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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 up-regulation 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).

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CONFLICT OF INTEREST DISCLOSURES

JDS is co-founder of Myeloma Health LLC and owns stock in the company; he receives royalties from Novartis, Genzyme, and Myeloma Health, and he is a paid consultant to Novartis, Myeloma Health, Genzyme, Array BioPharma, Onyx, Millennium and Celgene.

BB has received research funding from Celgene and Novartis. He is a consultant to Celgene and Genzyme. He has received speaking honoraria from Celgene and Millennium. Dr. Barlogie is a co-inventor on patents and patent applications related to use of GEP in cancer medicine.

SZU has served as a consultant to Celgene, Millennium, and Onyx. He has received research funding from Celgene and Onyx, and speaking honoraria from Celgene and Millennium.

AUTHORSHIP CONTRIBUTIONS

Conceptualized work: CJH, JS, JDS, FvR, JE, BB

Wrote paper: CJH, JS, JDS, SU, FvR, BB

Contributed patients and performed clinical research: FvR, BN, SW, YA, SU, BB

Supervised and discussed gene expression profiling analyses: JDS

Performed statistical analyses: JS, AH, JC, EH

Provided data management support: CB, NP

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.

Keywords

Myeloma; Glucocorticoids; Glucocorticoid Receptor; Total Therapy 2; Thalidomide

INTRODUCTION

Our Total Therapy 2 (TT2) protocol was a randomized phase-III trial evaluating the impact of the up-front 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 employed.

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.

METHODS

Protocol details and clinical outcomes have been reported previously.(1, 2) In brief, 668 patients with newly diagnosed multiple myeloma received two 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.

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. Due to its randomized trial design and grant support from the National Institutes of Health, 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 cut-off 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 employed 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 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. CR was defined according to the IMWG 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 five 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 to 3124), mid (3136 to 4284) and high (4301 to 12,158) *NR3C1* expression. *NR3C1* expression groups (low, mid, high) at relapse were defined using cutoffs of ≤ 2280 and ≥ 3885 . We also evaluated the effect of *NR3C1* groups at relapse defined by baseline cutoffs. This approach however performed worse than using *NR3C1* groups defined by gene expression at relapse and thus was excluded from further analysis (data not shown).

Baseline GEP data has 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 this 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 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 disease (*LB*) and MAF/MAFB (*MF*) subgroups (6) were under-represented. 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 (Supplemental Figure 1). 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 (Supplemental Figure 2).

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 two 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* expression did not affect OS (HR=1.01, $p=0.972$) (Figure 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, $p=0.552$) (Figure 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 $\beta 2$ -microglobulin (B2M), albumin, creatinine, lactate dehydrogenase (LDH), and hemoglobin, as well as metaphase cytogenetic abnormalities (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, **Panel A**). *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, **Panel B**), 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 greater than the median showed a trend towards a better PFS compared to 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 estimates of 63%, 53% and 42% respectively. There was no significant difference between low cumulative thalidomide dose and the control arm ($p=0.198$). There was also no significant difference in OS between the three groups (supplemental figure 3). 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%, logrank p -value= 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 Supplemental Table 1. 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 (Figure 2A) or in relation to type of salvage therapy (Supplemental Figure 4). 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 (Figure 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 (Figure 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 (Supplemental Figure 5). However, due to the small sample number in each group this last observation needs to be considered with some caution.

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, **Panel A**). In addition, many standard and newer genetic variables affected PRS. Age ≥ 65 yr, 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 while relapse *HY* subgroup designation conveyed superior PRS (Table 3, **Panel B**). 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, justifying their use in combination regimens.(13–16) However, only approximately one-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 up-regulation 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.

Due to 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 et al. recently showed improved short-term OS for patients treated with low-dose dexamethasone plus lenalidomide compared to high-dose dexamethasone plus lenalidomide.(29) The doses of glucocorticoids used in TT2 compare to the high dose arm in the study by Rajkumar et al. 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 to no thalidomide and showed a trend towards 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 reflect 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 immune-modulatory derivatives), reserving these agents for treatment of relapse, when *NR3C1* levels are lower, may prolong overall survival.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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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.

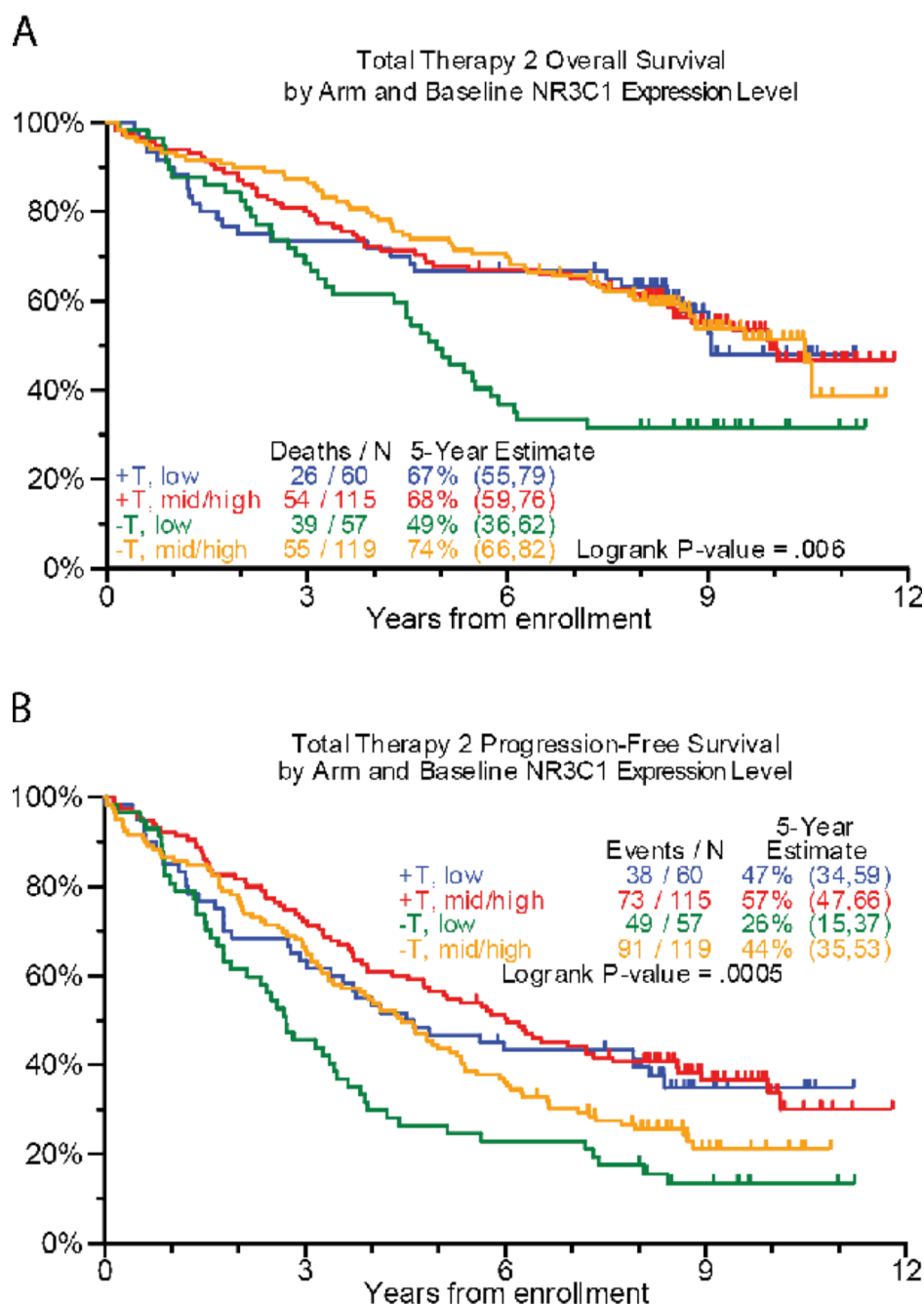


Figure 1. Survival outcomes with Total Therapy 2 by treatment arm and NR3C1 expression levels

5-yr OS/PFS 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: Overall survival 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 vs. yellow HR= 1.90, p=0.002; blue vs. green, HR=0.54, p=0.015

B: Progression-free survival A similar trend was observed for the control arm (-T) for progression-free survival.

Green vs. yellow, HR=1.47, p=0.03; blue vs. green, HR=0.54, p=0.004

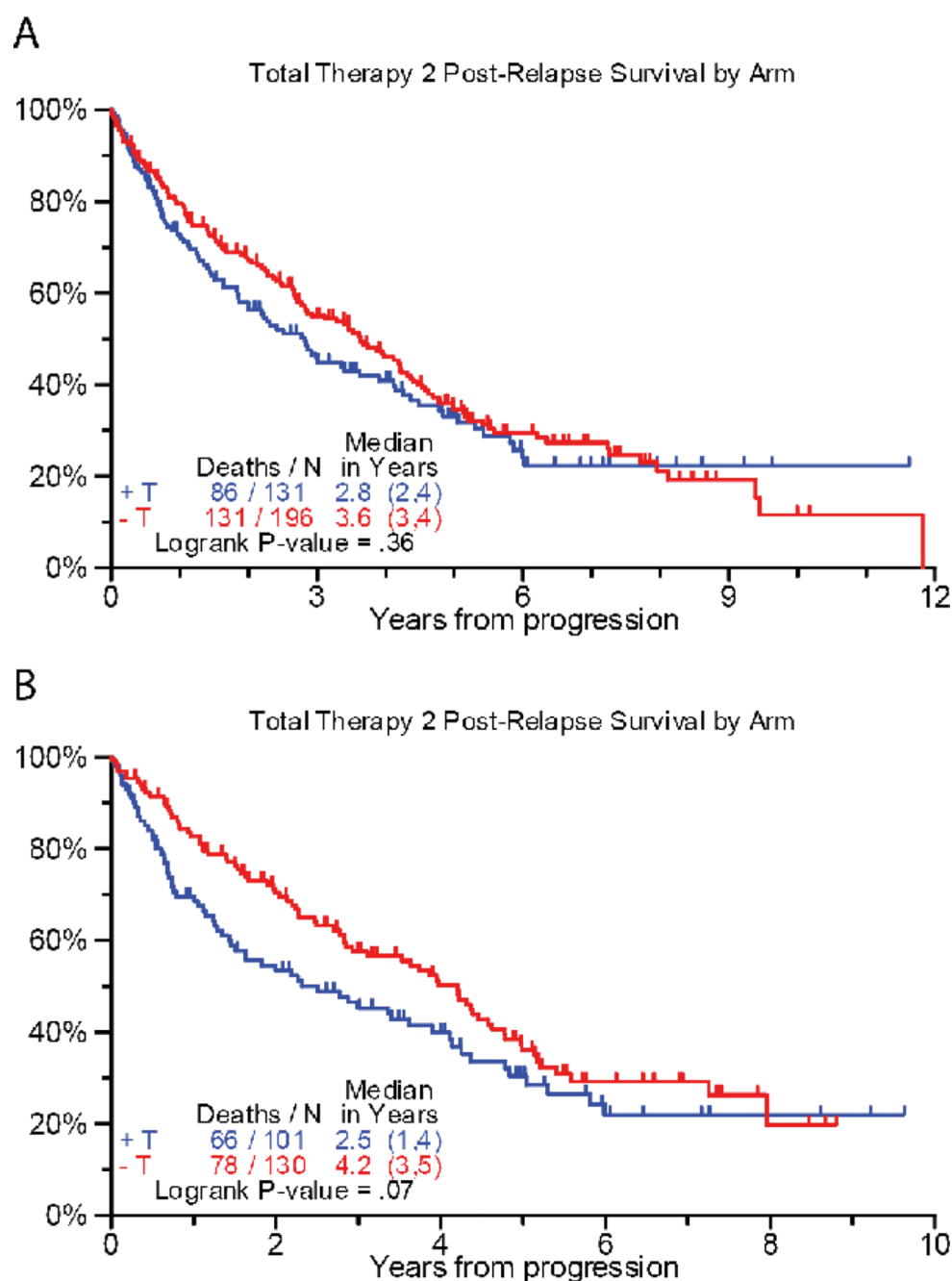


Figure 2. Post-relapse survival related to initial randomization to thalidomide (+T) or control arm (-T)

5-yr OS/PFS 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 Post-relapse survival was 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.

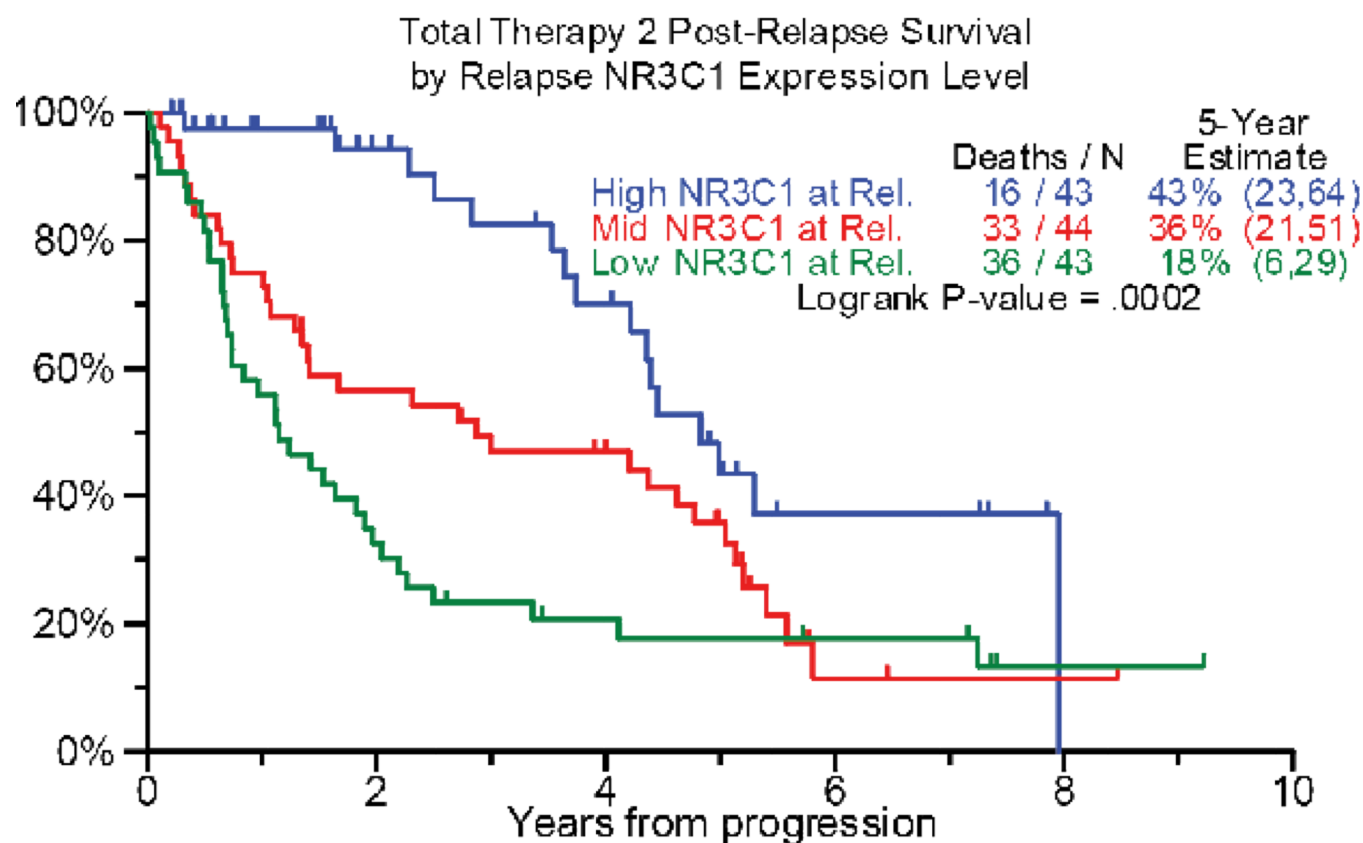


Figure 3. Post-relapse survival according to *NR3C1* levels at relapse

Graded adverse post-relapse survival effects with progressive loss of *NR3C1* expression levels at relapse. 5-yr OS/PFS are noted for each curve, values in parentheses represent the 95% confidence intervals.

P-values: high vs middle, $p=0.015$; high vs low, $p<0.001$; middle vs low, $p=0.123$; low vs mid/high, $p<0.001$; high vs mid/low, $p<0.001$

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

Factor	GEP <i>NR3C1</i> tertile			P-value
	Lower Tertile	Middle Tertile	Upper Tertile	
Age ≥ 65 yr	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 ≤ 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 ≤ 2 mg/dL	13/113 (12%)	10/116 (9%)	14/113 (12%)	0.630
CRP ≤ 8 mg/L	49/116 (42%)	38/116 (33%)	37/116 (32%)	0.189
Hb < 10 g/dL	28/117 (24%)	34/117 (29%)	36/117 (31%)	0.479
LDH ≤ 190 U/L	51/117 (44%)	33/117 (28%)	34/117 (29%)	0.020
Cytogenetic abnormalities	53/117 (45%)	30/117 (26%)	29/116 (25%)	<.001
GEP high-risk	26/117 (22%)	13/117 (11%)	7/117 (6%)	<.001
GEP <i>CD-1</i> subgroup	11/117 (9%)	12/117 (10%)	2/117 (2%)	0.020
GEP <i>CD-2</i> subgroup	27/117 (23%)	14/117 (12%)	9/117 (8%)	0.002
GEP <i>HY</i> subgroup	17/117 (15%)	46/117 (39%)	44/117 (38%)	<.001
GEP <i>LB</i> subgroup	5/117 (4%)	14/117 (12%)	29/117 (25%)	<.001
GEP <i>MF</i> subgroup	3/117 (3%)	1/117 (1%)	18/117 (15%)	<.001
GEP <i>MS</i> subgroup	23/117 (20%)	14/117 (12%)	11/117 (9%)	0.059
GEP <i>PR</i> subgroup	31/117 (26%)	16/117 (14%)	4/117 (3%)	<.001
GEP <i>TP53</i> 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%)	<.001
Randomization to thalidomide	60/117 (51%)	59/117 (50%)	56/117 (48%)	0.862

n/N (%): n- Number with Factor, N- Number with Valid Data for Factor

Table 2

Cox regression analyses to determine baseline and post-treatment events linked to decreased overall and progression-free survival.

PANEL A: Univariate Analysis (both arms combined)		Overall Survival from Enrollment		Progression-Free Survival from Enrollment	
Variable	n/N (%)	HR (95% CI)	P-value	HR (95% CI)	P-value
Age 65 yr	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
Hb < 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
Cytogenetic abnormalities prior to 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-value	HR (95% CI)	P-value
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

PANEL A: Univariate Analysis (both arms combined)		Overall Survival from Enrollment		Progression-Free Survival from Enrollment	
Variable	n/N (%)	HR (95% CI)	P-value	HR (95% CI)	P-value
Bolted variables indicate statistical significance (p < 0.05) and were considered for multivariate analysis. GEP molecular subgroups: <i>CD1</i> =cyclin D1; <i>CD2</i> =cyclin D2; <i>HY</i> =hyperdiploid; <i>MF</i> =MAF/MAFB; <i>MS</i> =MMSET/FGFR3, <i>PR</i> =Proliferation, <i>LB</i> = Low Bone Disease (6)					
PANEL B: Interaction Analysis (both arms combined, with other variables)		Overall Survival from Enrollment		Progression-Free Survival from Enrollment	
Variable	n/N (%)	HR (95% CI)	P-value	HR (95% CI)	P-value
Randomization to thalidomide	175/350 (50%)	0.98 (0.67, 1.44)	0.920	0.67 (0.49, 0.91)	0.011
GEP <i>NR3C1</i> 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 <i>NR3C1</i> 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)	<.001
LDH 190 U/L	118/350 (34%)	1.49 (1.08, 2.07)	0.015	1.11 (0.84, 1.46)	0.457
Cytogenetic abnormalities	112/350 (32%)	1.87 (1.35, 2.59)	<.001	1.54 (1.17, 2.03)	0.002
GEP70 high-risk	46/350 (13%)	2.52 (1.69, 3.75)	<.001	2.10 (1.46, 3.04)	<.001
GEP <i>MS</i> subgroup	48/350 (14%)	2.01 (1.36, 2.96)	<.001	1.88 (1.32, 2.67)	<.001
GEP <i>TP53</i> deletion	35/350 (10%)	2.46 (1.60, 3.77)	<.001	2.06 (1.39, 3.05)	<.001
Bolted variables indicate statistical significance (p < 0.05).					

Table 3

Post-relapse survival adjusted for initial treatment randomization, baseline and relapse variables.

PANEL A			Post-relapse survival	
Univariate Analysis	Variable	n/N (%)	HR (95% CI)	P-value
	Baseline age ≥ 65 yr	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 mos 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)	<.001
	Relapse GEP70 high-risk	30/88 (34%)	3.77 (2.18, 6.53)	<.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)	<.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	Variable	n/N (%)	HR (95% CI)	P-value
	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
<p>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.</p> <p>GEP molecular subgroups: CD1=cyclin D1; CD2=cyclin D2; HY=hyperdiploid; MF=MAF/MAFB, MS=MMSET/FGFR3, PR=Proliferation, LB= Low Bone Disease (6)</p>				
PANEL B			Post-relapse survival	
Interaction analysis with baseline GEP NR3C1 expression (with other baseline and relapse variables)	Variable	n/N (%)	HR (95% CI)	P-value

PANEL A			Post-relapse survival	
Univariate Analysis	Variable	n/N (%)	HR (95% CI)	P-value
	Randomization to thalidomide	38/88 (43%)	1.00 (0.47, 2.14)	0.992
	Baseline GEP <i>NR3C1</i> low	31/88 (35%)	1.10 (0.48, 2.49)	0.824
	Randomization to thalidomide and Baseline GEP <i>NR3C1</i> 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 <i>HY</i> subgroup	24/88 (27%)	0.31 (0.13, 0.72)	0.007
	Relapse GEP <i>NR3C1</i> low	27/88 (31%)	2.61 (1.24, 5.50)	0.012
Bolded variables indicate statistical significance (p < 0.05).				