid Receptor Gene *NR3C1*

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Abstract

Purpose: Because dexamethasone remains a key component of myeloma therapy, we wished to examine the impact of baseline and relapse expression levels of the glucocorticoid receptor gene *NR3C1* on survival outcomes in the context of treatment with or without thalidomide.

Experimental Design: We investigated the clinical impact of gene expression profiling (GEP)–derived expression levels of *NR3C1* in 351 patients with GEP data available at baseline and in 130 with data available at relapse, among 668 subjects accrued to total therapy 2 (TT2).

Results: Low *NR3C1* expression levels had a negative impact on progression-free survival (PFS; HR, 1.47; $P = 0.030$) and overall survival (OS; HR, 1.90; $P = 0.002$) in the no-thalidomide arm. Conversely, there was a significant clinical benefit of thalidomide for patients with low receptor levels (OS: HR, 0.54; $P = 0.015$; PFS: HR, 0.54; $P = 0.004$), mediated most likely by thalidomide's upregulation of *NR3C1*. In the context of both baseline and relapse parameters, post-relapse survival (PRS) was adversely affected by low *NR3C1* levels at relapse in a multivariate analysis (HR, 2.61; $P = 0.012$).

Conclusion: These findings justify the inclusion of *NR3C1* expression data in the work-up of patients with myeloma as it can significantly influence the choice of therapy and, ultimately, OS. The identification of an interaction term between thalidomide and *NR3C1* underscores the importance of pharmacogenomic studies in the systematic study of new drugs. *Clin Cancer Res; 18(19); 5499–506. ©2012 AACR.*

Introduction

Our total therapy 2 (TT2) protocol was a randomized phase III trial evaluating the impact of the upfront addition of thalidomide to a multi-agent chemotherapy and high-dose melphalan program supported by tandem autotransplants (1, 2). The long median overall survival (OS) of 10 years among 668 patients accrued to this protocol affords the unique opportunity to examine the contributions to clinical outcomes of added thalidomide in the context of baseline clinical and tumor-specific molecular variables and salvage strategies used.

Because dexamethasone remains an important component of myeloma therapy, we have studied and reported on the prognostic implications of the glucocorticoid receptor gene *NR3C1*, which is upregulated by both dexamethasone and thalidomide following test-dosing of both agents (3). Here, we examine the impact of baseline and relapse *NR3C1* expression levels on survival outcomes in the context of randomization to control or thalidomide treatment in TT2. As this required the availability of gene expression profiling (GEP) data of purified plasma cells, our analysis was limited to 351 TT2 patients with such baseline information and to 130 who had GEP data obtained at the time of relapse.

Materials and Methods

Protocol details and clinical outcomes have been reported previously (1, 2). In brief, 668 patients with newly diagnosed multiple myeloma received 2 cycles of intensive melphalan-based chemotherapy, each supported by autologous hematopoietic stem cell transplantation. A total of 323 were randomly assigned to receive thalidomide from the outset until disease progression or undue adverse effects and 345 did not receive thalidomide. Patients who were initially randomized to not receive thalidomide (control arm) had the opportunity to be treated with a thalidomide-based regimen after relapse.

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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doi: 10.1158/1078-0432.CCR-12-0019

Translational Relevance

Glucocorticoids have remained an important component of myeloma therapy, owing to their independent activity and synergism with most other agents currently in use. Results of our total therapy 2 trial, randomizing approximately half the patients to receive thalidomide, show that overall and progression-free survival are linked to expression levels of the nuclear glucocorticoid receptor, *NR3C1*, with low expression imparting poorer outcomes in the control (no-thalidomide) arm, which was overcome by the addition of thalidomide. In contrast, myeloma with high *NR3C1* levels did not benefit from the addition of thalidomide. Should these findings also apply to second- and third-generation thalidomide analogues (lenalidomide, pomalidomide), their application in high *NR3C1* myeloma should be reserved for salvage therapy as relapse is often associated with a decrease or even loss of *NR3C1* expression.

All patients signed an informed consent acknowledging the investigational nature of the protocol and agreeing to the ongoing research investigations. The protocol and its revisions were approved by the Institutional Review Board at our institution, which also received annual follow-up reports. Approximately 80% of all patient records have been audited by an independent team of investigators. Because of its randomized trial design and grant support from the NIH, a Data and Safety Monitoring Board was convened annually to review the protocol.

GEP samples were obtained as previously described (4), and both GEP-defined risk (5) and molecular subgroup designations were determined (6) in addition to *NR3C1* expression levels and GEP-derived *TP53* deletion status (7). To guard against bias, the subsets of patients with and without baseline GEP data were compared. This revealed no differences in progression-free survival (PFS), OS, or post-relapse survival (PRS; $P = 0.17$, $P = 0.40$, $P = 0.11$, respectively; data not shown). There were no differences in prognostic features between the GEP and no GEP baseline groups, such as age, albumin, B2M, CA. The analysis is based on data with a cutoff date of March 16, 2012.

OS and PFS were measured from the time of protocol enrollment. Events included death from any cause for OS and death, relapse, or progression for PFS. PRS was measured from time of relapse until death. Responses were defined according to International Myeloma Working Group criteria (8). Kaplan–Meier statistical methods were used for OS, PFS, and PRS plots, and the log-rank test was used for comparisons (9). Cox regression modeling (10) was used to determine which baseline and relapse parameters significantly affected the

Table 1. Comparison of patient characteristics by *NR3C1* expression levels

	GEP NR3C1 tertile			
Factor	**Lower tertile**	**Middle tertile**	**Upper tertile**	***P***
Age $\geq$ 65 y	21/117 (18)	24/117 (21)	24/117 (21)	0.850
Female	47/117 (40)	51/117 (44)	54/117 (46)	0.651
White	108/117 (92)	99/117 (85)	104/117 (89)	0.179
Albumin < 3.5 g/dL	27/115 (23)	21/116 (18)	8/116 (7)	0.002
B2M $\geq$ 3.5 mg/L	55/117 (47)	38/117 (32)	51/117 (44)	0.061
B2M > 5.5 mg/L	29/117 (25)	17/117 (15)	26/117 (22)	0.129
Creatinine $\geq$ 2 mg/dL	13/113 (12)	10/116 (9)	14/113 (12)	0.630
C-Reactive protein $\geq$ 8 mg/L	49/116 (42)	38/116 (33)	37/116 (32)	0.189
Hemoglobin < 10 g/dL	28/117 (24)	34/117 (29)	36/117 (31)	0.479
LDH $\geq$ 190 U/L	51/117 (44)	33/117 (28)	34/117 (29)	0.020
CA	53/117 (45)	30/117 (26)	29/116 (25)	<0.001
GEP high-risk	26/117 (22)	13/117 (11)	7/117 (6)	<0.001
GEP *CD-1* subgroup	11/117 (9)	12/117 (10)	2/117 (2)	0.020
GEP *CD-2* subgroup	27/117 (23)	14/117 (12)	9/117 (8)	0.002
GEP *HY* subgroup	17/117 (15)	46/117 (39)	44/117 (38)	<0.001
GEP *LB* subgroup	5/117 (4)	14/117 (12)	29/117 (25)	<0.001
GEP *MF* subgroup	3/117 (3)	1/117 (1)	18/117 (15)	<0.001
GEP *MS* subgroup	23/117 (20)	14/117 (12)	11/117 (9)	0.059
GEP *PR* subgroup	31/117 (26)	16/117 (14)	4/117 (3)	<0.001
GEP *TP53* deletion	15/117 (13)	13/117 (11)	7/117 (6)	0.192
Virtual karyotype: Chr 5q amp	25/117 (21)	57/117 (49)	62/117 (53)	<0.001
Randomization to thalidomide	60/117 (51)	59/117 (50)	56/117 (48)	0.862

Abbreviations: *n*/*N* (%): n, number with factor; *N*, number with valid data for factor.

aforementioned endpoints. Variables included in multivariate models were selected using stepwise selection techniques, requiring a significance level of 0.10 for entry into the model and 0.05 to remain. Complete remission (CR) was defined according to International Myeloma Working Group criteria (8).

NR3C1 expression was defined as gene expression of the probe 261321_s_at on the Affymetrix U133Plus2 microarray. There was no significant difference between the 5 probes representing the *NR3C1* gene. All samples were ordered according to their *NR3C1* expression level and then divided in 3 equal groups of 117 patients each with low (895–3,124), mid (3,136–4,284), and high (4,301–12,158) *NR3C1* expression. *NR3C1* expression groups (low, mid, high) at relapse were defined using cutoffs of $\leq$ 2,280 and $\geq$3,885.

Baseline GEP data have previously been published and deposited in the NIH Gene Expression Omnibus [GEO, National Center for Biotechnology Information (NCBI), http://www.ncbi.nlm.nih.gov/geo/] under accession number GSE2658. Relapse GEP data presented in the manuscript have been deposited in the NIH GEO under the accession number GSE38627.

Results

Table 1 compares patient characteristics according to *NR3C1* levels. Patients with low *NR3C1* expression were more likely to present with low albumin, high LDH, or to have high-risk features such as cytogenetic abnormalities (CA) or GEP-defined high risk. There was also a preponderance of GEP-defined *cyclin D2* (*CD-2*) and proliferation (*PR*) molecular subgroups in the low *NR3C1* group, whereas the hyperdiploid (*HY*), low bone (*LB*) disease, and *MAF*/*MAFB* (*MF*) subgroups (6) were underrepresented. There were fewer patients with an amplification of chromosome 5q to which *NR3C1* maps. Among patients randomized to the control arm, OS improved with the transition from low to mid or high *NR3C1* expression; no difference was noted in PFS between high- and mid-expression groups. Among patients randomized to thalidomide, OS and PFS were *NR3C1* expression–neutral (Supplementary Fig. S1). When examined within *NR3C1* tertiles, thalidomide benefited both OS and PFS in the low-expression group. PFS but not OS was extended by thalidomide in patients the mid-expression group, whereas in patients with high expression of *NR3C1*, no difference was observed between thalidomide and control arms (Supplementary Fig. S2).

This observation suggested an interaction between *NR3C1* expression levels and treatment arms. The presence of an interaction describes a situation in which the effects of 2 variables on a third are not simply additive. Thus, the presence of an interaction term would imply that the effect of thalidomide on survival outcomes varies as a function of *NR3C1* expression level. This was further examined and validated for both OS and PFS. In the control arm, low *NR3C1* expression significantly increased the hazard of death to 1.90 ($P = 0.002$) compared with mid and high receptor levels, whereas in the thalidomide arm, *NR3C1*

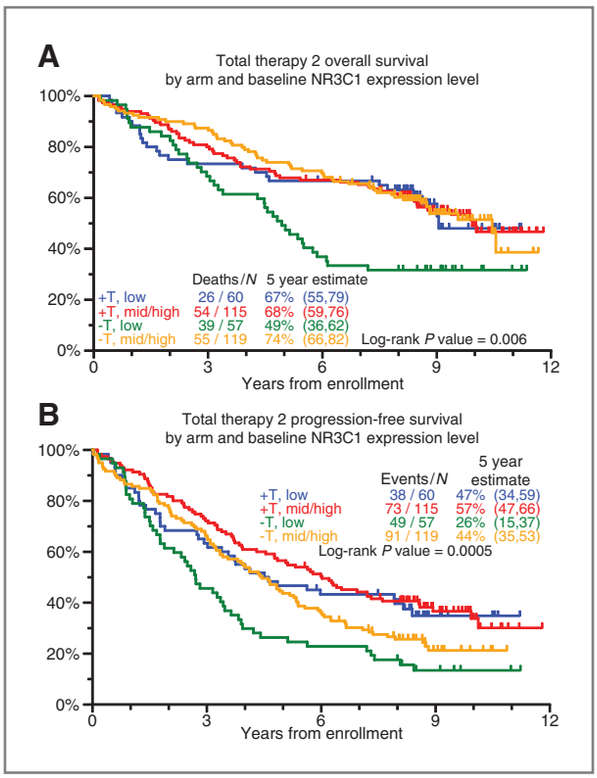

Figure 1. Survival outcomes with TT2 by treatment arm and *NR3C1* expression levels of 5-year OS/PFS rates are noted for each curve; values in parentheses represent the 95% confidence intervals. Green, low *NR3C1* on control arm; yellow, mid/high *NR3C1* on control arm; blue, low *NR3C1* on the thalidomide arm; red, mid/high *NR3C1* on the thalidomide arm. A, OS: low *NR3C1* levels conferred inferior survival in patients randomized to the control arm (−T), whereas in the thalidomide arm (+T), survival was *NR3C1* expression–neutral. Green versus yellow: HR, 1.90; $P = 0.002$; blue versus green: HR, 0.54; $P = 0.015$. B, PFS: a similar trend was observed for the control arm (−T) for PFS. Green versus yellow: HR, 1.47; $P = 0.03$; blue versus green: HR, 0.54; $P = 0.004$.

expression did not affect OS (HR, 1.01; $P = 0.972$; Fig. 1A). This trend was also seen for PFS where, in the control arm, low *NR3C1* expression significantly increased the hazard of progression or death to 1.47 ($P = 0.030$); in the thalidomide arm, low *NR3C1* did not significantly impact PFS (HR, 1.13; $P = 0.552$; Fig. 1B). CR frequency and CR duration were not affected by *NR3C1* levels (data not shown).

Univariate analysis of survival outcomes across treatment arms showed that many of the well-established prognostic features, including levels of β2-microglobulin (B2M), albumin, creatinine, lactate dehydrogenase (LDH), and hemoglobin, as well as metaphase CA, GEP-defined high-risk designation (5), deletion of *TP53*, and the GEP-defined *MMSET*/*FGFR3* (*MS*) subgroup (6) affected both PFS and OS adversely (Table 2, univariate analysis). *LB* and HY subgroup designation had a favorable effect on PFS and OS, respectively. Low *NR3C1* expression conferred inferior OS, whereas randomization to thalidomide prolonged PFS. On multivariate analysis, across treatment arms (Table 2, interaction analysis), the presence of CA, elevated B2M,

GEP-defined *TP53* deletion, high-risk status in the 70-gene model (11), and *MS* subgroup designation adversely affected the OS and PFS. Elevated LDH had an adverse effect on OS only. Patients with low *NR3C1* expression who were randomized to thalidomide (interaction term) had improved OS with a HR of 0.63 ($P = 0.007$).

We further investigated the effect of cumulative thalidomide dose on survival. For this, we calculated the cumulative thalidomide dose from protocol enrollment until the start of maintenance therapy. OS and PFS were measured from the beginning of maintenance therapy. Patients on the thalidomide arm who received a cumulative dose that was

Table 2. Cox regression analyses to determine baseline and posttreatment events linked to decreased overall and PFS

Univariate analysis (both arms combined)					
		OS from enrollment		PFS from enrollment	
Variable	**n/N (%)**	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Age ≥ 65 y	69/351 (20)	1.32 (0.92–1.89)	0.126	1.08 (0.80–1.47)	0.609
Albumin < 3.5 g/dL	**56/347 (16)**	1.43 (0.98–2.09)	0.063	**1.47 (1.07–2.02)**	**0.018**
B2M ≥ 3.5 mg/L	**144/351 (41)**	**2.17 (1.61–2.93)**	**<0.001**	**1.79 (1.40–2.30)**	**<0.001**
B2M > 5.5 mg/L	**72/351 (21)**	**2.14 (1.54–2.97)**	**<0.001**	**1.85 (1.39–2.47)**	**<0.001**
Creatinine ≥ 2 mg/dL	**37/342 (11)**	**2.25 (1.49–3.41)**	**<0.001**	**2.19 (1.52–3.16)**	**<0.001**
CRP ≥ 8 mg/L	124/348 (36)	1.07 (0.78–1.45)	0.690	0.89 (0.68–1.16)	0.384
Hemoglobin < 10 g/dL	**98/351 (28)**	1.36 (0.99–1.87)	0.055	**1.36 (1.04–1.78)**	**0.025**
LDH ≥ 190 U/L	**118/351 (34)**	**1.77 (1.31–2.40)**	**<0.001**	1.28 (0.99–1.66)	0.062
CA before enrollment	**112/350 (32)**	**2.24 (1.66–3.02)**	**<0.001**	**1.79 (1.39–2.32)**	**<0.001**
GEP high-risk	**46/351 (13)**	**3.64 (2.53–5.25)**	**<0.001**	**2.77 (1.97–3.90)**	**<0.001**
GEP *CD-1* subgroup	25/351 (7)	0.78 (0.42–1.43)	0.417	0.76 (0.46–1.27)	0.293
GEP *CD-2* subgroup	50/351 (14)	0.69 (0.42–1.12)	0.132	0.85 (0.59–1.23)	0.400
GEP *HY* subgroup	**107/351 (30)**	**0.67 (0.48–0.94)**	**0.022**	0.85 (0.65–1.11)	0.228
GEP *LB* subgroup	**48/351 (14)**	0.65 (0.40–1.05)	0.080	**0.63 (0.42–0.93)**	**0.021**
GEP *MF* subgroup	22/351 (6)	1.51 (0.88–2.62)	0.137	1.54 (0.97–2.46)	0.069
GEP *MS* subgroup	**48/351 (14)**	**2.05 (1.42–2.98)**	**<0.001**	**1.82 (1.30–2.56)**	**<0.001**
GEP *PR* subgroup	**51/351 (15)**	**1.69 (1.16–2.47)**	**0.006**	1.39 (0.98–1.95)	0.062
GEP *TP53* deletion	**35/351 (10)**	**2.63 (1.75–3.95)**	**<0.001**	**1.97 (1.34–2.89)**	**<0.001**
GEP *NR3C1* low	**117/351 (33)**	**1.40 (1.03–1.91)**	**0.031**	1.27 (0.98–1.65)	0.073
GEP *NR3C1* high	117/351 (33)	0.84 (0.61–1.15)	0.274	1.00 (0.77–1.30)	0.970
Randomization to thalidomide	**175/351 (50)**	0.82 (0.61–1.11)	0.193	**0.65 (0.51–0.83)**	**<0.001**
Interaction effects	***n/N* (%)**	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Randomization to thalidomide	**175/351 (50)**	1.02 (0.70–1.48)	0.931	**0.70 (0.52–0.96)**	**0.025**
GEP *NR3C1* low	**117/351 (33)**	**1.90 (1.26–2.87)**	**0.002**	**1.47 (1.04–2.08)**	**0.030**
Randomization to thalidomide and GEP *NR3C1* low (interaction term)	**60/351 (17)**	**0.53 (0.28–0.99)**	**0.046**	0.77 (0.45–1.29)	0.318
Interaction analysis (both arms combined, with other variables)					
Randomization to thalidomide	**175/350 (50)**	0.98 (0.67–1.44)	0.920	**0.67 (0.49–0.91)**	**0.011**
GEP *NR3C1* low	117/350 (33)	1.52 (1.00–2.32)	0.053	1.34 (0.94–1.91)	0.105
Randomization to thalidomide and GEP *NR3C1* low (interaction term)	**60/350 (17)**	**0.42 (0.22–0.79)**	**0.007**	0.60 (0.35–1.03)	0.065
B2M > 5.5 mg/L	**72/350 (21)**	**1.78 (1.25–2.52)**	**0.001**	**1.68 (1.24–2.28)**	**<0.001**
LDH ≥ 190 U/L	**118/350 (34)**	**1.49 (1.08–2.07)**	**0.015**	1.11 (0.84–1.46)	0.457
CA	**112/350 (32)**	**1.87 (1.35–2.59)**	**<0.001**	**1.54 (1.17–2.03)**	**0.002**
GEP70 high-risk	**46/350 (13)**	**2.52 (1.69–3.75)**	**<0.001**	**2.10 (1.46–3.04)**	**<0.001**
GEP *MS* subgroup	**48/350 (14)**	**2.01 (1.36–2.96)**	**<0.001**	**1.88 (1.32–2.67)**	**<0.001**
GEP *TP53* deletion	**35/350 (10)**	**2.46 (1.60–3.77)**	**<0.001**	**2.06 (1.39–3.05)**	**<0.001**

NOTE: Bolded variables indicate statistical significance ($P \leq 0.05$) and were considered for multivariate analysis.
Abbreviations: CI, confidence interval; CRP, C-reactive protein; GEP molecular subgroups: *CD-1, cyclin D1*; *CD-2, cyclin D2*; *HY,* hyperdiploid; *MF, MAF/MAFB*; *MS, MMSET/FGFR3* (6).

greater than the median showed a trend toward a better PFS compared with patients who received equal or less than the median dose ($P = 0.083$) and was significantly better than receiving no thalidomide at all on the control arm ($P = 0.002$), with 5-year survival rate estimates of 63%, 53%, and 42%, respectively. There was no significant difference between low cumulative thalidomide dose and the control arm ($P = 0198$). There was also no significant difference in OS between the 3 groups (Supplementary Fig. S3). The effect on PFS was even more pronounced when the analysis was limited to the patients with low expression of *NR3C1* (PFS: 73% vs. 53% vs. 44%, log-rank, $P = 0.05$; OS: not significant; data not shown).

In addition to initial PFS, PRS is an important component in determining the total length of OS. Salvage regimens are depicted in Supplementary Table S1. There was no difference between the treatment arms. With an overall median PRS of 3.4 years, there was no difference related to the initial treatment randomization when examined for all patients (Fig. 2A) or in relation to type of salvage therapy (Supplementary Fig. S4). Examining PRS in the subset of patients with available *NR3C1* data at baseline or at relapse an adverse PRS trend was apparent for patients randomized to thalidomide (Fig. 2B). We investigated the impact of *NR3C1* expression levels at relapse on PRS. PRS shortened progressively as the *NR3C1* levels decreased from high to mid to low levels (Fig. 3). For the 88 patients with available baseline and relapse GEP data, we also examined PRS in the context of both baseline and relapse *NR3C1* levels. Patients maintaining low *NR3C1* levels from baseline to relapse and those transitioning from mid-/high-expression to low-expression had the shortest PRS duration. Patients with high levels at relapse had the longest PRS regardless of *NR3C1* expression levels at baseline (Supplementary Fig. S5). However, because of the small sample number in each group, this last observation needs to be considered with some caution.

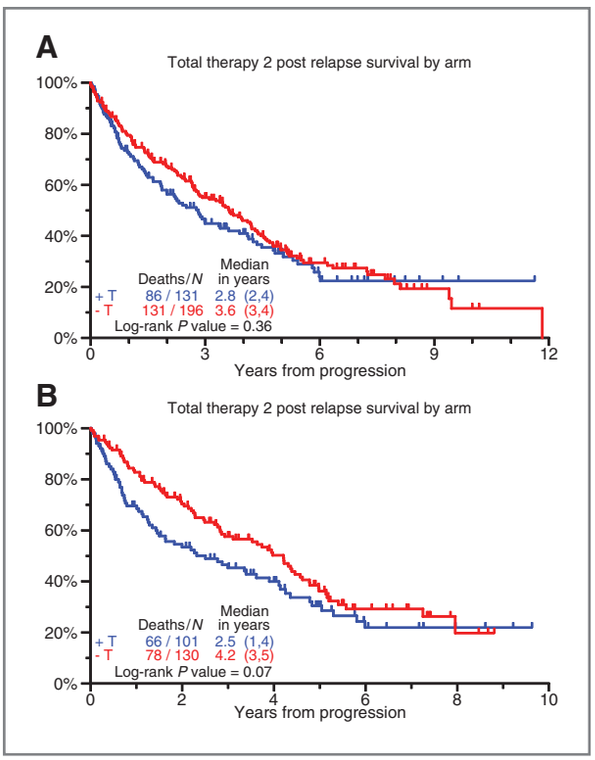

Figure 2. PRS related to initial randomization to thalidomide (+T) or control arm (−T) 5-year OS/PFS rates are noted for each curve; values in parentheses represent the 95% confidence intervals. Blue, patients randomized to thalidomide; red, patients randomized to the control arm. A, all patients regardless of *NR3C1* data PRS were independent of initial randomization to control arm (−T) or thalidomide arm (+T). B, limited to those with baseline or relapse *NR3C1*, this also applied to the subset with GEP information, although a trend was observed in favor of longer PRS among patients on the control arm.

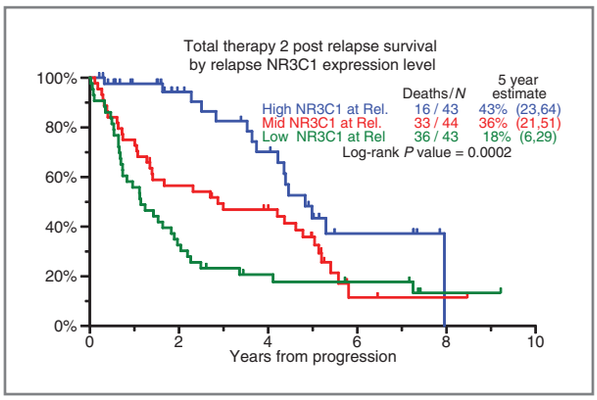

Figure 3. PRS according to *NR3C1* levels at relapse: graded adverse PRS effects with progressive loss of *NR3C1* expression levels at relapse. Five-yr OS/PFS rates are noted for each curve; values in parentheses represent the 95% confidence intervals. *P* values: high versus middle, $P = 0.015$; high versus low, $P < 0.001$; middle versus low, $P = 0.123$; low versus mid/high, $P < 0.001$; high versus mid/low, $P < 0.001$.

We also examined PRS in the context of potentially relevant prognostic baseline and relapse variables. On univariate analysis, whether taken at relapse or baseline, low *NR3C1* levels imparted short and high *NR3C1* levels longer PRS (Table 3, univariate analysis). In addition, many standard and newer genetic variables affected PRS. Age $\geq$ 65 years, elevated baseline B2M or LDH, GEP high-risk designation at baseline and relapse, *MS* subgroup classification at baseline, *PR* subgroup classification at baseline, and relapse and deletion of *TP53* were associated with shorter PRS. *CD-2* subgroup classification at baseline and *HY* classification at relapse were prognostically favorable. Adjusting for all individually significant baseline and relapse variables in a multivariate regression analysis, low *NR3C1* expression levels at relapse imparted inferior, whereas relapse *HY* subgroup designation conveyed superior PRS (Table 3, interaction analysis). GEP-defined high-risk status, whether examined at baseline or relapse, both conferred poor PRS.

Discussion

Glucocorticoids, such as dexamethasone, have marked anti-myeloma activity (12) and have been shown to act synergistically with most other anti-myeloma agents,

Table 3. PRS adjusted for initial treatment randomization, baseline, and relapse variables

Univariate analysis		PRS	
Variable	**n/N (%)**	**HR (95% CI)**	***P***
Baseline age ≥ 65 y	**38/189 (20)**	**1.69 (1.08–2.65)**	**0.022**
Baseline B2M ≥ 3.5 mg/L	**85/189 (45)**	**1.62 (1.12–2.35)**	**0.011**
Baseline B2M > 5.5 mg/L	**43/189 (23)**	**1.64 (1.08–2.49)**	**0.019**
Baseline LDH ≥ 190 U/L	**57/189 (30)**	**1.80 (1.23, 2.64)**	**0.003**
Baseline CA	**69/188 (37)**	**1.89 (1.30–2.75)**	**<.001**
Relapse CA	**108/188 (57)**	**1.57 (1.07–2.32)**	**0.022**
Any CA within 6 mo of relapse	**47/161 (29)**	**1.70 (1.12–2.56)**	**0.012**
Baseline GEP70 high-risk	**29/189 (15)**	**3.25 (2.10–5.03)**	**<0.001**
Relapse GEP70 high-risk	**30/88 (34)**	**3.77 (2.18–6.53)**	**<0.001**
Baseline GEP *CD-2* subgroup	**22/189 (12)**	**0.42 (0.20–0.90)**	**0.027**
Relapse GEP *HY* subgroup	**24/88 (27)**	**0.32 (0.15–0.68)**	**0.003**
Baseline GEP *MS* subgroup	**31/189 (16)**	**1.74 (1.12–2.69)**	**0.014**
Baseline GEP *PR* subgroup	**29/189 (15)**	**1.59 (1.01–2.49)**	**0.045**
Relapse GEP *PR* subgroup	**22/88 (25)**	**2.39 (1.35–4.23)**	**0.003**
Baseline GEP *TP53* deletion	**18/189 (10)**	**2.16 (1.27–3.68)**	**0.005**
Baseline GEP *NR3C1* low	66/189 (35)	1.45 (0.99–2.13)	0.053
Relapse GEP *NR3C1* low (cutoffs defined at relapse)	**27/88 (31)**	**2.61 (1.49–4.56)**	**<0.001**
Relapse GEP *NR3C1* high (cutoffs defined at relapse)	**27/88 (31)**	**0.44 (0.23–0.86)**	**0.016**
Relapse GEP *NR3C1* low (using baseline cutoffs)	**51/88 (58)**	**2.03 (1.13–3.67)**	**0.018**
Relapse GEP *NR3C1* high (using baseline cutoffs)	**20/88 (23)**	**0.42 (0.19–0.94)**	**0.035**
Randomization to thalidomide	80/189 (42)	1.29 (0.89–1.87)	0.184
Interaction effects			
Randomization to thalidomide	80/189 (42)	1.61 (1.00–2.60)	0.051
Baseline GEP *NR3C1* low	**66/189 (35)**	**1.90 (1.15–3.15)**	**0.013**
Randomization to thalidomide and Baseline GEP *NR3C1* low (interaction term)	30/189 (16)	0.52 (0.24–1.13)	0.097
Interaction analysis with baseline GEP NR3C1 expression (with other baseline and relapse variables)			
Randomization to thalidomide	38/88 (43)	1.00 (0.47–2.14)	0.992
Baseline GEP *NR3C1* low	31/88 (35)	1.10 (0.48–2.49)	0.824
Randomization to thalidomide and Baseline GEP *NR3C1* low (interaction term)	15/88 (17)	0.71 (0.22–2.25)	0.562
Baseline GEP70 high-risk	**17/88 (19)**	**2.10 (1.01–4.35)**	**0.047**
Relapse GEP70 high-risk	**30/88 (34)**	**2.33 (1.15–4.73)**	**0.018**
Relapse GEP *HY* subgroup	**24/88 (27)**	**0.31 (0.13–0.72)**	**0.007**
Relapse GEP *NR3C1* low	**27/88 (31)**	**2.61 (1.24–5.50)**	**0.012**

NOTE: Bolded variables indicate statistical significance ($P \leq 0.05$) and were considered for multivariate analysis. Randomization to thalidomide, baseline GEP *NR3C1* low, randomization to thalidomide, and baseline GEP *NR3C1* low (interaction term) were forced into the multivariate analysis.

Abbreviations: CI, confidence interval; CRP, C-reactive protein; GEP molecular subgroups: *CD-1, cyclin D1*; *CD-2, cyclin D2*; *HY,* hyperdiploid; *MF, MAF/MAFB*; *MS, MMSET/FGFR3* (6).

justifying their use in combination regimens (13–16). However, only approximately half of newly diagnosed patients respond to single-agent dexamethasone and CRs are infrequently observed (17, 18). Thus, steroid-resistant tumor cell subpopulations exist both *de novo* and in higher proportion at the time of relapse (19, 20).

Most glucocorticoid hormone effects are mediated by the glucocorticoid receptor. Although there is only one known gene encoding this receptor, *NR3C1* (located on chromosome 5q31.3), several receptor isoforms result from alternative splicing (21, 22). Poor clinical responses to glucocorticoid therapy associated with low expression of the receptor have been previously reported for multiple myeloma (23) and other malignancies (24). This correlation can be reproduced *in vitro* with glucocorticoid-resistant myeloma cell lines (25). Comparing gene profiles of glucocorticoid-sensitive myeloma cells (MM1.S) with those that are glucocorticoid-resistant (MM1.RE and MM1.RL) revealed a significant reduction in *NR3C1* mRNA, which was correlated with decreased expression of glucocorticoid receptor protein and glucocorticoid resistance (22).

The favorable survival effects of high *NR3C1* expression levels are consistent with a good prognosis linked to gains of chromosome 5q31.3 (26). This matches our observation

that amplification of 5q, identified by a virtual GEP-based karyotyping model (11) is significantly overrepresented in the group of patients with middle or high expression of *NR3C1*. In a previous analysis of myeloma GEP among patients enrolled in the TT3 protocol (27), high *NR3C1* levels were associated with superior and low levels with inferior survival outcomes. We also noted that cumulative glucocorticoid dosing during induction therapy extended both OS and PFS significantly when *NR3C1* expression was low; this favorable survival effect seen for thalidomide but was not observed with bortezomib (28).

Analyzing gene expression profiles after short-term exposure to various agents *in vivo*, we found that *NR3C1* was upregulated by both dexamethasone and thalidomide (3). In the current study, we show that added thalidomide in the TT2 protocol neutralizes the inferior prognosis of patients with low glucocorticoid receptor gene expression, implying that thalidomide compensates for the deleterious effect of low *NR3C1* expression by inducing upregulation of this receptor and that failure of exogenous glucocorticoids to induce apoptosis in tumor cells with insufficient glucocorticoid receptor levels can be reversed by the addition of thalidomide.

Because of the study design, we cannot evaluate the effect of thalidomide alone on PFS and OS and, therefore, are not able to determine whether the survival benefit for low *NR3C1* patients was due to the combination of thalidomide and dexamethasone or could have been achieved with thalidomide alone, nor can we evaluate the effect of different doses of dexamethasone on survival. Rajkumar and colleagues recently showed improved short-term OS for patients treated with low-dose dexamethasone plus lenalidomide compared with high-dose dexamethasone plus lenalidomide (29). The doses of glucocorticoids used in TT2 compare with the high-dose arm in the study by Rajkumar and colleagues. It is conceivable that patients with high *NR3C1* expression at baseline derive little benefit from the high dose of glucocorticoid and would possibly have done just as well if a lower dose was used. Conversely, using a lower dose of dexamethasone in patients with low *NR3C1* expression levels would have had a detrimental effect as high doses of glucocorticoid most likely partially overcome the adverse effect of low *NR3C1* expression levels.

In the current study, thalidomide extended OS and PFS in patients presenting with low baseline *NR3C1* expression levels. Among the patients treated with thalidomide, higher cumulative doses resulted in a significantly better landmarked PFS compared with no thalidomide and showed a trend toward significance in the comparison between high and low cumulative thalidomide doses, suggesting a dose-dependent effect. However, this could also be a reflection of other myeloma-related factors causing intolerance to thalidomide, thus making it *de facto* a more aggressive disease. In addition, relapse GEP features impacted PRS, with low *NR3C1* levels and GEP-defined high-risk having adverse roles and the *HY* subclass designation having a favorable impact. The latter most likely reflects the better prognosis of patients with gains of chromosome 5 and which coincides with mid and high *NR3C1* expression as mentioned earlier.

We believe our findings justify the inclusion of *NR3C1* expression data, or other means of glucocorticoid receptor quantification, in the work-up of patients. For example, if patients with high *NR3C1* expression levels do not benefit from upfront thalidomide (and possibly newer immunomodulatory derivatives), reserving these agents for treatment of relapse, when *NR3C1* levels are lower, may prolong overall survival.

Disclosure of Potential Conflicts of Interest

J.D. Shaughnessy Jr is cofounder of Myeloma Health LLC and owns stock in the company; holds ownership interests in Signal Genetics LLC and DNA BioPharma LLC; receives royalties from Novartis, Genzyme, and Myeloma Health; and is a paid consultant to Novartis, Myeloma Health, Genzyme, Array BioPharma, Onyx, Millennium, and Celgene. S.Z. Usmani has served as a consultant to Celgene, Millennium, and Onyx and has received research funding from Celgene and Onyx and speaking honoraria from Celgene and Millennium. B. Barlogie has received research funding from Celgene, Novartis, Millenium, Centocor, Johnson and Johnson, Onyx, and ICON; is a consultant to Celgene, Millenium, and Genzyme; and has received speaking honoraria from Celgene and Millennium. He is a coinventor on patents and patent applications related to use of GEP in cancer medicine and holds ownership interest in Signal Genetics. No potential conflicts of interest were disclosed by the other authors.

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Grant Support

The study was supported by CA 55819-15 grant from the National Cancer Institute, Bethesda, MD.

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Received January 6, 2012; revised May 31, 2012; accepted July 5, 2012; published OnlineFirst August 1, 2012.

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