Molecular predictors of response in patients with myeloid neoplasms treated with lenalidomide

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Lenalidomide (LEN) treatment leads to red blood cell transfusion independence in approximately two-thirds of anemic myelodysplastic syndrome (MDS) patients with del(5q). (ref. 1) Some patients also experience cytogenetic remissions while in others the del5q abnormality persists.(ref. 1) Furthermore, responses also occur in ~25% of MDS patients without del(5q).(ref. 2) This suggests that in addition to del(5q) other molecular lesions may also be associated with sensitivity to LEN. To date, apart from del(5q), no reliable predictive factors for LEN responsiveness have been reported, but clinically patients with early MDS, normal/low-risk cytogenetics, or anemia with preserved platelet and neutrophil counts, appear to be better LEN candidates than those with more advanced disease, complex cytogenetics and cytopenias.(ref. 3) On a molecular level, while initial attempts to prospectively identify responders based on gene expression patterns suggested response-specific signatures,(ref. 4) they have not been translated into an actionable test, and have not been validated in non-del(5q) cases.(ref. 5) Recently, the mechanism of action of LEN has been attributed to induction of ubiquitination and enhanced degradation of casein kinase 1A1 (CK1α) by the E3 ubiquitin ligase cereblon.(ref. 6)

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Conflict of Interest
MAS serves on an advisory board for Celgene, Corp. JPM has received speaking honoraria from Celgene.

To identify new biomarkers of sensitivity to LEN, using next generation sequencing, we determined whether recurrent mutations can help to predict response or resistance to LEN in patients with myeloid malignancies.

We have collected a unique cohort of patients with and without del(5) who underwent routine therapy with LEN and have adequate clinical and molecular annotation. Informed consent in accordance with Institutional Review Board-approved protocols of participating institutions was obtained for sample collection from 137 patients (Supplementary Tables 1, 2), of whom 108 patients were treated with LEN alone (Table 1) and 29 patients received LEN in combination with another agent. WHO classification (2008), IPSS-R, and International Working Group Criteria (2006) were used to define clinical characters. Patients treated at least 2 cycles of LEN were analyzed. Technical details regarding single nucleotide polymorphism array have been previously described.(ref. 7) Multi-amplicon deep sequencing (TruSeq; Illumina, San Diego, CA, USA) was performed for 45 genes most commonly mutated in MDS (Supplementary Table 3). Algorithms for identification of somatic lesions, exclusions of germ line alterations, and removal of technical artifacts were previously reported.(ref. 8) Logistic regression, Fisher's exact test and the Mann-Whitney U test were used to identify covariates that correlated significantly (two-sided α of 0.05) with response. Multivariate logistic regression models were identified using backward stepwise selection.

We identified 108 cases of myeloid neoplasms [MDS, MDS/ myeloproliferative neoplasms (MPN), or MPN] treated with LEN alone. The median duration of treatment was 5 months (range 2–66). Patients were sub-grouped into LEN-responder (n=55) and LEN-refractory (n=53) cases (Table 1). Initially, we combined isolated del(5q) (n=29), del(5q) with additional cytogenetic abnormalities (n=17), and non del(5q) (n=62) cases. Patient IPSS-R risks were grouped as either very low/low/intermediate (92%) or high/very high (8%). Hematologic improvement (HI), partial response (PR), complete response (CR, marrow CR), or any response (unknown detail) was achieved in 12 (22%), 5 (9%), 19 (34.5%), and 19 (34.5%) of the cases, respectively. In those treated with LEN alone, the del(5q) group overall response rate (ORR) was 65% (30 responded), while in non-del(5q) patients it was 40% (23 responded) (Supplementary Table 4 & 5). The relatively favorable response rate in the non-del(5q) cohort treated in these series may reflect the rational selection of likely responders based on empiric criteria, though this ORR was similar to that of previous studies.(ref. 1, 2) Analyses of clinical parameters, including age, absolute neutrophil counts, blast counts, hemoglobin and IPSS-R scores indicated that responders had significantly higher platelets, were more likely to be female, were treated for a longer period of time, had lower IPSS-R scores, and were less likely to have excess blasts (Table 1). The resultant multivariate model of ORR included as covariates platelets and the presence of isolated del(5q); using only the del(5q) cohort the covariates were MDS type and the positivity for PRC2 complex lesions. Within isolated low risk MDS cohort, del(5q) remained the only predictive covariate (Supplementary Table 6). When stratifying based on del(5q) status, in the del(5q) cohort, responders were less likely to have RAEB, and more likely to have lower IPSS-R scores, higher platelets, and a longer treatment period (Supplementary Table 4); in the non-del(5q) cohort, higher platelets and longer treatment duration also predicted response (Supplementary Table 5). When MDS patients were sub-grouped according to the

status of disease (low risk: MDS with IPSS-R very low/low/intermediate, MDS/MPN-u, and CMML-1 *vs.* high risk: MDS with IPSS-R (high/very high), all responses occurred in low risk category, responders likely to have del (5q), isolated del(5q), 5q-MDS, higher platelets and longer treatment duration, while non-responders have normal karyotype and CMML-1 (Supplementary Table 7 & Figure 1). Within cohorts defined by morphologic features such as the presence of ring sideroblasts, uni- *vs.* multi-lineage dysplasia, the responders were not enriched nor a mutational pattern emerged that would predict response (data not shown).

We next examined genomic differences between LEN responders and refractory patients. Cytogenetics, namely complex aberrations, -7/del(7q), +8, -20/del(20q), and -Y, were not associated with response to LEN but, as expected, del(5q) and isolated del(5q) was significantly associated with response: odds ratios (OR) of response in all patients were 2.1 (1, 4.7) *P*=.04 and 4.2 (1.5, 13.6) *P*=.003 for del5q and isolated del5q, respectively (Figure 1A) and 2.7 (1.2, 6.7) *P*=.02 and 5.5 (2, 19) *P*<.001 when focused monotherapy (Figure 1B & Supplementary Figure 2). Both del(5q) long (includes the commonly retained region, CRR) and short (excludes CRR) showed equal sensitivity to LEN (Figure 1A, 1B).

We also applied targeted sequencing (Supplementary Tables 3) or (in selected cases) whole exome sequencing to identify mutations (Supplementary Table 8) that constitute LEN resistance factors. Overall, focusing on OR, the number of mutational events *per* patient was not associated with responses (responder *vs.* non-responder; OR 1.8 *vs.* 2.3, *P*=.35). Subsequently, we analyzed types of alterations in all cases treated with LEN alone, or with combination therapy. *U2AF1* (OR < 0.5) was overrepresented in refractory cases, while rates for DEAD box RNA helicase combined (*DDX41, DDX54, and DHX29*), OR = 3.4 (1, 15.1) were significantly higher in responders (Figure 1A). Since the total cohort included patients with combination therapies, to avoid confounding factors, we focused our analyses on cases treated with LEN alone. *U2AF1* mutations were significantly associated with failure to respond to LEN (0% *vs.* 11%, *P* = .01; Figure 1B). When reverse analysis was performed for all the genes tested for mutations and deletions, *U2AF1* mutations were also less common among responders (0% response among $U2AF1^{MT}$ *vs.* 54% among $U2AF1^{WT}$ cases; OR < 0.8; Supplementary Figure 2) and 5/6 cases were found in non del(5q) cohort (0% response among $U2AF1^{MT}$ *vs.* 44% among $U2AF1^{WT}$ cases; OR < 1.5; Figure 1C). Previously, MDS patients with *U2AF1* mutations have been associated with a higher progression rate and poor prognosis. (ref. 9) Similarly, *TP53* mutations predict response failures suggesting that del(5q) MDS with $TP53^{MT}$ might be a clinically important molecular configuration.(ref. 10) However, 2 cases with *TP53* mutations were found in the context of del(5q) with complex karyotype (Figure 1B, C) and both, who received LEN alone, were refractory. In contrast, 3/4 (75%) *TP53* mutant or deletion cases who received LEN+Azacitidine combination showed clinical improvement (Figure 1A). When we explored additional LEN sensitivity factors in our del(5q) cohort, no individual significant somatic mutations were found (Figure 1C), though PRC2 complex genes (*EZH2, SUZ12,* and *DNMT3A*) were significantly associated with poor risk by multivariate analysis, and were consistent with the known unfavorable prognosis of *EZH2* mutations.(ref. 11) *EZH2* and *SUZ12* are components of the PRC2/EED-EZH2 complex; mutations reduce PRC2 histone methyltransferase activity.(ref. 12) Previously, *CSNK1A1* mutation has been shown to convey a poor prognosis in patients with the del(5q) abnormality,(ref. 13) but in our cohort, with a limited number of mutant

cases, no impact on response to LEN was noted (OR < 34). Recently, *DDX41* mutations have been reported to be associated with LEN sensitivity.(ref. 14) Among 7 patients with heterozygous *DDX41* mutations or deletions who received LEN alone, the response rate was 57%, OR = 1.3 (0.2, 9.4) (Supplementary Figure 2) and all *DDX41* mutants without deletions (*n*=3) were responders (Figure 1B). In the non-del(5q) cohort, DEAD box RNA helicase (*DDX41, DDX54,* and *DHX29*) mutations/deletions were significantly more common in responders (*P*=.02).

Grouping clone sizes to identify dominant likely ancestral events (*SF3B1, TET2, JAK2, GPR98*, *PRPF8, STAG2, DNMT3A,* and *ZRSR2)* did not yield significant results (Supplementary Table 9 & Figure 3). In a recent study of patients treated with LEN and erythropoietin, combined predictors that do not include mutation status were described, and analysis of clonal architecture showed effects on subclonal distribution, whereas loss of responsiveness was associated with the re-expansion of the dominant subclone.(ref. 15)

In conclusion, in addition to the presence of del(5q), gender, platelet counts, treatment period, MDS type, and IPSS-R, the *U2AF1* mutation in the entire cohort, mutations in *TP53* in the del(5q) cohort, and DEAD box RNA helicases in the non-del(5q) cohort grouped with responses to LEN.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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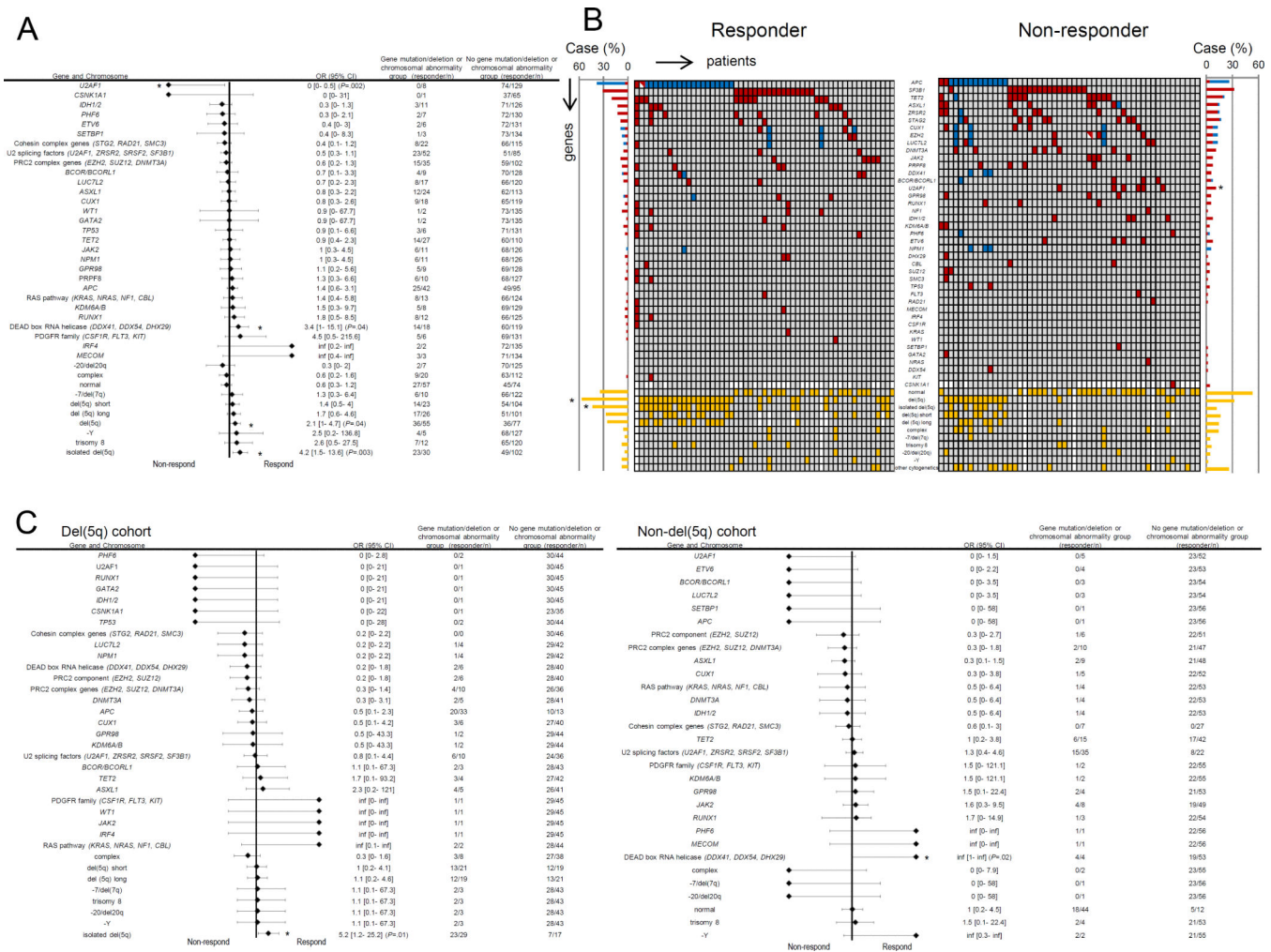
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Figure 1.
Correlations of gene mutation/deletion and cytogenetics abnormality with Lenalidomide response. (A) Response rate to LEN alone and LEN combination therapy in patients with gene mutations/deletions or cytogenetic abnormalities compared with wild type cases. (B) Spectrum of mutations and deletions in myeloid malignancy between responder (n=55) and non-responder (n=53) to Lenalidomide (LEN) monotherpay, * mutation or cytogenetic abnormality; p<.05. Red, blue, yellow, gray and white indicate mutations, deletions, cytogenetic abnormalities, wild type and no data respectively. Triangle indicated mutation +deletion of the corresponding genes affected by hypomorhic/loss of function mutations. (C) Response rate to LEN monotherapy in del(5q) and non-del(5q) patients with gene mutations/ deletions or cytogenetic abnormalities compared with wild type cases; * <.05. del(5q) short: excluding commonly retained regions (CRR; q11.1-q14.2 and/or q34-qter), del(5q) long: including CRR. Inf: infinity.

Table 1

Clinical Characteristics of patients of Myeloid malignancy treated with LEN monotherapy

	Responder	Non-responder	*P*
Sample size for sequencing	55	53	
Diagnosis			
MDS (*n*=91)	48	43	.2
Low risk (RA, RARS, RCMD, 5q-MDS)	42	29	.009 [*]
RA (FAB criteria)	8	5	.55
RARS	6	7	.78
RCMD	9	14	.24
5q-MDS	19	3	<.001 [*]
High risk (RAEB-1)	2	11	.008 [*]
MDS/MPN (*n*=10)	3	7	.2
CMML-1	0	4	.06
MDS/MPN unclassifiable	3	3	1
MPN (*n*=7)	4	3	1
Myelofibrosis	4	3	1
Demographics			
Age (*n*=40 *vs.* *n*=46)			
Median (range)	69 (20–90) years	72 (30–85) years	.95
Gender (*n*=41 *vs.* *n*=46)			
Female	25	17	.03 [*]
IPSS-R (*n*=48 *vs.* *n*=42)			
High risk (very high, high)	0	7	.008 [*]
Low risk (intermediate, low, very low)	48	35	.008 [*]
Blood counts before treatment (*n*=36 *vs.* *n*=38)			
Hemoglobin level, median (quartile 1–3)	8.6 (8–9.6) g/dl	8.9 (8.0–10.1) g/dl	.56
Neutrophil count, median (quartile 1–3)	1.8 (1.1–3.0) $\times 10^9$/l	1.8 (1.2–4.0) $\times 10^9$/l	.39
Platelet count, median (quartile 1–3)	284 (140–366) $\times 10^9$/l	85 (33–196) $\times 10^9$/l	<.001 [*]
Bone marrow (*n*=34 *vs.* *n*=37)			
Blast >5%	2	6	.15
Cyotogenetics (*n*=53 *vs.* *n*=50)			
Normal	18	26	.07
Any abnormal cytogenetics	35	24	.07
Del(5q) with or without other mutations	30	16	.02 [*]
Isolated del(5q)	23	6	<.001 [*]
Del(5q) short	13	8	.2
Del(5q) long	12	7	.2

	Responder	Non-responder	P
Complex	3	7	.19
-7/del(7q) with or without other mutations	2	2	1
Trisomy 8 with or without other mutations	4	3	1
-20/del(20q) with or without other mutations	2	2	1
-Y with or without other mutations	4	1	.36
Other	4	13	N/A
Treatment period (*n*=30 *vs.* *n*=36)			
Median (range)	12.5 (2–66) months	3.5 (2–19) months	<.001 [*]
Response			
Complete response (CR/ marrow CR)	12	N/A	N/A
Partial response (PR)	5	N/A	N/A
Hematologic improvement (HI)	19	N/A	N/A
Unknown detail	19	N/A	N/A

IPSS-R: Revised International Prognostic Scoring System, LEN: Lenalidomide, Aza: Azacytidine

MDS: Myelodysplastic syndrome, MPN: Myeloproloferative neoplasms, RA: Refractory anemia

RCUD: Refractory cytopenias with unilineage dysplasia, RARS: Refractory anemia with ring sideroblasts

RCMD: Refractory cytopenia with multilineage dysplasia, RAEB: Refractory anemia with excess blasts

CMML: Chronic myelomonocytic leukemia, MDS type is unknowen in 7 cases.

del(5q) short: excluding commonly retained regions (CRR; q11.1-q14.2 and/or q34-qter),

del(5q) long: including CRR, N/A; Not applicable,

[*] *P*<.05