haematologica
Journal of the Ferrata Storti Foundation

Expression levels of genes implicated in the working mechanism of lenalidomide predict treatment response in lower risk myelodysplastic syndrome patients

by Florentien E.M. in 't Hout, Thessa N. Scheele, Theresia M. Westers, Canan Alhan, Carolien Duetz, Eline M.P. Cremers, Heleen A. Visser-Wisselaar, Annelies Verbrugge, Dana A. Chitu, Bert A. van der Reijden, Aniek O. de Graaf, Arjan A. van de Loosdrecht, and Joop H. Jansen

Received: August 5, 2024.
Accepted: October 3, 2024.

Citation: Florentien E.M. in 't Hout, Thessa N. Scheele, Theresia M. Westers, Canan Alhan, Carolien Duetz, Eline M.P. Cremers, Heleen A. Visser-Wisselaar, Annelies Verbrugge, Dana A. Chitu, Bert A. van der Reijden, Aniek O. de Graaf, Arjan A. van de Loosdrecht, and Joop H. Jansen. Expression levels of genes implicated in the working mechanism of lenalidomide predict treatment response in lower risk myelodysplastic syndrome patients. Haematologica. 2024 Oct 10. doi: 10.3324/haematol.2024.286157 [Epub ahead of print]

Letter to the Editor

Expression levels of genes implicated in the working mechanism of lenalidomide predict treatment response in lower risk myelodysplastic syndrome patients

Florentien E.M. in 't Hout[1,2], Thessa N. Scheele[1*], Theresia M. Westers[3*], Canan Alhan[3], Carolien Duetz[3], Eline M.P. Cremers[3], Heleen A. Visser-Wisselaar[4], Annelies Verbrugge[4], Dana A. Chitu[4], Bert A. van der Reijden[1], Aniek O. de Graaf[1], Arjan A. van de Loosdrecht[3*], Joop H. Jansen[1*], on behalf of the HOVON89 study group.

[1]*Department of Laboratory medicine, Laboratory of Hematology, Radboud University Medical Centre, Nijmegen, The Netherlands*
[2]*Department of Hematology, Radboud University Medical Centre, Nijmegen, The Netherlands*
[3]*Department of Hematology, Cancer Center Amsterdam, Amsterdam UMC, Vrije Universiteit Amsterdam, The Netherlands*
[4]*HOVON Data Center, Department of Hematology, Erasmus MC Cancer Institute, Rotterdam*
[*]*Equally contributed*

Corresponding author
Prof. Dr. JH Jansen, Lab. of Hematology, Dept. Lab. Medicine
Joop.Jansen@Radboudumc.nl

Authors contribution
FH, AL and JJ contribute to the conception and design of the work and wrote the manuscript. TS acquired the laboratory data. FH, JJ, AdG, BvR, TM, TS, AL contribute to the analysis, and interpretation of data. CA, CD, EC, HV, AV, DC, AL, contribute to the accrual of the patients and interpretation of the data. All authors reviewed critically and approved the final manuscript.

Disclosures
AL: research support: Celgene/BMS, Roche, Alexion; advisory boards: Celgene/BMS, Amgen, Novartis, Pfizer, AbbVie, Syros; speakers fee: Celgene/BMS, Novartis, Takeda. PM: advisory boards: Novartis; speakers fee: Sobi. PB: advisory boards: AbbVie; speakers fee: Novartis. JJ: research support: Novartis, BMS. President, Apps for Care and Science, nonprofit. foundation supported by Amgen, Astellas, Daiichi-Sankyo, Janssen, Olympus, Incyte, BMS, Sanofi Genzyme, Servier, Jazz, Takeda. Honoraria: Abbvie, Novartis, Pfizer, Incyte. All other authors declared no conflicts of interest.

Data sharing statement
Clinical data is available in our previously published data[1]. All other datasets analyzed during the current study are available from the corresponding author on reasonable request.

Clinical trial details: Hovon-89 clinical trial[1], EudraCT2008-002195-10, www.trialregister.nl.

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal bone marrow malignancies characterized by ineffective hematopoiesis, morphological dysplasia, peripheral cytopenias and risk of evolution to acute myeloid leukemia (AML). Patients with a very low to intermediate risk according to the Revised International Prognostic Scoring System (IPSS-R) or very low to moderate low risk in the Molecular IPSS (IPSS-M)[2], have a low risk of progression to AML[2,3]. In those patients, treatment of anemia remains challenging and therapeutic options are limited[3-5]. Although, erythropoiesis-stimulating agents (ESAs) are the first line of treatment, this is insufficient in the majority of patients[3]. For subgroups of MDS patients, specific treatment can be beneficial, for example lenalidomide in patients with an isolated chromosomal 5q deletion (del(5q)) and luspatercept in MDS with ringsideroblasts[3-5]. Del(5q)-MDS is found to be sensitive to lenalidomide, with an erythroid response (hematological improvement erythroid, HI-E) of 60-80% and a cytogenetic remission in more than 50% of patients[1,4]. Also, in non-del(5q)-MDS-patients, lenalidomide was shown to initiate HI-E, however at a much lower response rate (±25-30%)[1,4,6-8]. Previously, we have shown that high percentages of bone marrow lymphocytes and progenitor B-cells, a low number of mutations, absence of ring sideroblasts, and the absence of *SF3B1* mutations were predictive for a response to lenalidomide in low and intermediate-risk MDS patients treated within the Hovon-89 clinical trial[1]. That study was approved and registered at www.trialregister.nl; NTR1825 (former ID); NL1715 (recent ID); EudraCT 2008-002195-10; METC: 2009/50 NL25632.029.08 and conducted according to the declaration of Helsinki. Here we assessed the expression levels of genes which are associated with the working mechanism of lenalidomide, and looked for correlations with regard to response to lenalidomide.

Lenalidomide binds to the E3-ubiquitin ligase cereblon (CRBN), altering its substrate specificity (figure 1). As a result, various proteins become targets for degradation by the proteasome. Amongst the de-novo targets are transcription factors Ikaros (IKZF1) and Aiolos (IKZF3), postulated to play a role in the response to lenalidomide in multiple myeloma, and casein kinase1A1 (CSNK1A1), which was shown to play a role in MDS[9]. As CSNK1A is located on chrom5q, haploinsufficiency in combination with enhanced degradation in MDS patients may have an additive effect which could explain the good response rate of lenalidomide particularly in cases with a del(5q)[10]. Degradation of CSNK1A1 induces P53-dependent growth inhibition[9] and *TP53* mutations are associated with lenalidomide resistance[11]. Murine double minute 2 (MDM2) enhances degradation of P53 and increased expression of MDM2 limits P53-dependent growth inhibition[12]. Another proposed mechanism of action of lenalidomide in MDS is activation of the calcium- and calpain-dependent pathway[13] (figure 1). *GPR68*, which encodes a G-protein-coupled-receptor implicated in calcium metabolism is also regarded as an important modulator of sensitivity to lenalidomide. Lenalidomide was shown to induce GPR68 expression via CRBN-induced IKZF1 degradation, resulting in increased cytostolic calcium levels and activation of a calcium-dependent Calpain (CAPN1) which leads to induction of apoptosis in MDS cells. Calpastatin (CAST) is an endogenous inhibitor of CAPN1. As the *CAST* gene is located on chrom5(q15), haploinsufficiency of CAST in del(5q)-MDS may contribute to the specific sensitivity of these patients to lenalidomide[13]. We assessed the expression levels of 7 genes (*CSNK1A1*, *CRBN*, *IKZF1*, *IKZF3*, *MDM2*, *CAPN1*, *CAST*), which are associated with the lenalidomide working mechanism, in low and intermediate-risk MDS patients treated within the Hovon-89 clinical trial[1]. This prospective phase II randomized multicenter study (EudraCT2008-002195-10, www.trialregister.nl) included low and intermediate-1 risk MDS patients with anemia with hemoglobin (Hb<100g/L) or transfusion dependency. A total of 200 patients were included and for

141 patients RNA was available. All patients received lenalidomide (10 mg/day/day 1-21, with or without ESA/G-CSF) for a minimum of 6 months until loss of response or disease progression. The primary endpoint was hematological improvement erythroid (HI-E) according to the IWG criteria for MDS 2006[14]. The mean age was 69.9 years (range 38-85) and the mean follow-up time was 39.2 months (range 1.4-110.2). Of the included patients, 27 (19%) patients had del(5q)-MDS (according to the 2022 WHO definitions) and 109 (77%) had a non-del(5q), for 5 patients (4%) cytogenetics were unknown. In total, 57 of the 141 (40%) patients reached HI-E upon lenalidomide treatment, being 85% within the del(5q)-patients and 29% within non-del(5q). Responders did not vary from non-responders with respect to clinical characteristics such as age, bone marrow blasts, or peripheral blood cell counts (supplementary table 1).

mRNA expression of 7 genes (*CSNK1A1*, *CRBN*, *IKZF1*, *IKZF3*, *MDM2*, *CAPN1*, *CAST*) implicated in the two proposed working mechanisms of lenalidomide[9,13], was determined in bone marrow mononuclear cells (MNCs). MNCs were isolated using Ficoll (GE Healthcare) density gradient isolation. cDNA was generated using M-MLV reverse transcriptase (Invitrogen). Q-PCRs were performed in duplicate using two independent primer/probe mixes for all genes except CSNK1A1 (Applied Biosystems, supplementary table 2). The matched primer/probe sets had a spearman correlation between 0.70-0.93 (supplementary table 3). CT values were normalized using *ACTB* (B-actin) and the average of the normalized mRNA expression of both Q-PCR assays was used for the assessment of a potential effect on lenalidomide response. Survival curves and univariate analysis were calculated by the Kaplan-Meier method and compared using the log-rank test. Cut-off point for mRNA expression analysis was selected conducting univariate analysis on quartiles and median. Those that demonstrated the most distinct statistically significant differences in the HI-E log rank test were selected for reporting.

Of the genes investigated, *CSNK1A1*, *CAST*, *IKZF1*, *MDM2* and *CAPN1* were significantly higher expressed in non-del(5q) patients compared to del(5q) patients (supplementary figure 1). Only patients with low expression of *CAST* (lowest 25%), *MDM2* (lowest 75%) or *CAPN1* (lowest 25%) were more likely to respond to lenalidomide (figure 2A, 1-year HI-E; 53% vs 33%, p=0.058; 44% vs 21%, p=0.043; 57% vs 34%, p=0.018, respectively). This effect was retained in the non-del(5q)-group specifically, however this was not significant (figure 2B; 1-year HI-E; 40% vs 25%, p=0.135; *MDM2* 34% vs 17%, p=0.125; *CAPN1* 45% vs 26%, p=0.081). When we combined *CAST*, *MDM2* and *CAPN1* in a response probability score (RPS), each gene receiving 1 point in case of low expression, we could stratify patients into 4 groups with response rates of 16.0% in the patients with high mRNA expression in *CAST*, *MDM2* and *CAPN1* (0 points), and 62.2% response to lenalidomide in patients with low expression *CAST*, *MDM2* and *CAPN1* (3 points). Patients with 1 or 2 points had 41.7% and 50.0% response rates, respectively (figure 2C, p=0.037). Stratification by RPS showed the same pattern in non-del(5q) patients, but this did not reach statistical significance (figure 2D, p=0.165). When incorporating RPS into a cox regression multivariate model including the number of mutations and age, RPS was an independent factor for reaching HI-E (Table 1A, p=0.035), also when taking *SF3B1* mutations into the equation (Table 1B, p = 0.043).

Lenalidomide is very effective in del(5q)-MDS to diminish transfusion dependency, but also to delay the time to transfusion dependency[4,15]. However, lenalidomide has also shown benefit in a subset of non-del(5q)-MDS patients[4,8]. Previously, we have identified high percentages of bone marrow lymphocytes and progenitor B-cells, a low number of mutations, absence of ringsideroblasts, and *SF3B1* mutations as predictive variables for a response to lenalidomide[1]. Here we show the additional

impact of mRNA expression of genes involved in the molecular mechanisms of lenalidomide response. We show that differences in expression of various of these genes at diagnosis correlates with HI-E. We incorporated the 3 genes (*CAST*, *MDM2* and *CAPN1*) which showed the most impact on response to lenalidomide in univariate model into a response probability score (RPS). This score allows stratification of patients into 4 distinct groups with a significantly different probability of attaining HI-E. The best group was 3 times more likely to respond to lenalidomide compared to patients with the lowest score. The RPS was an independent predictive factor in a multivariate model containing previously identified factors including *SF3B1* mutations, which we recently identified as a novel (negative) predictive factor for lenalidomide response. In non-del(5q) patients, the score still showed the same pattern, but significance was lost probably due to the small number of patients in each score group. *TP53* mutations are correlated with poor response to lenalidomide. In our cohort we found *TP53* mutations in 6 patient, 3 with a VAF>5%. None the patient with a *TP53* mutation with a VAF>5% reached HI-E. Two of these patient had a response probability score (RPS) of 1 and one patient had a RPS of 3. The patient with an RPS of 3 also had a del(5q), which could explain the high RPS. The *TP53* mutation probably contributed to its lack of response, however the numbers are too small to make any general comments on the gene expression patterns and *TP53* mutations.

In summary, based on the proposed working mechanism of lenalidomide, we identified several markers that may help to predict response to lenalidomide in MDS patients developing a RPS. This could help to further identify patients who are more likely to respond to lenalidomide treatment. This may broaden the use of lenalidomide in a disease where therapeutic options are limited.

References

1. van de Loosdrecht AA, Cremers EMP, Alhan C, et al. Determinants of lenalidomide response with or without erythropoiesis-stimulating agents in myelodysplastic syndromes: the HOVON89 trial. Leukemia 2024;38(4):840-850.
2. Bernard E, Tuechler H, Greenberg PL, et al. Molecular International Prognostic Scoring System for Myelodysplastic Syndromes. NEJM Evid. 2022;1(7):EVIDoa2200008.
3. Bazinet A, Bravo GM. New Approaches to Myelodysplastic Syndrome Treatment. Curr Treat Options Oncol. 2022;23(5):668-687.
4. Fenaux P, Platzbecker U, Ades L. How we manage adults with myelodysplastic syndrome. Br J Haematol 2020;189(6):1016-1027.
5. Sebert M. Next-generation therapy for lower-risk MDS. Hematology Am Soc Hematol Educ Program. 2023;2023(1):59-64.
6. Toma A, Kosmider O, Chevret S, et al. Lenalidomide with or without erythropoietin in transfusion-dependent erythropoiesis-stimulating agent-refractory lower-risk MDS without 5q deletion. Leukemia. 2016;30(4):897-905.
7. Raza A, Reeves JA, Feldman EJ, et al. Phase 2 study of lenalidomide in transfusion-dependent, low-risk, and intermediate-1 risk myelodysplastic syndromes with karyotypes other than deletion 5q. Blood. 2008;111(1):86-93.
8. Sibon D, Cannas G, Baracco F, et al. Lenalidomide in lower-risk myelodysplastic syndromes with karyotypes other than deletion 5q and refractory to erythropoiesis-stimulating agents. Br J Haematol. 2012;156(5):619-625.
9. Kronke J, Fink EC, Hollenbach PW, et al. Lenalidomide induces ubiquitination and degradation of CK1alpha in del(5q) MDS. Nature. 2015;523(7559):183-188.
10. Boultwood J, Pellagatti A, Cattan H, et al. Gene expression profiling of CD34+ cells in patients with the 5q- syndrome. Br J Haematol. 2007;139(4):578-589.
11. Mossner M, Jann JC, Nowak D, et al. Prevalence, clonal dynamics and clinical impact of TP53 mutations in patients with myelodysplastic syndrome with isolated deletion (5q) treated with lenalidomide: results from a prospective multicenter study of the german MDS study group (GMDS). Leukemia. 2016;30(9):1956-1959.
12. Moll UM, Petrenko O. The MDM2-p53 interaction. Mol Cancer Res. 2003;1(14):1001-1008.
13. Fang J, Liu X, Bolanos L, et al. A calcium- and calpain-dependent pathway determines the response to lenalidomide in myelodysplastic syndromes. Nat Med. 2016;22(7):727-734.
14. Cheson BD, Greenberg PL, Bennett JM, et al. Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. Blood. 2006;108(2):419-425.
15. Diez-Campelo M, Lopez-Cadenas F, Xicoy B, et al. Low dose lenalidomide versus placebo in non-transfusion dependent patients with low risk, del(5q) myelodysplastic syndromes (SintraREV): a randomised, double-blind, phase 3 trial. Lancet Haematol. 2024;11(9):e659-e670.

Table 1 Cox regression multivariate model on achieving hematologic improvement–erythroid (HI-E)

A. Cox regression multivariate model on achieving HI-E. Included variables: number of mutations, response probability score and age (>60 years).

	Wald	df	p	HR (to achieve HI-E)
Number of mutations	13,778	4	0,008	
1 mutation	5,473	1	0,019	0,395 (0,182 - 0,860)
2 mutations	4,462	1	0,032	0,423 (0,193 - 0,927)
3 mutations	9,959	1	0,002	0,138 (0,040 - 0,472)
>3 mutations	1,458	1	0,227	0,455 (0,127 - 1,632)
Response probability score	8,598	3	0,035	
1	5,101	1	0,024	3,435 (1,177 - 10-021)
2	3,973	1	0,046	3,699 (1,022 - 13,383)
3	8,594	1	0,003	5,592 (1,769 - 17,675)
Age (>60 years)	0,079	1	0,778	0,870 (0,329-2,298)

B. Cox regression multivariate model on achieving HI-E. Included variables: *SF3B1* mutation present, response probability score and age (>60 years).

	Wald	df	p	HR (to achieve HI-E)
***SF3B1* mutation**	2,787	1	0,095	0,575 (0,300-1,101)
Response probability score	8,152	3	0,043	
1	4,616	1	0,032	3,276 (1,109 - 9,622)
2	3,804	1	0,051	3,699 (0,994 - 14,536)
3	8,076	1	0,004	5,251 (1,673 - 16,479)
Age (>60 years)	0,250	1	0,617	0,781 (0,296-2,059)

Figure 1 Proposed mechanism of lenalidomide action.
Lenalidomide binds to the E3-ubiquitin ligase Cereblon (CRBN) altering substrate specificity. As a result, several proteins are targeted for ubiquitination and subsequent degradation by the proteasome. These de novo targets include casein kinase 1A1 (CSNK1A1), Ikaros (IKZF1) and Aiolos (IKZF3) leading to TP53 activation and apoptosis. IKZF1 also has a direct inhibitory effect on G Protein-Coupled Receptor 68 (GPR68) and degradation of IKZF1 leads to increased GRP68 expression which increases cytosolic calcium levels and activation of a calcium-dependent calpain (CAPN1) which leads to apoptosis. Calpastatin (CAST) is an endogenous inhibitor of CAPN1. CK1a and CAST are both located on chromosome 5q and may therefore be expressed at lower levels in patients with a del(5q) whiuch might cause enhanced vulnerability to lenalidomide.

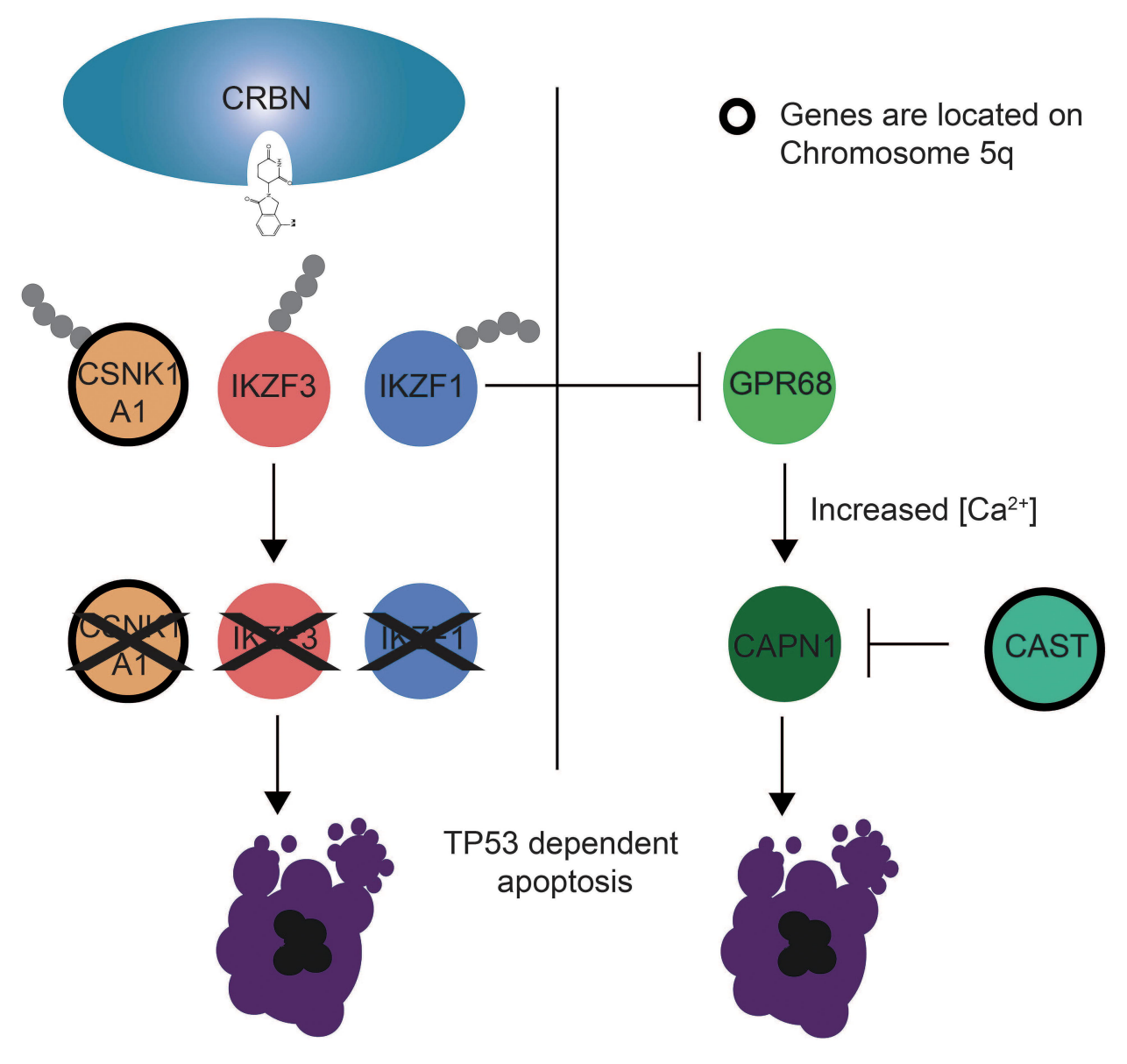
Figure 2 Hematological improvement–erythroid (HI-E) divided by mRNA expression

A. HI-E divided by *CAST* mRNA expression (lowest quartile n= 30, highest 75% n= 88), *MDM2* mRNA expression (highest quartile n= 29 , lowest 75% n= 88), *CAPN1* mRNA expression (lowest quartile n= 29 , highest 75% n= 87).

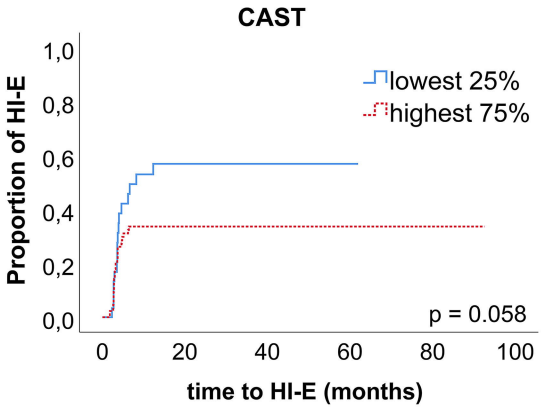
B. HI-E in non del(5q) MDS patients divided by *CAST* mRNA expression (lowest quartile n= 19 , highest 75% n= 74), *MDM2* mRNA expression (lowest 75% n= 67 , highest 25% n= 25), *CAPN1* mRNA (lowest quartile n= 19 , highest 75% n= 72). Quartiles are based on expression levels in the entire cohort.

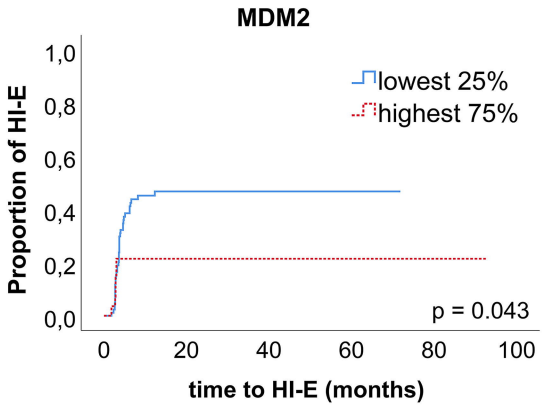
C. HI-E divided by response probability score. 0 points n= 26, 1 point n= 56, 2 points n=13, 3 points n=21.

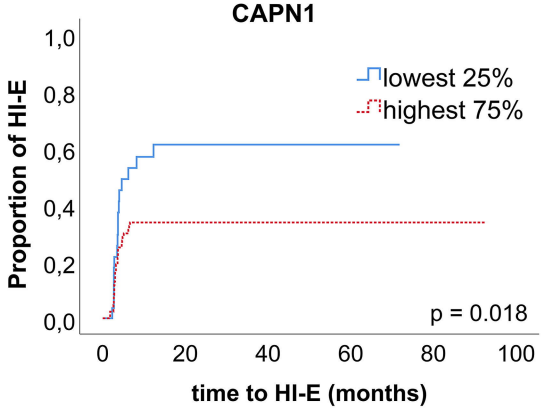
D. HI-E divided by response probability score in non-del(5q) patients. 0 points n= 23, 1 point n= 46, 2 points n= 9, 3 points n= 13.


CRBN
Genes are located on Chromosome 5q
CSNK1A1
IKZF3
IKZF1
GPR68
Increased [Ca2+]
CAPN1
CAST
TP53 dependent apoptosis

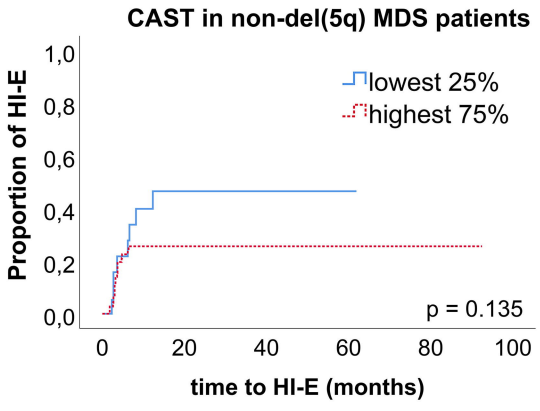
A

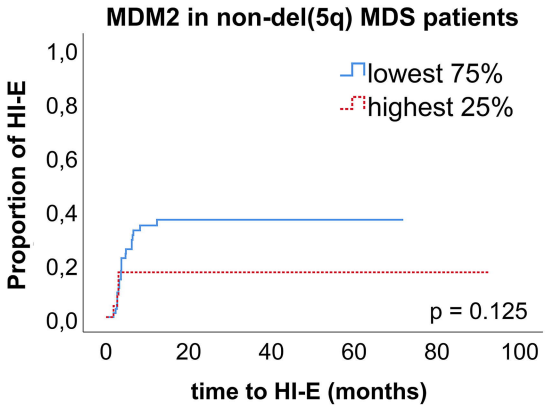

CAST
Proportion of HI-E
1,0
0,8
0,6
0,4
0,2
0,0
lowest 25%
highest 75%
p = 0.058
0
20
40
60
80
100
time to HI-E (months)

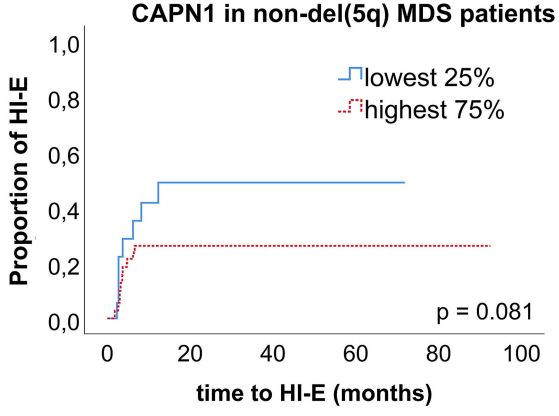

MDM2
Proportion of HI-E
1,0
0,8
0,6
0,4
0,2
0,0
lowest 25%
highest 75%
p = 0.043
0
20
40
60
80
100
time to HI-E (months)


CAPN1
Proportion of HI-E
1,0
0,8
0,6
0,4
0,2
0,0
lowest 25%
highest 75%
p = 0.018
0
20
40
60
80
100
time to HI-E (months)

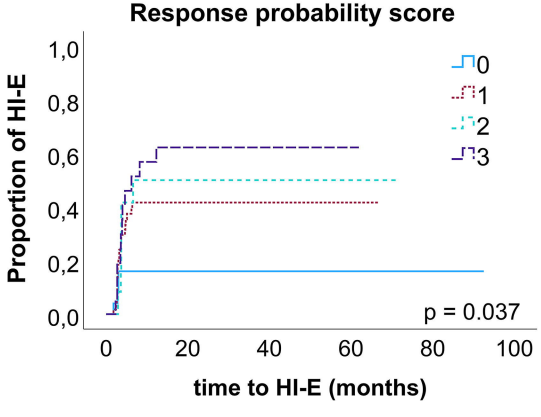
B


CAST in non-del(5q) MDS patients
Proportion of HI-E
1,0
0,8
0,6
0,4
0,2
0,0
lowest 25%
highest 75%
p = 0.135
0
20
40
60
80
100
time to HI-E (months)

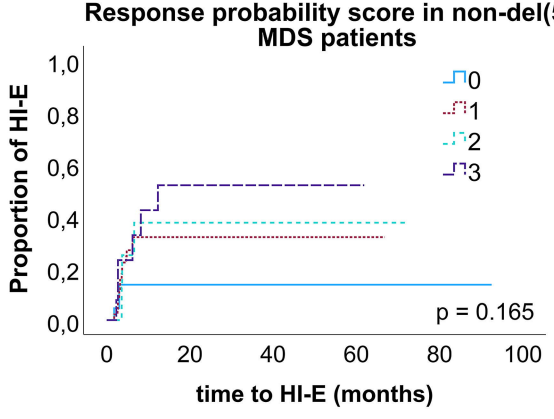

MDM2 in non-del(5q) MDS patients
Proportion of HI-E
1,0
0,8
0,6
0,4
0,2
0,0
lowest 75%
highest 25%
p = 0.125
0
20
40
60
80
100
time to HI-E (months)


CAPN1 in non-del(5q) MDS patients
Proportion of HI-E
1,0
0,8
0,6
0,4
0,2
0,0
lowest 25%
highest 75%
p = 0.081
0
20
40
60
80
100
time to HI-E (months)

C


Response probability score
Proportion of HI-E
1,0
0,8
0,6
0,4
0,2
0,0
0
1
2
3
p = 0.037
0
20
40
60
80
100
time to HI-E (months)

D


Response probability score in non-del(5q) MDS patients
Proportion of HI-E
1,0
0,8
0,6
0,4
0,2
0,0
0
1
2
3
p = 0.165
0
20
40
60
80
100
time to HI-E (months)

Supplementary data

Expression levels of genes implicated in lenalidomide's mode of action predict treatment response in lower risk myelodysplastic syndrome patients

Florentien E.M. in 't Hout[1,4], Thessa N. Scheele[1*], Theresia M. Westers[2*], Canan Alhan[2], Carolien Duetz[2], Eline M.P. Cremers[2], Heleen A. Visser-Wisselaar[3], Annelies Verbrugge[3], Dana A. Chitu[3], Bert A. van der Reijden[1], Aniek O. de Graaf[1], Arjan A. van de Loosdrecht[2*], Joop H. Jansen[1*], on behalf of the HOVON89 study group.

[1]*Department of Laboratory medicine, Laboratory of Hematology, Radboud University Medical Centre, Nijmegen, The Netherlands*

[2]*Department of Hematology, Cancer Center Amsterdam, Amsterdam UMC, Vrije Universiteit Amsterdam, The Netherlands*

[3]*HOVON Data Center, Department of Hematology, Erasmus MC Cancer Institute, Rotterdam*

[4]*Department of Hematology, Radboud University Medical Centre, Nijmegen, The Netherlands*

[*] *Equally contributed*

Supplementary Table 1 Clinical characteristics of patients who reached HI-E (responders) and patients who did not reach HI-E upon lenalidomide treatment (non-responders).
SD = standard deviation.
[a]indicates a patient with IPSS score 1.5: this patient was included in the study based on an initial diagnosis of RCMD with int-1 risk; renewed BM analysis > 3 months revealed additional chromosomal abnormalities which could be confirmed in the samples of BM at entry of the study. Patient continued in the study, but was upgraded to IPSS 1.5.

	Responders (n=57)	SD	non-responders (n = 84)	SD
Age (median)	71.2	9.8	71.8	8.3
Sex (male)	31 (54.4%)		50 (59.5%)	
IPSS score				
0	23 (40.3%)		36 (42.9%)	
0,5	24 (42.1%)		32 (38.1%)	
1	9 (15.8%)		16 (19.0%)	
1,5	1 (1.8%)[a]		0 (0.0%)	
Bone marrow blasts (%)	2.4	2.1	2.5	2.4
Hemoglobin (mean, g/dL)	8.64	1.1	8.52	1.0
White blood cell count (mean, $\times 10^9$/L)	4.8	2.7	5.1	2.9
Absolute neutrophil count (mean, $\times 10^9$/L)	2.8	2.2	3.5	3.4
Platelet count (mean, $\times 10^9$/L)	239	130.5	221	157.5

Supplementary Table 2 Primer probe sets used to quantify mRNA expression (applied biosystems).

CSNK1A1	Hs00740463_m1
CRBN	Hs00372266_m1
	Hs00372271_m1
IKZF1	Hs00958474_m1
	Hs00172991_m1
IKZF3	Hs00232635_m1
	Hs00918017_m1
MDM2	Hs01066930_m1
	Hs01066938_m1
CAST	Hs00156280_m1
	Hs00987946_m1
CAPN1	Hs00559804_m1
	Hs01548333_g1

Supplementary Table 3 Spearman correlations of mRNA expression of two independent qPCR primer/probe mixes for multiple genes proposed in the lenalidomide pathway.

Gene	Spearman r
CRBN	0.74
IKZF1	0.70
IKZF3	0.93
CAST	0.81
MDM2	0.88
CAPN2	0.92

Supplementary Figure 1 mRNA expression in del(5q) and non-del(5q) MDS patients.